



MASTERCLASS SERIES



Experiments

in

Animal

Behaviour



Cutting-edge research at trifling cost



**RAGHAVENDRA
GADAGKAR**



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INDIAN ACADEMY OF SCIENCES

Bengaluru

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Foreword

The Masterclass series of eBooks brings together pedagogical articles on single broad topics taken from *Resonance*, the Journal of Science Education, that has been published monthly by the Indian Academy of Sciences since January 1996. Primarily directed at students and teachers at the undergraduate level, the journal has brought out a wide spectrum of articles in a range of scientific disciplines. Articles in the journal are written in a style that makes them accessible to readers from diverse backgrounds, and in addition, they provide a useful source of instruction that is not always available in textbooks.

The Eighth book in the series, ‘Experiments in Animal Behaviour - Cutting-Edge Research at Trifling Cost’, is a collection of articles by Prof. Raghavendra Gadagkar, presently DST Year of Science Chair Professor at the Centre for Ecological Sciences, Indian Institute of Science, Bengaluru. These articles were originally published as a series in *Resonance* between 2018 and 2021, and provide an excellent introduction to many interesting phenomena and studies in animal behaviour while also exemplifying that cutting-edge research can often be done at relatively low cost, a consideration especially important in India. Prof. Gadagkar is a very distinguished researcher in animal behaviour, ecology and evolution, well known around the world for his research on the evolution of sociality in primitively eusocial wasps, and also the Founder President of the Indian Society of Evolutionary Biologists. He is also a former President of the Indian National Science Academy, New Delhi. His research is conceptually rich and experimentally elegant, yielding important insights with simple, creatively designed experiments. He is also very well known as a superb teacher and mentor. He was one of the founders of *Resonance* in 1996 and also served as an Associate Editor of *Resonance* for many years after its inception.

Foreword

As always, this book will be available in digital format, and will also be housed on the Academy website. Especially in view of the neglect of animal behaviour, and evolution and ecology, more broadly, as a discipline in India, it is hoped that this book will be valuable to both students and teachers as a resource with which to supplement textbook material on animal behaviour and its evolution. The book should also be useful to researchers as a convenient handbook on diverse aspects of experimental design in animal behaviour.

AMITABH JOSHI
Editor of Publications
Indian Academy of Sciences
March 2021

About the Author

Raghavendra Gadagkar is an eminent behavioural ecologist who is currently Year of Science Chair Professor (conferred by the Department of Science and Technology, India) and Honorary Professor at the Centre for Ecological Sciences, Indian Institute of Science, Bengaluru. He established an outstanding research programme at the Centre for Ecological Sciences to study insect sociobiology, which, over the last three and a half decades, has provided fascinating insights into the social evolution, social organisation, and behaviour of wasps, ants, and bees, especially the primitively eusocial wasp, *Ropalidia marginata*. These insights have resulted primarily from elegantly designed experiments, careful behavioural observations, theoretical models, and computer simulations, with genetic markers and biochemical techniques used when necessary. Therefore, it is fitting that Prof. Gadagkar has written this delightful book impressing upon the reader that cutting-edge research does not necessarily require expensive or sophisticated equipment.

Prof. Gadagkar's interest in the social behaviour of *Ropalidia marginata* began when he was an undergraduate student at Central College, Bangalore University. He completed B.Sc. (Hons) and M.Sc. in Zoology at Bangalore University, and joined the Microbiology and Cell Biology Department (MCBL) at the Indian Institute of Science (IISc), Bengaluru, in 1974 to carry out Ph.D. research on a *Mycobacteriophage*. Although he loved both molecular biology and animal behaviour, once he completed his Ph.D. in 1979, he chose to switch to animal behaviour (read the book to understand why) and has carried out world-class research using the locally present paper wasp, *Ropalidia marginata*. He demonstrated the importance of demographic factors in social evolution with his theory of Assured Fitness Returns, and the inadequacy of genetic relatedness alone in

explaining the social evolution of eusocial insects. He and his students have also found self-regulating age polyethism of workers, pheromonal inhibition of worker reproduction by queens, and seamless, conflict-free queen successions through queueing, suggesting that this wasp is more socially advanced than the typical primitively eusocial wasp.

Prof. Gadagkar's outstanding research has been recognized by numerous awards in India and abroad - the Shanti Swarup Bhatnagar Prize, B.M. Birla Science Prize, the Third World Academy of Sciences Award in Biology, and Cross of the Order of Merit of the Federal Republic of Germany – to name a few. He is an elected Fellow of the Indian National Science Academy, of which he was also President, and other Indian science academies, and International member of the National Academy of Sciences, USA, the American Academy of arts and Sciences and the German National Science Academy Leopoldina, among others. He is a Non-resident Permanent Fellow of the Wissenschaftskolleg zu Berlin. He is also Founding President and Fellow of the Indian Society of Evolutionary Biologists and has held numerous other academic and advisory positions.

Prof. Gadagkar is a teacher par excellence, cultivating logical and critical thinking and a love for science amongst students/audience in his classes and the hundreds of outreach talks he has given. Further, his book *Survival Strategies* simply and succinctly explains concepts in behavioural ecology to a wide audience. He was also involved in setting up the pedagogic journal *Resonance* to cater to undergraduate students. He has mentored a very large number of research students, many of whom have gone on to establish independent research groups. Prof. Gadagkar also pioneered the establishment of the Centre for Contemporary Studies at IISc to impart an appreciation for the interdisciplinarity of knowledge.

The present volume is a compilation of a series of articles by Prof. Gadagkar that appeared in *Resonance*. Although focused on experiments in animal behaviour, the message of the book applies to other subjects as well; all are encouraged to read it.

T N C Vidya, JNCASR, Bengaluru

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Preface

In *The Ten Most Beautiful Experiments* (2008) *, the science writer George Johnson laments that “Science in the twenty-first century has become industrialized. The experiments so often celebrated in the newspapers—sequencing the genome, proving the existence of the top quark, discovering a new planet by analyzing the wobble of a distant star—cost millions of dollars. They generate terabytes of data to be analyzed by supercomputers: calculating factories spewing so much heat that they are equipped with cooling stacks that consume the energy of small towns. The experiments are carried out by research teams that have grown to the size of corporations”.

Reviewing Johnson’s book in the *New York Times*[†] Peter Dizikes, then an independent writer, said “Johnson’s lively book nicely evokes the lost world of the tabletop experiment”, and asked “But are all remaining advances really beyond the reach of individual hands and minds, as he supposes? Might we still attribute major ideas to ingenious individuals, even if the ideas are tested by teams?”. Finally, Dizikes concluded sardonically that “Certainly, Johnson is entitled to his nostalgia. . . Still, if lone scientists rarely push knowledge forward today, they rarely impede it, either.”

I think that all of this pessimism is misplaced. Johnson’s description of 21st Century science is as true as it is wonderful and welcome. But it has one avoidable downside. It relegates the vast majority of humanity to the role of an awe-struck audience—constraining them to be consumers of knowledge without hope of becoming knowledge producers. But this need not be the case. Alongside the

*Johnson, George. 2008. *The Ten Most Beautiful Experiments*. 1st ed. New York: Alfred A Knopf. pp xi.

[†]Dizikes, Peter. 2008. “Book Review: ‘The Ten Most Beautiful Experiments’ (Published 2008).” *The New York Times*, April 16, 2008, sec. Arts.

“industrialised” science there is continuing room and need for simple, low-cost, ‘where ideas trump technology’ kind of science, that is open to all, young and old, rich and poor, professional and amateur. Simple experiments, propelled by ideas rather than technology and large grants, can be both the fountainhead as well as the cutting-edge of scientific research, well into the 21st century and beyond. The upside of this brand of science is that it can make knowledge production an inclusive and democratic enterprise and contribute toward constructing a more egalitarian world.

In the very last sentences of his book Johnson concedes that “As the twentieth century wears on, the pickings grow slimmer, with nature holding tightly to what secrets remain. The days when an unknown piece of the scaffolding could be exposed on a tabletop might be behind us. But you never know. The eleventh most beautiful experiment may be yet to come.” I would say, many more beautiful ‘tabletop’ experiments can come, if we play our cards right. My aim in this book is therefore to make cutting-edge research at trifling cost an attractive option for large numbers of scientists and convert an even larger number of aspiring scientists into practicing scientists. I have chosen the field of animal behaviour to illustrate the beauty and power of simple, low-cost experiments because this is my area of expertise. I have no doubt that similar endeavours can be made in many other areas of natural science within and outside of biology.

In this book I illustrate a number of “beautiful” experiments addressing different questions in animal behaviour, drawn from the work of many scientists, spanning from early 20th century to early 21st century. For me, a “beautiful” experiment is simple, elegant and clever, and evokes a “why didn’t I do that experiment” feeling. My choice of experiments here largely reflects my personal fascination and covers insects as well as all classes of vertebrates, fishes, frogs, snakes, birds and mammals. Five of the ten insect examples are from my own research group; throughout my career, I have tried to practice as well as espouse the cause of low-cost research. I conclude each chapter reflecting on what the experiments described can teach us more generally about the practice of science and the pursuit of low-cost research. I end with a final chapter on the importance of low-cost research for science, scientists and society and discuss the many barriers to low-cost research and how they may be overcome.

I am grateful to my friend and colleague T N C Vidya for suggesting and making it possible to publish each of the 16 chapters as stand-alone articles in *Resonance – journal of science education*, published by the Indian Academy of Sciences, Bengaluru. I thank my friend and colleague Amitabh Joshi, who is also

Preface

Editor of Publications at the Academy, for spearheading the Academy's effort in producing this book. I am delighted that, in keeping with the philosophy of low-cost, inclusive research, this book is being made available by the Indian Academy of Sciences as a free-to-download, open-access e-book in pdf and e-pub formats. The book can be downloaded from the Academy's website: https://www.ias.ac.in/Publications/e-Books/Experiments_in_Animal_Behaviour

My research, including the writing of this book has been supported by the Department of Science and Technology, The Council of Scientific and Industrial Research, The Department of Biotechnology and the Ministry of Environment and Forests, Government of India. I wrote some of the early chapters of the book in the excellent, interdisciplinary, intellectually vibrant atmosphere of the Wissenschaftskolleg zu Berlin and the rest at my home in Bengaluru, thanks to the Covid-19-induced lockdown! I thank Alok Bang, Anindita Bhadra, Anindita Brahma, Aniruddha Mitra, Annagiri Sumana, David Kikuchi, David Pfennig, Geetha Gadagkar, Harald Wolf, Hari Sridhar, Jean-Louis Deneubourg, Lars Chittka, Lee Alan Dugatkin, Mandyam Srinivasan, Maria Modanu, Michael Ryan, Nick Davies, Rüdiger Wehner, Ruchira Sen, Shakti Lamba, Souvik Mandal, Suhel Quader, Sujata Deshpande, T N C Vidya, Ullasa Kodandaramaiah and Ivan Chase for reading and commenting on one or more chapters and/or for many helpful discussions. I appreciate Milind Kolatkar for his expert help in drawing many of the figures. David W Pfennig and Nick Davies generously provided the photographs reproduced in Chapter 13 and 14, respectively. I am indebted to Thresiamma Varghese for being the chief entomologist and chief photographer of my research group. It is a pleasure to acknowledge Swarnalatha Chandran, S. Uma, Nutan Karnik and Milind Kolatkar for expert help in finding books and documents, typing, copy-editing and proof-reading. D Manjunath, S. Ganesh and Ponnanna took expert care of our study subjects in the lab and field and often of the researchers too. I am fortunate to have had the contribution of the fine artist, Shubhankar Biswas, for designing the cover and drawing the portrait of Louise Joséphine Bourgeois. I thank M Srimathi, Executive Editor and her team for producing the book with great care and competence. I appreciate the efforts of Partha P Majumder and N Maheshchandra, President and Executive Secretary respectively, of the Academy for striving to maintain the glorious academic and cultural traditions established by the founders and past Presidents of the Indian Academy of Sciences.

RAGHAVENDRA GADAGKAR
Bengaluru, January 2021

1

How Wasps Find Their Nests

In this book, I will introduce the reader to the science of ethology, somewhat indirectly by describing simple experiments, both old and new, designed to understand how and why animals behave the way they do. My emphasis will be on the design of the experiments and my goal will be to motivate readers not only to think about the design but also to come up with alternatives and improvements. Motivated readers can indeed replicate some of these experiments even if they end up replacing the study animal or the behaviours of interest with their own favourite choices. In this chapter, I describe how Niko Tinbergen – Nobel Laureate and one of the founding fathers of ethology (the science of animal behaviour) – designed remarkably simple experiments to successfully understand how digger wasps find their own nests in a complex habitat also consisting nests built by other wasps.

1.1 Ethology

I study animal behaviour and I am technically called an ‘ethologist’. Ethology, literally the study of ‘ethos’ or character, is not a very old discipline. Charles Darwin’s *The Expression of Emotions in Man and Animals* (1872) [1] may be considered as the first modern treatment of the subject. Notwithstanding the award

Resonance, Vol.23, No.8, August 2018, pp.871–884

of the Nobel Prize to three of the founders of modern ethology, Niko Tinbergen, Konrad Lorenz and Karl von Frisch in 1973, and the popular appeal of its subject matter, ethology does not always enjoy the prestige it deserves in the academia. For an aspiring ethologist, and one desirous of elevating its prestige, it is inspiring to read Peter and Jeanne Medawar, in a remarkable book entitled *A Philosophical Dictionary of Biology* [2], describe ethology in the following words:

“The word ‘ethology’ is not merely an alternate designation for the science of behaviour: it is a term that stands for a genuine revolution in biological thought. Ethology is rooted in observation of animal behaviour, an activity that only simpletons think simple. . . observation is a difficult and sophisticated process calling upon all the intellectual virtues: attention, patience, heightened awareness, caution in coming to conclusions, courage in framing expectations.”

1.2 Experiments

These words can, of course, be taken as praise, but budding ethologists would be better advised to rather take them as a challenge – to measure up to Medawars’ expectations. Let us focus on the process of observation, so elegantly described by them. I believe that we often need to perform ‘experiments’ prior to observation, to match the rigour that is being demanded. In this book, I will describe several experiments in ethology, both new and old. My focus will be on the ‘design’ of the experiments while the ethology learned in the process will be a collateral benefit. I will deliberately pick simple experiments that almost anyone can perform without requiring much instrumentation or other research infrastructure. The goal will be to use reasoning and logic rather than technology and automation and will require a passion for animals rather than for machines. I encourage readers to attempt to repeat these experiments, modifying them in any way they wish, guided by necessity and creativity, swapping the animals used and even the questions asked, with their own personal favourites [3]. As a general introduction to performing simple and elegant experiments, I encourage readers to study *Darwin’s Backyard: How Small Experiments Led to a Big Theory* by James Costa [4].

1.3 Wasps and Their Nests

Wasps are a diverse group belonging to the insect order Hymenoptera, along with ants and bees. Most wasps are solitary while a small number of them are social.

All social wasps build nests to lay eggs and raise their offspring and are carnivorous, preying on other insects or spiders (or any other meat if they can get hold of – they are known to steal meat from butchers’ shops), to feed their growing larvae. They themselves persist on the nectar and juices imbibed while masticating their prey, as they cannot ingest solid food on account of their characteristically narrow waists. Among the solitary wasps, many are egg parasitoids laying their own eggs in or on the eggs of other insects. Others lay their eggs on the larvae or adults of other insects (or spiders). Let us consider the life cycle of one such solitary wasp using the example of *Philanthus triangulum*, that was used in the experiments I will discuss in this chapter. The genus *Philanthus* consists of about 135 species that are often called the ‘beewolf’ because they usually hunt adult honeybees. Along with other wasps with similar habits, they are more generally called the ‘digger wasps’. *Philanthus triangulum*, male and female, emerge from their underground nests to begin a new life cycle. Males mate and die while the females have to do more to pass on their genes to future generations. When it is warm enough, the female wasps painstakingly and with much trial and error, locate suitably soft patches of ground and dig tunnels at angles of about 30° followed by several lateral branches that serve as brood chambers. Then they fly out to hunt adult honeybees and sting them, carefully maneuvering their posture so as not to be stung by the bees instead. They paralyze the bees with a neurotoxic venom and fill up the brood chambers with up to six honeybees per chamber, as food for their as yet unborn larvae, laying one egg per brood chamber. The brood chambers are built, stocked with prey, and supplied with an egg, sequentially so that a mother may be at work on a nest for several days. At the same time, other female *Philanthus triangulum* wasps are doing the same nearby and herein lies a problem. How does a wasp find her own unfinished nest among the many that do not belong to her? Watching many wasps rapidly fly in and out of their respective nest entrances made Tinbergen wonder. Spending what might seem like idle time outdoors and being a keen observer curious about how and why the natural world is what it is, forms the first part of being an ethologist. The second part requires the ability to ask questions, frame hypothetical answers, make predictions arising out of those answers and design simple experiments to test the predictions.

1.4 Niko Tinbergen

Niko Tinbergen, one of the founders of modern ethology and one of the recipients of the Nobel Prize in 1973 (as mentioned above), possessed all these traits. But

how did he come to possess them and how did he come to put them to good use? Here, I will quote a few passages from an essay by Tinbergen's first PhD student Gerard Baerends [5], indicating the environment that Tinbergen was born in:

“In the Netherlands, between 1930 and 1940, ethology grew from what was originally seen as a pleasant and harmless hobby, to a new biological discipline, recognized by the academic world. . . In the 1880s, coinciding with a growing awareness of the need for a more socially just society, cultural attitudes towards nature changed. Literature and the fine arts became increasingly interested in a realistic representation of nature. Writers and poets. . . and sculptors. . . began to deal with landscapes, plants and animals in a style that took as much care with the correctness of naturalistic details as with the emotional impressions felt by the observer. Entirely new methods were developed for the teaching of children in primary schools, aimed at making them aware of the life and work of people in different communities and professions, and with particular emphasis on informing urban children about rural life. . . Inspired by this atmosphere two schoolmasters. . . began writing a series of six popular books, each dealing with the life of plants and animals in a characteristic Dutch habitat and a field guide for identifying the more common animals and plants. . . As a consequence of the increasing interest in natural history, naturalist societies were formed all over the country. . . A unique feature of the Netherlands – and one that in my opinion was very important for the development of ethology in our own country – was that young naturalists, from 11 to 23 years old, formed societies of their own, quite separate from those of adults.”

1.5 Tinbergen's Experiments

Now, I will briefly describe six simple outdoor experiments performed by Tinbergen for his PhD thesis, in order to understand how the wasps located their nests [6].

Experiment 1

In the first experiment, Tinbergen tested the hypothesis that the wasps learned and remembered the visual landmarks around their nests to distinguish them from other nests. Between 8 and 10 a.m., he placed about 20 pine cones that were lying around in the general area around a wasp's nest. In the afternoon, by which time the wasp might have learned the new landmarks around its nest, he waited for the wasp to fly out on one of its hunting trips and moved the circle of cones about

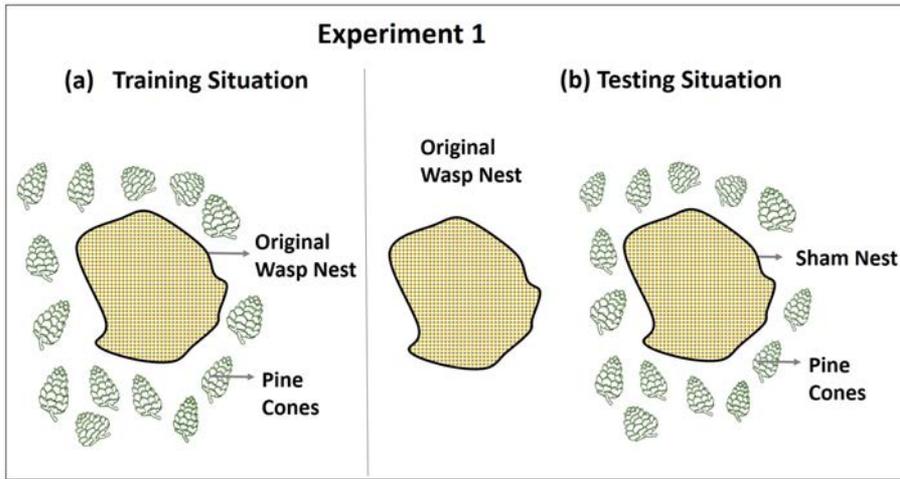


Figure 1.1 : Cartoon depicting the arrangements in Tinbergen’s first experiment. (a) Depicts the training situation during which Tinbergen had placed a circle of about 20 pine cones around the original nest in the morning. This allowed the wasp to learn these new landmarks for a few hours, during the course of its natural flights in and out of the nest. (b) Shows the test situation during which Tinbergen had moved the circle of pine cones from the original nest to a sham nest he had made about 30 cm away, leaving the original nest intact but without pine cones. Redrawn from Tinbergen (1932) (see [6]).

30 cm away from the original nest and around a sham nest made by “imitating fairly accurately the sandy spot and the slight depression indicating the (covered) entrance”, to use his own words (*Figure 1.1*).

The idea was to see whether the returning wasp would go to the real nest, now without the pine cones, or to the displaced circle of pine cones around the sham nest. If visual cues of the landmarks around the nest were guiding the homing behavior of the wasp, then she should return to the sham nest with the pine cones. But if some other cues were being used, then she should return to her own nest in spite of the missing pine cones. Since the returning wasps would make her choice only once and Tinbergen did not want to test any wasp more than once, he used a clever trick to increase his sample size. After the wasp had unambiguously demonstrated her preference for the real or the sham nest but before she actually landed and dropped the bee she was carrying, he shooed her away gently, making her fly away some distance and try again. In this manner, he made the wasp demonstrate her preference at least five times. After shooing her away once

more, he quickly relocated the circle of pine cones around the original nest and retested her preference, again several times. If visual cues were indeed involved, she should now switch her preference and go to her original nest, now with the pine cones returned. Tinbergen repeated the experiment with 17 different wasps, testing them five to twelve times each with pine cones around the sham nest (he called this the ‘experiment’), and five to six times with the pine cones returned to the original nest (he called this the ‘control’). His results (*Table 1.1*) could not have been more clear-cut. In 105 out of 105 trials, the wasps chose the sham nest when it had pine cones around it (experiment), and 86 out of 86 times, they chose the original nest when the pine cones were returned to it (control). This experiment showed clearly that the pine cones overwhelmingly decided the choice of the wasps. Tinbergen was well aware that this did not necessarily prove that the wasps had used visual cues, but only that they had used the pine cones.

Experiment 2

To rule out the possibility that the wasps had relied on the smell rather than the sight of the pine cones, Tinbergen did a second experiment. Now, during the training period, he placed along with the pine cones, two cardboard plates scented with pine-needle oil (*Oleum pini sylvestris*) that gives off smell characteristic of pine cones. The wasps could thus get accustomed to the sight of pine cones or the smell of pine cones around their nests, or both. During the test phase, he retained the scented plates around the original nest and moved only the pine cones to the sham nest. To mimic the visual cues of the scented cardboard plates he placed two identical cardboard plates but without scenting them, around the sham nest. Thus, the sham nest had all the visual cues and the original nest had the olfactory cues present during the training phase. After recording the choices of the returning wasps as in experiment 1, he created a control situation by interchanging the cues, i.e., he moved the pine cones and the unscented plates to the original nest and the scented plates to the sham nest and tested the wasps again (*Figure 1.2*). This time he used five different wasps and found that in 29 out of 29 trials, the wasps chose the sham nest when it had pine cones and unscented plates around it (experiment) and chose the original nest when it had the pine cones and unscented plates moved back to it (control) (*Table 1.2*). Clearly, the pine cones won over the scented plates. However, Tinbergen was quite aware of a potential design flaw in the experiment. The scent from the scented plates may have been too strong leading to their rejection because the wasps might have been more accustomed to the smell

How Wasps Find Their Nests

	Results of Tinbergen's Control <i>Training situation with the pine cones arranged around the original nest. See Figure 1.1 (a)</i>		Results of Tinbergen's Experiment <i>Training situation with the pine cones arranged around the sham nest. See Figure 1.1 (b)</i>	
Wasp Number	No. of times the wasp returned to the original nest	No. of times the wasp returned to the sham nest	No. of times the wasp returned to the original nest	No. of times the wasp returned to the sham nest
1	5	0	0	9
2	5	0	0	6
3	5	0	0	7
4	5	0	0	5
5	6	0	0	5
6	5	0	0	5
7	5	0	0	7
8	5	0	0	5
9	5	0	0	6
10	5	0	0	8
11	5	0	0	12
12	5	0	0	5
13	5	0	0	5
14	5	0	0	5
15	5	0	0	5
16	5	0	0	5
17	5	0	0	5
Total	86	0	0	105

Table 1.1 : The results of Tinbergen's study showing the importance of pine cones in the choice of the wasps. The original data was published by Tinbergen in his 1932 publication [6].

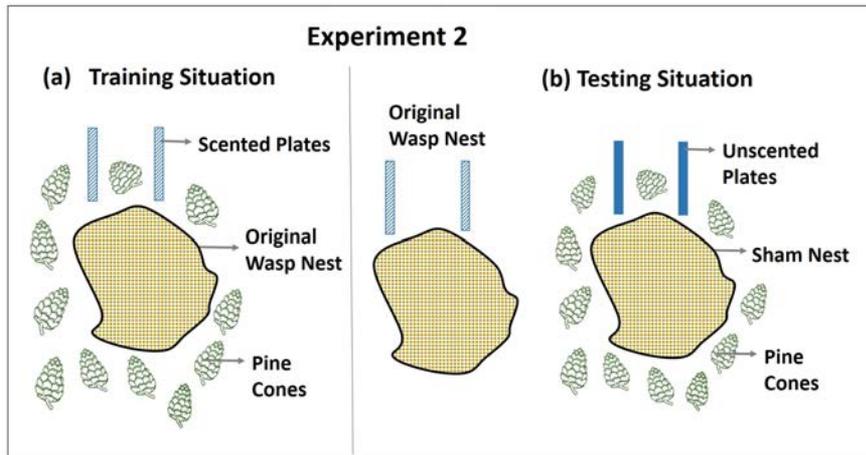


Figure 1.2: Cartoon depicting the arrangements in Tinbergen’s second experiment. **(a)** Shows the training situation during which Tinbergen placed a circle of about 20 pine cones as well as a pair of scented plates around the original nest in the morning and allowed the wasp to learn these new landmarks (visual and olfactory), for a few hours, during the course of its natural flights in and out of the nest. **(b)** Shows the test situation during which Tinbergen had moved the circle of pine cones (visual landmarks) but not the scented plates (olfactory landmarks), from the original nest to a sham nest. To make the visual landmarks around the sham nest similar to those around the original nest during the training situation, he placed an identical pair of unscented plates around the sham nest. Redrawn from Tinbergen (1932) (see [6]).

of the real pine cones. Thus, the wasps may have been sensing the real pine cones by their smell after all. The real problem with this design was that Tinbergen had set up a competition between visual and olfactory stimuli rather than eliminate one of them altogether. In the third experiment, he set out to do the latter.

Experiment 3

Tinbergen soaked the pine cones overnight in alcohol and dried them in the sun. Now he repeated experiment 1 taking care to train the wasps with fresh cones and test them with dried, presumably odourless cones. In 37 experimental trials and 30 control trials, the wasps never made a mistake – they always chose the cones. The operative phrase here is ‘presumably odourless’ which means he needed an even better experiment.

	Results of Tinbergen's Control <i>Training situation with pine cones and scented plates around the original nest and unscented plates around the sham nest. See Figure 1.2 (a)</i>		Results of Tinbergen's Experiment <i>Test situation with pine cones and unscented plates around the sham nest scented plates around and the original nest. See Figure 1.2 (b)</i>	
Wasp Numer	No. of times the wasp returned to the original nest	No. of times the wasp returned to the sham nest instead of original nest	No. of times the wasp returned to the original nest	No. of times the wasp returned to the sham nest instead of original nest
1	5	0	0	5
2	5	0	0	5
3	5	0	0	6
4	5	0	0	8
5	5	0	0	5
Total	25	0	0	29

Table 1.2 : The results of Tinbergen's study after training and testing the wasps with pine cones and scented plates. The original data was published by Tinbergen in his 1932 publication [6].

Experiment 4

With fine scissors and forceps, Tinbergen amputated both antennae of four wasps but of course only after the training period. To his delight, these antenna-less wasps flew about and performed their usual homing behaviour. In 20 experimental trials and 20 control trials, not one mistake! Wasps that had learned the presence of the cones when they possessed their antennae chose the cones even after they

had lost their antennae. It was clear that visual stimuli were enabling correct orientation of the wasps. Never throwing caution and modesty to the winds even as a PhD student, Tinbergen imposed on himself two caveats. First, these experiments may have shown that visual stimuli were adequate and even dominant, but they did not prove that the wasps were entirely incapable of using olfactory stimuli. Second, visual cues may have worked in his experiments, but he had only studied the role of visual stimuli in close proximity to the nests. How did the wasps get close enough to see the pine cones in the first place? Tirelessly, he set out to explore these caveats.

Experiment 5

To test whether the wasps could be trained to recognize their nest by odor alone, he first confirmed that the wasps could indeed smell the oil he was using. He did this by putting some oil near the nest entrance. He observed that the wasps reacted quite strikingly to the presence of oil, twitching their body and flying away for some time. Then he repeated his experiment 2 only with the scented and unscented plates and without the pine cones. During the training, he placed two scented odor plates at the original nest, and this time he trained the wasps for 2 to 3 days instead of 2 hours as before. During the experimental phase of the test he placed the scented plates around the sham nest and the unscented plates around the original nest. For the control phase, he interchanged the scented and unscented plates between the sham and original nests. He found that the wasps which appeared quite confident while landing on their nest of choice with the pine cones – be it sham or original – in all the previous experiments, now seemed a bit confused. In this experiment, the wasps, in spite of being trained with the scented plates, chose the original nest with the unscented plates and ignored the sham nest with scented plates 21 out of 24 times. In the control, they once again chose the original nest with the scented plates 19 out of 20 times. In other words, they chose their original nests, with or without the scented plates and were not distracted by the presence of the scented plates around the sham nest. Thus, it appeared that the wasps could not be trained to use odor for nest recognition.

Experiment 6

Tinbergen realized that his success in training the wasps to find their nest using visual cues is relevant only when the wasp is already close to the nest (proximate

orientation) but it doesn't explain how the wasps find the general areas where their nest is located (distant orientation). Admitting that distant orientation was very difficult to study experimentally in the field, he tried to determine the point where the distant orientation ended, and the proximate orientation began. To do this, he trained wasps with pine cones around their original nests at 30 cm as before. But in the test, he placed the cones at increasing distances around the sham nests, making bigger circles of pine cones, varying the diameter of the circle of pine cones around the sham nest from a diameter of 50 cm to up to a diameter of 200 cm (he had already tried 30 cm with success). The idea was that if the circle of pine cones was too big for proximate orientation then the wasp will not be able to find the sham nest (let us say, will not be distracted by it) and should search harder for their real nests. Although he was able to test fewer and fewer wasps at longer distances (due to bad weather), he found that the wasps always chose the sham nest with pine cones when the diameter was 50, 70 or 100 cm. But a single wasp that he was able to test at a diameter of 200 cm could not find the sham nest and went to the original nest after a long search. Tinbergen concluded that proximate orientation operated up to about 100 to 200 cm only, cautioning of course that this value will vary depending on the physical features of the environment.

Here, I will not describe the 4 other (rather inconclusive) experiments Tinbergen performed to test if the wasps used colour vision to find their nests.

In summary, Tinbergen concluded that “females of *Philanthus triangulum* are able to orient by means of visual landmarks once, through a yet unknown method of ‘distant orientation’, they have found the ‘nest surroundings’. These occupy a roughly circular area of 1–2 m diameter, within which they can be misled by displacement of the landmarks in the immediate vicinity of the nest entrance.”

1.6 Reflections

Let us now reflect on the set of six experiments as a whole. The first thing that comes to my mind is that Tinbergen vindicated Medawars' of any charge of exaggeration when they claimed that “observation is a difficult and sophisticated process calling upon all the intellectual virtues: attention, patience, heightened awareness, caution in coming to conclusions, courage in framing expectations.” There are several useful lessons to be learned from these set of experiments. Where we can emulate Tinbergen, we must do so, and where we cannot, we must at least reflect on why we cannot. Scientists, especially during the early stages of their careers, are unsure about how to choose a problem to work on. Today, science

has become such an ‘industrial’ and expensive activity that PhD students are not encouraged to and cannot afford to decide by themselves; their research problem is nearly always assigned by their thesis supervisors and research is almost always a collaboration between the student and the supervisor. But this was not how it always was and need not always be.

Tinbergen strolled around the woods and his curiosity about how the wasps managed to find their nest hole among so many others, was aroused. He framed hypotheses and proceeded to test them, designing the simplest possible experiments using what was readily available – a method that is sometimes affectionately called ‘quick and dirty’. Tinbergen did not take detailed photographs of the nest surroundings and he did not try to reproduce the exact features of the nests around the sham nests. He did not apply for a big research grant nor did he try to make his research appear sophisticated. His experiments were not more sophisticated than absolutely necessary. They were literally playful. And yet, his experiments were designed very thoughtfully, with precision and imagination, yielding clear-cut results.

Tinbergen’s six experiments illustrate how we learn from our failures. When the wasps could not be trained with scented plates, he tried with de-scented cones, and when that failed he tried with antenna-less wasps. Even when he was successful with the circle of pine cones, he kept on increasing the diameter of the cone circles until he failed to train the wasps. Unfortunately, today it has become fashionable to discard negative results.

Tinbergen’s modesty and caution come through clearly and are in stark contrast to the prevailing standards today. In his paper, he constantly refers to previous researchers, not just by way of introducing the subject but with a clear intention of giving credit where it is due. In his concluding remarks, he says less about what he has discovered and more about what he has not – “a yet unknown method of distant orientation”, “did not succeed in demonstrating colour vision”, “this in no way implies that *Philanthus* is unable to perceive colour”, “wasps orient themselves to... a complex of stimuli, which I have so far not analysed”, “attempt to train the wasps to use olfactory stimuli was not successful” (rather than that the wasps cannot learn olfactory stimuli), “these results may not apply to other digging wasps”, “my observations cannot decide whether *Philanthus* is able to register and remember the number of turns made on the way out”.

I would like to recommend that readers reflect on the design of Tinbergen’s six experiments, attempt to find flaws, come up with alternative designs which are just as good or perhaps better. In addition to reflection, I encourage my young readers

to try their hands at designing and carrying out simple experiments of their own, using animals and questions, driven by their own imagination and curiosity. In this chapter, wasps were the protagonists and they hunted and paralysed honeybees. In the next chapter, I will restore the glory of the honeybees by making them the protagonists!

Suggested Reading

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- [6] N Tinbergen, On the Orientation of the Digger Wasp *Philanthus triangulum*, *Fabr. Zs. 'Uber die vergl. Physiol.*, 16, pp.305–334, 1932. [Translated from the original German into English and published in: N Tinbergen, *The Animal in Its World: Explorations of an Ethologist 1932–1972. Vol.1, Field Studies*, Harvard University Press, Cambridge, Massachusetts, USA. pp.103–127].

2

Do Bees Have Colour Vision?

In this chapter, I will describe how the young Karl von Frisch, later to become another founding father of ethology and Nobel Laureate, defied established authority to design simple, logical and clever experiments to show that honey bees indeed have colour vision. His experiments forever changed our view of animals and also the way experiments in animal behaviour are designed. It might interest readers to know that Karl von Frisch's experiments described in this part inspired Tinbergen's experiments described in chapter 1.

2.1 The Ability to Ask Questions

Children are born with a natural curiosity and incessantly ask questions, much to the irritation of their parents and teachers. Both parents and especially teachers work overtime to kill this curiosity; indeed, our entire system of child rearing and education are willy-nilly designed to abolish curiosity. We force children to shift their focus from questions to answers. We associate social prestige with knowing answers and shame with not knowing them. We leave no room for pride in asking questions. That is the reason why our students don't ask questions in the classroom, and when pressed to say why they did not ask even though they did not know the answers, the standard reply is that they thought it might be

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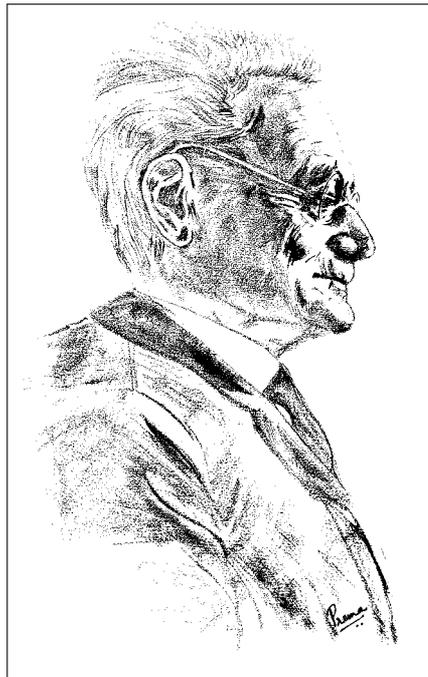


Figure 2.1 : Karl von Frisch (1886–1982, Nobel Laureate, 1973).

a stupid question, meaning the answer is already known to someone. A child (before education, if we can still find any) does not share this definition of a stupid question. My repeated assertions in the classroom that no question can be stupid by definition, and that only answers can be stupid, are often met with a polite, unconvinced smile! If there is indeed such a thing as a stupid question, it is one that is in principle, unanswerable, certainly not one that is answerable, and most certainly not one that has already been answered.

2.2 Karl von Frisch's Question

Karl von Frisch (*Figure 2.1*), Nobel laureate, another founding father of ethology, and one of the two protagonists of this second instalment of the series on how to design experiments in animal behaviour (the other protagonist being the honey bee) asked a simple question: why are flowers brightly coloured? He surmised that flowers must be brightly coloured in order to attract bees. But this was counter to

the view prevailing at that time because one of the most distinguished scientist of the time and an authority in visual science, the ophthalmologist C von Hess (indeed Sir von Hess), had concluded on the basis of his own experiments, that all invertebrates including honey bees and even fish were colour blind. For von Frisch, the logical deduction that flowers must be brightly coloured to attract honey bees and that bees must, therefore, have colour vision, was so compelling that he was unimpressed by the authority of Sir von Hess. He therefore decided to ignore established knowledge and conduct his own experiments to re-examine the question of colour vision in honey bees. Recall “courage in framing expectations” as one of the descriptors of ethology, which I quoted from *A Philosophical Dictionary of Biology* by Peter and Jeanne Medawar, in chapter 1. In 1949, Donald Griffin, famous for his discovery of echolocation in bats, then at Cornell University, USA, arranged for von Frisch to tour the USA and give a series of lectures. These lectures were subsequently published by Cornell University Press in the form of a highly readable little book entitled *Bees: Their Vision, Chemical Senses and Language* [1]. This book permits us to hear it from the horse’s mouth: “. . . von Hess asserted, fishes and invertebrates, and in particular bees, are totally color-blind. If this were true, the colors of flowers would have no biological significance. But I could not believe it, and my scepticism was the first motive which led me to begin my studies of bees about 1910. I tried to find out whether bees have a color sense” [1].

2.3 The Experiments

Although von Frisch worked on many animals during his lifetime including fish, honey bees were his most favourite study objects. It is useful to contrast the experimental designs of von Hess on the one hand and those of von Frisch on the other. Von Hess’s paper in German remains untranslated into English, and we have to, therefore, depend on other scientists who have described his experiments. Bert Hölldobler, a renowned authority on ants and who, along with Martin Lindauer (his PhD supervisor and who in turn was student of von Frisch), edited a volume in memory of von Frisch [2], gives us the essence of von Hess’s error: “Von Hess placed honey bee workers into experimental chambers, where environmental factors could be controlled, and presented them with two light spots simultaneously, each of a different colour and varying in light intensity. In this situation, the bees were invariably attracted to the brightest spot, whatever the colour was, which seemed to prove [to von Hess] that bees are colour blind and react only to different light intensities” [2].

In contrast, von Frisch ensured that he trained and tested his bees in their natural habitat. He tested them in a situation where they would be expected to be motivated to learn and to display their ability to distinguish different colours if they have the capability to do so. Ironically, von Frisch's experiment was much simpler than that of von Hess's. His experiment was as simple as his description of it. Let's listen to him again: "By the scent of a little honey it is possible to attract bees to an experimental table. Here, we can feed them on a piece of blue cardboard, for example. They suck up the food and, after carrying it back to the hive, give it to the other bees. The bees return again and again to the rich source of food which they have discovered. We let them do so for some time, and then we take away the blue card scented with honey and, put out two new, clean pieces of cardboard at the site of the former feeding place – on the left a blue card and on the right a red one. If the bees remember that they found food on blue, and if they are able to distinguish between red and blue, they should now alight on the blue card. This is exactly what happens" [1].

In addition to their simplicity and originality, von Frisch's experiments gave birth to a whole new field of study. Hölldobler says "Experimental behavioural ecology can be said to have begun around 1911, when the young Karl von Frisch wondered why flowers are colourful" [2]. But it is the simplicity of his experiments that I wish to dwell on some more here. Indeed, they are so simple that they have been repeated countless times not only to confirm von Frisch's original results but also to test the sensory and learning abilities of many animals under many different conditions. Indeed, von Frisch's experiments are a great way to train young students in designing experiments and to get them hooked to science, a much better way than merely lecturing to them in the classroom. This I can testify from personal experience.

Tenth class students in the Kendriya Vidyalaya (Central School) attached to my Institute are required to do a science project for one month in their vacation. Some of them come to my lab, and I get them to repeat von Frisch's experiment, which they do so with great enthusiasm and success. We have adapted von Frisch's experimental design to be able to work on the terrace of my laboratory with little danger of being stung by bees. We have designed a cardboard box in which we hide a bottle of sugar solution. The box has only two openings through which the bees can potentially enter to reach the sugar solution. At one of the entrances we place a blue cardboard disc, and at the other we place a green cardboard disc (*Figure 2.2*). During the training period, we keep only one of the discs, say the blue one open and the other (green) closed. Because the bees can enter through the



Figure 2.2 : Cardboard box with blue and green discs used to train and test honey bees. *Photo courtesy: Aniruddha Mitra*

blue discs and get to the sugar solution successfully, we refer to ‘blue as plus (+)’ and because they cannot do so through the green disc we refer to ‘green as minus (-)’. Following von Frisch, we train bees from a nearby hive which we maintain, to come to our feeding station rather than go to nearby real flowers. This we accomplish by adjusting the concentration of the sugar solution, to compete with the varying attractiveness of the surrounding flora. If our sugar solution is weak compared to the competition, the bees will abandon us and go elsewhere; if it is too strong, we may get too many bees. Indeed, we can get just the number of bees we want by carefully modulating the concentration of our sugar solution.

First, we place our sugar solution (feeder) close to the bee hive so that the bees find it accidentally. Then, when they are hooked, we gradually move the feeder closer to our desired place and finally into the cardboard box. I must say that such a procedure can achieve remarkable results. With care and persistence, bees can be lured to fly several kilometres, go around trees and buildings and even enter your lab through the window and alight on your microscope stage! In the current experiment, however, we only needed the bees to fly a few hundred meters and reach our feeder hidden inside the cardboard box. On reaching the target site, the bees quickly discover that they can enter through the openings provided. In

one experiment for example, we kept the opening with the blue disc open and the opening with the green disc closed: ‘blue (+) and green (-)’. Bees entered the cardboard box through the blue disc and drank the sugar solution. A great advantage of working with honey bees (of course, these are workers who do all the foraging, not the queen nor the drones) is that they are perpetually hungry as they do not immediately ingest the sugar solution all by themselves but deliver it to the waiting unloader bees back home and return for more. In fact, they also recruit more forager bees to help them transport the sugar we provide if it is attractive enough. Following several entries by the bees through the blue disc, we were ready to test them. For the test, we removed both discs and replaced them with similar fresh discs so that the bees didn’t use the smell they might have left on the discs during training. Now we closed both the discs and counted the number of times they searched at the blue and green discs respectively.

The results of one of the experiments by the students is shown in *Figure 2.3*. The upper panel represents the experiment with blue (+) and green (-), as described above. If the bees have learnt that the blue disc rather than the green gives them the reward of sugar solution, and if they can discriminate between the colours blue and green, they should search at the blue rather than the green disc during the test phase when both discs are closed. As you will see from the bar chart at the left of the upper panel in *Figure 2.3*, the bees searched 104 times at the blue disc and only twice at the green disc. Note that this is the sum total of searches by several bees and we have not here provided data for individual bees. The difference between 104 and 2 is quite obvious. Nevertheless, we can perform a statistical test, such as a chi-squared test, for example, to ascertain that the higher number of searches at the blue disc is not simply by chance. Such a test may be critically important if the numbers are less drastically different than the ones obtained here. In this case the ‘ $P < 0.001$ ’ indicated at the bottom of the bar chart means that such a large difference as that between 104 and 2 could have been obtained by mere chance, less often than 1 in 1000 trials, i.e., if the bees had actually not learned anything and were simply going to the blue and green discs randomly. Does this mean that we can safely conclude that the bees have indeed learned that blue is the rewarding colour and green is not? Now, remember another descriptor of ethology in the quote from Peter and Jeanne Medawar’s book – “caution in coming to conclusions”.

We must exercise abundant caution before concluding from this experiment that bees have learned blue versus green, let alone that they have colour vision. We know from experience that bees are very good learners and that they will learn

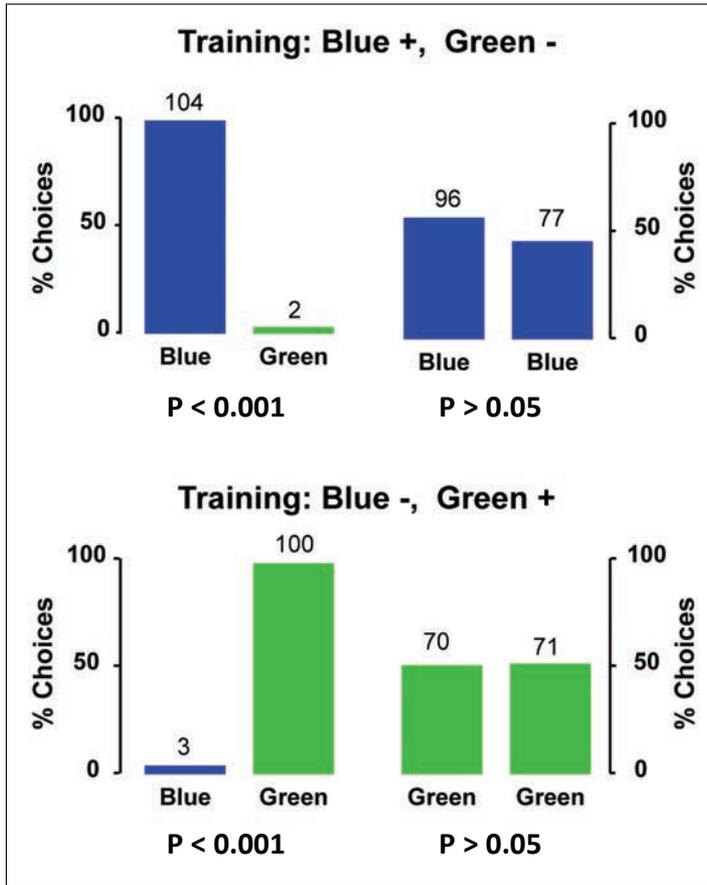


Figure 2.3: Results of experiments designed to assess colour discrimination by honey bees. See text for a detailed description.

anything that is different between the two discs, not just their colours. In this case, they might have learned, for example, that the disc on the left is rewarding and the one on the right is not rewarding. To rule out this possibility, the bees are tested repeatedly by placing closed blue discs on both sides and counting the numbers of times they search at the left and right blue discs respectively. The results plotted in the bar chart on the right side of the upper panel in *Figure 2.3*, show that the bees chose the blue disc on the left side 96 times and the blue disc on the right side 77 times. 96 is not sufficiently greater than 77, and such a difference could have

been obtained by chance alone, i.e, without the bees having learned that left is better than right. This is indicated by a similar statistical test with the result ' $P > 0.05$ ', meaning that there is a more than 5% probability that this is a chance event. There is much more than 5% probability in this case, but 5% is used as a cut-off so that we do not take anything seriously if there is more than 5% probability that it could have been obtained by chance alone. So, the bees have not merely learned left versus right but have learned blue versus green.

Can we conclude that the bees go preferentially to the blue disc because they have learned that blue is rewarding, and green isn't? What if the bees simply like blue and hate green? In that case, they would go the blue disc irrespective of the reward. So, my students repeated the experiment, this time making green rewarding and blue unrewarding. The results show that the bees trained with green positive choose green over blue, and once again not as left versus right (see bottom panel of *Figure 2.3*). Thus, when rewarded at blue, the bees choose blue and when rewarded at green they choose green. Can we finally conclude that bees have colour vision? Not quite yet, I am afraid. Von Frisch realized of course that such experiments merely demonstrate that bees can distinguish the blue and green discs from each other but this doesn't necessarily prove true colour vision. Even a colour-blind person can tell the two discs apart, just as we can tell them apart on a black and white photograph because the two discs appear as two different shades of grey. To rule out the possibility that his bees were merely distinguishing colours as different shades of grey, von Frisch performed yet another experiment. This time he trained the bees to distinguish blue colour from several shades of grey, ranging from black to white. When offered a choice between blue and different shades of grey, the bees correctly chose blue over all the other offerings. Only then did von Frisch conclude that bees have colour vision.

2.4 Happy Ending

There was a happy ending to this story but not before some more hiccups. Professor Lars Chittka, of the Queen Mary University in London, an expert in colour vision and learning abilities in honey bees and bumble bees, describes vividly the drama that followed, in his forthcoming book *The Mind of the Bee*: "... von Hess was infuriated – having caught wind of the young scientist's experiments, he rushed to publish his own account before von Frisch could even get his work into print. . . his paper was entitled 'Experimental investigations about the alleged color sense of honey bees'; in it, he blustered (in 1913): "It was possible to demonstrate

that the older claims of Lubbock. . . , as well as recent ones of von Frisch, according to which bees can be “trained” to certain colors, are wrong altogether. . . not a single fact is known that could even make plausible the notion of a color sense in bees. . . through my investigations, this assumption is terminally refuted.” [3]. Although von Frisch put up a brave front to the world, Chittka quotes from a moving letter that von Frisch wrote to his mother saying, “I have the uncomfortable feeling that I now have a real enemy in the world out there, the first one, and someone who could really damage me” [3]. There was more drama to come as von Frisch was targeted by the Nazis because his grandmother was of Jewish origin, but he was just about spared (actually his incarceration was merely planned to be postponed until the Germans won the war!) because his knowledge of honey bees was suspected to be of use in the war effort. Read more about the interplay between science, society and politics in Chittka’s forthcoming book [3]. In the end, however, the world came to accept von Frisch’s clear-cut experimental evidence, so that von Hess was all but forgotten and von Frisch was awarded the Nobel Prize.

2.5 Bees Have Trichromatic Colour Vision

Not only did von Frisch’s evidence from behavioural evidence show without doubt that bees have colour vision, but subsequent experiments by von Frisch and others showed that honey bee colour vision is quite different from that of humans, and that bees are blind to red colour. Many years later, von Frisch’s successor in the University of Munich, the physiologist H Autrum obtained direct evidence of (trichromatic) colour vision in bees in 1962 by inserting microelectrodes into single photoreceptor cells in the bees’ eyes and recorded their electrical signals in response to lights of different wavelengths. It was the first time that such a feat was accomplished in any animal. The spectral sensitivities of receptors in humans and honey bees are shown in *Figure 2.4*. While humans recognise three primary colours – red, blue and green, bees also recognise three primary colours, but these are ultraviolet, blue and green. Thus, the visible spectrum of bees is a bit shifted from that of human beings so that bees cannot see as far into the red as we humans and we cannot see UV. This has interesting consequences for our (in)ability to judge what the bees can see. When photographed with a UV filter, we can visualize that bees can see remarkable patterns on flowers some of which guide the bees straight to the pollen-bearing anthers, something to which we are blind. James L Gould said it beautifully once of Karl von Frisch: “His pioneering work inspired the discovery of several otherwise unimaginable sensory systems in

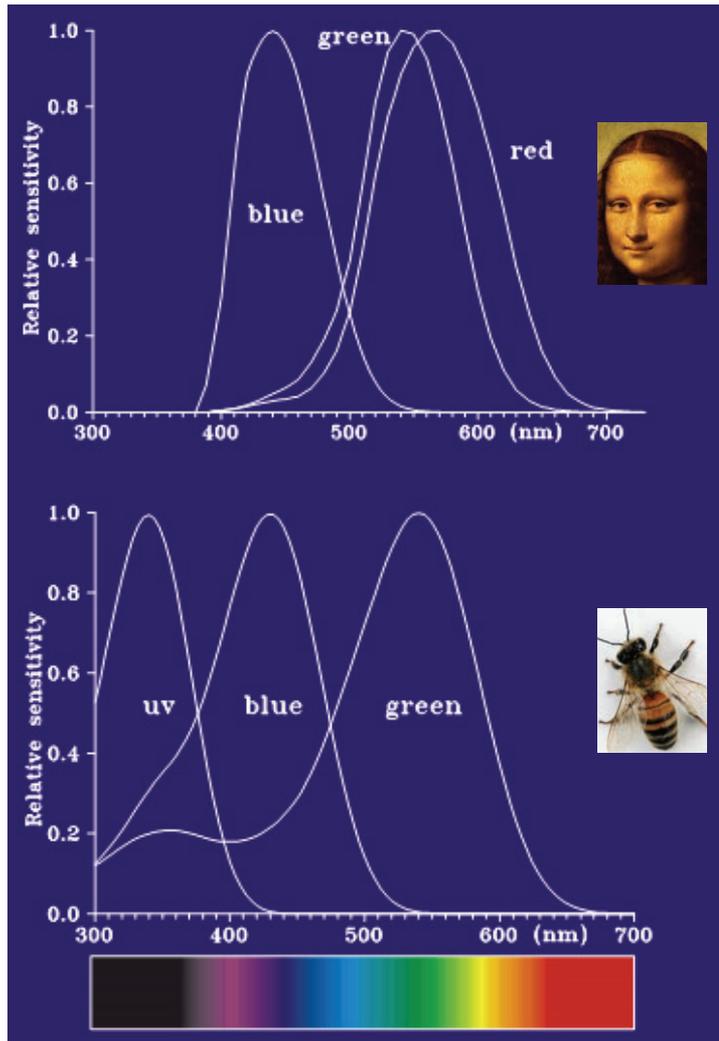


Figure 2.4: Spectral sensitivity curves of humans (top) and bees (bottom). (modified from images kindly provided by Professor Lars Chittka).

animals: infrared detectors in night-hunting snakes, ultrasonic sonar in dolphins and bats, infrasonic hearing in birds, and magnetic field sensitivity in a variety of animals. Doubtless, other systems are still to be discovered. The lesson is a melancholy one: We are blind to our own blindness, and must not try to read our



Figure 2.5 : Prof. Lars Chittka (1963–), Queen Mary University, London.

own disabilities into the rest of animal kingdom”. Autrum’s expensive and sophisticated recordings of the spectral sensitivities of the honey bee receptors are correctly regarded as the direct and final proof of honey bee colour vision, but it is von Frisch and his simple behavioural experiments that are correctly credited with the original discovery that bees have colour vision. As Professor Chittka has perceptively remarked, “. . . one should not fetishize technology – many of today’s academics, and many journal editors and funding agencies, confuse using fancy (and often expensive) technology for entirely predictable outcomes with the process of scientific discovery. To this date, many of the most important discoveries in biology are made with careful observation and the simplest experimental tools imaginable, while armies of scientists equipped with fashionable ‘omics’ tools confirm decades-old knowledge with novel methods, with marginal gains in terms of pushing the boundaries of science” [3].

2.6 Why do Bees Have Colour Vision?

Karl von Frisch asked the obvious question as to why flowers are brightly coloured and proposed the obvious hypothesis that it must be to attract honey bees, which implied that bees must have colour vision. As we have seen, he then set out to prove this hypothesis. Conversely, we can ask why bees have colour vision

and hypothesize that it must be to locate flowers efficiently and harvest pollen and nectar. The idea that colour vision in bees and brightly coloured flowers coevolved, reinforcing each other, making bees with better colour vision better at seeking out flowers and brightly coloured flowers better at attracting bees – a win-win situation – is indeed an attractive one. Research by Professor Lars Chittka has shown us that an attractive hypothesis is not necessarily a correct one [5]. By mapping the spectral sensitivities of the photoreceptors of different insects onto a phylogenetic tree of arthropods, he has shown that the spectral sensitivities of arthropod lineages that diverged from those of bees even before flowers had evolved are very similar to those of bees. In other words, bees had evolved their trichromatic colour vision even before flowers evolved. Thus, bees do not have colour vision to find flowers; instead, flowers seem to have evolved colours to match the spectral sensitivities of bees. And why bees evolved colour vision in the absence of flowers is a mystery that is waiting to be solved – take note, readers! You can read more about the evolution of colour vision in insects in a review article by Briscoe and Chittka [5].

2.7 Reflections

There are many takeaways from all that we have seen so far from Karl von Frisch's experiments and those that followed. The spotlight is on simple experiments, clever experimental designs, courage in framing expectations, caution while coming to conclusions, disrespect for authority, and not being enamoured by attractive hypotheses. I also emphasized in chapter 1 of this book that readers should not only reflect on the design of the experiments being discussed but also attempt to design, and if possible, perform their own experiments, swapping animals, questions and methods as need be. Here, I have the opportunity to demonstrate how this can indeed be done. My high school students showed the way. They have replaced coloured discs with pairs of discs with contrasting patterns, for example, a black circle on one disc and a black square or rectangle on the other, vertical stripes on one disc and horizontal stripes on the other, and have shown that the bees can efficiently learn the differences in patterns and shapes. In some years they decided to test the limits of the learning abilities of the bees. This experiment was performed in my laboratory when Professor M V Srinivasan, then at the Australian National University, Canberra, was visiting me (you will hear more about him in chapter 4). In one experiment they placed the bee hive also in a cardboard box with two openings surrounded by blue and green discs. The hive, so

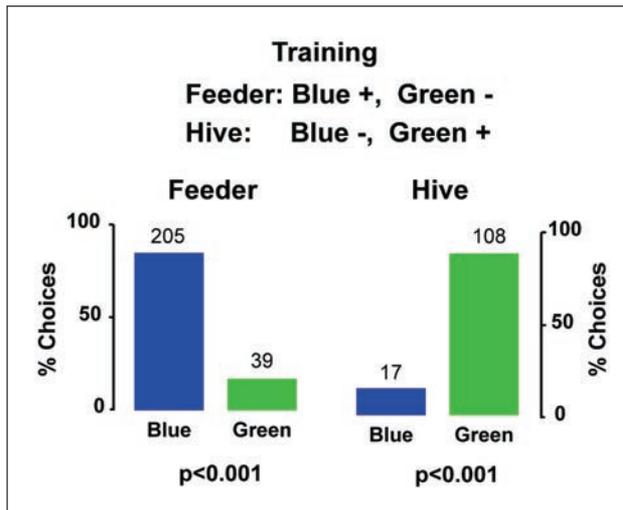


Figure 2.6: Context-dependent learning and recall. Bees can learn to choose one colour while entering the feeder and a different colour while entering their hives. See text for details.

enclosed in a cardboard box was placed only a few meters from the feeder which was, as before, enclosed in a cardboard box with blue and green discs. The bees were trained to fly back and forth between their hive and feeder. Students being mischievous by nature, decided to trick the bees. At the feeder they kept the blue open and green closed while at the hive they kept the blue closed and green open. The challenge for the bees was to choose blue over the green at the feeder and within a few seconds, choose green over blue at the hive entrance. Do bees have the ability to learn and recall the correct colours in such a context-specific manner, and that too in rapid succession? On another occasion, they enclosed only the feeder in the cardboard box with blue and green discs but kept the blue disc open and green disc closed in the morning and the blue disc closed and green disc open in the afternoon. The challenge was that the same bees which were successfully choosing blue over the green in the morning should reverse their preferences at the same feeder in the afternoon. Do bees have the ability to associate their preferences with the time of the day and make a choice after consulting their biological clocks? In both cases, the bees came out with flying colours! They can learn and recall both in a context-specific manner as well as in a time-dependent manner (*Figures 2.6 and 2.7*). This associative learning paradigm in which the bees associate something (colour in this case) with a reward, first perfected by Karl von Frisch,

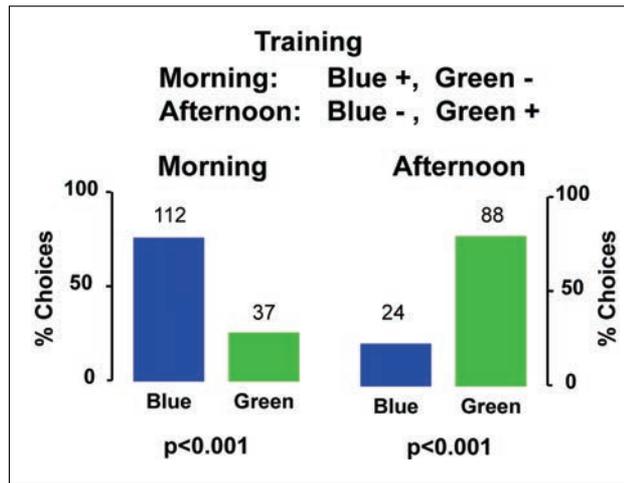


Figure 2.7: Bees can associate with different colours at different times of the day. See text for details.

has since been used thousands of times and is of boundless utility. Finally, I think you will agree that, as promised in chapter 1, I have indeed restored the glory of the honey bees!

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3

How Do Ants Find the Shortest Path?

In this chapter, I will describe how a simple, curiosity-based experiment to understand how ants are smart enough to choose the shortest path led the exploration of self-organization and swarm-intelligence and resulted in major applications in computer science and optimization algorithms. The focus will be on curiosity, simplicity, interdisciplinarity, and being unmindful of immediate applications.

3.1 The Usefulness of Useless Knowledge

In a 1939 influential essay entitled ‘The Usefulness of Useless Knowledge’, Abraham Flexner, Founding Director of the Institute for Advanced Study in Princeton, and one who recruited the likes of Albert Einstein and gave them the freedom to do what they wished, said “A poem, a symphony, a painting, a mathematical truth, a new scientific fact, all bear in themselves all the justification that universities, colleges and institutes of research need or require” [1]. In a brilliant companion essay written for the new reprint of Flexner’s essay in book form, Robert Dijkstra, the current Director of the Institute for Advanced Study in Princeton, says “Just as in Flexner’s time, the progress of our modern age, and of the world of tomorrow, depends not only on technical expertise, but also on unobstructed cu-

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riosity and the benefits—and pleasures—of traveling far upstream, against the current of practical considerations.” [2]. Nevertheless, we often hear from misguided politicians, bureaucrats, and (sadly) from scientists who happen to be doing the so-called applied research, that taxpayers’ money cannot be wasted on basic, or blue-sky research, especially in a developing country like India. This, I have come to accept as arising from political and selfish motives, rather than from ignorance. What I am more concerned about is the genuine and often very touching need, almost a moral imperative, felt by many young people to do good to the society through their scientific research. We cannot find fault with this. Unfortunately, the problem, however, is that we cannot usually predict what kind of research will do the maximum good to society. It is the unpredictability of the possible uses of knowledge that makes it necessary that we pledge to do good research of all kinds, whether its ultimate use is currently obvious or not. We must keep adding nuggets of the highest quality to the well of collective human knowledge so that we can proceed rapidly when the utility of any kind of knowledge becomes obvious, and feasible to apply, to the problems of society. How then should we choose our research topic? I will make two points. First, I think we will have a natural advantage if we follow our curiosity and work on the topics that we most love. Second, we are more likely to succeed if we choose, from among the topics we love, those that present the least external obstacles to achieving the best of our potential. For these reasons, I have chosen to illustrate the theme of how to design experiments, with examples of experiments that attempt to answer intrinsically interesting questions and at the same time require almost nothing in terms of money, equipment and other facilities. In this chapter, we will have the opportunity to also illustrate the usefulness of useless knowledge.

In a classic 1989 paper [3] that started it all, published in a somewhat obscure journal, Jean-Louis Deneubourg and his colleagues, begin by observing that “It is evident that finding the shortest route is extremely important not only for Roman road builders, thirsty rugby men and applied mathematicians working on this very problem, but also for any animal that must move regularly between different points. How can an animal with only limited and local navigational information achieve this?” So, they asked how ants find the shortest path to food – as good an example of blue-sky research as any.

3.2 Ants

Ants are probably the most familiar social insects, occurring everywhere – from the arctic tree line to the tip of South Africa and Tasmania. They are especially



Figure 3.1 : Jean-Louis Deneubourg (1951–).

familiar because they walk about on the ground, often in intriguingly disciplined moving columns. Some 13,000 species of ants have been catalogued, named and described by scientists who call themselves myrmecologists, although this number is expected to be no more than a half or a third of the number of ant species out there, waiting for you. Finding new ants without having a scientific name yet and describing them and thus adding to the well of collective human knowledge is a charming hobby, easily cultivated and one that can be especially rewarding in a poorly studied place like India. Wondering how and why they (named or not yet) move about in columns and do so many other amazing things can be equally fascinating and rewarding, enough to prevent you from ever growing out of your hobby and pursuing other ‘traditional careers’. There are many charming accounts of ant watchers that can entice you into such an adventurous life [4, 5].

3.3 The Experiments

Jean-Louis Deneubourg (*Figure 3.1*), one of the protagonists of this chapter is a theoretical chemist but has always been interested in animal behaviour. He was registered for a PhD in the Université de Bruxelles in Belgium, with the famous Viscount Ilya Romanovich Prigogine, physical chemist and Nobel laureate, famous for his work on complex systems, and sometimes called the ‘Information Philosopher’. But Jean-Louis wanted to work on ants! So he teamed up with, to use his own words, “three brilliant biologists: J M Pasteels who was the Head of our university lab working on social insects, one of his PhD students S Aron, and



Figure 3.2: Argentine ants *Linepithema humile* (previously known as *Iridomyrmex humilis*). (Image purchased from Shutterstock: <https://goo.ggl/BPa6UT>)

a British biologist working in our university S Goss. One result of our discussions is the paper Goss *et al.*” [3]. Deneubourg, being a chemist rather than a field biologist (there is room for everyone’s curiosity), was more intrigued by the columns of moving ants rather than their diversity. He chose to study the Argentinian ant, then known as *Iridomyrmex humilis* (more recently renamed as *Linepithema humile*), that has become notoriously invasive in Europe and other places, having been inadvertently introduced there by humans (*Figure 3.2*). Deneubourg and his colleagues decided to conduct laboratory experiments to understand how these ants choose the shorter of two available paths. Deneubourg says he was interested in shortcuts because he thought “time is money”, and that must be true for the ants too. Biologists would say, for ants, time is Darwinian fitness.

Thus, they put an ant colony in one box and some food in another box and connected the two boxes with a bridge with two branches – a long one and a short one – and let the ants forage. You will get an idea of the experimental setup that Deneubourg and his colleagues used from *Figure 3.3* (which shows the setup that my undergraduate students Ankan and Jadeera used in trying to repeat this experiment). As expected, the ants began by randomly using either branch but soon, nearly all the traffic was on the shorter branch. How did the ants manage to achieve this feat? A reasonable line of inquiry, and one that I think a biologist is more likely to have made is to assume that the ants measure and compare distances

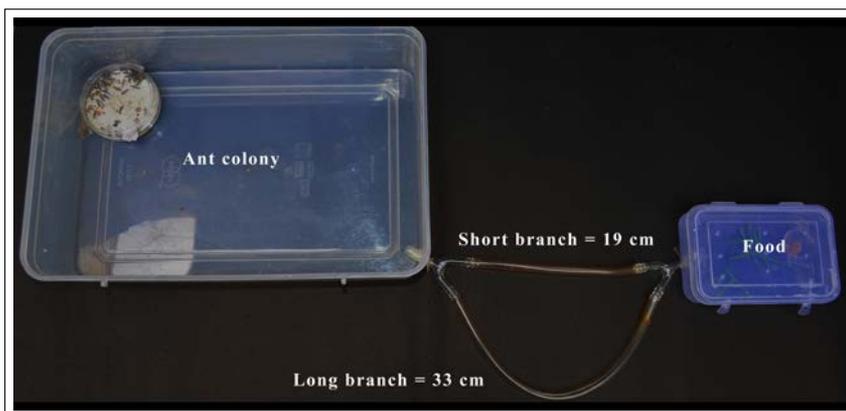


Figure 3.3: Experimental setup used by my undergraduate students Ankan and Jadeera to study if ants can choose the shorter of two paths provided between their nest and the food source. *Photo credit:* Dr. Thresiamma Varghese

and that natural selection would have selected for ants that choose the shortest path. Ants and bees can indeed measure distances, and this will be the subject of the next chapter of this book. But being a chemist, Deneubourg appears to have followed his natural instinct to pursue a physicochemical explanation, rather than a biological one, and to his credit, he appears to have persuaded his two biologist colleagues to give it a shot.

It is well known that many ant species including the Argentine ants deposit volatile chemicals, known as ‘pheromones’, as they walk from their nest to the food and from the food to the nest. This behaviour scent marks the route taken by the ants so that other naïve ants can follow the trails laid by the successful ants. This is the method by which scout ants recruit naïve ants from their colonies to efficiently transport food that they may have found, back to the nest. Deneubourg built up a logical argument (hypothesis) that did not involve any intelligence or ability to measure or compare distances. Instead, it required the ants to obey only two simple rules, unconnected to the lengths of the branches – (i) lay trail pheromones as you walk and (ii) follow the trails of others. A crucial clue to build such an argument comes from a preliminary experiment, a kind of null experiment in which they had both branches of equal length. Now, the question was whether half the ants will use one branch and the other half will use the other branch, or whether one branch will nevertheless win over and get all the traffic. These two possible outcomes will lead us to very different logical paths of hypothesis construction.

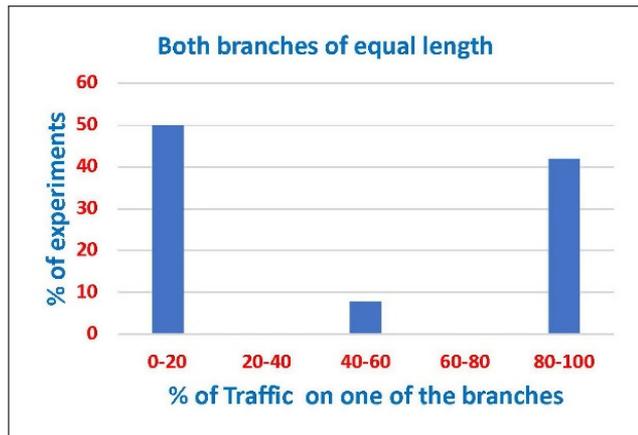


Figure 3.4: When Argentine ants are given a bridge with two equal branches, most of the traffic in most of the experiments is on or the other branch and on both branches in only a few experiments (see text for a detailed description). Adapted by permission from Springer Nature Customer Service Centre GmbH, Springer, *Naturwissenschaften*, Self-organized Shortcuts in the Argentine Ant, S Goss, S Aron, J-L Deneubourg, and J M Pasteels, 76, pp.579–581, 1989.

As it happened, one of the two branches always won over the other so that in most trials of the experiment, most of the traffic was restricted to one of the two branches, even though both branches were of the same length (*Figure 3.4*). *Figure 3.4* may seem a bit confusing so let us go over it carefully. Percentage of traffic is counted on any one of the branches, chosen randomly, say the left branch. The tall bar on left shows that 0–20% of the traffic was on the left branch in 50% of the experiments. This means that 80–100% of the traffic was on the right branch. Thus, most of the traffic in 50% of the experiments was largely restricted to one of the two branches, namely the right branch. In other words, one of the branches (the right one) had won over the left branch, even though the two branches were of the same length. The short bar in the middle shows that 40–60% of the traffic was on the left branch in only 8% of the experiments, meaning that the traffic was roughly equally divided between the two branches. The relatively tall bar on the right shows that in 42% of the experiments, 80–100% of the traffic was restricted to the left branch (the one we are counting) meaning that only 0–20% of the traffic was on the right branch. In other words, most of the traffic was restricted on one branch (the left one) in 42% of the experiments. In summary, most of the traffic was restricted to one of the two branches (either the right or the left) in 92% of

the experiments while the traffic was distributed roughly equally between the two branches only in 8% of the experiments.

So, we must look for a mechanism by which one branch wins over the other even when the two branches are of equal length. This makes us think very differently because we are no longer thinking of the biological advantage of choosing the shorter of the two paths. Deneubourg and his colleagues imagined the following scenario. In the very beginning, the ants choose randomly between the two branches. In course of time, as the ants begin to move between the nest and the food and back, repeatedly, and keep laying pheromone all the time, pheromone concentrations build upon both the branches. Due to small stochastic variations in the amount of traffic on one of the two branches, there will be small stochastic variations in the amount of pheromone on the two branches. Now, the branch with the slightly higher concentration of pheromone will be slightly more attractive to the ants leading to slightly more traffic on that branch. This will result in even higher pheromone concentration on that branch and the process will runaway due to positive feedback resulting in that branch winning completely over its counterpart. Which of the two branches will win, however, is completely random. Now let us consider the experiment in which one branch is shorter than the other. As in the previous experiment, the ants will choose one of the two branches randomly in the beginning. In this experiment, there will, of course, be more traffic per unit time on the shorter branch because ants choosing the shorter branch will reach the food sooner and return sooner and thus make more trips per unit time. Here there will be more pheromone build up on the shorter branch and through a similar positive feedback, the shorter branch will win over the longer branch. See from *Figure 3.5* that 80–100% of the traffic was on the shorter branch in 97.5% of the experiments and only in 2.5% of the experiments, was there less traffic on the shorter branch, but even here there was 60–80% traffic on the shorter branch and only 20–40% of the traffic on the longer branch. Thus, the ants can choose the shorter of the two available paths merely by each individual ant choosing two simple rules unrelated to the final outcome – lay pheromone and follow the trails of others.

Impressive, no doubt! But we have a result and we have a hypothesis that can explain the observed result. There is a correlation between the prediction of the hypothesis and the behaviour of the ants. However, correlation is not always evidence of cause and effect. There is always the possibility that the behaviour of the ants was caused by some mechanism other than what we have postulated. We, therefore, need better evidence to prove that the mechanism we postulate is

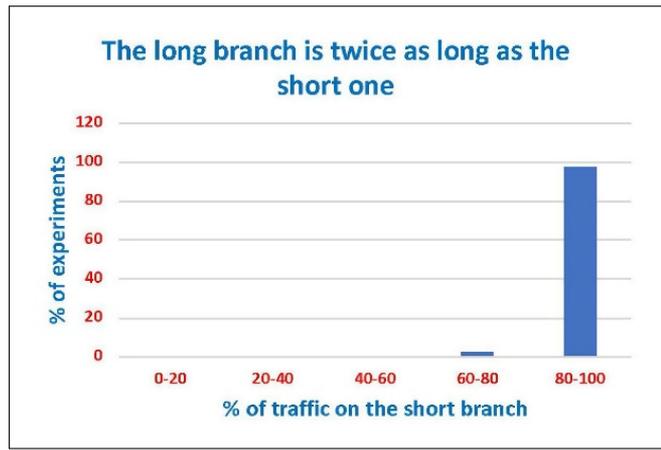


Figure 3.5: When Argentine ants are given a bridge with two unequal branches, most of the traffic in most of the experiments is on the shorter branch (see text for a detailed description). Adapted by permission from Springer Nature Customer Service Centre GmbH, Springer, *Naturwissenschaften*, Self-organized Shortcuts in the Argentine Ant, S Goss, S Aron, J-L Deneubourg, and J M Pasteels, 76, pp.579–581, 1989.

indeed at work. Deneubourg and his colleagues marshalled two further lines of evidence in support of their hypothesis. First, they built a stochastic mathematical model of differential equations which defined the probability that an ant chooses branch A (for e.g.) as:

$$(k + A_i)^n / [(k + A_i)^n + (k + B_i)^n],$$

where A_i and B_i are the numbers of ants that have previously chosen the branch A and B respectively. The parameters k and n are meant to decide how slowly or quickly one branch wins over the other and they determined that $k = 20$ and $n = 2$ best described the rate at which the short branch won over, in their experiments. Using this equation, they used Monte Carlo simulations to obtain the rate at which traffic on the short branch should increase gradually, starting from about 50% (random) to plateau at some 80–100%. This rate closely matched the corresponding rate observed in the experiments (*Figure 3.6*) [6]. Although the values of k and n were empirically determined from the experiments, the close fit between the results from the simulation of the model and the behaviour of real ants gives confidence that the simple mechanism envisaged in the model is sufficient for the ants to choose the shorter of the two branches. This is encouraging but not quite sufficient.

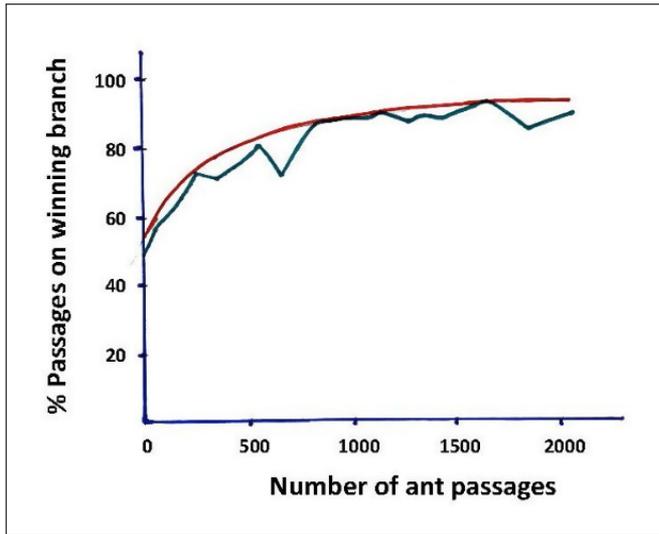


Figure 3.6: Percentage of ants passing on the winning branch (Y-axis) as a function of the total number of ants passing on the two branches put together. 20 experiments, in colonies of 150–1200 workers. The green curve represents the average values calculated for every 100 ant passages. The red curve represents the average of 200 Monte Carlo simulations, with $n = 2$ and $k = 20$. Adapted by permission from Springer Nature Customer Service Centre GmbH, Springer, *Journal of Insect Behaviour*, The Self-organizing Exploratory Pattern of the Argentine Ant, J-L Deneubourg, S Aron, S Goss, and J M Pasteels, Vol.3, pp.159–168, 1990.

Their clinching evidence came from another simple but really clever experiment. They modified the original experiment in such a way that the mechanism we have in mind will fail, and then they checked whether the ants also failed to choose the short branch. They argued that if they opened the long branch first and allowed the ants to forage using only this branch for some 30 minutes before they opened the short branch, then so much pheromone would have been built upon the long branch by the time the short branch was opened, that it would not be able to win over the long branch. This idea was also supported by their model, which showed that the short branch would not win if it was opened 30 minutes later [6]. This is precisely what they also found when they did the experiment accordingly. See from *Figure 3.7* that when the short branch was opened 30 minutes later, there was less than 50% of the traffic on the short path in more than 79% of the experiments (sum of the two left-most bars). This is clinching evidence [3].

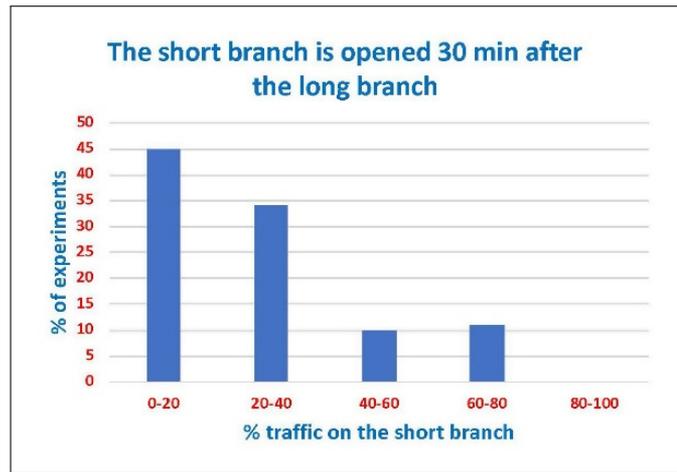


Figure 3.7: When Argentine ants are given a bridge with two unequal branches, but the short branch is opened 30 minutes after the ants have been using the long branch. Now the ants fail to choose the short branch (see text for a detailed description). Adapted by permission from Springer Nature Customer Service Centre GmbH, Springer, *Naturwissenschaften*, Self-organized Shortcuts in the Argentine Ant, S Goss, S Aron, J-L Deneubourg, and J M Pasteels, 76, pp.579–581, 1989.

Thus, a colony of ants can choose the shorter of two branches available, a task that at first appears to be cognitively challenging, by nothing more than individual ants following two simple rules, with no apparent connection to the ultimate goal. This process has come to be known as ‘self-organization’. Other telling phrases that are often used to describe such phenomena include emergent properties, bottom-up control, distributed intelligence and swarm intelligence [7, 8]. The last phrase comes from ‘a swarm of bees’, the name given to an aggregation of bees; bees, of course, are also social insects which provide many similar instances of complex behaviour arising through self-organization. Self-organization is today the preferred explanation for a variety of phenomena including, pattern formation in slime moulds and bacteria, feeding aggregations of bark beetles, synchronized flashing among fireflies, fish schooling, nectar source selection by honey bees, trail formation in ants, the swarm raids of army ants, colony thermoregulation in honey bees, comb construction by honey bees, wall building by ants, termite mound building, construction algorithms in wasps, and dominance hierarchies in paper wasps [8]. Self-organization, with its distributed intelligence and bottom-up control, appears to have permitted social insects, in spite of their small brains, to



Figure 3.8: Marco Dorigo (1961–).

accomplish extremely complex tasks. It is perhaps for this reason that they have achieved unprecedented ecological dominance and evolutionary success – some three-quarters of all animal biomass in some tropical forests is made up by ants and termites.

3.4 Enter Marco Dorigo

Marco Dorigo, now Director of IRIDIA (*Figure 3.8*), the Artificial Research Intelligence Laboratory in the University of Brussels, was a young student pursuing his PhD when Deneubourg and his colleagues were performing these, now classic, experiments about how ants choose the shorter of two paths. Marco Dorigo realized that the mechanism proposed by Deneubourg and his colleagues for the efficient behaviour of their ants lends itself to an application in developing efficient computer algorithms for solving many discrete optimization problems. The simple mechanism by which ants choose the shorter of two paths, discovered by Deneubourg and his colleagues, has since been put to mind-boggling practical use, the extent of which is hard to believe, for a biologist, even after the fact. Inspired by the behaviour of real ants as discovered by Deneubourg and colleagues, Dorigo and colleagues considered the problem of how artificial ants can move around on graphs of varying complexity, and find the shortest path between any two points. Although the behaviour of real ants is the starting point, the behaviour of the ar-

tificial ants can be made progressively more complicated as per the needs of the task. We know that even real ants can behave in more complex ways if required. For example, when presented with two different qualities of food at the same distance, ants can efficiently choose the better of the two, by a slight modification of their behaviour namely laying more pheromone when exploiting the better food source [9]. Real ants may have evolved many other additions to their behaviour, but the initial discovery of Deneubourg and colleagues was enough to set computer scientists on a path of exploration of their own by making their artificial ants increasingly complex as per the needs of their problems. Indeed, one can imagine a sort of competition between the designing of artificial ants and the discovery of the behaviours of different real ants – a healthy competition between nerds and naturalists!

Dorigo and colleagues realized that using difference equation with discrete time steps, while mimicking the behaviour of Deneubourg's ants and their differential equations, are more amenable for further development of their algorithms. The main reason for this is that while the continuous model reflects the stochastic behaviour of individual ants, the discrete model describes the average behaviour of a finite number of ants. Thus, they first developed an algorithm they called a 'simple ant colony optimization' or S-ACO and used to understand in detail, how ant colony optimization works. For a biologist, it is pleasing to see how much of the ant metaphors they have retained in exploring the workings of S-ACO. Before meaningful application, they (and by now many more computer scientists had jumped in) had to develop an ant colony optimization metaheuristic. A heuristic is a kind of self-learning strategy based on trial and error or on some very simple rules, and a metaheuristic is a master strategy that guides individual heuristics. The 'ant colony optimization metaheuristic' and related ant, wasp or bee-based algorithms have since been used to solve a number of real-life problems. In a remarkable lucid and reader-friendly textbook (even reprinted as an inexpensive 'Eastern Economy Edition' for the Indian market) [10], Marco Dorigo develops the idea of ACO from its historical roots beginning with the experiment by Deneubourg and his colleagues all the way to its development as an everyday application and shows us how ACO-based algorithms are outperforming other routing methods for the internet and telecommunication and how ant-based models have been used by South West Airlines for example, for efficient routing of their air-cargo. In *Table 2.1* of this book, they list more than 30 real-life problems including the travelling salesman problem, adaptive routing in communication networks, distributed algorithms for data clustering, dynamic resource sharing, graph

colouring, machine scheduling, vehicle routing, sequence learning, and machine learning for which ACO has been applied [10]. There are also a number of other useful and very accessible accounts of this story, going from mere curiosity to real-life applications [11].

3.5 Reflection

What are the general lessons one learns from this experiment? Clearly, the spotlight is on curiosity, simplicity, interdisciplinarity, and being unmindful of immediate applications.

Curiosity: How ants choose the shortest path is the kind of curiosity-based question that any person should be able to ask; it does not take training of any kind. Indeed, it takes training not to ask such questions or at least think that it is pointless to ask such questions and to believe and make others believe that there are better, more useful and responsible ways to spend one's time. That is the kind of training that we must try to eliminate, students can do so by staying away from such training, and educators must find ways of abolishing such training.

Simplicity: There is so much social prestige these days in sophisticated and complicated scientific research that simplicity has become unfashionable. Instead, we must create prestige for simple, inexpensive experiments, of the kind that anyone can do and anyone can understand. We must once again make it fashionable to say that simple is beautiful.

Interdisciplinarity: Another feature of modern science is that it has become very specialized, leading to the mistaken belief that it is essential to become an expert in the subject, and gain mastery of all that is already known, before you can discover new things. This is not always true. Some of the best discoveries are made precisely because of being naïve in the field one is dabbling in.

Practical Utility: Perhaps the biggest lesson we learn from these experiments is that apparently, useless knowledge can have unexpected uses. Who would have thought that understanding how ants find the shortest path can lead to a revolution in computer science! The more we make our research simple and low-cost, the more we can avoid the need to guarantee practical utility.

Suggested Reading

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4

How Do Bees Estimate the Distance Flown?

This chapter describes simple experiments that show that honey bees estimate the distance they have flown, by means of ‘optic flow’, i.e., the extent of image motion experienced by their eyes. The estimated distance is then communicated to the bees at home through the tempo of their dance (number of dance circuits in 15 s) or the duration of the waggle phase in each circuit. The experiments also provide strong evidence against the previously held view that distance is estimated by the amount of energy consumed during the flight. These experiments illustrate how cutting-edge research is possible with little or no facilities, equipment or money, by asking the right questions, optimizing the design of the experiments and regarding previously fashionable theories with an appropriate degree of scepticism.

4.1 Why are Social Insects Ideally Suited for Experiments in Animal Behaviour?

Insects, especially social insects, such as ants, bees and wasps, prominently feature in most of the experiments I will describe in this book. And there are very good reasons for this. Insects are everywhere, easy to observe and handle, and luckily, no one cares very much if you do some playful experiments with them.

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They are, therefore, ideally suited to fulfil the objectives of this book: doing simple, curiosity-driven experiments with little or no expense. Social insects are even better – they live in groups, often very large groups, facilitating experiments that require many trials and large samples. Besides, social insects construct elaborate nests in which they spend most of their time, store food and rear their young. For this reason, they regularly travel to and away from their nests in a predictable manner – they are tellingly called ‘central place foragers’. Not surprisingly, social insects have helped us understand how insects and other animals perceive the world around them and respond in adaptive ways. Honey bees and ants have been especially helpful in this regard, and in the next two chapters, we will see how they have helped us understand how animals can estimate the distance they have travelled. In this chapter, we will see how simple experiments have helped us understand how honey bees estimate the distance they have flown, and in the next chapter, we will see how equally simple experiments have helped us understand how ants estimate the distance they have walked.

4.2 How do Honey Bees Assess How Far They Have Flown?

We saw in the second chapter that Karl von Frisch discovered that honey bees have colour vision. von Frisch is even more famous for discovering that by means of a dance-language, honey bees can communicate the distance and direction of the food source they have found to naïve bees back home. The better known aspect of the honey bee dance language is that the bees determine the direction of the food source by measuring the angle subtended at the hive, between the azimuth of the sun and the source of food, and convey this angle to bees at home as the angle at which they perform their waggle dance, relative to the line of gravity [1]. The less-celebrated aspect of the dance-language is that the bees somehow estimate the distance they have flown and communicate that too, by the duration of their waggle dance. von Frisch produced a classic graph plotting the distance from which he had trained his bees to seek a reward, against the waggle duration [2]. He measured the waggle duration (or the inverse of it) by simply counting the number of dance circuits the bees performed in a 15-second interval, a level of accuracy that was both feasible and adequate, at the time. Today, and as we shall see below, we can measure waggle duration much better and much more precisely but as even Aristotle knew, “It is the mark of an instructed mind to rest satisfied with the degree of precision which the nature of the subject permits and not to seek an exactness where only an approximation of the truth is possible”, or sufficient,

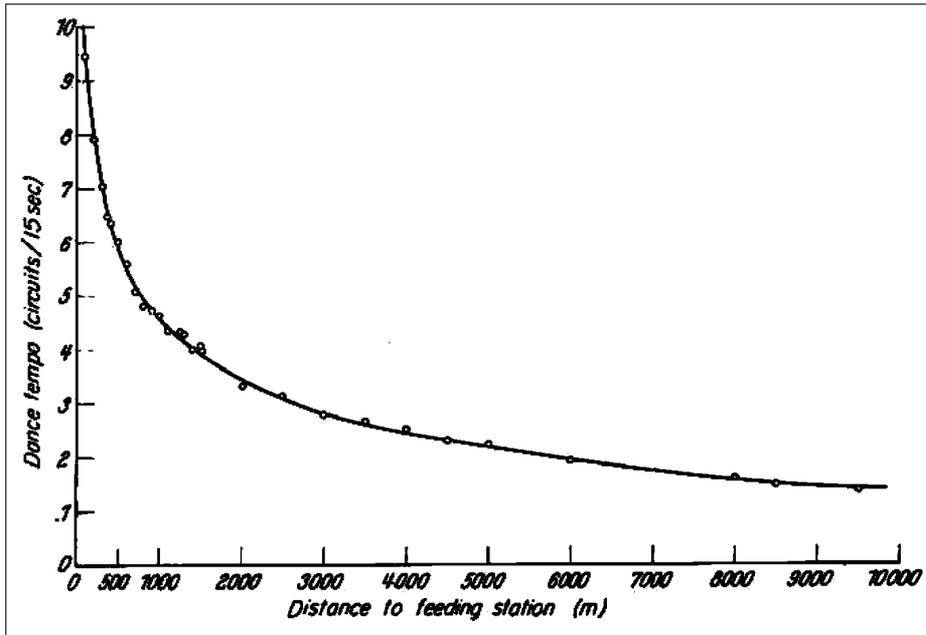


Figure 4.1: The tempo of bee dances decreases with increasing distance being conveyed. Reproduced with permission *The Dance Language and Orientation of Bees* by K von Frisch, translated by Leigh E Chadwick, Cambridge, Mass.: The Belknap Press of Harvard University Press, Copyright 1967, 1993 by the President and Fellows of Harvard College.

I would add, von Frisch termed the number of dance circuits in 15 seconds as the ‘tempo’ of the dance and showed that the tempo of the dance decreased with the distance of the food source (*Figure 4.1*). This inverse relationship between the tempo and distance makes sense as it conveys a sense of urgency and greater enthusiasm for nearby sources of food.

But how do bees estimate the distance they have flown, in the first place? von Frisch observed that bees flying against the wind indicated longer distances, while those flying with the wind indicated shorter distances, and those flying on windless days indicated intermediate distances. He (and others) also observed that bees with lead weights glued to their backs indicated longer distances compared to the normal bees. From these observations, von Frisch proposed the ‘energy hypothesis’ suggesting that bees estimate the distance they have flown by the energy expended in flying to the source of food. Many subsequent experiments, however,

have failed to uphold the energy hypothesis. Nobel laureates can also be wrong of course. We must remember that science is always a work in progress and that all conclusions are provisional. Anyone, however great or small, can call to question, anything in science, using the scientific method, at any time. And so, others have called von Frisch's energy hypothesis into question and proposed and supported an alternate hypothesis, the so-called 'optic flow hypothesis'.

4.3 What is the Optic Flow Hypothesis and How Do We Know It Works?

The optic flow hypothesis states that bees estimate distance by the image motion experienced by them, i.e, by measuring the extent to which the image of the surrounding landscape has moved on their eyes. This is an attractive, almost common-sense idea. A good way to illustrate this is to recall what Sherlock Holmes told Watson in *Silver Blaze*, a remarkable tale of Holmesian deduction in the collection, *The Memoirs of Sherlock Holmes* by Sir Arthur Conan Doyle. Let me quote the relevant passage:

"And so, it happened that an hour or so later I found myself in the corner of a first-class carriage flying along en route for Exeter, while Sherlock Holmes, with his sharp, eager face framed in his ear-flapped travelling-cap, dipped rapidly into the bundle of fresh papers which he had procured at Paddington. We had left Reading far behind us before he thrust the last one of them under the seat and offered me his cigar-case."

"We are going well," said he, looking out the window and glancing at his watch. "Our rate at present is fifty-three and a half miles an hour." "I have not observed the quarter-mile posts," said I. "Nor have I. But the telegraph posts upon this line are sixty yards apart, and the calculation is a simple one."

The idea of estimating the distance from image motion has the added advantage of being independent of the speed of the flight. But do the bees actually do this? Can they do this? Interestingly, it is von Frisch who first seems to have considered 'optic flow' as a hypothesis. He observed that bees flying over a water surface which is unlikely to give as strong an optic flow as the ground or vegetation indicated a shorter than expected distance. Nevertheless, he favoured the energy hypothesis over the optic flow hypothesis but without actually ruling out the latter from playing at least 'a modest part in estimation of the distance', as he put it. The optic flow hypothesis was formally and more confidently proposed by Herold E Esch and John E Burns of the University of Notre Dame in 1996 [3]. An early experiment that supported the optic flow hypothesis showed that bees from hives

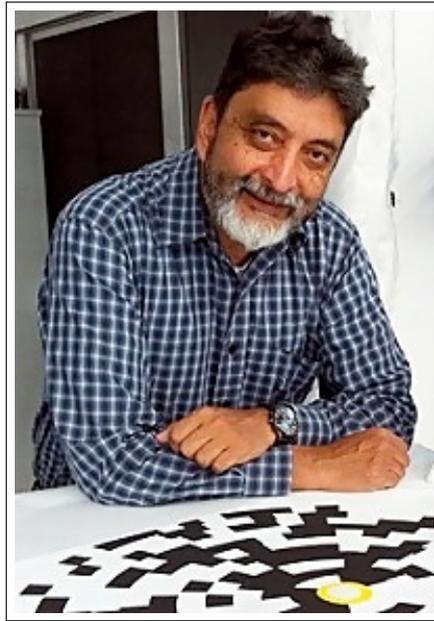


Figure 4.2 : Mandyam Veerambudi Srinivasan (1948–). *Image Credit:* M V Srinivasan.

placed on one tall building foraging from feeders placed on another tall building indicated much shorter distances than bees flying a similar distance at the ground level. Bees flying at a higher altitude would be expected to experience much less optic flow on account of the ground and vegetation being farther away. Esch and Burns [3] provide an interesting survey of various experiments performed over the years, to test the energy hypothesis, and explain the circumstances under which they proposed the optic flow hypothesis.

4.4 Definitive Experiment

The definitive experiment to test the optic flow hypothesis was performed by Mandyam Srinivasan (*Figure 4.2*) and his colleagues at the Australian National University, Canberra. Mandyam Srinivasan, who studied electrical engineering for his BSc from Bangalore University and MSc from the Indian Institute of Science, Bangalore, has performed some of the simplest and most elegant experiments on honey bees, and the one I will describe below is exceptional even by his



Figure 4.3 : The simple and elegant experiment by Srinivasan *et.al* made it to the cover of *Science* magazine (see [4]). *Credit:* Coverpage, *Science*, Vol.287, No.5454, 2000.

standards. Notice that the experiment in which the bees had to fly from one tall building to another, the optic flow was reduced resulting in their underestimation of the distance. Srinivasan and colleagues increased the optic flow by training their bees to fly through a narrow tunnel lined with a random visual texture and asked if the bees overestimated the distance. They constructed a wooden tunnel 6.4 m long, 11 cm wide and 20 cm high. While the floor and the two walls of the tunnel were lined with a random visual texture, the top of the tunnel was covered with a black insect-screen cloth so that the bees had a view of the sky, and the experimenters could observe the bees from the top. The far end of the tunnel was closed so that the bees could only enter and leave from the end closer to the hive. This is all the experimental apparatus they needed, and they performed just four experiments. For each experiment, they trained about 6 individually marked bees to fly into the tunnel, collect a reward of sugar solution and return to their hive (Figure 4.3).

In the first experiment, they placed the tunnel described above, 35 m away from the hive (see Figure 4.4 for a diagrammatic representation of the 4 experiments). Although the tunnel was 6.4 m long, the feeder from where the bees

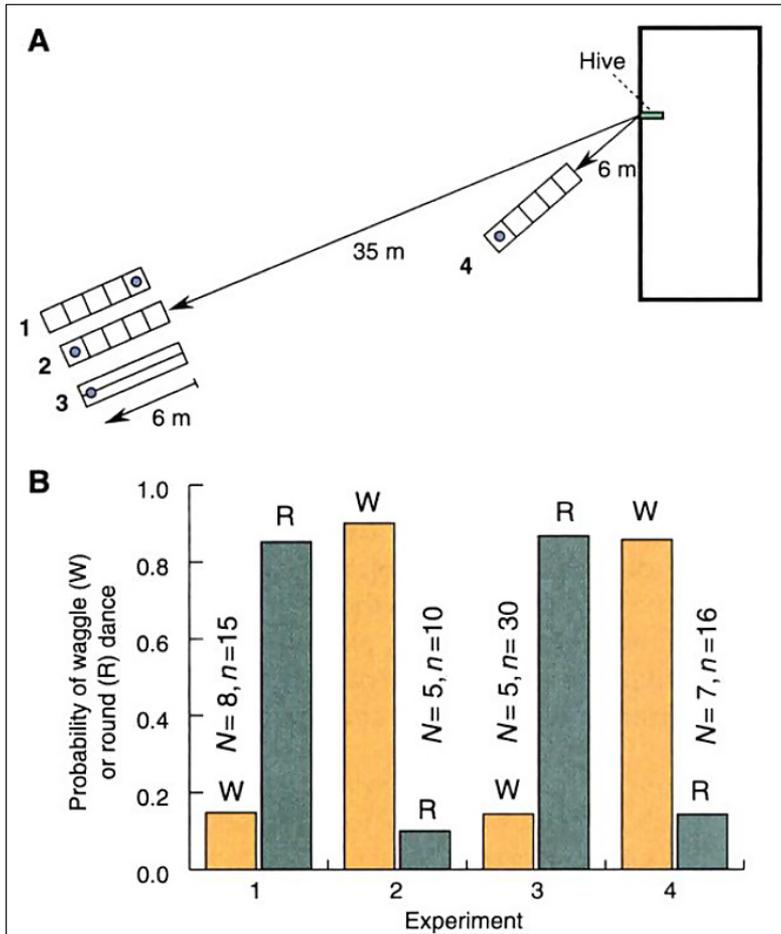


Figure 4.4: Four experiments using tunnels. **(A)** Layout for experiments using tunnels. Each tunnel represents a separate experiment (1, 2, 3, or 4). The dot in the tunnel shows the position of the feeder in each case. **(B)** Probability of waggle (W) or round (R) dance for experiments 1 to 4. N and n represent the numbers of bees and dances analyzed, respectively, in each experiment. Reproduced with permission from Mandyam V Srinivasan, Shaowu Zhang, Monika Altwein and Jürgen Tautz, Honeybee Navigation: Nature and Calibration of the Odometer, *Science*, The American Association for the Advancement of Science, Vol.287, No.5454, pp.851–853, 2000.

could collect their reward was placed right at the entrance to the tunnel. This was a control experiment because the bees essentially flew outside the tunnel and were rewarded immediately upon entering the tunnel. Thus, the bees performed a normal outdoor flight for 35 m. Bees are known to perform round dances if they have travelled about 50 m or less. As expected, the probability of a round dance, as opposed to a waggle dance (which the bees perform when they have flown more than about 50 m from the hive) was 85.2% in this experiment. In the second experiment, they placed the feeder at the far end of the tunnel, 6 m away from the entrance. Now the bees had to fly a total of 41 m (35 m outside and 6 m inside the tunnel) in order to get the reward. If the optic flow hypothesis is valid, the bees should overestimate the 6 m flown inside the tunnel because the random visual pattern on the walls and floor of the tunnel was much closer than the ground or vegetation would be during the outdoor flight. If the bees did not overestimate the distance flown inside the tunnel, then they should perform predominantly round dances to indicate their flight of 41 m. If the bees did overestimate the distance of their flight in the tunnel, then they should perform predominantly waggle dances. It turned out that in this experiment, the probability of waggle dance was 90%, clearly indicating that the bees had overestimated the distance of their flight inside the tunnel. So far, this is merely a reconfirmation of what Esch and Burns had found previously – when bees fly at a greater height, there is less optic flow, and they underestimate the distance flown, while when they fly in a narrow tunnel, there is greater optic flow and they overestimate the distance flown. What makes this study the definitive one, providing clinching evidence in favour of the optic flow hypothesis is the next experiment.

4.5 Clinching Evidence

At first, it might appear that the results of the experiments where bees flying above tall buildings underestimated the distance and bees flying in a narrow tunnel overestimated the distance are adequate to uphold the optic flow hypothesis. It is not easy to imagine what else could explain these results. But here we should not rely on our ability or inability to imagine alternative explanations for the observed results. We should accept that any number of hard-to-imagine confounding factors might explain the result – flying at abnormal heights, in narrow tunnels, a mixture of outdoor and tunnel flights, who knows what else can confuse the bees? In the case of the tunnel experiment, at the very least, we should ascertain that the tunnel did not somehow cause the overestimation, independent of increased optic flow.

If we can show these bees flying in the tunnel will not overestimate distance in the absence of increased optic flow, that would be clinching evidence for the role of optic flow, independent of the tunnel.

How do we delink the tunnel from increased optic flow? Actually, it is easy. Srinivasan and colleagues performed a third experiment in which they placed the feeder 6 m away from the entrance of the tunnel as before, but they provided axially oriented stripes (parallel to the flight direction of the bees), which would be expected to produce almost no optic flow. If image motion is the real cause of the distance overestimation in experiment two, then the bees in experiment three should not overestimate the distance and should show predominantly round dances. Indeed, they do – the probability of round dances in experiment three was 86.7%. They then performed a fourth experiment, which was not critical in the same sense that the third experiment was, but one which made the results of experiment two more dramatic while also providing a replicate. In the fourth experiment, they repeated experiment two, but placed the tunnel itself closer to the hive, at a distance of only 6 m, as compared to 35 m in experiments 1 to 3. Now the bees flew a total of 12 meters, 6 outdoors and 6 in the tunnel (with random visual texture). And yes, even though the bees had flown only 12 m, the probability of waggle dances in this experiment was 87.5%. Taken together, the results of the four experiments provide strong, indeed, clinching evidence in favour of the optic flow hypothesis. Nevertheless, their concluding statement is modest: “We conclude from these experiments that distance flown is inferred on a visual basis, the primary cue being the extent of image motion experienced by the eye”. It is obvious that these experiments not only provide strong evidence in favour of the optic flow hypothesis but also strong evidence against the energy hypothesis. The phrase “distance flown is inferred on a visual basis” is meant to make that distinction and is a definitive statement. Note, however, that the second part of the conclusion namely, “the primary cue being the extent of image motion” is less definitive and leaves open the possibility other visual cues (other than image motion per se) may play, a role, even if a minor one. This reflects the previously mentioned idea that some other, as yet unimaginable, confounding factor may exist and cannot be completely ruled out.

By any standard, this was an exciting outcome and enough to justify rushing to publication. But Srinivasan and colleagues had the patience to reflect a bit more and realise that by doing a simple calculation, they could extract much more information out of their data. Since they had video-recorded the dances of the bees back at the hive in order to determine the percentage of round and waggle dances,

they could also measure the actual duration of the waggle phases of their dances. Thus, their data consisted not only of the percentage of round and waggle dances, which specifies near versus far but also the waggle durations which indicate the distance flown as a continuous variable.

4.6 Calibrating the Honey Bee Odometer

The video records indicated that bees in experiment 2 that had flown a distance of 41 m (35 outdoors and 6 in the tunnel) showed a waggle duration of 529 ms and the bees in experiment 4 that had flown a distance of 12 m (6 outdoors and 6 in the tunnel) showed a waggle duration of 441 ms. To know what distances these waggle durations indicated to the bees, we need to know the waggle duration of bees flying outdoors, to known distances. Hence Srinivasan and colleagues performed a series of separate experiments with two colonies of honey bees, where they recorded the waggle durations of bees flying 60, 110, 150, 190, 225, 340, 350 m in an outdoor habitat. These data permitted them to produce a ‘standard graph’ with the distance plotted on the X-axis and waggle duration on the Y-axis (*Figure 4.5*). The function of a standard graph is that if we know any value of either the X or Y-axis, we can read off the expected value on the other axis. Thus, we can see from *Figure 4.5* that the waggle durations of 529 ms (in experiments 2) corresponds to 230 m of outdoor flight. Since the bees in this experiment had flown 35 m outdoors and 6 m in the tunnel, they seem to have perceived a flight of 6 m in the tunnel as equivalent to $230 - 35 = 195$ m of outdoor flight. Similarly, the 441 ms waggle dances of bees in experiment 4 corresponds to 184 m of outdoor flight. Since these bees had flown for 6 m outdoors and 6 m indoors, they seem to have perceived a flight of 6 m in the tunnel as equivalent to $184 - 6 = 178$ m of outdoor flight. Taking the average of these two overestimates, bees perceive a 6 m flight in the tunnel as equivalent to 186 m of outdoor flight, a 31-fold overestimate. Since bees are known to fly in the middle of the tunnel (think of how they might use optic flow to stay in the middle), the calibration of their odometer is a simple calculation, and I shall describe it more or less in the words of Srinivasan *et. al.*

- The distance to each wall is 5.5 cm. Thus, 1 cm of forward motion in the tunnel would cause the image of the wall to move backward by an angle of 10.3° in the lateral visual field.
- Therefore, 6 m of forward motion in the tunnel would generate 6180° of image motion.

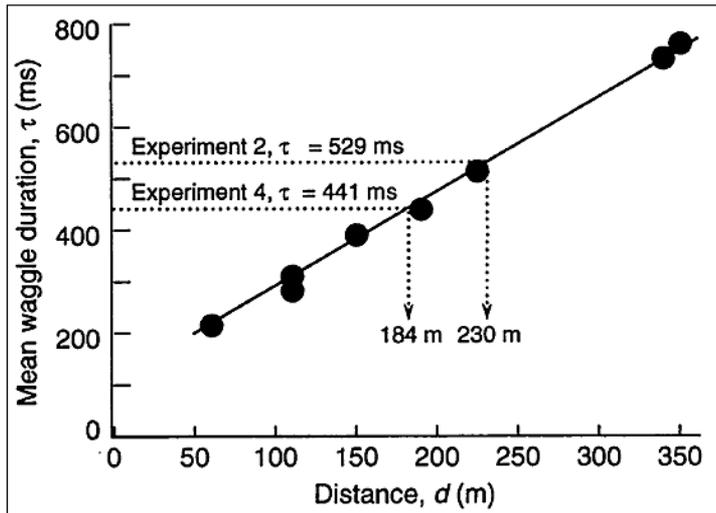


Figure 4.5 : Distance–waggle duration calibration during outdoor flights. Mean waggle durations of dances elicited by outdoor feeders at various distances d . The straight line is a linear regression on the data, defined by the expression $\tau = 95.91 + 1.88d$. Also shown are the mean waggle durations measured in the tunnel experiments (experiments 2 and 4) and their equivalent outdoor flight distances as read off from the regression line. Reproduced with permission from Mandyam V Srinivasan, Shaowu Zhang, Monika Altwein and Jürgen Tautz, Honeybee Navigation: Nature and Calibration of the Odometer, *Science*, The American Association for the Advancement of Science, Vol.287, No.5454, pp.851–853, 2000.

- A 6 m flight in the tunnel corresponds to a flight of 186 m in natural outdoor environment as we have seen above.
- 186 m of outdoor flight is encoded by a waggle duration of 350 ms (*Figure 4.5*).
- Therefore, 1 ms of waggle in the dance encodes $(6180/350) = 17.7^\circ$ of image motion in the eye.

By means of a few simple experiments, Srinivasan and colleagues were not only able to support the optic flow hypothesis and disprove the energy hypothesis, but they were also able to produce a definitive calibration of the honey bee odometer. Today it is widely accepted that honey bees estimate distance flown by assessing the extent of image motion in their eyes [5, 6].

4.7 Reflections

The simplicity of these experiments and their use of inexpensive locally available material to do cutting-edge research admirably illustrate the theme of this book – no one should feel that they are incapable of doing great science due to the lack of facilities. I cannot imagine any educational institution in India, whether it is a high school, undergraduate college or University, let alone a research institute, that is so impoverished of resources that these experiments could not have been done there and that the discovery that honey bees use optic flow to estimate distance flown, could not have been made there. We must realise that our institutions are not so much impoverished of resources as they are of an intellectual and academic environment that is conducive to free inquiry into the laws of nature unfettered by notions of immediate utility and so-called ‘national importance’. We must also inculcate in ourselves and our students, the idea that science is always a work in progress and that even the ideas of Nobel laureates can and should be called into question.

Suggested Reading

- [1] R Gadagkar, The Honeybee Dance-Language Controversy: Robot Bee Comes to the Rescue, *Resonance – journal of science education*, Vol.1, No.1, pp.63–70, 1996.
- [2] K von Frisch, *The Dance Language and Orientation of Bees*, Cambridge, MA: Harvard University Press, pp.566, 1967.
- [3] H E Esch and J E Burns, Distance Estimation by Foraging Honey Bees, *The Journal of Experimental Biology*, Vol.199, pp.155–162, 1996.
- [4] M V Srinivasan, S Zhang, M Altwein and J Tautz, Honeybee Navigation: Nature and Calibration of the “Odometer”, *Science*, Vol.287, pp.852–853, 2000.
- [5] S Mandal, How Do Animals Find Their Way Back Home? A Brief Overview of Homing Behaviour With Special Reference to Social Hymenoptera, *Insectes Sociaux*, Vol.65, pp.521–536, 2018.
- [6] H Wolf, Odometry and Insect Navigation, *The Journal of Experimental Biology*, Vol.214, pp.1629–1641, 2011.

5

How Do Ants Estimate the Distance Walked?

In this chapter, I will describe experiments designed to understand how ants estimate the distance they have walked. They rival in their simplicity, the experiments described in chapter 4, designed to understand how bees estimate the distance flown. Although ants can also use optic flow to estimate distance, in the absence of optic flow cues and of pheromone/chemical trails, as may sometimes be the case in the desert ants, *Cataglyphis*, ants estimate the distance walked, not by the energy expended but, believe it or not, by ‘counting’ (or integrating) the number of steps they have taken. This was proved by showing that ants on stilts (elongated legs) overshot the required distance to return home while ants on stumps (shortened legs) undershot the required distance.

5.1 How Do Ants Assess How Far They Have Travelled?

Ant workers walk rather than fly, and this has made them perhaps even more attractive model systems than flying insects for studying navigation. Being so-called central place foragers, foraging ants have to regularly return to their nests, and being social, large numbers of workers find and bring back food to the colony [1]. Many species of ants lay pheromone trails as they walk and use these to guide

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them on their return path. It is well-known that naïve ants will rely almost entirely on pheromone trails laid by themselves or their nestmates, but experienced ants can augment this with visual landmark cues. But what if the ants are incapable of laying chemical trails, as is true for many species, and there are no sufficiently conspicuous visual landmarks? Would the ants in such a situation be able to successfully navigate their way back to the nest? The answer is yes, and many species do so. That foraging ants in such situations successfully return back to the nest from long distances is astonishing enough. But even more astonishing is the fact that, while they may follow complicated, circuitous search routes on the way out, they return by a short straight path to the nest, at the end of their foraging effort. To accomplish this feat, they must somehow keep track of every turn they make and the distance they travel before making every new turn. Such a method of returning to the starting point by the shortest, straight path from the endpoint is called ‘dead reckoning’, a technique and term long used by humans in marine navigation.

When animals use the method of dead reckoning, it is called ‘path integration’. To successfully perform path integration, the ants must continuously keep track both of their angular displacement (turns) as well as their linear displacement (distance travelled). It is well-known that ants track their angular displacement using a celestial compass. On the other hand, how they measure distance was not clear until recently. As in the case of the honey bees, the energy hypothesis, suggesting that the ants estimate distance travelled by the energy consumed in the process, was a favourite candidate. But as in the case of the honey bees again, there has been growing evidence against the energy hypothesis. For e.g., copper or lead weights attached to the ant’s bodies have no effect on their distance estimation. The hypothesis that ants might simply estimate distance by the time elapsed, assuming that they walk at a constant speed throughout the entire round-trip journey, has also not found favour in empirical research. What remains is an audacious idea, first proposed by the French psychologist H Pieron that the ants might estimate distance travelled by counting the steps they take in the process. Of course, they need not literally count every time they take a step, the value of a continuous variable might be incremented by a fixed quantity. In other words, ants are hypothesized to use cues not derived from the environment, but those derived from the movement of their own bodies, legs in this case. Such cues are known as ‘idiothetic’ cues, in contrast to ‘allothetic’ cues derived from outside oneself [2].

In an attempt to test this ‘pedometer’ hypothesis (also known as ‘stride integrator hypothesis’), Matthias Wittlinger and Harald Wolf from the University of Ulm

in Germany, and Rüdiger Wehner from the University of Zürich in Switzerland (*Figure 5.1*), decided to study the Saharan desert ant *Cataglyphis fortis*, which is an efficient navigator without pheromone trails and without visual landmarks in its desert environment. Their experiment to test this hypothesis is as audacious as the hypothesis itself. They reasoned that ants walking on stilts (with elongated legs) should have increased stride lengths compared to normal ants and should, therefore, underestimate distance travelled. Conversely, ants walking on stumps (with shortened legs) should have decreased stride lengths compared to normal ants and should, therefore, overestimate distance travelled.

Hence, they elongated the legs of the ants by attaching pig bristles to the tips of their legs with superglue and shortened their legs by clipping off their tips. The effective change in leg length was about 2 mm in either case (*Figures 5.2 and 5.3*).

Remarkably, the operated ants not only survived but also resumed their foraging duties and successful navigation [3]. See for yourself how well the ant on stilts walk [<http://bit.ly/2YVLAJp>] or go the Supporting Material in [3].

5.2 The Experiments

Ants were trained to walk from their nest to a feeder kept 10 m away. The walking was performed inside a channel 7 cm wide with walls 7 cm high. The open-top allowed the ants a view of the sky, to facilitate their use of the celestial compass. Even though it was thought at that time that optic flow, which we encountered in the honey bee experiment [see chapter 4], plays a rather small role in these ants in this environment (the ants can estimate distances accurately even in featureless environments and even in total darkness), great care was taken to further minimise optical flow cues. The floor of the channel was coated with fine grey sand to provide traction for walking, but the sand particle size was carefully chosen to be below the ants' optical resolution. The walls of the channel were painted with matt grey varnish to provide a featureless environment. It must be noted however that the same ants can estimate distance travelled entirely by means of optic flow under the right conditions. Hence it was all the more important to deprive them of optic flow cues in the present experiment. After a day of training during which the ants walked up and down this channel, they were put to a test. Ants reaching the feeder were transferred to a different channel placed a little away from the original channel. These ants were given a piece of biscuit to increase their motivation to return home, and they promptly began walking in the new channel in the homeward direction.



Figure 5.1: Photos of the authors and the apparatus. Top: Matthias Wittlinger with the experimental apparatus (photo: Harald Wolf), middle: Harald Wolf (left) and Rüdiger Wehner (right) (photo: Sibylle Wehner) and bottom: Rüdiger Wehner (photo: Sibylle Wehner), at the study site in Mahrès in Tunisia.



Figure 5.2 : Photo of *Cataglyphis fortis* ants used in the current experiment, right, with normal legs, middle, on stilts and left, on stumps (left) (Photo: Matthias Wittlinger).

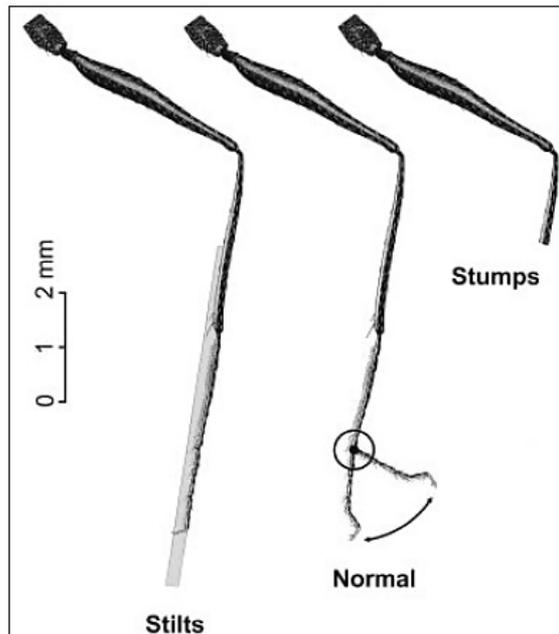


Figure 5.3 : Images of the normal and manipulated ant legs. Left, elongated legs (stilts) due to the attached pig bristles, middle; normal unmodified legs, with approximate range of tarsus movement indicated; right, shortened legs (stumps). The right hind leg is shown from anterior, in all cases. Reproduced with permission from *An Ant Odometer: Stepping on Stilts and Stumps* by Matthias Wittlinger, *et al.*, *Science*, 312, pp.1965, 2006, American Association for the Advancement of Science.

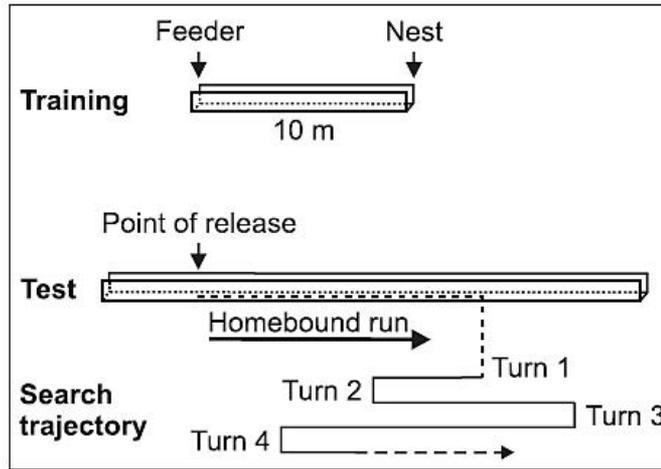


Figure 5.4: Schematic representation of the training and testing layout. Reproduced with permission from *An Ant Odometer: Stepping on Stilts and Stumps* by Matthias Wittlinger, *et al.*, *Science*, 312, pp.1965, 2006, American Association for the Advancement of Science.

After walking a certain distance, presumably their estimate of where the nest ought to be, and not finding it, they abandoned their straight and steady homeward run and began to search for the missing nest (goal) (Figure 5.4). The point of the experiment was to see how far the ants will walk in the homeward direction before beginning to search for the missing nest. This will tell us what the ants had estimated as the distance they had walked from the nest to the feeder and hence the distance they needed to walk back to reach the nest. In such an experiment, normal (un-operated) ants walked up to 10.2 m before abandoning the straight path and beginning to search, as might be expected because they had walked a distance of 10 m to reach the nest in the first place.

In the first experiment with operated ants (which the authors call test 1), ants reaching the feeding station were collected and their legs were elongated or shortened as described above. After the operation, the ants were briefly rested in a separate chamber and offered biscuits. When they picked up a piece of biscuit, it was assumed that they were ready to go home and were transferred to the second channel. These ants now began their straight homeward run. As expected from the pedometer hypothesis, ants walking on stilts (elongated legs) overshot the distance and walked up to 15.3 m before beginning to search for the missing nest. On

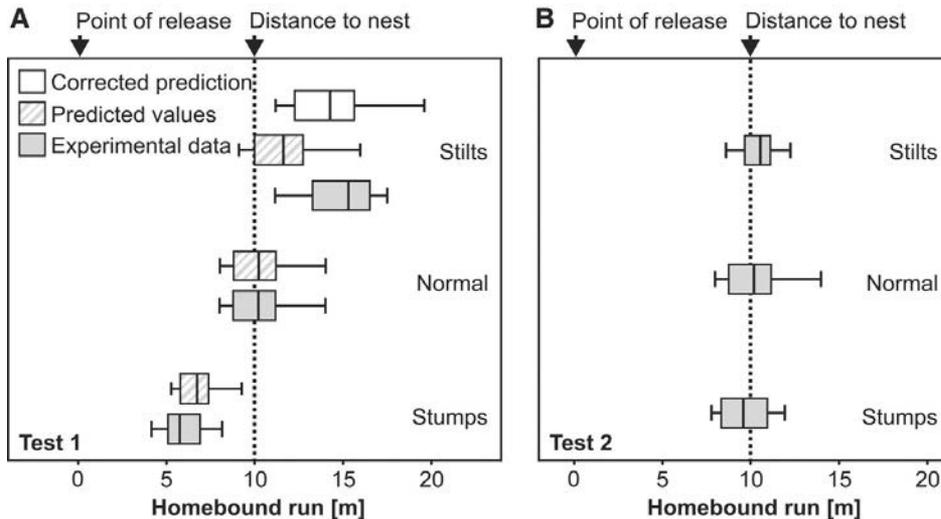


Figure 5.5: Comparison of homing distances of normal ants and of those whose leg lengths had been modified at the feeding site. (Panel A) Leg lengths were normal during the outbound journey but manipulated during the homebound run, resulting in different homing distances. (Panel B) Ants tested after re-emerging from the nest after manipulation so that the leg lengths were the same during outbound and homebound runs, for each kind of ant. Box and whisker plots show median, inter-quartile margins, and 5th and 95th percentiles of the homing distances recorded for 25 ants per experiment. The hatched box plots in (A) illustrate the homing distance predicted from the high-speed video analyses of stride lengths in normal and manipulated animals. The open box represents the prediction further corrected for slow walking speed of ants on stilts. Reproduced with permission from *An Ant Odometer: Stepping on Stilts and Stumps* by Matthias Wittlinger, *et al.*, *Science*, 312, pp.1965, 2006, American Association for the Advancement of Science.

the other hand, ants walking on stumps (shortened legs) undershot and began to search after walking 5.75 m. The values of 10.2 m, 15.3 m and 5.75 m mentioned above, are just the median values (*Figure 5.5*). To see whether the differences between normal ants and those on stumps and stilts are statistically significant, the experimenters needed to measure the variation around these median values. This they did by dividing the running channel into 10 cm bins and noting how often ants that had left the straight homebound path and begun searching for the missing nest were seen in each bin.

From this data, they calculated a search density distribution for 25 ants of each type and plotted them as box-and-whisker plots indicating the medians, interquar-

tile range (boxes) and the 5th and 95th percentile values (whiskers). They found that the lengths of the straight homeward runs of the normal ants, ants walking on stilts and those walking on stumps were all statistically significantly different from each other. These results clearly support the pedometer hypothesis. Thus, ants must estimate distance travelled by ‘counting’ the number of steps needed to cover the distance.

5.3 The Clinching Experiment

Abundant caution never hurts. As I have emphasized before, we must always worry about potential confounding factors. Even though ants with elongated and shortened legs behaved in opposite ways as expected, could the modified legs have influenced the behaviour of the ants in some other way? To rule out this possibility, the authors performed a second, clinching experiment (which they call test 2). They reasoned that in test 1 the ants on stilts and stumps overshot and undershot respectively because they had estimated the distance from the nest to the feeder with normal legs, but had to travel back from the feeder to the nest with altered legs. Thus, if the ants also walk from the nest to the feeder with altered legs then they should not make the same error of over- or under-shooting. So, they let the ants on stilts and stumps rest in the nest and then walk back from the nest to feeder in the first channel, and finally transferred them to the second channel. Now all the ants, normal as well as those on stilts and stumps walked the same distance before searching; ants on stilts now walked 10.55 m (instead of the previous 15.3 m), and those on stumps walked 10.25 m (instead of their previous 5.75 m). These new distances travelled by ants on stilts and stumps are not significantly different from each other nor are they significantly different from the 10.2 m of the unmanipulated ants. This experiment strongly reinforces support for the pedometer hypothesis because it eliminated the possible confounding factors associated with the operation of the ants.

5.4 Can One Be Even More Cautious?

What might constitute an even better proof of the pedometer hypothesis? One must always think along these lines, rather than worry that another experiment might just rock the boat and spoil the party. The goal should be to try one’s best to disprove a hypothesis, and only failing to do so should one accept the hypothesis and always only for the time being. In the present case, it would be even more

convincing if we can count the number of steps made by the ants and show that in test 1, the number of steps taken during the return homeward run are the same, whether the ants are normal, on stilts or on stumps, hence providing the basis for the error in distance estimation. In test 2 however, the number of steps while walking from the nest to the feeder should be different for each kind of ant – normal, those on stilts, and those on stumps; and on the return journey, each kind of ant should take the same number of steps that it made in the outbound journey – hence the absence of overshooting and undershooting. Despite recording high-speed videos of the ants, we are told that it was technically impossible to actually count the number of steps the ants took during these experiments (test 1 and test 2) across the whole distance of 10 m.

The next best thing they could do was to check if the exact extent of undershoot and overshoot was what one might predict due to the exact increase or decrease in the length of the legs. To predict the extent of overshoot and undershoot, we need to know the changes in the stride lengths due to the operation. Hence, they measured the stride lengths for normal as well as manipulated ants, in a separate set of experiments. This creates its own problem because stride length is expected to be influenced by the body size in addition to the leg length. But this problem is solvable – the researchers corrected for differences in body size by normalizing the stride lengths obtained for variations in body size. This means that they divided the stride lengths of each ant by a measure of the body size of that ant in all cases. After such normalization, they found that normal ants had stride lengths of 13.0 ± 1.98 mm, ants on stilts had stride lengths of 14.8 ± 2.5 mm and ants on stumps had stride lengths of 8.6 ± 1.73 mm. These three values are significantly different from each other. Now, one could make predictions about how far the ants should walk on the way to the nest. From the altered stride lengths, they made predictions about how far the ants of stilts and stumps should have travelled homeward in test 1, before beginning to search for their missing nests, if they were guided by their pedometers i.e., they took the same number of steps as they had taken on the outbound journey with normal lengths.

These predictions further upheld the pedometer hypothesis because the predicted homeward travel distances of the modified ants were in general agreement with the observed values. In other words, the ants behaved as if they were counting the number of steps in the outbound journey and taking a similar number of steps in their homebound journey. But the authors were not satisfied with a ‘general agreement’. Instead, they focussed on tiny differences between the observed and predicted values, comparing them statistically. They found that while the dif-

ferences between the observed and predicted values were not significant in the case of ants on stumps, ants on stilts overshot significantly more than predicted. Now, why should this be so? If a satisfactory explanation cannot be found for this subtle quantitative discrepancy between the observed and predicted overshoots, the entire interpretation may be suspect, or so the authors fearlessly reasoned.

To fathom this discrepancy, they first reasoned that the operation performed on the ants could not be held responsible because, rather than be somehow incapacitated, the ants on stilts walked longer than predicted. Besides, they observed that the operated ants (on stilts and stumps), were making successful foraging trips several times a day for several days – not a sign of incapacitation. The other possibility is that altered walking speeds may account for the discrepancy. Stride length is expected to be dependent on both leg length as well as walking speed. To test this possibility that altered walking speeds might be responsible for the discrepancy, they determined the walking speeds of normal and both kinds of operated ants. They did this both by recording the time required to walk a distance of 3 m, using a stopwatch as well as using a high-speed video camera. Normal ants walked at a speed of 0.31 m/s. As expected from their shorter legs and smaller strides, ants on stumps walked at a slower speed of 0.14 m/s. However, ants on stilts, rather than walking at higher speeds than normal ants, on account of their longer legs and greater strides, walked at a speed of 0.29 m/s, slower than normal ants. This was probably on account of the extra weight of the pig bristles attached to their legs. Now, the prediction about how far the ants on stilts should walk in a straight homebound direction before beginning their search was corrected for this altered walking speed and the corresponding changes in stride length. They did this using a standard graph relating the stride length to walking speed obtained for each kind of ant [4]. Thus, they finally obtained a value not significantly different from the observed value. Only at this point, did the authors put their weight behind the pedometer hypothesis and claim that *Cataglyphis* ants estimate distance travelled by ‘counting’ the steps. As the authors point out, these simple experiments open up exciting opportunities to understand how the ant brain, tiny as it is, counts steps and directs the legs to take the desired number of steps to go back home. And let us not forget, the brain has to perform path integration in between, to arrive at the number of steps required to trace a straight path back to the nest after having made a meandering outward journey. It is perhaps worth reiterating that the ants need not literally count their steps, the value of a continuous variable might be incremented by a fixed quantity every time they take a step, based for instance on the movements of the leg muscles.

Today we know that these desert ants can also use optic flow to estimate the distance. In another remarkable experiment, ants which did not walk but were carried by their nestmates were also shown to have correct information about the distance between their current position and their nest. The clinching experiment here was to show that when the carried ants were blindfolded, and thus could not gather optic flow information, they were quite lost. Ants can, therefore, estimate distance both with the help of their stride integrator and optic flow; they can use either, but anyone will do [5]. There are many more intriguing suggestions from similar simple behavioural experiments. E.g., distance information gathered from optic flow during the outbound journey (by carried ants that are not blindfolded) cannot be used to work out the number of steps to walk back (by walking ants that are blindfolded). And yet distance estimated by both methods can be integrated to arrive at the best possible estimate of the distance to walk back. I will not go into these details here, but readers will surely enjoy reading about them [5, 6]. I also recommend a delightful account of 50 years of research by Rüdiger Wehner's group on *Cataglyphis* ants in the Tunisian deserts near Mahrès [7] and his recently published elegant and comprehensive monograph, *Desert Navigator – the journey of an ant* [8].

5.5 Reflections

As the reader would be familiar by now, the broad message of this book is that cutting-edge science can be done with little or no access to large sums of money and sophisticated laboratory facilities. The aim of spreading this message is to make the production of scientific knowledge and not merely its consumption, as democratic and widespread an activity as possible. This will not just be good for large numbers of people in less endowed circumstances, but the participation of a large and diverse set of actors with many different perspectives would be good for science as a whole. As might be expected, I am choosing examples of experiments in animal behaviour that are especially appropriate to illustrate this message. Hence the lessons to be drawn from each experiment are much the same. Nevertheless, the experiments with ants on stilts and stumps does have something unique to ponder.

First, I would like to draw attention to the audacity, both of the hypothesis being tested and the experiment used to test it. Try telling an intelligent, educated person with no knowledge or interest in insects that you think that ants might measure distance travelled by counting steps, and you will likely get a look suggesting

that you must be mad to think so. Try telling a scientist, even someone who works on insects that you plan to cut the legs of an insect or add pig bristles to its legs and study its behaviour, and you will most likely get a similar look. And yet, as JBS Haldane put it brilliantly, “The Universe is not only queerer than we suppose, but queerer than we can suppose.” And that is why the Medawars included “courage in framing expectations” in their description of ethology, as you will recall from the first chapter [7].

Second, I would like to draw attention to the simplicity of the experiments and the fact that very little was needed by way of instruments, money, or other facilities – pig bristles, super glue, some simple channels and some sand. It is true, however, that they used a high-speed video camera for filming the ants. It is not so unreasonable to imagine that you could borrow or rent a high-speed video camera for a small fraction of the cost of buying one. Moreover, notice that they also measured walking speed by recording the time taken to cover a distance of 3 m using a stopwatch. I do not know whether measurement of walking speed with a stopwatch would have been sufficient in this case, but I wish to make a general argument about the precision of measurements. How precisely should we measure something in any experiment? My answer would be, as precisely as is necessary for answering the question at hand. Unfortunately, many people insist that it should be as precise as possible. I find this unconvincing, counterproductive and dangerous.

I find utterly unconvincing, the secondary argument that greater precision than needed today may be useful for others tomorrow. If the cost of doing the experiment minus the unnecessarily great precision is a small fraction of the extra effort to achieve more precision than necessary today, it makes more sense to repeat the experiment with greater precision whenever required. The real danger of demanding greater precision than necessary for answering the question at hand just because greater precision is possible at extra cost is that it privileges better-funded scientists and deprives poorly-funded scientists and students with bright ideas but without a research grant. If we are to democratize the production of scientific knowledge, then we, especially journal editors and reviewers, must adopt the principle that precision should be based on necessity, not capability.

Finally, we must reflect on the fact that the authors of this study were not easily satisfied. Recall “caution in coming to conclusions” in Medawars’ description of ethology, mentioned in the chapter 1 [7]. They did not stop after obtaining the remarkable result that as expected from the pedometer hypothesis, ants on stilts overshot their target during the homeward run and that ants on stumps undershot.

Even though it was not possible to count the number of steps, they attempted to examine if the extent of overshoot and undershoot were consistent with the extent of change in leg length. Even here, they were unsatisfied with a general agreement and specifically focussed on a tiny discrepancy between the observed and predicted values, even though the predicted values were in the correct direction. This permitted them to understand the reason for the discrepancy and to show that the discrepancy increased confidence in the pedometer hypothesis rather than cast doubt on it. In order to emulate the authors in this regard, we must guard against falling in love with the hypothesis we are testing, even if it is our own. We must be prepared to let ugly facts demolish beautiful theories. An important reason that often prevents people from being more detached from their hypotheses is that we consider a positive result supporting a hypothesis as a success worthy of a reward and a negative result as a failure, not even worthy of publication, let alone a reward. Here again, publishing policies must change. After all, a negative result can lead you to the correct hypothesis, and even if not, it spares future researchers repeating those ‘unsuccessful’ experiments. There is a great deal we can do to elevate the enterprise of science through simple changes in our mindset. And that is much more likely to happen if we make the practice of science more inclusive and open it up to as wide and diverse a circle of practitioners as possible.

Suggested Reading

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6

Why are Male Wasps Lazy?

In this chapter, we move from sensory physiology to psychology and consider the proverbially lazy drone. I will describe how some simple experiments permitted us to understand why males in the Indian paper wasp *Ropalidia marginata* do no work in the colony even during the time they live in it. Taking the behaviour of feeding larvae as an example of work, we show that male wasps normally do not feed larvae, not because they are incapable of doing so, but because they do not have access to enough food and also because female wasps are so much better at this job. As a confirmation of this conclusion, we could cure the males of their laziness, i.e., get them to feed the larvae by providing them with excess food and leaving them in the presence of hungry larvae, without the presence of females.

6.1 From Sensory Physiology to Psychology

In the first five chapters of this book, we focussed on how animals perceive their environment using their senses of vision and smell, and modulate their behaviour appropriately to solve complex problems necessary for their survival. We used their behaviours as reporters of their sensory capabilities. We saw how simple experiments permitted us to conclude that digger wasps use vision to learn the

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features of the landscape around their nests, that honey bees have well-developed colour vision, that ants use pheromone trails to choose the shortest path to their feeding sites, that honey bees use optic flow to estimate distance flown and that some ants count their steps to estimate the distance they have walked. Of course, sensory inputs were processed in the brains of the insects before they produced the appropriate behaviour. Nevertheless, there was a relatively more direct connection between the sensory input and the motor output. From such sensory physiology, we will now consider phenomena more in the realm of psychology, where the cause-effect relationship between sensory input and motor output will be more indirect, delayed and complex. In this chapter, we will consider the ‘laziness’ of male wasps and see how similarly simple experiments can help us conclude that male wasps are lazy, understand why they are lazy and, incredible as it may sound, cure them of their laziness.

6.2 The Saga of the Honey Bee Queen

Every high-school student today knows that honey bees live in populous colonies consisting of a single large queen bee, a few lazy drones and thousands of worker bees that can give you a mildly painful sting if you mess with them. But our understanding of the honey bee queen has a surprising history. Honey bees have been known to humans and have sweetened their lot with their honey, since long before recorded history. However, our wise men (females having been forcibly excluded from the intellectual affairs of society), could neither imagine nor accept that the honey bee colony could be ‘headed’ by a female bee! Aristotle pronounced that honey bee colonies are led by a King. Without realizing that the honey bee colony’s ‘leader’ is a female bee, one John Knox published a treatise in 1558 entitled *First Blast of the Trumpet Against the Monstrous Regiment of Women* in which he argued against the rule of women such as Queen Elizabeth, on the grounds that “Nature hath in all beasts printed a certain mark of dominion in the male, and a certain subjugation in the female.” The cleric Charles Butler, however, seems to have made amends in 1609 by referring to the honey bee colony as a ‘feminine monarchy’ but he must have only guessed rather than actually known the correct sex of the “leader”. It was only in the seventeenth century that the Dutch anatomist Jan Swammerdam demonstrated that the ‘King’ bee contained ovaries with eggs [1, 2]. If this was not irony enough, it turned out that the male bees, rather than have ‘a certain mark of dominion’, are lazy, i.e, they do not participate in colony labour, and are at the mercy of the female bees for their food,

until they leave the nest of their birth and die in the act of mating or be dragged by their legs and ejected from the colony by their sisters (for overstaying!). But why are the males so lazy?

6.3 One Question, Two Answers

Why indeed are the males lazy? When a biologist asks ‘why’, there are two distinct kinds of answers possible and both are appropriate, and neither is more nor less valid than the other. Consider, for example, the following ‘why’ question in the context of a very different kind of animal and a very different kind of phenomenon. Every year Siberian cranes migrate 6400 kilometres from their breeding grounds in Siberia and arrive in the Bharatpur bird sanctuary in Rajasthan in North-Western India. Now why should they do something so risky and audacious? One kind of answer to this ‘why’ question has been painstakingly put together with much research over many years using a variety of different migratory birds. The birds migrate because, at the onset of winter, the shortening day lengths in the northern latitude are sensed by their pineal gland which in turn leads to hormonal changes in the birds, ultimately leading to what is tellingly called ‘migratory restlessness’ and produces the urge to migrate. The migratory routes appear to be genetically fixed, as research on another bird, the blackcap, has shown. This is a perfectly legitimate answer to the ‘why’ question and is championed by physiologists and others who revel in unravelling the mechanistic causes of behaviour. Such answers are labelled as ‘proximate’ answers or mechanisms. On the other hand, more evolutionary minded biologists seek to understand why natural selection has favoured such unusual behaviour over simply staying back in Siberia during the harsh winters. They focus on the possible advantages of better survival and feeding in the relatively warmer climate in Bharatpur, and attempt to evaluate whether these advantages offset the disadvantages of the costly and risky journeys involved. Such answers are equally (but no more) legitimate and are labelled as ‘ultimate’ or ‘evolutionary’ answers or mechanisms [3].

Similarly, some researchers have attempted to provide an ultimate answer to the question of why the ant, bee and wasp males are lazy. In other words, they argue that the altruistic sterile worker strategy is likely to yield more evolutionary fitness for females than it does for males. Relatively speaking, males get more fitness by going off to reproduce on their own by mating with females from other nests. This has to do with the peculiar haplodiploid genetics of the Hymenoptera where males are haploid, and females are diploid. But I will not go into that here



Figure 6.1 : A typical nest of the Indian paper wasp *Ropalidia marginata*, showing several adults and cells with brood in various stages of development. (Photo: Dr Thresiamma Varghese).

both because it is a somewhat complicated theoretical argument and also because it cannot be verified by simple experiments. Instead I will focus on possible proximate answers to the question of male laziness. And, to do so, I will take you to the wonderful world of the Indian paper wasp *Ropalidia marginata*.

6.4 The Primitively Eusocial Wasp *Ropalidia marginata*

I joined the Central College in Bangalore to pursue BSc (Hons) and MSc in zoology in 1969. The first thing I saw was that every window of the zoology and botany department buildings had one or more nests of the social wasp *Ropalidia marginata* (Figure 6.1). I was surprised that my zoology teachers could tell me nothing about these insects – they were only knowledgeable about the exotic species described in our textbooks, which were mostly written (or copied from) textbooks written in the UK or USA. These living, local insects fascinated me much more than those that resided in my textbooks, and I have never stopped

watching and studying them till today. *R. marginata* is a primitively eusocial wasp that is widely distributed in peninsular India. They are called paper wasps because they build their honeycomb-like nests from paper, which they manufacture by scraping cellulose fibres from plants, adding some salivary secretions and making a fine pulp. I will have the opportunity to give several interesting facts about this species in the next few chapters of this book. For the purpose of this chapter, all we need to know is that their colonies comprise several females, only one of which lays eggs and is called the 'Queen'. The remaining female wasps function as sterile workers, cleaning and guarding the nest and making trips away from the nest to bring back small insects and spiders to feed the larvae, and cellulose fibres to build the nest. There may also be a few males about which we will see more below, but make no mistake, *R. marginata* is as much a feminine monarchy as Charles Butler's honey bee colonies [4].

6.5 Are Males of *R. marginata* Lazy?

When we study these wasps, we mark all individual wasps with one or more spots of non-toxic, odourless and quick-drying paints of different colours on different parts of their bodies to individually identify every wasp. We mark the wasps without capturing them, but by patiently waiting for them to be busy doing something when we opportunistically apply small spots of paint on them with a fine toothpick or broomstick. We have developed a system to thus uniquely mark and name thousands of wasps that would otherwise look alike.

This allows us to follow the behaviour of individually identified wasps for many days, and even over their entire lifespan. During the marking process, we encounter the occasional male wasp. We can easily distinguish males from female wasps because of their yellow faces as compared to the browner faces of the females (*Figure 6.2*). If you watch a nest containing marked male and female wasps, your first impression would be that the female wasps are usually very busy working or fighting, but the males are doing nothing. But such first impressions are not good enough to conclude that male wasps are lazy. If we follow the fates of male and female wasps since their eclosion from their pupal cases, we find that female wasps remain on their nests of birth for 27 ± 23 (range = 1–106) days. However, the male wasps stay on their nests of birth only for 6 ± 2.6 (range = 1–12) days, during which they never leave the nests and after which they leave permanently, never to return and never to visit any other nests. They spend the rest of their lifespan leading a nomadic life and mating with females that may be



Figure 6.2 : The brown faces of *R. marginata* females (top) and yellow faces of the males (bottom). The mandibular plate that shows the clearest colour variations between the sexes is called the clypeus. (Photo: Dr Thresiamma Varghese).

out foraging. This behaviour of mating away from the nest must have evolved to avoid inbreeding. So, if males are hardly present on the nest, how can we accuse them of laziness. Thus, we must specifically focus on males at the time they are still present on their nests, i.e, before they leave. But these males may not work because they are too young to work. The appropriate test then is to compare the rates of work performed by males while still living on the nest with the corresponding rates of work performed by the similarly aged female wasps.

I had the good fortune of being joined by a student passionate about understanding why males are lazy, although we certainly had no idea at that time that we might actually be able to cure them of their laziness. It is perhaps not so surprising that the student passionate about this topic was a female student. Indeed, Ruchira Sen (*Figure 6.3*), who joined my laboratory to do her PhD in 2002, took the unusual step of devoting her entire PhD work to males of *R. marginata*. This was quite unusual for any research group studying social Hymenoptera and was a first for my group. Not surprisingly, female ants, bees and wasps have been the focus of most attention – after all, they are the stuff of feminine monarchies! But I think Ruchira chose to study males because she was bold enough to take the path less trodden, but I also suspect that male laziness did not appeal to her female sensibilities. Either way, I lucked out by having a student passionate about



Figure 6.3 : Ruchira Sen (left) with the author (right).

her research – passion is what drives most successful scientists and sustains them through back-breaking work that most friends and family do not appreciate or understand. Ruchira and I decided to focus on the behaviour of feeding larvae as the example of work. Feeding larvae is one of the most conspicuous and important tasks that adult wasps perform on the nest because the larvae are completely helpless and at the mercy of adults who have to insert food into their mouths several times a day.

Ruchira promptly set about comparing one-week-old female wasps with one-week-old male wasps in the rates at which they both fed larvae. This is not as simple as it sounds. The behaviour of feeding larvae is easy enough to identify – it involves the adult wasps with solid food in their mandibles inserting their heads into larval cells and rapidly vibrating their wings; the absence of wing vibration suggest that they changed their minds and did not feed that larva. The real problem is to get unbiased estimates of feeding rates by males and females. In studies of animal and human behaviour, it is much easier than you might think, to (sub-consciously) bias your observations to find the pattern you expect to see or that you approve of. Observing animal behaviour may sound easy, but converting the observations into reliable quantitative data is fraught with difficulties. Recall the description of ethology by the Medawars, quoted in the chapter 1, “*Ethology is rooted in observation of animal behaviour, an activity that only simpletons think simple. . . Observation is a difficult and sophisticated process. . .*”. At the very be-

ginning of my research career, when I decided to devote myself to the study of the behaviour of *R. marginata*, I standardised a set of sampling methods designed to provide as unbiased estimates of behaviour as possible. These include instantaneous scans, recording all occurrences of rare behaviours, focal animal sampling and focal behaviour sampling. An important trick to reduce subconscious bias is to reduce the number of individual or behavioural events that have to be recorded, per unit time. If we try to record more than is easily possible, then our mind will pick what it likes. The instantaneous scans (scans, for short) which involve scanning the nest and recording only the very first behaviour we see each wasp performing leaves little room for bias. In recording all occurrences of rare behaviour ('all occurrences', for short), again there is less chance of subconscious bias because rare behaviours are just that – rare. In the focal animal and focal behaviour sampling, we randomly pick just one animal or just one behaviour to observe at any given time, once again, reducing the opportunity for our minds to pick [4]. Using these methods, Ruchira proceeded to measure and compare the rates at which 1–6-day-old male wasps and 1–6-day-old female wasps fed the larvae. As we had expected from previous more casual observations, males hardly ever fed the larvae, while the females fed the larvae at significantly higher rates. This striking difference between male and female behaviour was made even more striking by the fact that the males fed themselves at about the same rates as the females did (*Figure 6.4*). So, male *R. marginata* are lazy indeed [5].

6.6 Three Hypotheses for Male Laziness

Having confirmed that the male wasps are indeed lazy, we proposed three testable hypotheses that can potentially explain why they are lazy. Let me reiterate that these hypotheses are meant to provide proximate (and not ultimate) explanations, as discussed above.

Hypothesis A

Males are simply incapable of feeding larvae. Feeding the larvae with solid food may require a certain amount of skill, involving judging larval hunger levels, the size of the food bolus that needs to be offered, the appropriate duration of feeding, etc. It is, therefore, not unreasonable to imagine males do not have these skills, especially because males in solitary wasps also do not feed larvae.

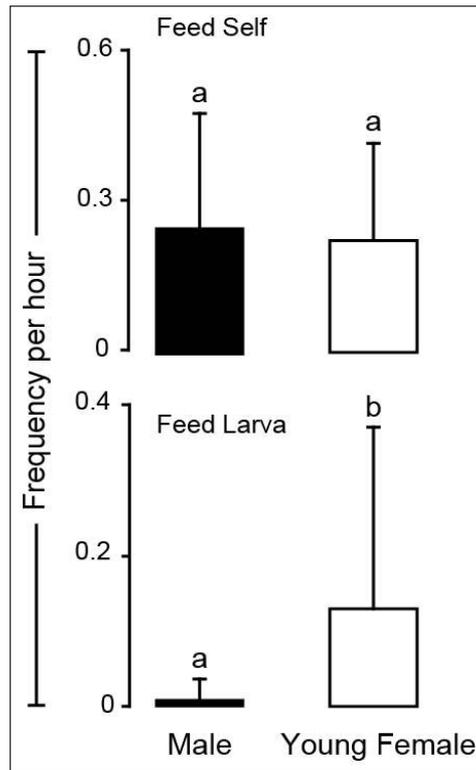


Figure 6.4: A comparison of male wasps (who stay on the nest for only about 6 days after eclosion) and 1–6-day female wasps of *R. marginata*. While male and female wasps feed themselves at comparable rates (top), males almost never feed larvae but females feed larvae at a significantly higher rate (bottom). Bars with different letters are significantly different from each other ($P < 0.007$, Mann–Whitney U test). (Redrawn with permission from [5]).

Hypothesis B

Males never get access to enough food to satisfy themselves and have something leftover to offer to the larvae. This is quite plausible because males do not forage while on the nest in this species, as in all social Hymenoptera, and thus have to depend on the females for their supply of food. In some species, such as honey bees, females are known to withhold food from males in times of shortage. Besides, males are not aggressive enough to be able to forcefully snatch food from females.

Hypothesis C

Females are so much more efficient at feeding the larvae that they leave no opportunities for the relatively inefficient males to do so. This may sound cheeky, but it is easy to imagine that larvae prefer to be fed by the more efficient females. More importantly, it is an eminently testable hypothesis, as you will see below.

6.7 Testing the Hypotheses

We set up the following experimental design to test these three hypotheses. We collected 14 naturally occurring nests of *R. marginata* from in and around Bangalore and transplanted them along with the brood and all adult wasps into our Vespiary. Early in my research career, I was able to design and construct a simple but very special laboratory for housing and studying the wasps which I call the Vespiary. This laboratory is nothing but a room measuring approximately 10 m × 6 m × 5 m and is covered on all four sides by a wire mesh with openings 0.75 cm × 0.75 cm (*Figure 6.5*). The mesh size is such that it allows *R. marginata* to fly in and out but keeps out its slightly larger predators namely the hornets *Vespa tropica* and *Vespa affinis*. Inside the Vespiary, we can keep the wasps in small plastic boxes, wood and wire mesh cages of various sizes and simply transplant them in the open, so that wasps can fly in and out. In the open transplants, the wasps forage on their own and are free to leave and build their nests elsewhere. Other wasps are also free to fly in and build their nests inside. Other than the useful convenience that they are protected from their main predators, these are essentially natural colonies. Much of our research is carried out in the Vespiary. The 14 nests brought in for this study were transplanted in the open so that the colonies were free foraging. As is our usual practice, all wasps in all the colonies were marked for unique identification.

The 14 nests were arbitrarily assigned to one of four types of experimental treatments.

In Type 1, we did nothing more, i.e., the wasps had to find food on their own and do as they please. We worried that the rates at which these wasps might find and bring food back to their nest and feed their larvae would be subject to the vagaries of the environment. If we were unlucky, we might not record enough instances of larval feeding behaviour, even by the female wasps, let alone by the male wasps, to be able to get statistically meaningful data.



Figure 6.5 : A view from inside the Vespiary showing little plastic boxes and small and large cages used to rear wasps. Notice that the whole room is enclosed with a wire mesh screen instead of walls. For the experiments described here (and for most experiments) we leave the doors of the cages open so that the wasps are at liberty to come and go as they like and have to forage for themselves. (Photo: Dr Thresiamma Varghese).

Therefore, in Type 2 nests, we provided them with a food supplement. This comprised 10 *Corcyra cephalonica* larvae, honey, and water placed in a petri dish 3 cm away from the nest every day. *C. cephalonica* is the common pest moth that infests stored rice and is easy to culture in the laboratory. We feed the moth larvae to the wasps kept in closed cages, where they thrive and reproduce well on this diet. Such a predictable supply of food close by, we hoped, would give us more opportunities to witness larval feeding behaviour.

In Type 3 nests, we removed all the female wasps and left the larvae with only the male wasps. This we hoped would encourage the male wasps to feed the larvae, if they could. The problem, of course, was that male wasps do not forage on their own and depend on the females to bring them food. To overcome this problem, we decided to hand-feed the male wasps ourselves. At first, we were not sure if this is possible. But Ruchira, with her patience and skill, succeeded admirably. She would take small pieces of *C. cephalonica* larvae with a long thin stick and hold them close to the male's mouth. At first, the males were afraid to approach but when they sensed that food was available without any harm, they

readily accepted the food. She thus fed them to satiation, i.e, until they accepted no more. We hoped that this procedure would leave them with enough food and more so that they might feed the excess food to the larvae if they could do so.

Type 4 nests had a very different purpose. If males could indeed feed larvae and did so in Type 2 or at least in Type 3 nests, we wanted to study male feeding behaviour in detail and compare it with female feeding behaviour. After all, we would witness, if we were lucky, a very rare behaviour and the opportunity to describe it in detail was not to be missed. One of our hypotheses was that males are incapable of feeding larvae. In the event of having to reject this hypothesis, we had to be fully ready to capitalize on the opportunity that males could feed larvae. Given that males rarely feed larvae, their method of feeding, when they did so under special circumstances, needed to be compared with the corresponding behaviour of females who feed larvae as a matter of routine. The chances of males feeding larvae in Type 2 nests were small, but we had pinned our hopes on their doing so in Type 3 nests. In order to potentially compare the method used by males to feed larvae in Type 3 nests, where they were hand fed, we similarly hand-fed female wasps with pieces of *C. cephalonica* larvae in Type 4 nests.

To estimate the rates at which male and female wasps performed various behaviours, including feeding self, feeding larvae, masticating food, etc., Ruchira used scans and ‘all occurrences’ methods. But to provide a detailed description of the behaviour of feeding larvae by males and females in Type 3 and Type 4 nests respectively, she used the focal behaviour sampling, focussing only on feeding larvae and associated behaviours. Here, she began her observation when an adult wasp, male or female as the case may be, obtained food and continued focussing on that individual until all its food disappeared. Thus, Ruchira could obtain 325 h of observational data from the 14 nests put together.

6.8 Males Can Feed Larvae and They Do Even Better in The Absence of Females

In Type 1 nests, males, as expected, did not feed the larvae, but females did so at a small rate. In Type 2 nests, with a food supplement, both males and females increased their rates of feeding larvae although the male rate was not statistically significantly different from zero. In Type 3 nests where there were no females, and the males were hand-fed and exposed to hungry larvae, male wasps fed larvae at a significantly higher rate, which is not statistically significantly different from the female feeding rates in Type 2 nests (*Figure 6.6*). We were convinced that

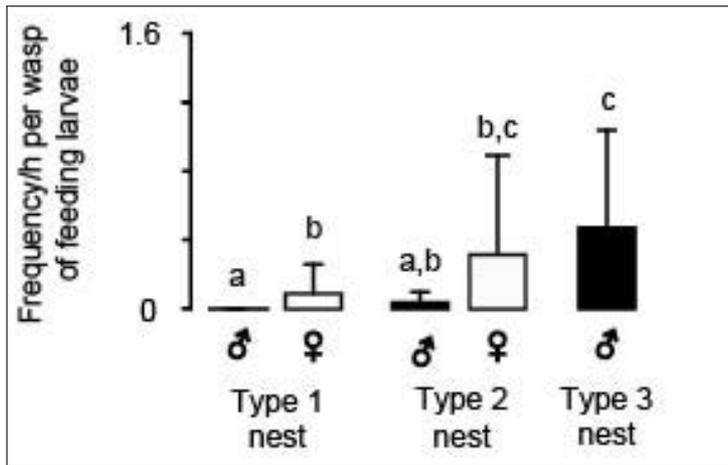


Figure 6.6 : Mean and standard deviation of the rates at which male and female wasps feed larvae in Type 1 nests (no food supplement), Type 2 nests (with food supplement) and Type 3 nests (males were hand-fed and females removed). Bars with different letters are significantly different from each other (Mann–Whitney U test, with Bonferroni correction, $P < 0.05$). [Redrawn from [6]].

the males were not simply getting rid of the food that they did not want but were actually seeking out the most appropriate larvae to be fed. For one thing, males did not simply dump unwanted food on the floor but delivered it to the cells in the nest. Second, they never delivered food to empty cells, egg cells or even to cells with very small larvae. Ruchira observed the males feeding larvae 345 times, and in 340 of these, they fed the largest class of larvae. Finally, in order to feed the larvae, males (in Type 3 nests), like the females (in Type 4 nests), performed a complex series of behaviours – they moved about the nest with a piece of solid food in their mandibles, checking out various cells and their contents with their antennae, apparently searching for cells bearing large larvae, and having found one, they inserted their head and thorax into the chosen cell, holding on to the food at one end, in a way that made it possible for the larvae to grab the food at the other end, and only then did they let go of the food.

Clearly, hypothesis A, that males are incapable of feeding larvae, can be rejected. Since males began to feed larvae in Type 2 nests while they did not do so at all in Type 1 nests, hypothesis B, that males rarely feed larvae because they rarely have access to enough food, is upheld. Because males fed larvae at higher rates in Type 3 nests (in the absence of females) compared to their own rates in

Type 2 nests (in the presence of females), hypothesis C that males do not feed larvae because females do a much better job, is also upheld. Well, at least males did not feed larvae much when females were present and were feeding the larvae. Although this is not explicit proof that females do a much better job, it suggests that females, for one reason or another, inhibited the males from feeding the larvae. But do the males really do a bad job?

6.9 A Twist in the Tale

It was heartening to know that males are not incapable of feeding larvae and that they indeed fed larvae when they have enough food and especially when the females are absent. Not only did we discover the reason for male laziness, but we could cure them of their laziness, by providing them excess food and leaving them in the presence of hungry larvae and with no females. We were delighted to publish a paper with the title ‘Males of the social wasp *Ropalidia marginata* can feed larvae, given an opportunity’ [6]. But alas, there was a twist in the tale, or a sting in the tail, as Roald Dahl would have put it. Many of the larvae in the exclusive care of the males, died. Now, why should this be so? We obtained some additional fascinating insights by making a detailed comparison of the behaviours of males and females feeding larvae in Type 3 and Type 4 nests, respectively. Upon acquiring solid food, adult wasps move from one larval cell to another, feeding bits of it to different larvae, all the while masticating it and imbibing some of the juices themselves. We defined a feeding bout as the time between an adult wasp acquiring a bolus of food and the food completely disappearing from its mandibles. Such a bout may have several episodes of feeding larvae interspersed with episodes of masticating, the latter representing self-feeding. We calculated the total duration of such feeding bouts for males and females as well as the duration of mastication within the feeding bouts. Males had significantly longer feeding bouts than females but more interestingly, males spent significantly more time masticating the food than did females. While females spent about a third of the feeding bout in masticating and the remaining time feeding the larvae, males spent over 90% of the bout masticating and less than 10% of the time feeding the larvae. Males thus masticated more and fed larvae less (*Figure 6.7*). Since adults imbibe juices in the food for themselves during mastication, we suspect that males drink most of the juices and feed much less nutritive fibre to the larvae. This may be why the larvae in their care died. But of course, males may have weaker mandibles and may require more time to masticate. At present, we do not have direct proof that

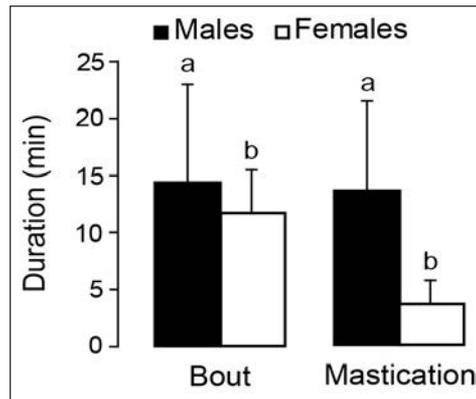


Figure 6.7 : Mean and standard deviation of the durations of feeding bouts and mastication by male and female wasps. Statistical comparisons are by t test, separately for bout duration and mastication. Bars carrying different letters are significantly different ($P < 0.05$).

males feed nutritionally impoverished food to the larvae, but this can be tested. Ruchira has found ways to snatch the bolus of food, from both males and females, just before they were about to offer them to the larvae. I am in search of a student to perform a comparative nutritional analysis of what males and females offer to the larvae and test this hypothesis. Until then, I better not pronounce the males ‘guilty’!

6.10 Reflections

In reflecting on the experiments described in this chapter, my student Ruchira’s passion for research, her confidence and courage in walking the less-trodden path during her PhD, her skill and patience in hand-feeding the wasps, and her female sensibilities in tackling the question of male laziness, all come to mind. In addition, I wish to reflect on two additional features of scientific research that these experiments draw our attention to – the source of hypotheses, and funding for research.

The Source of Scientific Hypotheses

In science, the source of a hypothesis is not important as long it is testable and potentially falsifiable. I have already mentioned that our hypothesis C, namely

that male wasps may not feed larvae because females are so much better at it, may seem a bit far-fetched but the source of the hypothesis is irrelevant as long as they are testable. Let us dwell on this a bit more. The misleading way in which we write our scientific papers is responsible for the erroneous impression among many scientists and especially among the public that all scientific hypotheses are generated by a logical, scientific process. In a thought-provoking essay, Peter Medawar (1961) asked “Is the scientific paper a fraud?” and answered in the affirmative [7]. Since this is a serious charge, let me quote him verbatim.

“As to what I mean by asking ‘is the scientific paper a fraud?’ – I do not of course mean ‘does the scientific paper misrepresent facts’, and I do not mean that the interpretations you find in a scientific paper are wrong or deliberately mistaken. I mean the scientific paper may be a fraud because it misrepresents the processes of thought that accompanied or gave rise to the work that is described in the paper. That is the question, and I will say right away that my answer to it is ‘yes’. The scientific paper in its orthodox form does embody a totally mistaken conception, even a travesty, of the nature of scientific thought. . . Hypotheses arise by guesswork. That is to put it in its crudest form. I should say rather that they arise by inspiration; but in any event they arise by processes that form part of the subject-matter of psychology and certainly not of logic, for there is no logically rigorous method for devising hypotheses.”

This is a very short excerpt – the full essay is well worth reading. In short, hypotheses can come from anywhere, even from your dreams, but they must be capable of being put to the test, and it must be possible to disprove them, at least in principle. We must only label an idea as a scientific hypothesis if there is a way to show that it is wrong.

Funding for Science

As it should be obvious, the research described in this chapter, required no money or any sophisticated laboratory facilities. Anybody could have performed these experiments. And yet these experiments have shown for the very first time that a proverbially lazy hymenopteran male can work, given an opportunity. They also show why, at least in one species, the males rarely work, and this paved the way to experimentally cure them of their laziness. The fact that they could be cured of their laziness is further proof of our conclusion about why they rarely work. The twist in the end, that larvae nevertheless die under the care of males, opens up avenues for new lines of research. No one is, therefore, justified in claiming

that they cannot do cutting-edge research due to lack of funds or facilities. And yet this is the almost universal excuse given by most people who should be doing good research but are not.

I am sometimes chastised for giving away the secret that cutting-edge research can be conducted with little or no money. The argument used against me is that if funders and politicians get wind of my claims, they will reduce funding for science. This imagined fear should not drive most of us to do expensive research just to prove that we need more money. Simply getting and spending more money should not be the source of satisfaction, prestige, or power. Different kinds of research need different levels of funding and different kinds of facilities. Research that doesn't require much money or facilities should not be made less fashionable. Indeed, we should all look for ways in which we get the most out of our research for the least amount of money.

One way to achieve and promote such behaviour is to devise metrics that divide research output by the money spent on it. Instead, today we flaunt grants received as an index of our success. This attitude may not be so easy to change as money is power, and the lure of large grants is irresistible to many. But at the very least, I wish to see that those who fail to get large research grants, for one reason or another, do not give up doing good science. Moreover, the distribution of grant wealth of researchers will inevitably be highly skewed with most of us having to make do with relatively small amounts of money. It is important for this large majority of researchers with relatively small grants, to nevertheless find ways of doing innovative research and disproving the belief that the quality of research is proportional to the grants received. And what about students who are yet largely outside the grant getting networks? Should they also be outside the knowledge-producing networks? In many cases, grant-free research may actually be easier, what with all the time saved by not having to write and defend grant proposals, battle the institutional bureaucracies to spend the money and with accounts and finance officers to comply with innumerable mindless rules. It might be good for researchers to begin their careers doing grant-free research and for grant earning scientists to deliberately plan and execute small research projects that require no money. We need to attach social prestige to grant-free or inexpensive research. We must introduce cost-effectiveness into the vocabulary and consciousness of scientists. The goal is not to reduce funding for science as a whole but to produce more out of the funding received and to continue to do cutting-edge research even when the funds have dried up.

Suggested Reading

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7

How Do Wasps Decide Who Would Be the Queen? – Part 1

In this, and the next few chapters, we will continue to explore the social biology of the primitively eusocial wasp *Ropalidia marginata* through simple experiments. Since each wasp colony has a single fertile queen and several sterile workers, and since all or most wasps are capable of taking on both roles, the wasps have to decide who will be the queen and who will be the worker/s. Such a decision has to be made both when new colonies are being initiated as well as when an old queen in a mature colony has to be replaced by a new one. Here, I will describe a simple laboratory experiment that reveals that in the context of new nest initiation, wasps decide who will be the queen by fighting—the winner becomes the queen and the loser/s become the worker/s. The same experiment, in addition to revealing the proximate mechanism of the division of reproductive and non-reproductive labour, also throws light on the advantages of such division of labour.

7.1 The Paper Wasp *Ropalidia marginata*

I have already introduced the Indian paper wasp *Ropalidia marginata* in chapter 6. We need to know a few more facts about this fascinating species, for the purpose of this chapter (*Figure 7.1*). New nests are started either by a lone fe-



Figure 7.1: A mature nest of the paper wasp *Ropalidia marginata* (left), where some but not all of the wasps are marked for individual identification, and newly initiated nests with one, two and three foundresses (right). [Photo credits. Mature nest: Souvik Mandal; Newly initiated nests: Thresiamma Varghese.]

male (single foundress nests) or by a small group (multiple foundress nests). In single foundress nests, the lone female lays eggs and also performs all the tasks connected with building the nest and caring for the brood. In multiple foundress nests, one of the foundresses becomes the queen and lays eggs while the remaining become workers and perform nest building and brood care. Eggs develop into adults, going through the larval and pupal stages, in about 62 days. Eclosing males stay on their parental nests for about a week, after which they leave and lead a nomadic life, mating with females from other colonies, out on foraging trips. Female wasps, however, have at least four different options (Figure 7.2). Option 1 would be to leave their natal nests and start their own new single foundress nests. Option 2 would be to leave their natal nests but to join other female wasps (usually from the same nest but sometimes from other nests as well) to start new multiple foundress nests. Option 3 would be to stay back in the parental nest and spend their whole lives as non-reproducing workers, helping to rear the queen's brood. Option 4 would be to stay back and work for a while, but then to seek an opportune moment to take over the colony of their birth as its next queen. This may become possible because old queens may age and die or may become too weak and susceptible to challenges by a prospective new queen. Such queen turnover

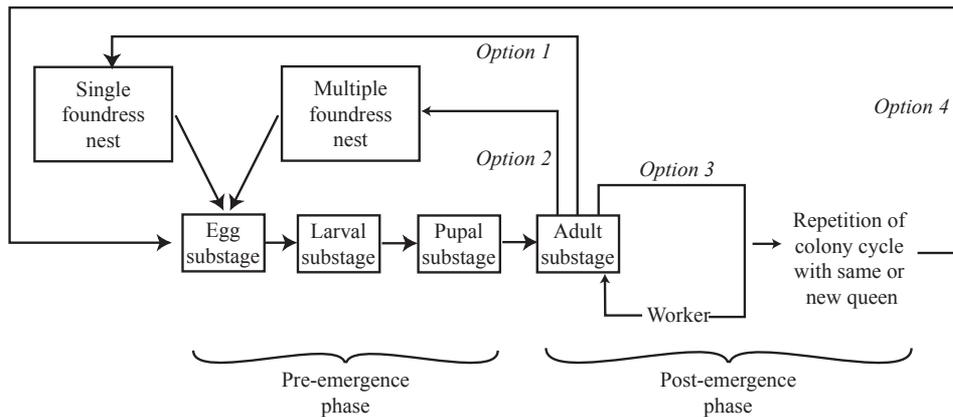


Figure 7.2: The perennial, indeterminate nesting cycle of *R. marginata*. For schematic convenience, the egg, larval, and pupal stages are shown as being distinct. In reality, there is considerable overlap between them, especially when several colony cycles are repeated on the same nest. Similarly, change of queens can take place at any time in the colony cycle. Note also that new colonies may be initiated at any time of the year and may also be abandoned at any time of the year and at any stage in the colony cycle. Female wasps have at least four different options. (Redrawn with permission from R Gadagkar. The evolution of eusociality, including a review of the social status of *Ropalidia marginata*. In: *Natural History and Evolution of Paper-Wasps*, (Eds.) S Turillazzi and M J West-Eberhard, Oxford University Press, Oxford, pp.248–271, 1996.)

happens often enough, but at some random, unpredictable times, and we are yet to make a systematic study of this most interesting phenomenon—we know rather little about when, how and why a new individual, let alone which individual, replaces the old queen and becomes the new queen. The founding of new single or multiple foundress nests as well as queen turnovers in mature colonies may happen anytime of the year and nests may be abandoned at anytime of the year, so that we say that *R. marginata* has an almost aseasonal, perennial, indeterminate nesting or colony cycle; ‘almost aseasonal’, because colonies, both small and large, are more abundant in the summer, though not entirely absent at other times of the year [1].

7.2 Caste Differentiation

One of the reasons we classify *R. marginata* as a primitively eusocial species is because queens and workers are not morphologically differentiated. Mature

colonies consist of a single, fertile queen and several non-reproducing workers. There is a clear reproductive division of labour such that queens lay eggs and do little else while workers perform all the other tasks required for the functioning of the colony—cleaning, building, feeding the larvae, guarding, and foraging for food and building material. Whether an individual becomes a queen or a worker is not already fixed at eclosion as in the case of highly eusocial species such as honey bees and ants, but is rather flexible, allowing individuals to switch roles in the adulthood, as suitable opportunities become available or are lost. Such switching is biased—workers frequently switch to queen roles but the reverse seldom happens, although it is not impossible. Workers switch to queen roles under two circumstances, in option 4 described above, when they may replace their previous queens and take over the queen role in the same colony, or in option 2, when they may leave their present colonies and start new ones in which they function as queens. There is a little complication here. Single foundresses are alone in their new colonies and, therefore, have to combine the dual roles of queens and workers, but we will leave that out of consideration for the present [1].

A defining feature of social insect colonies is division of labour, a feature that is thought to be at the root of their tremendous ecological and evolutionary success. Division of labour can be of two kinds—reproductive division of labour, resulting in queens and workers and non-reproductive division of labour resulting in sub-groups of workers specializing in specific tasks such as nursing, foraging or guarding. Groups of individuals specializing in specific tasks are referred to as ‘castes’. Thus, we have the queen caste, the worker caste, the forager caste, the soldier caste, etc. A more basic division of non-reproductive labour among the workers is often between those who work at home (intranidal workers) and those who work outdoors (extranidal workers). Soldiers or intranidal workers are sometimes referred to as sub-castes, indicating that this represents a secondary division of labour after the primary division of labour between the queens and workers. The divergence of identical individuals into castes or sub-castes is labelled as caste determination or caste differentiation. How and why does caste differentiation happen in the first place? Recall the distinction between the proximate and ultimate answers to the same question, we encountered in the chapter 6 [2]. What are the behavioural or physiological mechanisms which bring about the divergence of identical individuals into queens and workers or into intranidal and extranidal workers? Alternatively, what are the evolutionary advantages, or fitness consequences of differentiation into different castes? In this chapter, we will see that a single, simple experiment can help us answer both these questions.

But first, I will focus on the proximate question because that helps answer the question raised in the title of this chapter, namely, ‘Who would be the queen?’. In the end, I will show, as a bonus, that the same experiment also helps answer the ultimate question regarding the advantage of caste differentiation.

The proximate mechanism of caste differentiation depends on whether we are focussing on a primitively eusocial species or a highly eusocial species. In highly eusocial species, as we saw above, eclosing adults are already differentiated into queens and workers and cannot reverse their roles anymore. Thus, caste differentiation is said to be ‘pre-imaginal’. The adult insect in such holometabolous insects (i.e., those that go through egg, larval and pupal stages before eclosing as adults) is called an ‘imago’. Thus, pre-imaginal caste differentiation takes place in the early larval stages and is, therefore, a physiological and developmental phenomenon. We know that differential nutrition leads to differential hormone synthesis and differential development of the female reproductive system. But in the primitively eusocial species, adults are born nearly or entirely totipotent. Here, caste differentiation is post-imaginal, happening in the adult stage—it is, therefore, a behavioural and social phenomenon. Since *R. marginata* is a primitively eusocial species, we are concerned here with the social and behavioural mechanism of caste differentiation. So how do *R. marginata* wasps decide who would be a queen and who would be a worker? This decision has to be made in two contexts—one, when a group of females initiate a new nest and two, when one of the workers has to replace a dead or weak queen in a mature colony. In this chapter, we will direct our attention to the first context, namely new nest foundation.

7.3 The Experiment

Our goal here is to understand the proximate mechanism of caste differentiation in *R. marginata*, in the context of new nest foundation. In other words, how do the small group of wasps that found a new nest decide which one of them would function as the queen and which one/s would function as non-reproducing workers? This is not a decision that the wasps might be expected to take lightly as queens and workers would be expected to have very different evolutionary fitness. We already know that because *R. marginata* is a primitively eusocial species, caste differentiation is expected to be post-imaginal and mediated through behaviour and social interactions. What we, therefore, need to do is to observe the behaviour of the wasps during the early stages of new nest foundation. This, however, is not as easy as it sounds. In nature, newly initiated nests are hard to find and when we



Figure 7.3 : Souvik Mandal (left), Anindita Brahma (center) with the author (right). [Photo: Threesiamma Varghese]

do find them, it is possible that the crucial behavioural interactions that decide who would be the queen has already taken place. It is probably impossible to observe them before they ever interact. And what if they have already interacted and made their decisions before showing up at the new nest? This is precisely the kind of situation where a simplified, artificial and controlled experiment is called for. And that is exactly what my former student Anindita Brahma performed, with help from another former student Souvik Mandal (*Figure 7.3*). They collected naturally occurring nests with large numbers of pupae and brought them to the laboratory, leaving the adult wasps behind. Here they removed all the eggs and larvae and waited for the pupae to eclose into adult wasps. Soon after eclosion, adult wasps were isolated into individual holding boxes and prevented from interacting with each other. Then they assigned the wasps to three treatments—singletons, pairs and triplets. Thus one, two or three randomly chosen wasps were introduced into transparent acrylic boxes and provided with food (*Corcyra cephalonica* larvae), honey, water and some building material (a piece of soft wood). We know that under these conditions, at least some wasps, whether single or in groups, will initiate a nest, lay eggs and rear them successfully to adulthood. In other words, these conditions simulate new nest foundation in nature. This is quite remarkable

because all these wasps are still virgin. Fortunately for us, *R. marginata* females, although they can mate, appear not to require mating to develop their ovaries and lay viable, haploid, male-destined eggs.

7.4 Nest Foundation in the Lab

Anindita and Souvik set up 77 boxes with one wasp each (singletons), 34 boxes with two wasps each (pairs), and 30 boxes with three wasps each (triplets). They regularly monitored each box for the presence of a nest and, after a nest was built, they kept records of the contents of the nest. They also recorded the behaviour of the wasps in each box, using a video camera, for 10 h before nest initiation and for 30 h after nest initiation (*Figure 7.4*). At the end of the experiment, i.e., after the nest produced at least one adult offspring or one of the wasps died, they collected all the wasps and dissected them to measure their ovaries. Nests were not initiated in all boxes, but 39 singletons, 23 pairs and 20 triplets, initiated nests and laid eggs. This is consistent with our many earlier experiments in which all isolated wasps do not initiate nests; some die without doing so. Moreover, in the present experiment, we discarded pairs and triplets if even one of the wasps died. We are not sure why some wasps do not initiate nests under the same conditions under which others do. The numbers that don't initiate nests are quite substantial. So, we have previously postulated that not all wasps are born totipotent and that there is a certain amount of pre-imaginal caste bias [3] so that some wasps cannot lay eggs, but the remaining can become either queens or workers. It would be nice to understand why some of them failed to lay eggs, but I have not yet been able to come up with a suitable experiment to do so. So, for the time being, we proceed with our experiments, ignoring the dead wasps and working with the living wasps who appear to be totipotent.

7.5 Emergence of Cooperation and Division of Labour

A study of these 82 boxes (singletons, pairs and triplets put together) with successful nest initiation reveals several remarkable features [4].

Cooperation

In all the 23 boxes with pairs and 20 boxes with triplets, nest building and brood care was cooperative—both the wasps (in pairs), and all the three wasps (in the



Figure 7.4: Anindita Brahma, with the observation and recording set-up. Acrylic boxes containing 1, 2 and 3 wasp nests are faintly visible behind the camera stand. [Photo credit: Souvik Mandal]

triplets), invariably participated in these activities. It was never the case that one of the wasps sat idle in a corner and let the others do all the work. Nor was it ever the case that the non-working wasp/s interfered, much less, destroyed the work of the others. And we never had each wasp build its own separate nest; there was always only one nest in each box. This spontaneous display of cooperative

behaviour is truly remarkable. We do not expect such cooperation to be displayed if we put two or three beetles, bugs or spiders together. There is clearly an innate tendency to cooperate, in this social species, even though, as we will see below, such cooperation comes at a significant cost to all but one of the wasps in each box. This observation begs the question of what is it in the genetic make-up of this and similar species that makes them behave in this manner? I hope we can find ways to answer this question in the future.

Reproductive Division of Labour

In all pairs and triplets, there was a clear reproductive division of labour. One and only one individual developed her ovaries and laid eggs. A comparison of the levels of ovarian development of the singleton (which of course developed its ovaries and laid eggs), the two wasps in the pairs and the three wasps in the triplet can be seen in panel A of *Figure 7.5*. Only one wasp in each box had well-developed ovaries, while the other one or two wasps, as the case may be, had significantly poorly developed ovaries. This is the process of reproductive caste differentiation; recall that we began by asking how this came about—how do the wasps decide who will be the queen and who will be a worker? Before we answer that let us marvel at another phenomenon.

Non-Reproductive Division of Labour

In the triplets, reproductive caste differentiation resulted in one wasp with well-developed ovaries (queen) and two wasps with poorly developed ovaries (workers). We observe that the two workers display a non-reproductive division of labour between themselves, with one specializing in working at the nest (intranidal worker) and the other specializing in working away from the nest (extranidal worker). This can be seen from panels B and C in *Figure 7.5*. Note that in this and in *Figure 7.6*, we use the following convention to label the wasps. Queens are labelled Q1, Q2 and Q3, in the boxes with one, two and three wasps respectively. Workers in boxes with two wasps are labelled as W2. The two workers in the triplets are labelled as IW3 and EW3, the IW and EW referring to intranidal worker and extranidal worker respectively, and the subscript 3, referring to the fact that they are in the triplets. The queen still does more intranidal work compared to her two workers, but the intranidal worker does significantly more intranidal work than the extranidal worker. And the extranidal worker does significantly more ex-

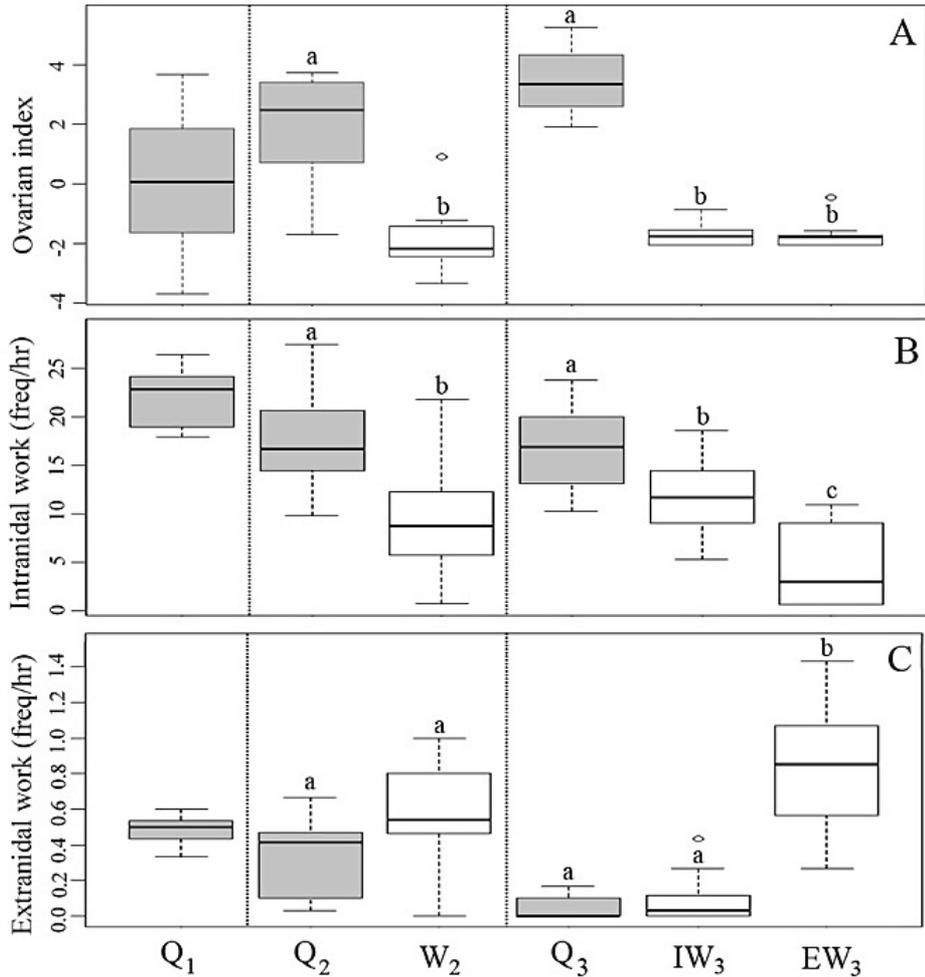


Figure 7.5: Comparisons between queens in solitary, pairs, and triplets and between queens and workers in pairs and triplets; shaded bars show queens and unshaded bars show workers. Letters above the bars represent comparisons between queens and workers in the same panel; bars carrying different letters are significantly different from each other. (Redrawn from A Brahma, S Mandal and R Gadagkar, Emergence of cooperation and division of labor in the primitively eusocial wasp *Ropalidia marginata*, *Proc. Natl. Acad. Sci.*, USA. 115, pp.756–761, 2018).

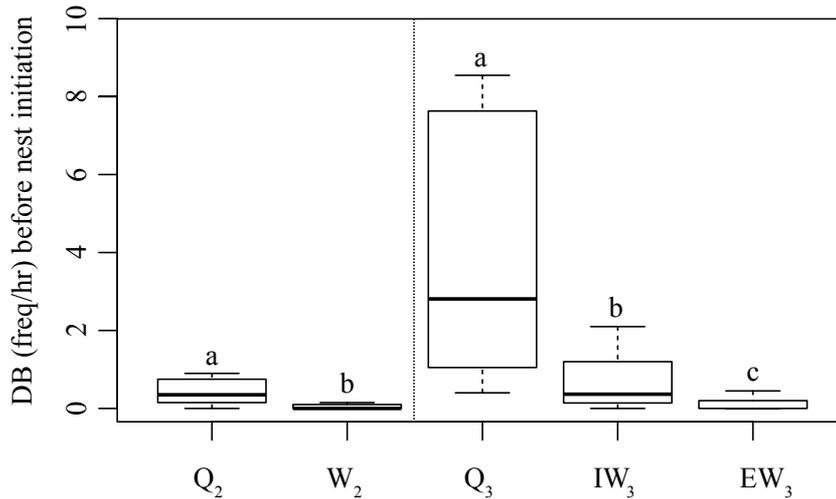


Figure 7.6 : Comparison of rates of dominance behaviour between queens and workers in pairs and triplets. Queens show more dominance behaviour than workers in pairs (left panel), and queens show the highest dominance behaviour and extranidal workers show the least, with intranidal workers being in between (right panel). Bars carrying different letters are significantly different from each other; statistical comparisons are only within each panel. (Redrawn from A Brahma, S Mandal and R Gadagkar, Emergence of cooperation and division of labor in the primitively eusocial wasp *Ropalidia marginata*, *Proc. Natl. Acad. Sci.*, USA, 115, pp.756–761, 2018.)

tranidal work than either the queen or the intranidal worker. We expect that the queen will stop doing intranidal work altogether with the addition of more wasps to the colony, an experiment that we are planning for the future. Just as in the case of the differentiation into queens and workers, we can also inquire into the proximate mechanism of such non-reproductive division of labour—how do the two workers decide who will specialize in the less risky intranidal work and who will specialize in the riskier extranidal work?

7.6 Why Do Wasps Fight?

The many interesting behaviours that we see in the wasp colony can be roughly classified into three kinds—those that benefit the nest and its brood, for example, building the nest, cleaning it or feeding the larvae; those that involve cooperation between the wasps, such as exchange of food or building material, mutual groom-

ing, etc., and finally those that appear, at least at first sight, to be an expression of conflict between the wasps and can be thought of as acts of aggression, although of varying intensities. Wasps may attack, nibble, peck, chase, or immobilize each other. These behaviours are collectively labelled as ‘dominance behaviour’ while the reciprocal behaviours of being attacked, nibbled, pecked, being chased or being immobilized, are labelled as ‘subordinate behaviour’. The expression of such dominance–subordinate behaviour is a distinct feature of primitively eusocial insects and was first discovered by the Italian zoologist, Leo Pardi. In our study, we measure the number of times per hour at which the wasps show each of the dominance or subordinate behaviours. Adding up the frequencies of the individual types of dominance behaviours such as attack, peck, etc., we get a composite number that we refer to as the frequency per hour of dominance behaviour (DB). But why do the wasps show dominance behaviour—why do they fight?

7.7 The Mechanism of Division of Labour

Remarkable as it may seem, the answers to the two questions we have been seeking are intertwined. The wasps fight in order to decide who would be the queen, and who would be a worker. Put in another way, they decide who would be the queen and who would be a worker by fighting. In the pairs, the winner becomes the queen and the loser becomes the worker. Here we define a winner as the one who shows more dominance behaviour and the loser as the one who shows less dominance behaviour (*Figure 7.6*, left panels). The two workers in the triplets also decide who would be the intranidal worker and who would be the extranidal worker by fighting. While queens show the highest levels of dominance behaviour, extranidal workers show the lowest levels of dominance behaviour, and the intranidal workers are in between (*Figure 7.6*, right panel). Notice that these different rates of dominance behaviour have been obtained from the video recordings before nest initiation. This simple experiment has answered the question raised in the title, namely, how do wasps decide who would be the queen? Indeed, it has also answered the question of how the worker wasps decide who would be the intranidal worker and who the extranidal worker, and the question of why the wasps fight. More technically speaking, this experiment has shown that fighting or dominance behaviour is the proximate mechanism of both reproductive and non-reproductive division of labour. Once the division of labour has been established, dominance behaviour reduces and what remains begins to serve an altogether different function, as we will see in the next chapter.

7.8 The Evolutionary Advantage of Division of Labour

Not only has this simple experiment answered the question of how the wasps decide who would be the queen and who would be the workers, and the question of who would be the intranidal workers and who the extranidal workers, but the very same experiment also answers the ultimate (evolutionary) question of the advantage of division of labour. This was possible because, although it was not strictly necessary for the original question, Anindita and Souvik kept careful records of the growth of the nests and their contents, until the nests were abandoned or were successful in producing at least one adult offspring. We define productivities of the nests by adding up the numbers of eggs, larvae of different stages and pupae contained in them on the day of eclosion of the first adult offspring. Since eggs, different stages of larvae and pupae correspond to very different amounts of work that have gone into their production, we cannot consider them as equal in computing total productivity. As a first approximation, we multiplied different developmental stages of the brood with increasing numbers (weights), as they became more advanced in age (and, by implication, needed more effort to produce). Thus, we computed total productivity as the number of eggs multiplied by 1.0, plus the number of larvae multiplied by 2.0, 3.0, and 4.0 depending on the stage of development, plus the number of pupae multiplied by 5.0. Comparing across singletons, pairs and triplets, we find clear evidence of the advantage of non-reproductive division of labour—triplets had significantly higher levels of total productivity as compared to singletons and pairs (*Figure 7.7*). Triplets did not just have significantly higher productivity than singletons and pairs on the last day of the experiment. They showed a consistent trend of being more productive than singletons and pairs throughout the experiment (*Figure 7.8*). Thus, two wasps are adequate to cooperatively build a nest and achieve reproductive division of labour, but a significant benefit in terms of increase in productivity needs at least three wasps, and the associated division of non-reproductive labour—it needs one wasp to specialize in intranidal work and another in extranidal work. Remarkably, the division of non-reproductive labour and increase in productivity is achieved as soon as there are three wasps, the minimum number required for non-reproductive division of labour. In other words, three wasps are both necessary and sufficient for the emergence of non-reproductive division of labour and the associated increase in productivity. This is an important result because, while there are many theoretical arguments and models which postulate the advantages of non-reproductive division of labour, this little experiment provides a rare and clear empirical evidence in support of these theoretical claims.

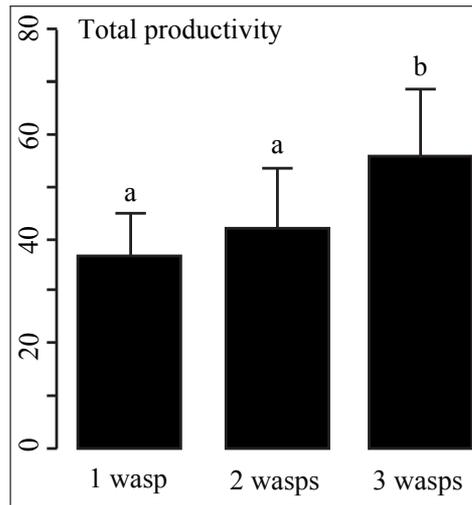


Figure 7.7: Comparison of boxes with 1, 2 and 3 wasps in their total productivity. Total productivity is measured as the sum of the numbers of eggs, larvae and pupae (including those eclosed) on the day of the eclosion of the first adult wasp from the nest. Bars carrying different letters are significantly different from each other by the Mann–Whitney test, $P < 0.01$). There is no significant difference in total productivity between one and two wasps but total productivity in three-wasp nest is significantly greater than that in one- and two-wasp nests, demonstrating the advantage of non-reproductive division of labour. (Redrawn from A Bhadra, S Mandal and R Gadagkar, Emergence of cooperation and division of labor in the primitively eusocial wasp *Ropalidia marginata*, *Proc. Natl. Acad. Sci., USA*, 115, pp.756–761, 2018.)

7.9 Reflections

The very simple experiment described here required no sophisticated equipment, nor any facilities that are hard to put together. Even the video recording required no more than a simple and inexpensive webcam costing about Rs. 4000. Not having to spend time and effort for obtaining funds and setting up laboratory facilities, we can focus more carefully and completely on the design of the experiment itself. Here I want to highlight two different themes.

Context

We must exercise caution in asking questions and drawing conclusions about the function of animal behaviour. Animal behaviour often tends to be context-

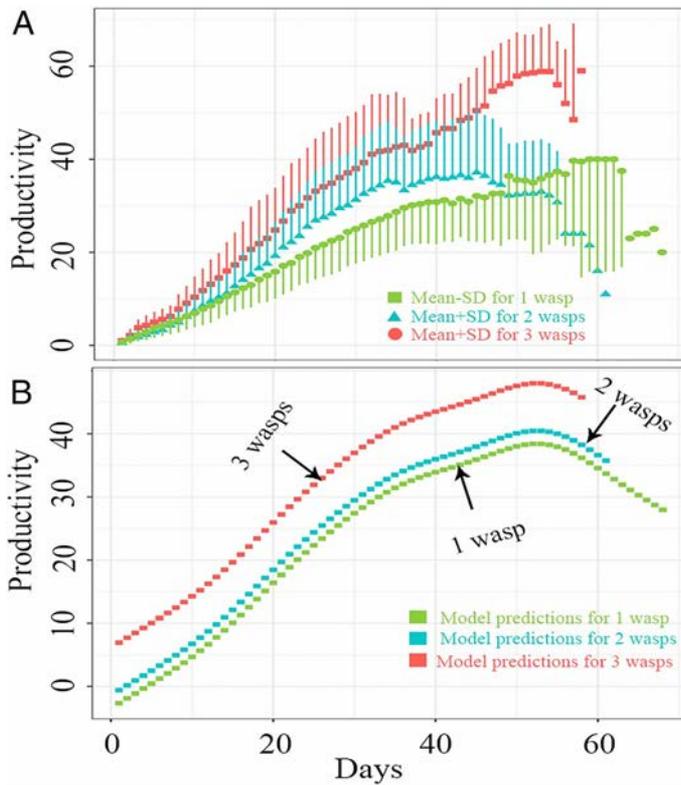


Figure 7.8: Rate of change of productivity with time in singletons, pairs and triplets. Mean plus or minus standard deviation of daily productivity (upper panel) and predictions of the mean daily productivity, using a generalized additive mixed model (lower panel) See [4] for details. (Redrawn from A Brahma, S Mandal and R Gadagkar, Emergence of cooperation and division of labor in the primitively eusocial wasp *Ropalidia marginata*, *Proc. Natl. Acad. Sci.*, USA, 115, pp.756–761, 2018.)

dependent—the same behaviour may have different functions in different contexts. Hence, we should be aware of the context before deciding the possible function of different behaviours. In this experiment, we asked questions such as how do the wasps decide who would be their queen, and why do they fight? We studied the wasps in the context of their building new nests and concluded that the wasps decide who would be the queen and who would be the worker, by fighting and that the function of fighting is, therefore, to settle the important question of who would be the queen. This conclusion is only in the context studied here, namely,

new nest foundation. Wasps also have to decide which one of them would be the queen in another context, namely, when the queen of a mature colony dies and has to be replaced by one of the workers. Although we see that the wasps show the same kinds of dominance–subordinate behaviours in mature colonies, we should not conclude that queen succession in mature colonies is also decided by fighting, nor that the function of fighting in mature colonies is to decide their next queen. We should not jump to such a conclusion even if this is indeed the correct answer in other species that have been studied so far. We will see in the next chapter that dominance–subordinate behaviour in mature colonies of *R. marginata* indeed has a different function and that we must, therefore, ask how wasps decide who would be the queen, all over again in that context. Recall “caution in coming to conclusions” in the definition of ethology by Peter and Jean Medawar, that has been serving us as a guiding beacon, throughout this book (see chapter 1).

Natural versus Artificial

An important feature of the experiment described here is that it was conducted in rather artificial conditions. We believe that by putting wasps in acrylic boxes and giving them food and building material, we have simulated the conditions for new nest foundation in nature. Nevertheless, these conditions are very artificial. The wasps are confined to a small box and have no choice of what to eat and what to build their nest with. More importantly, they have neither the choice of being alone or being in groups nor of the choice of group size. Even more importantly, they have no choice in the matter of who their partners and cooperating companions would be. We randomly assigned wasps to live as singletons, pairs or triplets. Besides, the wasps were virgin, which may not usually be the case when they set out to build new nests in nature. Is so much artificiality permissible? Is the experiment doomed from the start? There are many discussions and misunderstandings in the literature about how natural the experimental conditions should be and how much artificiality we can afford. The answer really depends on the questions we are asking and the tolerance of the experimental animal to the artificiality being imposed.

Consider the virginity of the wasps we used. We know from extensive previous research that in *R. marginata*, mating is not essential for a wasp, to develop her ovaries, lay viable haploid eggs, and become the sole egg layer of a colony, even superseding other mated wasps. When a virgin and a mated wasp are kept together in small boxes, we find they each have equal probabilities of becoming the queen

and workers—presumably, their fighting abilities are unaffected by their virgin or mated status. Hence, even though wasps building new nests in nature might be mated rather than virgin, we decided that it is perfectly reasonable to use virgin wasps for the experiment described here. To use only mated wasps would have made our experiment much more difficult, and would certainly have introduced other, even more undesirable artificialities.

One of the reasons we artificially confined randomly chosen wasps in small enclosures immediately after their eclosion is that we wanted to observe all their behavioural interactions from the beginning. This would not have been possible in nature. In spite of obtaining such clear-cut results about how the wasps decided who would be the queen and who would be the worker, we were constantly in search of ways of confirming these results under more natural conditions. More recently, we have succeeded in doing so. The problem in studying new nest foundations in nature, apart from the difficulty of finding them, is that we do not know where wasps seen at new nests came from. We do see wasps leaving mature colonies, but we do not know where they go to build new nests. In our closed laboratory cages, we seldom observe new nest foundation, presumably because there is insufficient space for the co-existence of multiple nests. We have now overcome these problems in a simple way. I constructed large walk-in cages measuring 1.75 m × 1.75 m × 1.95 m inside which I could place both the nest and my student Anindita (*Figure 7.9*)! As I had hoped, we immediately began to observe new nest initiations by wasps leaving their parent nests. Anindita and Souvik collected nine large nests from nature and transplanted each one of them into such walk-in cages and observed a total of 29 new nest initiations [5]. They were able to record a detailed timeline of events in each cage. Thus, we were able, for the first time, to study the process of wasps leaving their nest of birth and found new nests.

Of the 29 new nests initiated, nine were single foundress nests and 20 were multiple foundress nests. Now, in this more natural situation, we were able to understand who left the parent nest and why, who went with whom to the new nest and how they sorted themselves into queens and workers in the new nest. The most interesting result, from the point of view of the more ‘artificial’ experiment described in this chapter, is that small groups of wasps aggregated outside their parent nests and indulged in dominance–subordinate interactions even before they converged on a new place to build a new nest. The most dominant wasps in these off-nest aggregations, i.e., those showing the highest rates of dominance behaviour, became queens in the new nest, and those showing significantly



Figure 7.9 : Anindita Brahma making observations inside the walk-in cage. [Photo credit: Souvik Mandal].

lower rates of dominance behaviour became workers, thus confirming the result of the present experiment that dominance–subordinate behaviours are used to decide who would be the queen (*Figure 7.10*). It also confirmed our suspicion that the wasps might have interacted with each other and made their decisions before arriving at the new nesting site. The more natural, but more difficult, walk-in cage experiment with small sample sizes validated our more artificial, easier experiment with larger sample sizes. We were, therefore, justified in doing the artificial

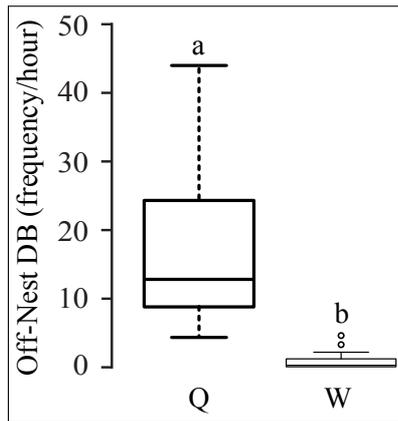


Figure 7.10 : Dominance behaviour in off-nest aggregations in the walk-in cages, prior to new nest initiation. Future queens show significantly higher rates of aggression compared to future workers, in the off-nest aggregations in the walk-in cages, as indicated by different letters above the bars (Redrawn with permission from A Brahma, S Mandal and R Gadagkar, To leave or to stay: direct fitness through natural nest foundation in a primitively eusocial wasp, *Insectes Sociaux*, 66, pp.335–342, 2019).

experiment after all. And we kept thinking of how to make it more natural, and we did. One might argue that the walk-in cage experiment is also not entirely natural—it will do for the time being, but we will keep working for ever on better experiments.

The Future

The ‘artificial’ experiment in acrylic boxes gave us the answers to our question. But they also motivated us to verify the answers in more natural settings. The walk-in cages did just that. Now they motivate us to ask the next set of questions using the ‘artificial’ acrylic boxes. If two wasps are adequate to produce cooperation and reproductive division of labour and three wasps are adequate to also produce non-reproductive division of labour and increase in productivity, what would be the effect of the 4th, 5th or 6th wasp on the nest? In nature, newly initiated nests can contain up to 22 wasps. We now propose to make careful studies of the behaviour of 4, 5, 6 and more wasps and hope to literally witness the emergence of social complexity, under our very eyes. The best experiments are those that while answering one question, raise one or more new ones.

Suggested Reading

- [1] R Gadagkar, *The Social Biology of Ropalidia marginata: Toward Understanding the Evolution of Eusociality*, Harvard University Press, Cambridge, Massachusetts, USA, 2001.
- [2] R Gadagkar, *Survival Strategies: Cooperation and Conflict in Animal Societies*, Harvard University Press, Cambridge, Massachusetts, USA and Universities Press, Hyderabad, India, 1997.
- [3] R Gadagkar, C Vinutha, A Shanubhogue and A P Gore. Pre-imaginal biasing of caste in a primitively eusocial insect, *Proceedings of the Royal Society of London B*, 233, pp.175–189, 1988.
- [4] A Brahma, S Mandal and R Gadagkar, Emergence of cooperation and division of labor in the primitively eusocial wasp *Ropalidia marginata*, *Proc. Natl. Acad. Sci.*, USA, 115, pp.756–761, 2018.
- [5] A Brahma, S Mandal and R Gadagkar, To leave or to stay: direct fitness through natural nest foundation in a primitively eusocial wasp, *Insectes Sociaux*, 66, pp.335–342, 2019. <https://doi.org/10.1007/s00040-019-00702-2>.

8

How Do Wasps Decide Who Would Be the Queen? – Part 2

Continuing to explore the fascinating world of the Indian paper wasp *Ropalidia marginata*, in this chapter, we will ask how wasps choose their queens in another context. In chapter 7, we saw how a simple experiment revealed that wasps fight, i.e., indulge in dominance-subordinate interactions, and the winner becomes the queen and the loser becomes the worker. This was in the context of new nest foundation. But context matters. When the same wasps once again have to decide who will be their next queen if the first one dies or is experimentally removed, the same rules do not hold. The wasps in a mature colony continue to show dominance-subordinate interactions and can even be arranged in a dominance hierarchy, but the dominance ranks of the wasps do not predict who their next queen will be. How they choose their next queen in this context continues to be an enduring mystery. In this chapter, I will describe four simple experiments that have helped us come close to nailing the culprit, although I must confess that we have not yet found the smoking gun—the chase is on, and we are hot on the trail—please join in!

In describing the four experiments below, I will use the following pattern. For each experiment, I will begin by asking a question, provide some necessary background information, outline the experimental design, state the predictions, describe the results and finally, answer the question raised at the beginning.

Resonance, Vol.24, No.11, November 2019, pp.1287–1310



Figure 8.1: A large nest of *Ropalidia marginata*, showing how we uniquely mark the wasps for individual identification, using spot of paint of different colours on different parts of their body [Photo: Souvik Mandal].

8.1 Experiment 1

Question

How does the queen signal her presence to her workers?

Background

We have already seen in chapters 6 and 7 that *Ropalidia marginata* wasps live in colonies consisting of a single fertile queen and several non-reproducing workers. This species is classified as primitively eusocial because the queens and workers cannot be distinguished morphologically (Figure 8.1). In other primitively eusocial species, the queen is usually a very aggressive and highly interactive individual, showing dominance behaviour and bullying her workers. It is believed that by such bullying, queens inhibit their workers from reproducing and ensure that they work for the colony. In *R. marginata*, however, the queen is a remarkably meek



Figure 8.2: Photo gallery of my five PhD students and one post-doc, who conducted the four experiments described in this chapter. Upper row, from left to right: Annagiri Sumana, Sujata Deshpande and Anindita Bhadra; lower row, from left to right: Aniruddha Mitra, Kannepalli Chandrasekhar and Alok Bang.

and docile individual who seldom shows any dominance behaviour and rarely interacts with her workers. We can only be really sure who the queen is when we see her lay eggs. This raises many interesting questions. Why do the workers accept her as their queen? How does she manage to inhibit worker reproduction and maintain reproductive monopoly? How does she ensure that the workers actually work and do not laze away? Indeed, how do the workers even know that they still have a queen and a healthy one at that? In other words, how does the queen signal her presence to her workers, if she is not actually bullying them from time to time? The experiment that I will describe in this section is meant to answer this last question. This was a rather massive and tedious experiment that was conducted by my PhD student Annagiri Sumana, with help from two other PhD students Sujata Deshpande and Anindita Bhadra (*Figure 8.2*, upper row). We hypothesized that the queens of *R. marginata* signal their presence to their workers by means of pheromones. Queens in highly eusocial species are known to do so. A honeybee queen with tens of thousands of workers does not of course physically interact with them or bully them; instead, she produces volatile pheromones which the

workers smell and thus detect the presence of their queen, and indeed, her health. Then why not the same in *R. marginata*? Even though it is a primitively eusocial species, *R. marginata* queens may produce pheromones, and the species may be intermediate between primitively and highly eusocial levels of organization. In order to find and study such pheromones, it would be helpful to know whether they are volatile or non-volatile; both kinds of pheromones are known in other animal species.

We do know that workers in *R. marginata* are aware of the presence of their queen because they change their behaviour within minutes of the experimental removal of the queen from their nest—one of the workers becomes hyper-aggressive and begins to show very high levels of dominance behaviour toward all the remaining workers. This hyper-aggressive worker will immediately stop being aggressive and go back to work if the queen is returned. If the original queen is not returned, however, she will reduce her aggression gradually and become the next queen of the colony in about a week. Hence, we have labelled such a hyper-aggressive individual as the potential queen (PQ). We have exploited this very striking response of the workers to the presence or absence of their queen to understand how they might sense her.

Experimental Design

Sumana, Sujata and Anindita brought 22 naturally occurring nests of *R. marginata* to the Vespiary and transplanted each of them into closed, wood and wire mesh cages of dimension $30 \times 30 \times 30$ cm, fed them with *Corcyra cephalonica* larvae, honey and water, and offered them a piece of soft wood to build their nests (*Figure 8.3*). The wasps do well under these conditions, display behaviour indistinguishable from that in natural nests, and produce both male and female adult offspring. As is our usual practice, they uniquely marked all the wasps with spots of coloured paint (see *Figure 8.1*), censused the population and maintained a map of the nest and its brood every day. After the nests had settled well in their cages, the student trio began the experiment, which lasted for three days for each nest.

On day 1, they conducted behavioural observations for six hours to record the various behaviours shown by the wasps. Following methods standardized in the lab, they used a set of unbiased sampling methods to record the behaviour of every wasp [1]. Their special interest was to compute the frequency per hour at which each wasp shows dominance behaviour to every other wasp. These observations also allowed them to identify the queen of the colony. If the queen does not lay



Figure 8.3 : Sujata Deshpande at work using the laboratory cage used in experiment 1.

an egg and thus give her identity away when we are making observations, we generally remove one of the eggs with a fine forceps, and this usually motivates her to lay an egg in that cell.

On day 2, they temporarily removed all the adult wasps, placed them in individual glass vials, and cut the nest in two halves, taking care to leave a roughly equal amount of brood in each half. They then inserted a wire-mesh between the two nest halves so that the wasps could not move across the mesh. Next, they reintroduced all the workers, one by one, on one or the other side of the mesh, by tossing coins and randomly assigning them to the left or the right side. Finally, they similarly tossed a coin and introduced the queen randomly to one of the two sides. Thus they now had two nest fragments, one with half the workers and the queen (the queen-right side) and the other with the remaining half of the workers but without the queen (the queen-less side). Following this procedure, they repeated six hours of observation, paying equal attention to all the wasps on both sides of the mesh.

On day 3, they moved the queen from its original side to the opposite side, leaving the workers undisturbed and repeated another day's observations as before.

Predictions

In designing this experiment, we had clear-cut predictions that would unambiguously distinguish between the two possibilities—whether the queen pheromone is volatile or non-volatile.

Prediction 1: If the queen pheromone is volatile, it should freely diffuse across the mesh partition, so that wasps on both sides of the mesh should be able to smell their queen as on the previous day, and both nest fragments should thus behave like normal queen-right colonies.

Prediction 2: If the queen pheromone is non-volatile, wasps on the queen-less side should behave as if they have lost their queen, i.e. one individual should become hyper-aggressive and display PQ-like behaviour. If this happens, then, as a further confirmation, the behaviour of the wasps should change on day 3. The new queen-less side which was queen-right and had no PQ on day 1, should now behave like a queen-less colony and have a PQ. The new queen-right side that was queen-less on day 2, should now behave like a queen-right colony—the previous day's PQ should reduce her aggression and go back to work (*Figure 8.4*).

Results

The results could not have been more clear-cut. In all experiments, prediction 2 was upheld. On day 1, a queen was identified in every colony, and there was no PQ. On day 2, the queen-right side behaved like a normal colony, with a normal queen and no PQ. The queen-less side, however, had a clearly hyper-aggressive individual, who was indistinguishable from hyper-aggressive PQ's from previous experiments where the queen was removed altogether—let us call her PQ1. On day 3, the previous day's queen-less side now behaved like a queen-right nest and the previous day's PQ (PQ1) lost her aggression, as expected. And, also as expected, the previous day's queen-right side now behaved like a queen-less colony, with a clearly hyper-aggressive PQ—let us call her PQ2. Once the particular individuals that became hyper-aggressive (PQ1 and PQ2) on the two sides, on days 2 and 3 respectively, we could retrospectively examine their levels of dominance behaviour when they were not behaving like PQs. Thus nobody showed high levels of dominance behaviour (hereafter I will use 'dominance behaviour' and 'DB', interchangeably) on day 1, not the queen, not the day-2's PQ1 nor the day-3's PQ2. On day 2, only PQ1 showed significantly high levels of DB and not the queen or PQ2. On day 3, only PQ2 showed significantly high levels of DB, not the queen, nor the PQ1 (*Figure 8.5*) [2].

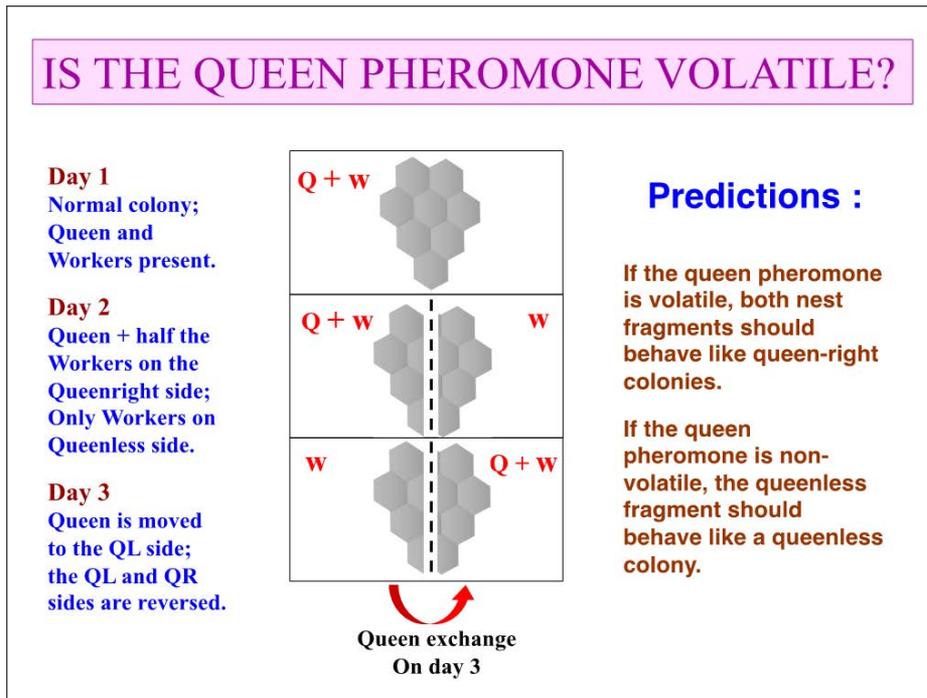


Figure 8.4 : Schematic representation of Experiment 1.

Answer

The queen signals her presence to her workers by producing a non-volatile pheromone.

8.2 Experiment 2

Question

How do the wasps detect the queen's non-volatile pheromone?

Background

If the queen pheromone in this species is non-volatile, how do the workers detect it? The honey bee queen pheromone is volatile and the air in the colony is impregnated with it, making it easy for workers to detect the presence of their queen.

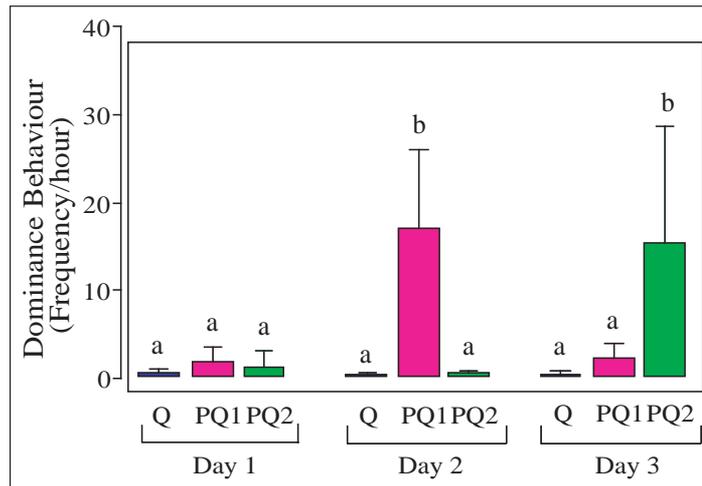


Figure 8.5: Mean and standard deviation of the frequency per hour of dominance behaviour of the queen, potential queen 1 (PQ1) and potential queen 2 (PQ2) on day 1 (unmanipulated colony), day 2 (after dividing the colony) and day 3 (after exchanging the queen) ($n = 12$ colonies). Comparisons are by Wilcoxon matched-pairs signed-ranks test and significantly different values ($P < \alpha$, where α is set to 0.0023 after Bonferroni correction) are indicated using different letters. (Redrawn with permission from A Sumana, S A Deshpande, A Bhadra and R Gadagkar, Workers of the primitively eusocial wasp *Ropalidia marginata* do not perceive their queen across a wire mesh partition, *J. Ethol.*, Vol.26, pp.207–212, 2008).

The attraction of the workers to the volatile queen pheromone of the honey bee queen is so impressive that you can remove the queen and place her a little away from the hive and see that the workers will move over to where she now is. This is used by some people to conjure up a trick and grow a bee beard—they place the queen bee in a cardboard box and tie the box to their chin, and workers will automatically come and settle around the cardboard box and make a beard on the wearer’s face! More profitably, other people chemically synthesise the queen pheromone and spray it on orchards, making nearby bees to visit the orchard in search of their queen and incidentally pollinate the flowers there. This can result in impressive increases in the yield of fruits from the orchards and whopping increases in profits [3]. But in *R. marginata*, the queen pheromone is non-volatile. The first hypothesis we considered was that it is transferred from the queen to the potential queen by direct physical contact. This requires that the PQ interacts with the queen more often than it takes for her to realize the queen’s absence. The

PQ behaves in a manner that she realizes the absence of her queen in no more than 30 minutes. But the queen being such a non-interactive individual, her rates of interaction with the PQ are inadequate to account for the rapidity with which the PQ realizes the queen's absence. Maybe the queen pheromone reaches the PQ through a relay from other workers who may interact with the queen more frequently. A computation of the shortest path from the queen to the PQ shows that even this is not adequate to account for the rapidity of the PQ's change in behaviour from a quiet individual to a strikingly hyper-aggressive one [4]. Another hypothesis is that the queen applies her pheromone to the nest surface. Indeed, the queen frequently rubs her abdomen on the nest. But where is the pheromone produced? We hypothesized that it is produced in the Dufour's gland residing in the queen's abdomen and delivered through a duct that opens on the underside of the abdomen. Everything seemed to fit our imagination, but of course, there was no evidence. It is precisely to test these speculations and find evidence, one way or the other, that my research team which included my PhD students Anindita Bhadra, Sujata Deshpande, Aniruddha Mitra and Annagiri Sumana, and post-doc Kannepalli Chandrasekhar (Chandu) (*Figure 8.2*) designed and conducted Experiment 2.

Experimental Design

The experiment we designed is called a bio-assay. In a bio-assay, we confirm the presence of a chemical of interest by showing that it induces the expected biological response. Here, the chemical in question was expected to be contained in the Dufour's gland. So we prepared a crude extract of the Dufour's gland, by dissecting the wasps, removing their Dufour's gland and macerating it in 30 μ l insect Ringer's solution (a mild salt solution). And the biological response we assayed was the behaviour of the PQ. We have seen that the PQ becomes hyper-aggressive upon removal of the queen and reduces her aggression upon return of the queen. The question we asked was whether the PQ would similarly reduce her aggression in a queen-less colony, if we applied a crude extract of the Dufour's gland, instead of returning the queen. In other words, does the crude extract of the queen Dufour's gland mimic the queen? Such experiments always need safeguards to prevent being misled by the expected result caused by something other than what we have postulated. One must build in such safeguards no matter how unlikely that something else may appear and even we cannot postulate 'something else' that might lead to the same result. These safeguards are called controls. We

planned two types of controls. The first control involved repeating an identical experiment but applying just the Ringer's solution instead of the Dufour's gland extract. This would rule out any unexpected and unknown effect of the experimental procedures that might make the PQ reduce her aggression. The second, even more important control, involved applying the crude extract of the Dufour's gland dissected from a worker rather than a queen. Another important safeguard that I must emphasize has to do with avoiding human bias while making behavioural observations. All the observations were done in the blind, i.e., the observer did not know whether it was the queen extract, the worker extract or the Ringer's solution that had been applied during any given trial. The experiment involved 3 sessions lasting 36 minutes each.

Session 1: Observe a normal colony (with all the wasps marked for individual identification as usual) and identify the queen.

Session 2: Remove the queen identified in session 1 and record the dominance behaviour of all the wasps to identify the PQ.

Session 3: Apply either the queen's Dufour's gland extract, the worker's Dufour's gland extract or the Ringer's solution and observe the behaviour of all the wasps (not just the PQ).

Only one thing can be applied at any given time and what is to be applied at any given is best decided randomly. And a nest can be used only once. We used 25 separate nests in all, eight for applying the queen extract, nine for applying the worker extract and another eight for applying the Ringer's solution. The reader can imagine that conducting this experiment required expert coordination and time management, not unlike the situation in an operation theater—observe the colony, remove the queen and a random worker, observe the queen-less colony during which time, also dissect the removed queen or worker and prepare the Dufour's gland extract, apply the extract and observe again, with no time to be lost. Anindita, Sujata, Aniruddha and Chandu braced themselves up to the task, with an efficient division of labour among themselves—Anindita would make the behavioural observations while Sujata, Aniruddha and Chandu would perform the dissections and prepare the required extracts in time and apply them to the nest. Yes, it worked, as we will see below.

Prediction

Our predictions were straightforward. The hyper-aggressive PQ should drop her aggression in response to the application of the queen's Dufour's gland but not

upon the application of the worker Dufour's gland extract, nor upon the application of the Ringer's solution.

Results

Our results were no less clear-cut. Nobody showed dominance behaviour in session 1. In session 2, the PQ significantly increased her rate of dominance behaviour compared to session 1. And in session 3, she reduced her rate of dominance behaviour significantly compared to session 2, only in the experiments where the queen extract was applied and not when either the worker extract or the Ringer's solution was applied (*Figure 8.6*) [5].

Answer

1. The queen's Dufour's gland is the source of her pheromone.
2. The workers detect the non-volatile pheromone of the queen because she applies it to the nest surface (and we can do it too!)

8.3 Experiment 3

Question

Do the wasps know who their next queen would be?

Background

Perhaps the most fascinating observation we have made on *R. marginata* is that as soon as we remove the queen, one of the workers reveals herself as the queen's next successor. First, she becomes hyper-aggressive and soon, she will become a full-fledged queen and start laying eggs and maintain reproductive monopoly, until she is in turn replaced by someone else. There are two remarkable features of this phenomenon. One is the predictability of the act of queen replacement and the other ironically enough, is the unpredictability of the identity of the individual who will replace the queen. While we can be sure of the identity of the queen's successor within 30 minutes of removing the queen, we cannot predict who will take on that role as long as the original queen is present. The predictability of the phenomenon of queen succession is on solid grounds. In all healthy colonies,

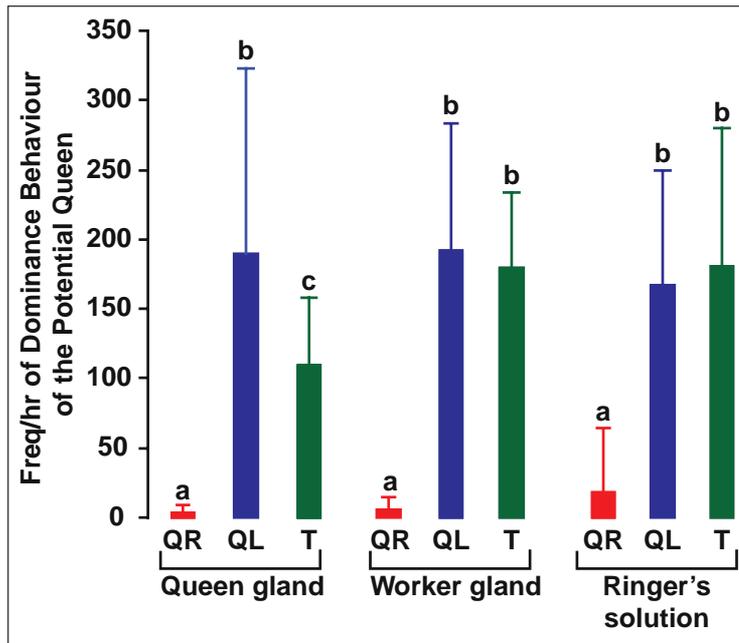


Figure 8.6 : Mean and standard deviation of the frequency per hour of dominance behavior exhibited by the potential queen from *Ropalidia marginata* nests in the three sessions of the bioassay (Queen-right, Queen-less and Treatment; $n = 8, 9,$ and $8,$ respectively) when the nest was exposed to queen Dufour's gland macerate, worker Dufour's gland macerate or Ringer's solution. Comparisons are by Wilcoxon matched-pairs signed-ranks test among the three sessions within each treatment. Different letters denote significant differences among bars ($P < 0.05$). (Reproduced with permission from A Bhadra, A Mitra, S A Deshpande, K Chandrasekhar, D G Naik, A Hefetz and R Gadagkar, Regulation of Reproduction in the Primitively Eusocial Wasp *Ropalidia marginata*: on the Trail of the Queen Pheromone, *J Chem Ecol*, Vol.36, pp.424–431, 2003).

one of the workers will invariably become hyper-aggressive upon removal of the queen. In many experiments, we have confirmed that such hyper-aggression is a reliable proxy for who will become the real egg-laying queen a week later. The unpredictability of the identity of the successor, during the presence of the old queen, is also, unfortunately, on pretty solid ground! We have performed many experiments with the explicit goal of predicting the successor before the removal of the queen, but so far, we have drawn a blank. The PQ, we always learn in retrospect, is a fairly non-descript individual. She is not unique by any criteria, not by her behavioural profile, even when plotted in multi-dimensional space of

many behaviours, not by her position in the dominance hierarchy of the colony, and not even by the residual development of her ovaries (some workers show some rudimentary development of their ovaries, not in any way comparable to an egg-laying queen, though). I must confess that I feel a certain thrill in this mystery and entertain a certain amount of pride in these wasps that I have been familiar with for over 50 years now—they are tough wasps, worthy of any investigator! In the face of this defeat, we asked a different, what seemed to many, as an even tougher question. We do not know who the next queen would be, but do the wasps themselves know who their next queen would be? Experiment 3, that I will describe below, was an attempt to answer this seemingly impossible question. And the challenge was taken up by my PhD student Anindita Bhadra, whom we have already encountered in both experiments 1 and 2, described above.

Experimental Design

On the face of it, the question we have asked sounds like it may be beyond the reach of science—at least present-day science. ‘Do the wasps know?’. Can we know what they know? Can we know the mind of a wasp? The answer I think is yes, but let us break the question in two parts. Is there an heir-designate who will take over as the next queen? Does everyone in the colony know who she is? If so, only the heir-designate, and no one else, should respond to the loss of the queen by becoming hyper-aggressive. The hyper-aggression of the PQ should be unidirectional, i.e, nobody should fight back, nobody should show dominance to the PQ—after all she is the heir-designate, and they know it. We already know that the hyper-aggression of the PQ is unidirectional, but is there an heir designate in the first place? We considered many potential strategies to design an experiment to answer this question. Finally, one idea seemed worth testing. Remove the queen, identify the PQ and put the queen back. We know that the PQ will lose her aggression and go back to being a worker. Now remove the queen again, the following day. If the individual who became the PQ on the previous day was indeed the heir-designate, the same individual should become the PQ again. We performed this experiment and found that the same individual becomes the PQ upon the second removal of the queen. However, we soon realized that this result has an alternate explanation and does not prove that there is an heir-designate. Imagine that there is no heir-designate and that a randomly chosen individual becomes the PQ the first time the queen is removed. Now, it is possible that this individual who became the PQ for a short period is no longer the same. She may

have some advantage over the others on account of her experience and this by itself may make it more likely that she will become the PQ when the queen is removed again. So, we needed a better experiment that does not permit alternate explanations. And here's what we hit upon.

First, let us assume that there is an heir-designate and that all the wasps know who she is. Now, let us predict the behaviour of the wasps in response to our experimental manipulation. Our idea was to make a 'wrong' wasp (who is not the heir-designate) think that she is the heir-designate and then make her realize that she is not the heir-designate, by bringing her face-to-face with the real heir-designate. To accomplish this, we resorted once again to the mesh experiment used in Experiment 1 and separated half the randomly chosen workers and the queen on one side of a wire mesh partition from the remaining workers (without the queen), on the other side. If the heir-designate happens by chance, to be on the queen-less side, she should become hyper-aggressive (PQ) and should not be challenged by anybody from her side. Let us call her PQ1. But she is supposed to be the heir-designate for the whole colony and not just for her side only and everybody, even on the other side of the mesh, should know that. So if we make the PQ1 and the queen to exchange sides, the PQ1 should now face no challenge from anybody on the other side either. She should remain an unchallenged PQ on both sides as long as we keep the queen away from her. But this can happen by chance alone in only about half the experiments. If we repeat this experiment several times, then in about half the experiments, the heir-designate should end up on the queen-right side, by chance alone. Now she cannot become hyper-aggressive because the queen is present on her side. On the queen-less side, however, the 'best' individual (however she is defined) should become a PQ and be unchallenged on her side. Let us call her PQ1 again. This PQ1 however, is not the heir-designate for the whole colony. The real heir-designate is sitting on the opposite side. Now if we make the queen and the PQ1 switch sides and thus bring the PQ1 face-to-face with the true heir-designate, in the queen's absence, the PQ1 should lose her aggression and the true heir-designate should become hyper-aggressive and be unchallenged by anybody from her side, including the PQ1. Let us call her PQ2. But that is not the end of the story. The acid test for the true heir-designate is that she should not be challenged on the opposite side either when we move her there and bring the queen back to her original side. If the wasps will tolerate all this messing around with them, we could test our assumptions that there is an heir-designate and that all the wasps know who she is. Anindita was confident and went right ahead and did the experiments. But let us state our predictions more clearly.

Predictions

Prediction 1: In half the experiments, the first wasp to become hyper-aggressive (PQ1) should be acceptable to both sides and there should be no PQ2. In the remaining half of the experiments, PQ1 should be unacceptable to the opposite side, and a second PQ (PQ2) should emerge on that side.

Prediction 2: PQ2 should be acceptable to both sides and we should never see a PQ3—there should be only two PQs if we cut the nest in two parts.

Results

These experiments are not easy to perform, but Anindita managed to complete eight experiments successfully. And her results were clear. In three of her eight experiments, there was no PQ2. PQ1 remained unchallenged on both sides. This must correspond to the situation where the heir-designate happened to be on the queen-less side. But in the remaining five experiments, a PQ2 emerged when she moved the PQ1 to the opposite side. This must correspond to the situation where the heir-designate was originally on the queen-right side. And she never saw a PQ3 (*Figure 8.7*). Thus, both the predictions were upheld. Perhaps the most important result is not revealed by the statistics. It was truly remarkable that the PQ2 was unchallenged—she did not receive a single act of aggression when she emerged, not from the rest of the workers and not even by the PQ1 who had recently been hyper-aggressive herself, and not on the opposite side, which had recently witnessed a different hyper-aggressive individual. This is what gave us confidence that all the wasps, even the PQ1, knew who the real heir-designate was.

Answer

There is an heir-designate and all the wasps know who she is. We labelled the heir-designate as a ‘cryptic successor’ and we were proud to publish a paper with the title “We know that the wasps ‘know’: cryptic successors to the queen in *Ropalidia marginata*” [6].

8.4 Experiment 4

Question

Is there a reproductive queue?

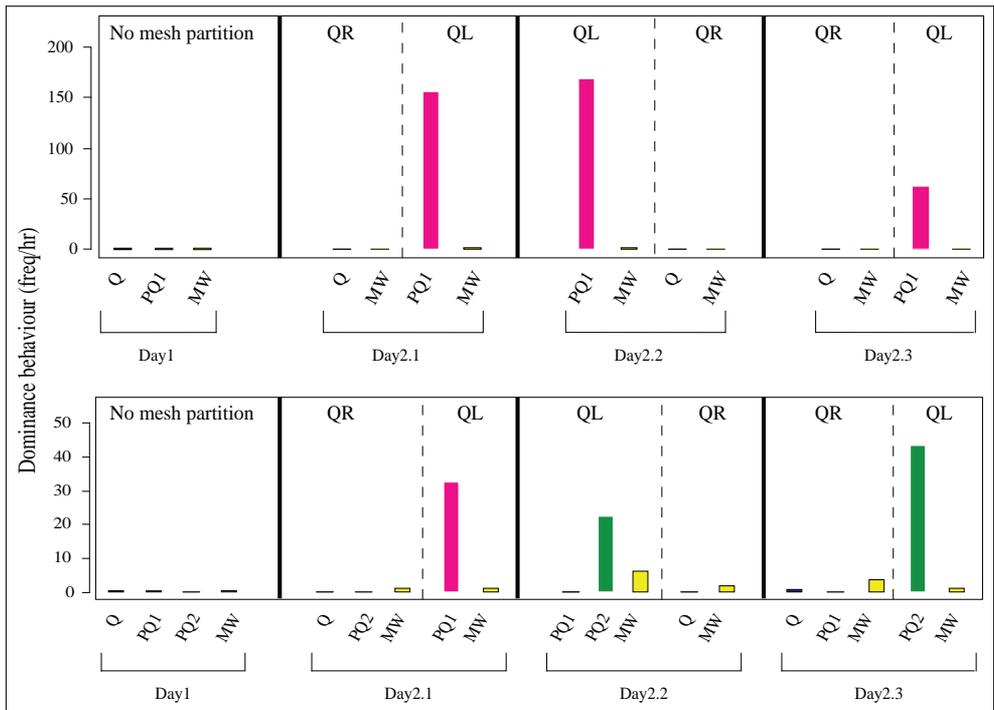


Figure 8.7: The queen Q–PQ exchange experiment. (Upper) A typical experiment in which the PQ1 was the cryptic successor. The frequency per hour of dominance behavior exhibited by the queen, PQ1, and Max worker (MW, defined as the worker showing maximum aggression) on day 1 in the normal colony and on the queen-right (QR) and queen-less (QL) fragments in the 3 sessions on day 2 are shown. (Lower) A typical experiment in which the PQ2 was the cryptic successor. The frequency per hour of dominance behavior exhibited by the queen, PQ1, PQ2, and Max worker on day 1 in the normal colony and on the queen-right and queen-less fragments in the 3 sessions on day 2 are shown. (Redrawn with permission from A Bhadra and R Gadagkar. We know that the wasps ‘know’: cryptic successors to the queen in *Ropalidia marginata*, *Biol. Lett.*, Vol.4, pp.634–637, 2008, Copyright 2008, The Royal Society).

Background

The results of the three experiments described so far paint the picture of a very well-organized society. The queen does not physically bully her subordinates, but simply signals her presence and health by producing a non-volatile pheromone, which she applies to the nest surface so that her workers need not even have to

come in contact with her. From an evolutionary point of view, this suggests that it is also in the interest of the workers to know if they have a healthy queen and obey her. It is surely the mark of an efficient society if the workers work on their own and do not need top-down control. More remarkable still, the workers have a Plan B, if the queen dies. They have organized themselves so as to have agreed on an heir-designate who will take over as the next queen, unchallenged. My next PhD student Alok Bang (*Figure 8.2*, lower right panel) and I wondered whether this society is so well-organized that they have agreed not just on one heir-designate but several of them, each of whom know when their turn would come, or is this too much wishful thinking? There is no better way to find out than to experiment.

The question we were asking was a simple one. When we remove the queen, we can identify the PQ by her hyper-aggression within 30 minutes. What would happen if we immediately removed this PQ also? One possibility is that the colony would not be ready for this calamity—the loss of two successive queens, and even before the second one starts laying eggs, may not be so common in nature. Hence, there might be chaos and some kind of scramble competition with several individuals trying to become queens or the wasps would just remain queen-less and abandon the nest. If our wishful thinking was correct, however, the colony would be prepared for such a severe test of their organization and would throw up a previously agreed upon, and therefore, unchallenged PQ2. Stretching our luck, we might even find that the immediate removal of the PQ2 would yield an unchallenged PQ3, and so on.

Experimental Design

The experimental design here was rather simple. Identify the queen, remove her, identify the PQ1, remove her, identify the PQ2, remove her, identify the PQ3 and so on.

Prediction

Our prediction, based on everything we had known by then about the efficient social organization of this species and some wishful thinking, was thus. Upon removal of a PQ within 30 minutes or so after she becomes hyper-aggressive, the colony will not descend into chaos, with scramble competition and much aggression and egg-laying by many individuals; nor will it simply stop functioning efficiently and get abandoned. Instead, we predicted that upon removal of a PQ,

the colony will behave exactly as it does after removing the queen, namely that one and only one of the remaining individuals will become hyper-aggressive and remain unchallenged. We hoped that, in this manner, we will be able to identify several successive PQs and thus discover a long, well-disciplined reproductive queue.

Results

Alok used 19 nests for each of which, after marking all individuals for identification, he spent two days observing the behaviour of all the wasps and also identified the queen. On the third day, he removed the queen at about 8:30 in the morning and continued behavioural observations for 30–90 minutes to see if a PQ became evident. He used a strict and objective criterion to decide whether there was a PQ and if so, who she was—she had to show at least 5 acts of dominance behaviour and at least twice as many acts of dominance than the next most dominant individual. Detection of PQs, it turned out, was easy and unambiguous—PQs showed 50–100 acts of DB per hour and about an order of magnitude more than the next most dominant individual, within the 30–90 minutes of observation (*Figure 8.8*). More tellingly, PQs rarely received any dominance behaviour from anybody in the colony (*Figure 8.9*). In this manner, he was able to identify five successive PQs in each colony. Perhaps there were more PQs lurking in the colony but he stopped at five, partly because it was too late in the day to continue the experiment and continuing the experiment after an overnight gap may introduce other unwanted variables. Alok took another important precaution while doing this so-called serial PQ removal experiment. We know that the hyper-aggression of the PQ is a good proxy for future queenship. We have seen repeatedly (at least with 13 PQs) that the hyper-aggressive individual we label as the PQ indeed goes on to become the next sole egg layer of the colony if the original queen is not returned. But can we be sure that this is also true for all subsequent PQs, PQ2 to PQ5? We need to be cautious here. We were not sure that removal of the PQ would lead to more unchallenged PQs in a similarly orderly fashion. That is why we did the experiment in the first place. PQ1 might be quite a different phenomenon, having had much more time to be properly designated as the heir-designate, by due process (whatever that might be). But subsequent PQs – PQ2 to PQ5 – emerged within 30–90 minutes of their predecessor PQ being removed and may have, therefore, arisen by quite a different route, without due process, if we can use that convenient phrase, for a process that we do not understand yet. Hence Alok explicitly confirmed that

hyper-aggression is as good a behavioural proxy for the future queenships of all subsequent PQs, as it is for PQ1. Using an independent set of 16 colonies, he allowed four each of PQ2, PQ3, PQ4 and PQ5s to mature into egg-laying queens. In all 16 cases, the PQs identified by the behavioural proxy of hyper-aggression went on to become the sole egg layers of their respective colonies [7].

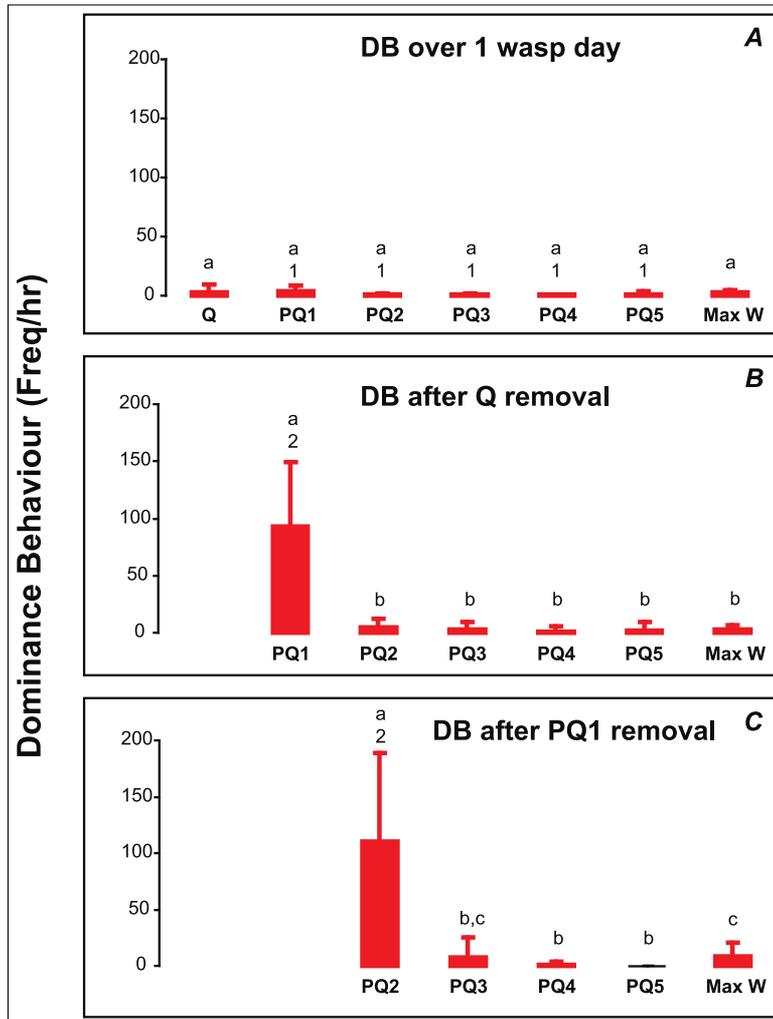


Figure 8.8 continued...

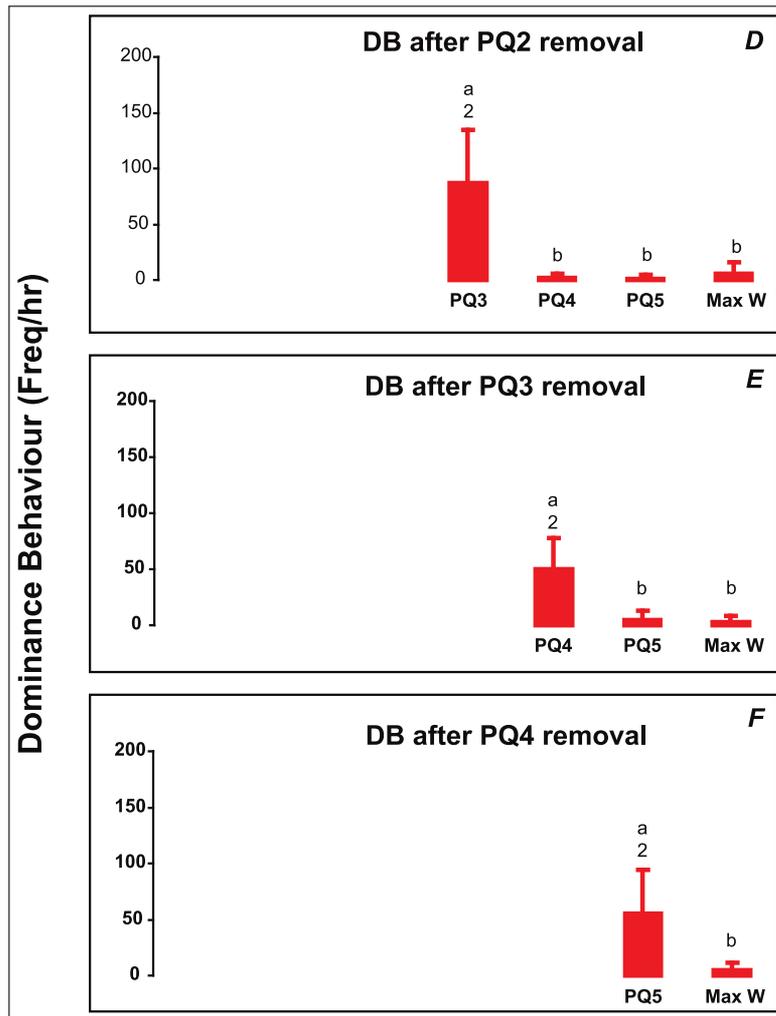


Figure 8.8 : Evidence for a reproductive queue in *R. marginata*. Means and SDs of frequencies per hour of behavioral dominance of the queen, five PQs, and the max worker (the individual that showed maximum behavioral dominance in the colony apart from the queen and the five PQs) in normal queen-right colonies (A) and the PQs and the max workers in the absence of the queen and the preceding PQs (B–F) ($n = 19$ colonies). Bars that carry different letters are significantly different from each other within each panel (Wilcoxon matched-pairs, signed-ranks test. See [7 for more details]. (Redrawn from A Bang and R Gadagkar, Reproductive queue without overt conflict in the primitively eusocial wasp *Ropalidia marginata*, *Proc. Natl. Acad. Sci., USA*. Vol.109, pp.14494–14499, 2012).

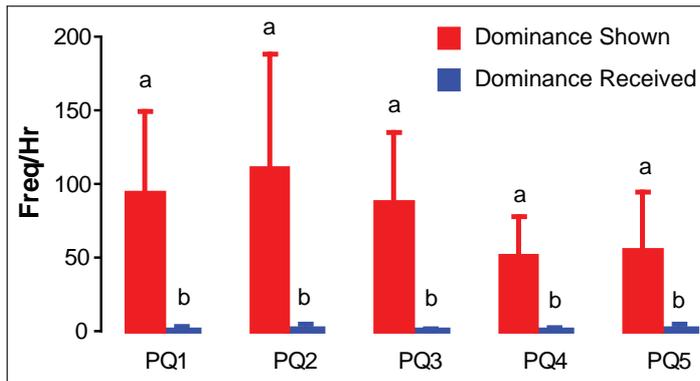


Figure 8.9: Behavioral dominance shown and received (mean \pm SD) by different PQs in the absence of the queen and previous PQs (Wilcoxon matched-pairs, signed-ranks test; $n = 19$ colonies). Note that each PQ showed significantly higher behavioral dominance than what she received. On an average, dominance shown by each PQ was \sim 40-180-fold higher than the dominance she received. DB, behavioral dominance shown; S-, behavioral dominance received. (Redrawn from A Bang and R Gadagkar, Reproductive queue without overt conflict in the primitively eusocial wasp *Ropalidia marginata*, *Proc. Natl. Acad., Sci. USA.* 109, pp.14494–14499, 2012).

Answer

There is a reproductive queue of at least five PQs, but perhaps all the wasps are lined up in a queue. What is even more remarkable is that they take charge in an orderly manner and refrain from becoming hyper-aggressive out of turn.

8.5 So, How Do Wasps Decide Who Would Be Their Next Queen?

I wish to return briefly to the main question raised in the title of this chapter. As I said in the opening passage, how the wasps in a mature colony choose their next queen is a mystery, but we are gathering helpful clues. Experiment 1 told us that queens advertise their presence by means of non-volatile pheromones. Experiment 2 told us that the queen applies her pheromone to the nest surface. Experiment 3 told us that even though we cannot identify the PQ in the presence of the old queen, the wasps themselves seem to know who she is—there is a cryptic heir designate known to the wasps. And, experiment 4 has told us that there is a long reproductive queue of PQs waiting their turn to become future queens. Experiment 4 also somewhat unexpectedly gave us another vital clue. The serial PQ

removal experiments gave us a sample of 100 PQs with data on their behavior before removing the old queen. Alok also measured the productivities of some PQs in the experiment where he tested whether the individuals identified as PQs by their hyper-aggression were really future queens. We had data on their dominance rank, their rates of displaying dominance behavior, the rates at which they built the nest, fed the larvae, the proportion of time they spent away from their nests, their body size, their state of ovarian development, their productivity and their age. Incorporating all these data into a statistical model, we asked whether we could predict the position of an individual in the reproductive queue, i.e., which individuals would become PQ1, PQ2, PQ3, PQ4 and PQ5. Among all the variables used only the age of the individual was a significant predictor of the position of an individual in the reproductive queue. Does this mean that we have solved the problem? Is it simply the age of the wasp that decides who would be the next queen? More caution is required before we jump to that conclusion. Age is certainly a statistically significant predictor of the position of an individual in the reproductive queue, but is it a perfect predictor? Can we use simply age to line up workers in a queue, even before removing the queen, and predict which wasps will successively take over the role of the queen during a serial PQ removal experiment? The answer, unfortunately, is that age is not a perfect predictor of the position of an individual in the reproductive queue. They often violate the theoretically predicted queue based purely on age—individuals often jump the age-based queue. We counted how many times this happened in what we called a queue-jumping analysis. Only in 10 out of the 30 cases studied did the oldest individual in the colony become the next PQ upon removal of her predecessor. Of the remaining 20 cases, 3 individuals became PQs in spite of the presence of equal-aged individuals and 17 PQs bypassed individuals older than themselves to become the PQ out of turn, so to speak [7]. PQs seem to be chosen from among the oldest individuals in the colony but which one from the cohort of old individuals actually becomes the next PQ, remains a mystery. As I said in the opening passage, we have not yet found the ‘smoking gun’ but the hunt is on.

8.6 Reflections

Let us reflect on what made these experiments possible and successful. None of these experiments needed any significant amount of money or sophisticated laboratory facilities. Each one of them could have been done at any Indian home or school, with a garden and a kitchen table; Perhaps a toy microscope would have

been helpful. What they needed instead was a band of dedicated and passionate students, and much background information. In this case, it was very helpful that my research group has been studying this locally available paper wasp species for decades, building up a vast and detailed knowledge, on every aspect of the biology of *R. marginata*. The cumulative impact of this persistent effort is reflected in the ease with which we can ask difficult questions and design clever experiments to answer them [8]. There is often a mistaken notion that we need to find a virgin territory and an unnecessary worry that everything would have been discovered by the time we get to the scene. Nothing could be farther from the truth. It is said that as a child, Francis Crick was worried that everything would have been discovered by the time he grew up. He need not have worried—even the double-helical structure of DNA was waiting for him to be discovered! True scientific knowledge does not annihilate the body of unknowns—it multiplies them. The more we know, the more there remains to be known and the more we can know. Only if you are devoid of imagination should you worry that everything will soon be already known. Imagination is one of the most important tools for scientific research. All children are born with a rich capacity for imagination, which seems to be systematically killed by parents and teachers. I often encourage my students to read as much fiction as they can to retain and sharpen their skills of imagination. Let us imagine, my students and I said, that even though *R. marginata* is a primitively eusocial species, the queen might have evolved a pheromone. Let us imagine we said that the queen rubs her pheromone on the nest. Let us imagine with some courage we said, that the wasps know who their queen is, even though we do not know. Let us be bold to imagine we said, that there is a long and disciplined queue of potential queens patiently awaiting their turn to become queens. And our bold imaginations paid off. It helps to be a bit irreverent toward textbooks, established knowledge and received wisdom. But the question of how the wasps choose their next queen remains a mystery. Does that worry me? Not at all. The thrill is also in the journey, not only in the destination. I am prepared to perform a hundred clever experiments and learn more and more about wasps even if the final answer to the big question keeps eluding us. That is why science is more thrilling than a whodunit!

Suggested Reading

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9

Why Do Wasps Fight? – Part 1

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 chapter 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 chapter 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 chapter that workers continue to show dominance-subordinate behaviour in mature colonies. What is the function of this aggression displayed by the workers? In this chapter, 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.

9.1 How Do the Wasps Fight?

For the reader who might read this chapter without having read the previous three chapters in this book, I should say briefly that in the fourth consecutive chapter, 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*, 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 chapters, 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 chapter, 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 chapters in this book, 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.

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 chapter, so that we can now quickly get back to discussing fighting behaviour of the wasps.

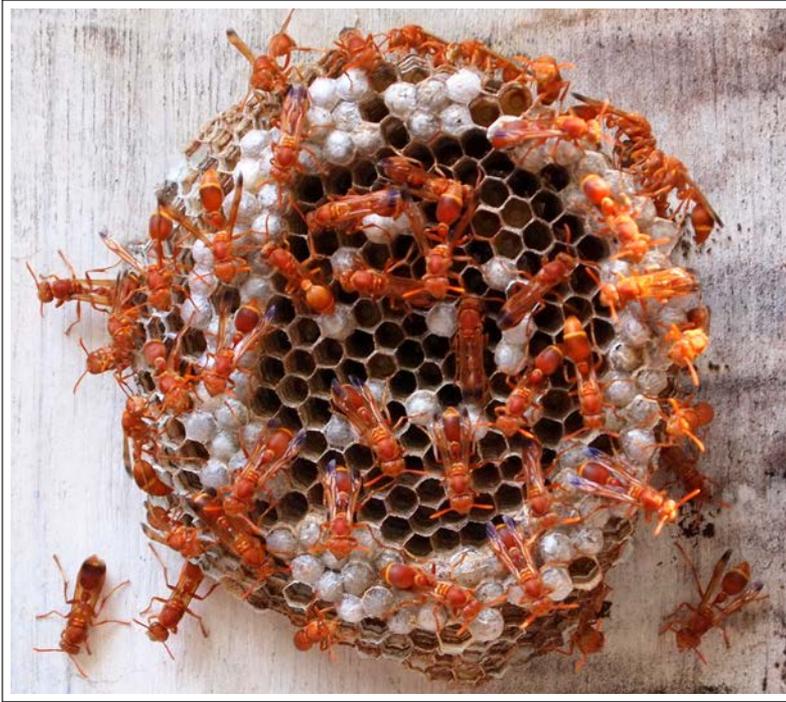


Figure 9.1 : A large nest of the Indian paper wasp *Ropalidia marginata*, showing the nest, brood and adults; the white capped cells are the pupae, a nearly full-grown larva can be seen at the nest periphery at the 1 o'clock position. (Photo: Dr Thresiamma Varghese.)

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

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).

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, for example. 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 [1]. In this chapter, 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 chapter, we will examine the possible functions of dominance behaviour in mature colonies, as opposed to the context of new nest foundation.

9.2 Why Do the Wasps Fight?

Background

We have already seen in previous chapters 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 [see chapter 7]. We have also seen from another previous chapter 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 [see chapter 8], although the potential queen becomes hyper-aggressive—but that is after she be-

comes 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 chapter 8 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?

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 [2]. 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.

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.

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 9.2*, 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.



Figure 9.2 : A Photo gallery of my students who conducted the experiments described in this chapter. **(From left to right, upper row):** Nadia Bruyndonckx, Sujata Deshpande; **(lower row):** Shakti Lamba, Kannepalli Chandrasekhar.

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

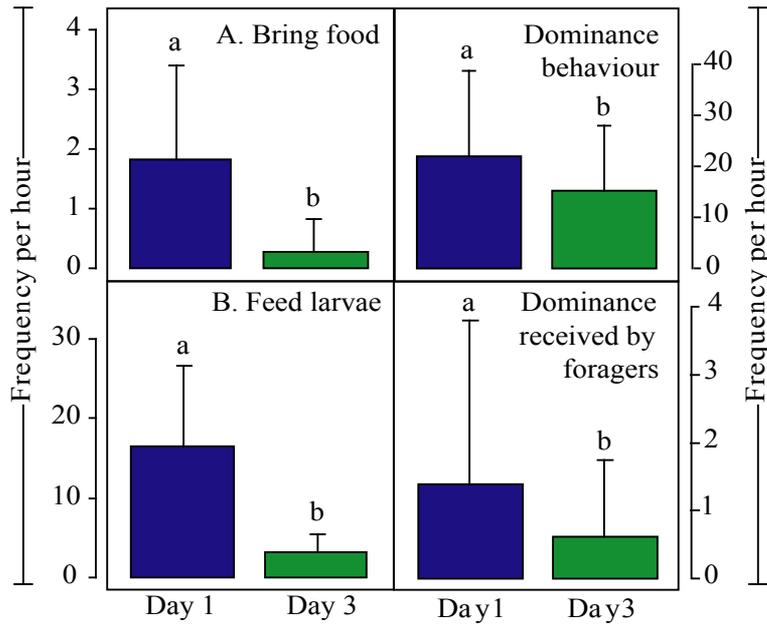


Figure 9.3: Excess feeding experiment. Comparisons of the rates of bring food, feed larvae, and dominance behaviour among workers and dominance received by foragers, on day 1 (normal colony) and day 3 (1 day after excess feeding). Bars shown are the means and SDs across 11 nests. For all variables, values 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).]

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 9.3*). 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 dominance behaviour is used to regulate foraging—workers do seem to signal colony hunger

levels to the foragers by directing dominance behaviour towards them [3]. 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 9.2*, 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 and 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 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 9.4*). 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 [4].

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 intra-nidal workers convey information about the hunger levels in the colony by showing dominance behaviour toward 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 workers 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.

9.3 Reflections

As has been my practice, I will attempt some reflection at the end of describing these experiments. The two experiments described in this chapter exemplify the various themes that I have been exposing in this series—low-cost or no cost, no

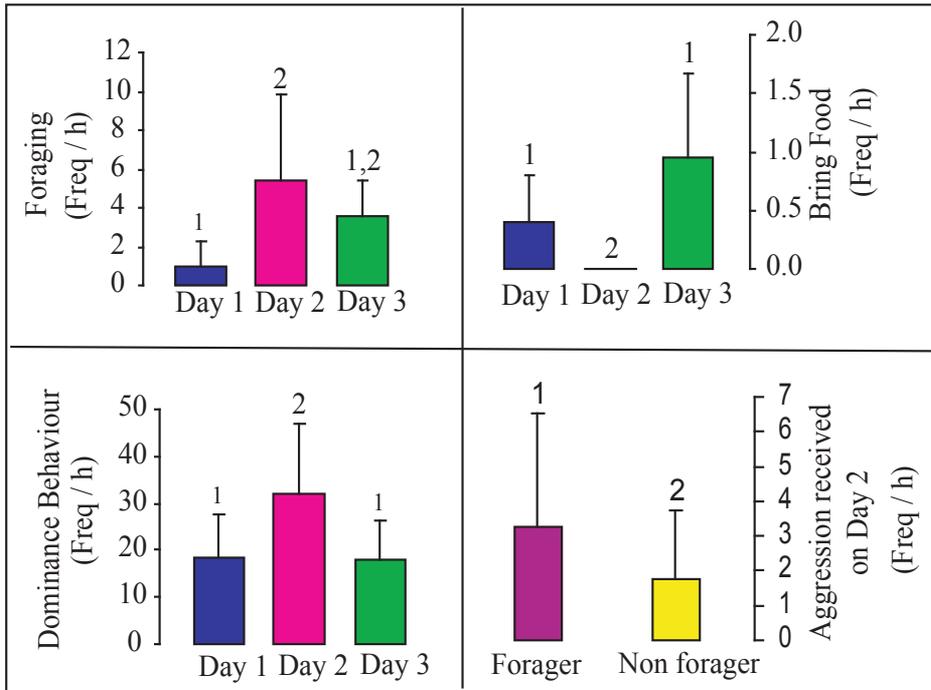


Figure 9.4: Food deprivation experiment. Comparison of mean and SD of frequency per hour of dominance behaviour, foraging attempts, and bring food on day 1 (normal colony), day 2 (food deprived by preventing foraging), and day 3 (foraging permitted). Different numbers on the bars indicate a significant 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).]

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 chapter 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 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 for-

agers. 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 decentralized self-organized manner.

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

Suggested Reading

- [1] R Gadagkar, *The Social Biology of Ropalidia marginata: Toward Understanding the Evolution of Eusociality*, Harvard University Press, Cambridge, Massachusetts, USA, 2001.
- [2] T Seeley, Social foraging in honey bees: How nectar foragers assess their colony's nutritional status, *Behav. Ecol. Sociobiol.*, Vol.24, pp.181–199, 1989.
- [3] 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*, Vol.72, pp.100–103, 2006.
- [4] S Lamba, K Chandrasekhar and R Gadagkar, Signaling hunger through aggression—the regulation of foraging in a primitively eusocial wasp, *Naturwissenschaften*, Vol.95, pp.677–680, 2008. doi: 10.1007/s00114-008-0369-9

10

Why Do Wasps Fight? – Part 2

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.

In the last few chapters, we have been using the Indian paper wasp *Ropalidia marginata* (Figure 10.1) to illustrate the design of experiments. Along the way, we have learnt many interesting facts about this remarkable insect society. In the sixth chapter, 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



Figure 10.1 : 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)

larvae, if they have access to enough food and if they have the opportunity to do so. In the seventh chapter, we saw that in the context of founding new nests, the wasps fight to decide who will be the queen and who will be the worker. As might be expected, the winner becomes the queen, and the loser becomes the worker. In the eighth chapter, 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 chapter. In this final chapter 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.

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

Background

We have seen from a previous chapter, 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 [see chapter 8] [1]. 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 [2]. The question that 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 chapter 9, Anindita Bhadra whom we met in chapter 8, and two undergraduate students Yasmin Claire Kazi from Bangalore and Meghana Natesh from Delhi (*Figure 10.2*).

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.



Figure 10.2: A Photo gallery of my students who conducted the experiments described in this chapter. (From left to right, upper row) Shakti Lamba, Yasmin Claire Kazi, Sujata Deshpande; (lower row) Meghana Natesh, Anindita Bhadra, Arun Venkataraman.

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 chapters. 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 [see chapters 8-9] [1]. 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.

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 [1]. 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 (*Figures 10.3a,b,c*). 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.

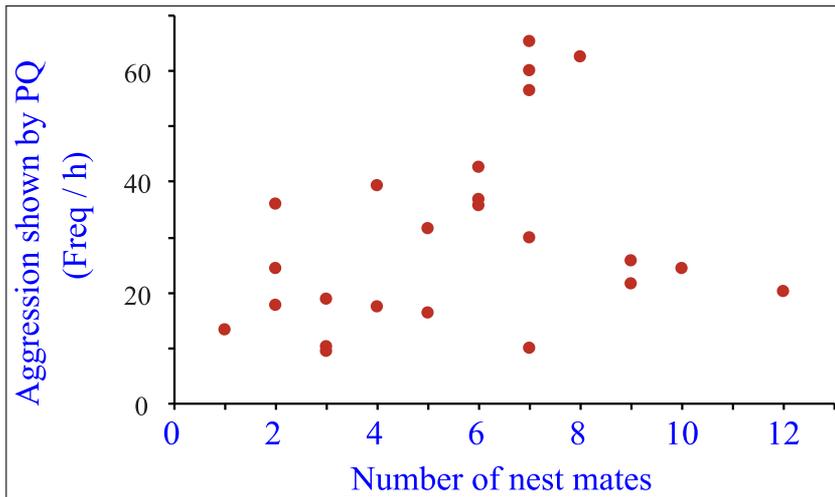


Figure 10.3a: Aggression shown by PQ, plotted against the number of her nestmates. Slope of the linear regression isn't significantly different from 0 ($p = 0.17$; $r^2 = 0.085$, $N = 24$).

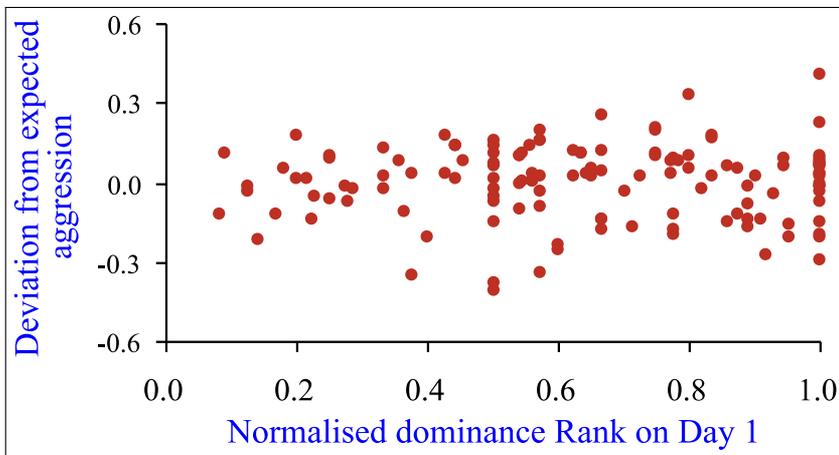


Figure 10.3b: Deviation from expected aggression for each wasp plotted against her normalised dominance rank on day 1. Note that a numerically lower rank corresponds to higher dominance status. Slope of the linear regression isn't significantly different from 0 ($p = 0.82$; $r^2 = 0$, $N = 135$).

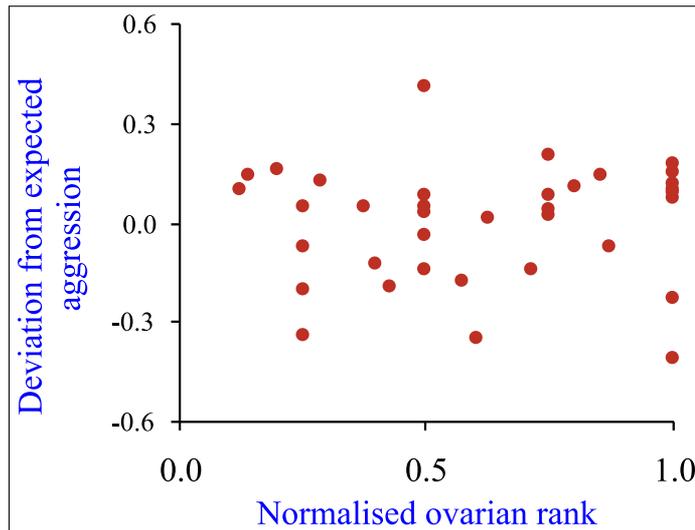


Figure 10.3c : Deviation from expected aggression for each wasp plotted against her normalised ovarian rank (where a numerically lower rank corresponds to better developed ovaries). Slopes of the linear regressions are not significantly different from zero (ovarian index: $p = 0.62$; $r^2 = 0.007$, $N = 36$; 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*, Vol.74, pp.351–356, 2007.]

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 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 10.4a*). 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 10.4b*) [3].

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 sup-

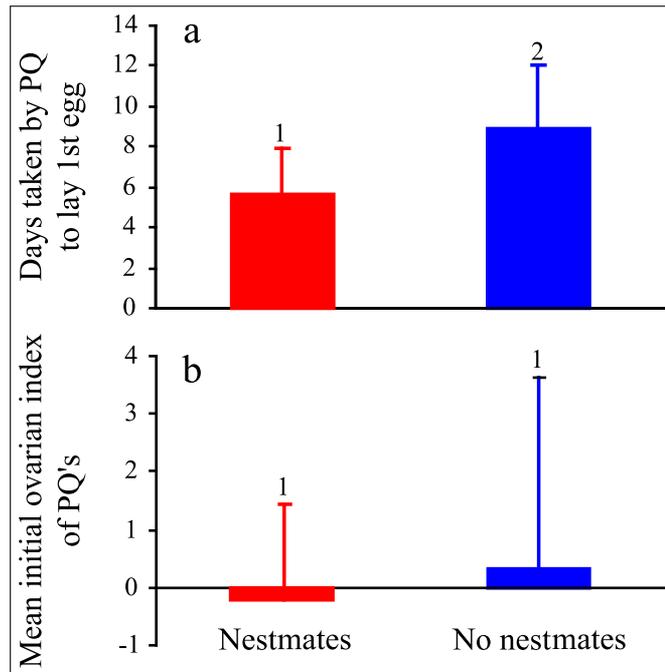


Figure 10.4: Means and standard deviations of (a) the time taken to lay their first eggs, by PQ's with nestmates (red bar: $M = 5.62$, S.D. = 2.29, $N = 13$), and those without nestmates (blue bar: $M = 8.85$, S.D. = 3.18, $N = 7$) and (b) ovarian indices of PQ's with nestmates (red bar: $M = -0.23$, S.D. = 1.69, $N = 8$), and without nestmates (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 [4] 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.)

press 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 counter-intuitive. 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 counter-intuitive hypothesis made a counter-intuitive 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 counter-intuitive 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*’ [3]. 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 counter-intuitive 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.

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

Background

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 chapter 8 are strikingly peaceful. 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 [4]. 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 10.2*, 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 chapters [see chapters 7,8,9] [1]. 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 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 [5] 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

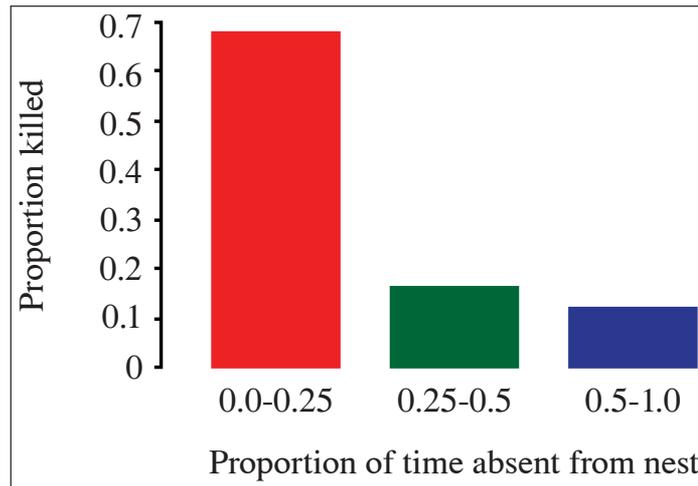


Figure 10.5: Proportion of wasps killed in each class. The classes are based on the proportion of their time that the wasps spent in being absent from their natal nests prior to introduction. The proportion of animals killed is a function of the class to which they belong ($\chi^2 = 9.05$, $P < 0.05$). 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.)

being killed compared to those wasps who had spent more time away from their own nests (*Figure 10.5*). 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 [10].

In the context of our discussion in the last few chapters, 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 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, 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.

10.3 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 chapter, 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* [7].

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 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

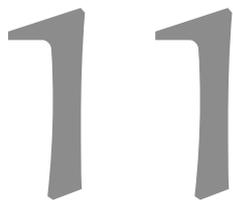
prestigious. More importantly, we should use the method that is suitable to the domain we are working in. If everybody is in a mad rush to do what is more prestigious, and what is more expensive, then several problems arise—some nascent areas of science get neglected (think taxonomy and natural history); 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.

This chapter concludes the set of five chapters 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 chapters 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 chapters to experiments conducted on vertebrates, from fish to humans.

Suggested Reading

- [1] R Gadagkar, *The Social Biology of Ropalidia marginata: Toward Understanding the Evolution of Eusociality*, Harvard University Press, Cambridge, Massachusetts, USA, 2001.
- [2] A Mitra and R Gadagkar, Road to royalty: Transition of potential queen to queen in the primitively eusocial wasp *Ropalidia marginata*, *Ethology*, 118, pp.694–702, 2012, doi: 10.1111/j.1439-0310.2012.02059.x
- [3] S Lamba, Y C Kazi, S Deshpande, M Natash, 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, pp.351–356, 2007.
- [4] R Gadagkar, Choosing a new queen: Consensus without conflict in a social wasp colony in *Landscapes of Collectivity in the Life Sciences*, edited by Snaith B Gissis, Ehud Lamm, and Ayelet Shavit, The MIT Press, Cambridge, Massachusetts, London, England, pp.67–75, 2017.
- [5] H S Arathi, M Shakarad and R Gadagkar, Social organization in experimentally assembled colonies of *Ropalidia marginata*: Comparison of introduced and natal wasps, *Insectes Soc.*, 44, pp.139–146, 1997.
- [6] A B Venkataraman and R Gadagkar, Differential aggression towards alien conspecifics in a primitively eusocial wasp, *Current Science*, 64, pp.601–603, 1993.
- [7] R Gadagkar, Interrogating an insect society, *Proc. Natl. Acad. Sci. USA.*, 106, pp.10407–10414, 2009.



Fighting Fish—Does Experience Matter?

Wonderful as they are, insects do not by any means exhaust the possibilities of suitable organisms to conduct fascinating, cutting-edge, low-cost research, especially in animal behavior. Having seen how insects can be used to this end, in all the previous chapters, I will now deliberately choose examples from studies done on vertebrates, starting with fish and navigating through the evolutionary tree of life, all the way to humans. In this chapter, we will see how simple, clever experiments can reveal that when fish fight, the outcome is not only based on their intrinsic fighting abilities but also on extrinsic factors such as prior winning and losing experiences, and indeed, on a sophisticated interaction between intrinsic and extrinsic factors. In particular, we will study the phenomenon of winner-effects and loser-effects and learn that this is a near-virgin field of research waiting to be exploited and eminently suitable for cutting-edge research at a trifling cost.

11.1 Fighting Again, But This Time in Fish

Insects were the protagonists of all the previous ten chapters in this book. But, as I said in chapter 10, I do not want to leave you with the impression that such experiments can only be done with insects. As promised, we will now consider the

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design of similarly simple and low-cost experiments that can be performed with equal ease with higher animals namely, vertebrates. We will begin with fishes in this chapter and navigate through the evolutionary tree of life, all the way to humans, in subsequent chapters. Here, we will focus on fighting in fish. Readers of this book may begin to get a bit wary of my apparent obsession with fighting in animals. I promise to make an attempt to focus on behaviours not involving fighting in some of the future chapters. But I must say in my defense, that fighting is a ubiquitous and important component of the social life of animals. Moreover, we will see in this chapter that a little bit of fighting helps to reduce much subsequent fighting. This is because animals seem to gain experience during fights and use that experience to decide whether or not to indulge in subsequent fights.

As is surely true in humans, in a variety of animal species too, individuals seem to gain confidence if they win a fight and lose confidence if they lose a fight. This can be inferred from the observation that winners of a fight are more likely to win their next fight and losers of a fight are more likely to lose their next fight. These phenomena are referred to as winner-effects and loser-effects. In other words, experience gained in fighting, whether it be a winning experience or a losing experience, plays a significant role in future fighting behavior, fighting decisions and fighting outcomes. We can design simple low-cost experiments to detect the presence or absence of winner- and loser-effects in different animal species. It turns out that fish species have been a favourite choice for such experiments, and we will see several examples. While these experiments may indeed require little by way of facilities and equipment, they nevertheless, need great care in designing the experiments lest we draw erroneous conclusions, and that is another reason why I have chosen to illustrate this topic.

11.2 Winner-Loser Effects

Experimental Design

Self-selection

To detect winner- and loser-effects, we first stage a contest between two randomly chosen individuals, identify the winner and loser and separate them. To do this, it is best to pair individuals as closely matched as possible in their fighting abilities, based on body size, age or whatever we know about the species. This is especially useful if our sample sizes are likely to be small. Then we can stage a second contest separately for both the winner and the loser, with other randomly

chosen individuals who have not had any recent winning or losing experience. If the winner in the 1st contest wins the 2nd contest more often than expected by chance alone, then we have discovered a winner effect. Similarly, if the loser in the 1st contest loses the second contest more often than expected by chance alone, we have discovered a loser effect. But what is expected by chance alone? Many investigators have assumed that winners and losers in the first contest have equal probabilities of winning or losing the 2nd contest, in the absence of winner/loser effect, i.e., by chance alone. Thus, they have inferred winner and loser effects when winners and losers in the 1st contest have won and lost their 2nd contest respectively with a probability that is significantly greater than 50%.

Let us now describe this more formally. Let us represent a win by W and a loss by L. At the end of the first contest, we will have identified winners and losers which we can label as W and L respectively, based on their respective fighting histories. At the end of the second contest, we will have individuals which we can label as WW and WL, based on their two fighting histories, when we are trying to find a winner effect. The null hypothesis has often been assumed to be that the frequency of WW = the frequency of WL, so that if the frequency of WW was significantly greater than the frequency of WL, then a winner effect has been inferred. Similarly, in an attempt to detect a loser effect, the null hypothesis has been assumed to be that the frequency of LW = the frequency of LL and hence, if the frequency of LL was significantly greater than the frequency of LW, then a loser effect has been inferred.

Unfortunately, the null hypothesis of 0.5, assumed by many researchers is wrong. In this experimental design, the experimenter does not decide who will win and who will lose the 1st contest. Hence, winners and losers entering the 2nd contest have not been randomly chosen. The contestants that entered the 1st contest were chosen randomly, but the experimenters had no control over who will become the winner and who will become the loser. This was decided by the animals themselves. Hence this experimental design is called 'self-selection'—here, the 'self' refers to the animals and not to the experimenters. The problem with such self-selection is that even though the contestants for the 1st contest were chosen randomly, the winners and losers are not random individuals, i.e., they are not drawn randomly from the distribution of fighting abilities in the population. It is reasonable to assume that the winners in the 1st contest are among those that have relatively better fighting abilities and the losers in the 1st contest are among those with relatively poorer fighting abilities. As a consequence, winners in the 1st contest have a better than even chance of winning the 2nd contest with a random

individual, and losers in the 1st contest have a greater than even chance of losing the 2nd contest with a random individual. It has been shown mathematically by Bégin *et.al.*, (1996) [1] that because of such self-selection, winners and losers in the 1st contest have a 2/3rd probability of winning and losing their second contests, respectively. Hence the null expectation should be 67% and only if WW has a significantly higher probability than 0.67 and LL has a significantly higher probability than 0.67, should we infer winner- and loser-effects.

It is easy enough to re-do the statistics for already published studies and confirm whether winner- and loser-effects were erroneously inferred or whether they are still valid under the new criterion, and that is what Bégin *et.al.*, (1996) [1] have done for some studies published prior to their 1996 paper in which they also mathematically proved the appropriateness of the 0.67 criterion and the inappropriateness of the 0.5 criterion. They examined five previously published studies and found that four out of five studies, including two of their own studies, had wrongly inferred winner/loser effects using the 0.5 criterion, and their results did not satisfy the 0.67 criterion; only one of the five studies they tested (fortunately their own!) held up in spite of the 0.67 criterion. As Bégin *et.al.*, (1996) emphasize, self-selection is not wrong but the null hypothesis of equiprobability of WW and WL in the 2nd contest, in the case of winner effects, and the equiprobability of LL and LW in the 2nd contest in the case of loser-effects is not valid. Instead, the outcome of the 2nd contest should be judged against a null hypothesis of 2/3rds probability of WW or LL, for inferring winner- and loser-effects, respectively.

Random-selection

Although self-selection seems easy and the most natural way to select winners and losers (in the 1st contest) to test for winner and loser effects (in the 2nd contest), there is another way to design the experiment. This is called ‘random-selection’. Here, we pick a random member of the population and ensure that it has a winning or losing experience, as per our choice (see below). Now we pair such randomly chosen animals with the experience of winning or losing, with other randomly chosen individuals that have no recent experience of winning or losing. If we do this with several randomly chosen individuals, it is likely that both winners and losers (in the 1st contest) will indeed be randomly distributed in their fighting abilities and hence, they will be expected to win or lose their 2nd contest with an equal probability, if there are no winner or loser effects. Thus, our null expectation now will legitimately be 0.5 and therefore, if WW is significantly more probable than WL, we can infer a winner effect and similarly, if LL is significantly more probable than LW, we can infer a loser effect.

But how do we ensure that our randomly chosen individual has a winning or losing experience at our will? This can be done in different ways depending on the species being studied. In some species fighting ability is strongly linked to body size and/or age of the individuals. In such cases, any randomly chosen individual can be paired with another individual who is smaller and/or younger than itself so that the randomly chosen individual is sure to win. If many randomly chosen individuals are thus paired with partners who are smaller and/or younger than them, we can obtain a number of individuals with a winning experience who themselves span the whole distribution of fighting abilities. Now, when these winners (in the 1st contest) are paired with randomly chosen individuals with no winning or losing experience, we can separate the effect of intrinsic fighting ability and the effect of experience. With such a 'random-selection' procedure, we can set a cut-off of 0.5 in the 2nd contest. Thus, we can infer a winner effect if WW occurs significantly more than WL and a loser effect if LL occurs significantly more often than LW. In many species of fish, residents (individuals in their territory) are more likely to win fights with intruders (individual outside their own territory and the opponent's territory). Hence, we can also pair randomly chosen individuals in their own territory with intruders and ensure that the randomly chosen individual has a winning experience. Conversely, we can pair our randomly chosen individual with an opponent in the opponent's territory to ensure that the randomly chosen individual has a losing experience. Of course, it is best to stage the final contest in neutral territory.

In some species, there may not be any obvious external markers of fighting ability such as body size or age. Nevertheless, there may well be internal correlates of fighting ability such as hormone levels, or, what is more generally called 'aggressiveness' which includes a willingness to initiate a fight or escalate an already initiated fight. There is a clever way to deal with this situation. We need to pair randomly chosen individuals with habitual losers so that we can guarantee that our randomly chosen animal has a winning experience. Similarly, we need to pair our randomly chosen individual with habitual winners so that we can guarantee a loss for the randomly chosen individual. In essence, we need to identify individuals with low and high fighting abilities by actual, repeated performance, in the absence of external markers. Thus, we can identify habitual winners and habitual losers by staging repeated, serial contests and choose those individuals who never lose or never win, as our habitual winners and habitual losers respectively.

Whether we plan to use the method of self-selection or random-selection, it is very useful, indeed essential, to have a good knowledge about what traits deter-

mine fighting ability in the chosen study species. And these vary greatly between species and may include body size, age, being in or out of one's territory, and so on. Prior familiarity with the study animal, especially its natural history and general behaviour patterns, particularly its social behavior will be very helpful. Sometimes, researchers pay great attention to the experimental design, sample sizes, statistical analysis and modelling, but inadequate attention to the biology of the species, treating different species as interchangeable black boxes. Any attempts to answer specific questions or test specific hypotheses are best embedded in long-term studies of the natural history, ecology and ethology of the study species, even if the experiments are conducted in an 'artificial' laboratory setting. Long-term familiarity with the biology of the study species is often evident when researchers spontaneously refer to them as 'my species'!

I will now describe four different studies that examined the possible presence of winner-and/or loser-effects and their ramifications.

11.3 Pumpkinseed Fish *Lepomis gibbosus* – Is There a Winner-effect and How Long Does it Last?

Ivan D Chase and Constanza Bartolomeo of the departments of Sociology and Ecology and Evolution respectively, State University of New York at Stony Brook, and Lee Alan Dugatkin, then at the H Morgan School of Biological Sciences, Lexington, Kentucky, USA used the pumpkinseed sunfish, *Lepomis gibbosus* (family: Centrarchidae, order: Perciformes) (*Figure 11.1*), a very common fish in north-eastern North America, to test for winner-effects (they did not explicitly test for loser-effects) [2]. They employed the method of random-selection by taking advantage of the fact that fish outside their territory are most likely to lose fights with territory owners. As they state in their paper, they simply collected some fish from a pond in their University campus, brought them to their laboratory and conducted the experiments. But before conducting the experiments, they had to solve a tricky problem—all fish look alike—and they needed a way to individually identify the different fish in their experiments—how else would they know if winners win again and again.

Individual identification of animals in behavioural experiments is a crucial part of the methodology. There is no universal way of achieving this. As we saw in several previous chapters, my students and I uniquely mark individuals of the Indian paper wasp *Ropalidia marginata* by applying spots of quick-drying enamel paints of different colours to different parts of their bodies. Researchers



Figure 11.1: Pumpkinseed fish *Lepomis gibbosus*, a very common fish in north-eastern North America used by Chase, Bartolomeo, and Dugatkin (1994), to demonstrate the winner-effect and its decay time (*Image Source:* Shutterstock.com).

studying birds often put coloured rings on their legs. I am envious of my friends who study monkeys or dogs, not only because they are often able to find adequate naturally occurring quirks on the bodies of their study subjects that permit them to uniquely identify almost all individuals, but also because they are able to give them interesting and mnemonic names, such as ‘bent-ear’, ‘broken-tail’, etc. One must, however, take care that the names we give to the animals do not bias our observations about who wins and who loses a fight. We, therefore, need to have clear-cut criteria to decide the winners and losers of fights. Fish are generally more tricky when it comes to marking them for individual identification, not the least because they are aquatic. In this case, the researchers identified their subjects by cutting small but unique notches in the non-vascularized outer margins of their caudal fins, ensuring that there were no injuries leading to infection. More generally, observations should be done in the blind, i.e., the observer should not know which individual was the winner and which was the loser in the 1st contest.

There is another problem that they had to solve before staging any contests between different fish. How does one decide who is the winner and who is the loser? When two pumpkinseed fish are put together in a tank, they fight, i.e., show aggressive behaviour. In this species, the aggressive acts comprise four different behaviours—displacement (chasing the opponent), nip (biting the opponent), butt (thrusting the closed mouth against the opponent’s body) and a fourth kind that they call ‘attacks-no-responses’ (nip or butt but the opponent does not move or respond, unlike in the three previous acts of aggression). They declared a fish as the

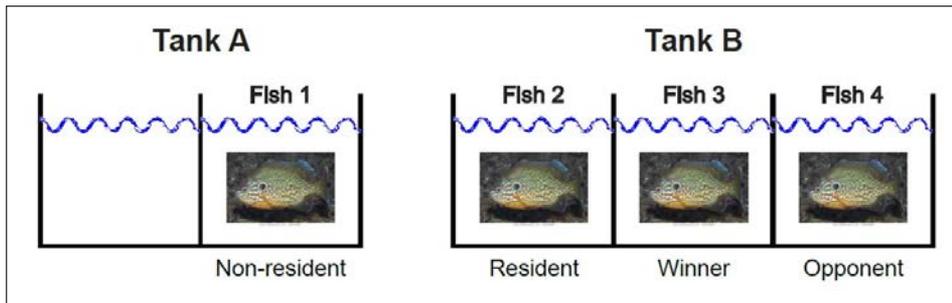


Figure 11.2: The experimental design used and the location of the four fish in the two tanks. See text for explanation (Reprinted with permission from D Chase, C Bartolomeo, and L A Dugatkin, *Aggressive interactions and inter-contest interval: How long do winners keep winning?* *Animal Behaviour*, Vol.48, pp.393–400, 1994.

winner of a contest between two individuals if one of them showed 20 consecutive acts of any of the above mentioned four kinds of aggressive behaviours towards its opponent, without the opponent ever retaliating. Armed with individually identified fish and a criterion to declare winners and losers, the researchers conducted 41 trials involving four different fish in each experiment.

For every trial, they used the following configuration of fish tanks. They used two fish tanks; let us call them Tank A and Tank B. Tank A was smaller and had two compartments, while tank B was bigger and had three compartments (*Figure 11.2*). For each experiment, they randomly chose four (never before used) fish and placed one of them in one of the compartments of the smaller tank and the other three, one each, in the three compartments of the larger tank. Let us call the four fish as 1, 2, 3 and 4 as in *Figure 11.2*. The other compartment of the smaller tank housed a different fish to be used for a different experiment, of no interest for us here (and hence is shown as a blank in *Figure 11.2*).

In order to use the method of random-selection, they needed a guaranteed loser so that the randomly chosen fish in the first contest will have a winning experience. They produced a guaranteed loser in the following way. They transferred fish 1 (from tank A) to the partition housing fish 2 in tank B. In this situation, fish 1 is an intruder, outside its own territory while fish 2 is a resident, inside its own territory. As we noted above, it is very common that fish lose fights when they are in an alien territory and win fights when they are in their own territory, regardless of their intrinsic fighting abilities. So, as expected, fish 1 lost the fight, and fish 2 won the fight. At this point, they removed the winning fish 2 and let the losing

fish 1 remain in that part of tank B. A fish that has just lost a fight is very likely to lose the next one too. This argument, you will realise assumes the existence of a loser-effect. Both winner-effects and loser-effects were already widely known (or expected) when this study was done, and so they could use that knowledge to design a suitable experiment to study winner-effects. Thus, fish 1 is now the almost guaranteed loser they needed.

At this point, they staged the first of two consecutive contests necessary to demonstrate the existence of a winner-effect. This they did by simply removing the partition between fish 1, which had remained in tank B, and its new neighbour, fish 3. As expected, fish 3 won the contest and fish 1 lost again. Their goal was to see if this winning experience that fish 3 got in this first contest would help it to win the next contest as well. In order to test this, they staged a second contest by re-introducing the partition between fish 1 and fish 3, removing the partition between the winning fish 3 and the naïve fish 4, and recorded the consequences.

Since they had used the method of ‘random-selection’, they could compare the numbers of wins and losses in the 2nd contest against a null hypothesis of 0.5. They did so by what is called a binomial test which tells you how much confidence you can have that the proportions of wins are greater than the proportions of losses. Notice that small imbalances in the proportions of wins and losses can, of course, occur by chance alone, especially when the sample sizes are small, as is often the case in these experiments. The binomial test computes the probability with which the observed deviations from 0.5 in the proportion of wins would be expected to occur by chance alone, i.e., in the absence of any winner effect.

Results

In the first 18 experiments, they staged the 2nd contest immediately after the 1st contest so that there was no delay that could bring about a decay in any winner effect, if it existed. The winner in the 1st contest again won the 2nd second contest in 14 out of these 18 trials (*Figure 11.3*). The binomial test indicates that the probability of getting this ratio of WW:WL to be 14:04, by chance alone, i.e., without any winner-effect, is 0.012 (or 1.2%) (traditionally represented as $P = 0.012$). This is quite a small probability, less than 0.05 which is the traditional cut-off point for rejecting the null hypothesis that there is no winner-effect and accepting the alternate hypothesis that there is a winner-effect. Hence they concluded that there is a winner effect in pumpkinseed fish *Lepomis gibbous*. The goal of their experiment was not only to demonstrate a winner effect but to see how long it will last or,

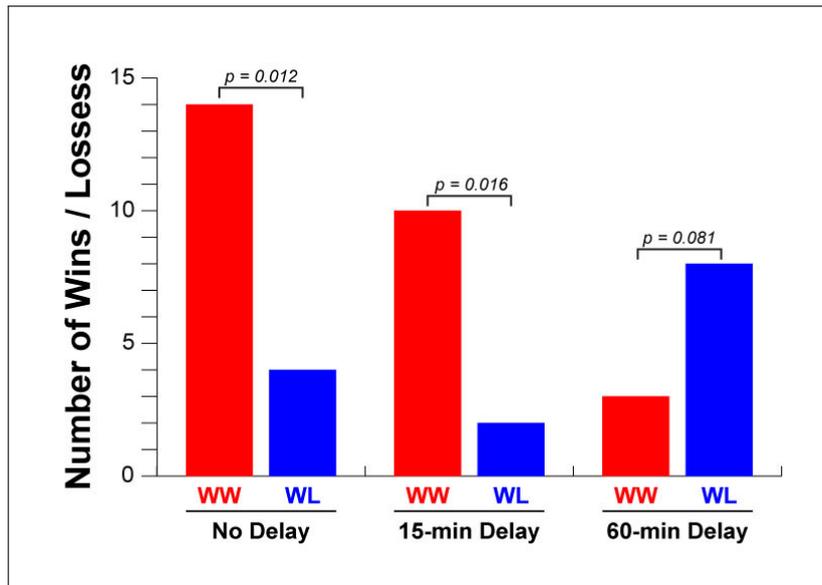


Figure 11.3: Results of the 2nd contest using winners of the 1st contest and pairing them with a randomly chosen naïve individual. The 2nd contest was staged immediately, after a delay of 15 minutes or a delay of 60 minutes. WW = winner of the 1st contest also won the 2nd contest; WL = winner of the 1st contest lost the 2nd contest. See text for details. (Figure drawn by the author using the numerical data from D Chase, C Bartolomeo, and L A Dugatkin, Aggressive interactions and inter-contest interval: How long do winners keep winning? *Animal Behaviour*, Vol.48, pp.393–400, 1994.)

how quickly might the winner-effect decay. Hence they conducted 12 new trials where they introduced a 15-minute delay between the first contest and the second contest. Now they obtained a WW:WL ratio of 10:02 which corresponds to $P = 0.016$ (Figure 11.3). Thus they concluded that the winner effect lasts for at least 15 minutes. Finally, they conducted another set of 11 trials with a delay of 60 minutes, always with fresh fish of course, and here they obtained a WW:WL ratio of 03:08, corresponding to $P = 0.081$ —clearly, not significant (Figure 11.3). The winner effect had decayed within the hour.

Summary

Pumpkinseed fish *Lepomis gibbosus* display a clear winner-effect which lasts less than an hour.



Figure 11.4: *Rivulus marmoratus*, now renamed as *Kryptolebias marmoratus*, a common, small mangrove killifish that occurs in brackish or marine waters along the coast of Florida, Mexico, Central and South America, used by Y Hsu and L L Wolf (1999), to see if multiple experiences are integrated. (Image Source: Wikimedia Commons).

11.4 Mangrove Killifish *Rivulus marmoratus*—Are Multiple Experiences Integrated?

Background

Yuying Hsu & Larry L Wolf of the Department of Biology, Syracuse University, New York, USA, used the mangrove killifish *Rivulus marmoratus*, now renamed as *Kryptolebias marmoratus*, to study winner-loser effects. *Kryptolebias marmoratus* (family: Rivulidae, order: Cyprinodontiformes) is a common, small mangrove killifish that occurs in brackish or marine waters along the coast of Florida, Mexico, Central and South America (Figure 11.4). They can be amphibious and often hermaphroditic, and are often used as aquarium fish, growing to no more than about 4 cm. The name killifish sounds like they may be dangerous but they are not; their name comes from *killi* which means a ditch in Dutch! Hsu and Wolf conveniently obtained their experimental animals from laboratory stocks maintained in the department for many generations.

Experimental Design

To test for winner- and loser-effects, they also used the method of random-selection but via a different route as compared to Chase and colleagues (in the previous ex-

periment with pumpkinseed fish), who you will recall, obtained the necessary guaranteed losers and winners by staging contests between pairs of fish, one inside its territory and the other outside its territory. In this study, the authors instead used the method of providing winning and losing experiences to the desired fish by pairing them with what they call 'standard losers' and 'standard winners'. They obtained standard winners by staging a series of contests among several large fish and choosing the one that won all contests. Similarly, they obtained standard losers by staging a series of contests among several small fish and choosing the one that lost all contests. Thus, a randomly chosen fish was guaranteed to get a winning experience when paired with a standard loser, conversely, a randomly chosen fish was guaranteed to get a losing experience when paired with a standard winner. Now they could check whether such randomly chosen fish with winning or losing experiences would have higher than expected chances of winning and losing respectively, in their next contests, with other randomly chosen individuals.

Yuying Hsu and Larry Wolf, as the title of their paper [3] indicates, were interested not only in the effect of the most recent past experiences but in seeing how the fish might integrate the combined effects of multiple prior experiences, as they would likely have to do in nature. For starters, they decided to study the effects of two consecutive prior experiences on the final contest. They labelled the two consecutive prior experiences as 'penultimate experience' in the case of the first one and 'recent experience' for the second one, both before the final contest. By pairing randomly chosen individuals with standard winners and/or standard losers in two successive contest, they obtained individual with different experiences, such as WW, WL, LW, and LL, i.e., individuals that had experienced 2 successive wins, a win and a loss, a loss and a win and 2 successive losses. These were then engaged in a final contest with a different randomly chosen individual to study the effects of multiple prior experiences.

As in the previous study by Chase *et.al.*, [2], Hsu and Wolf [3] had to set criteria to declare winners and losers in a contest. This will necessarily depend on the species being used and experimenters should make a careful study of their model species under non-experimental conditions to decide the most appropriate criteria for their species. In this case, a fish was declared a winner if it chased and/or attacked its opponent for 20 minutes without retaliation. By this criterion, they obtained clear winners and losers in every contest, in one hour. As in the pumpkinseed fish experiment, they clipped the non-vascularized, outer margins of caudal fins and made sure there were no infections. Each experiment lasted 3 consecutive days during which the fish were given their 'penultimate' experience

on day 1, their ‘recent’ experience on day 2 and the final contest was held on day 3. Recall that these authors used the method of random-selection and so they could compare their results against a null hypothesis of 0.5.

Results

They conducted 27 trials pairing WW individuals with LW individuals, i.e., individuals who had received a penultimate winning experience and also a recent winning experience with individuals who had received a losing penultimate experience and a winning recent experience. Notice that both individuals had a recent winning experience but differed in their penultimate experience. It turned out that WW individuals won significantly more contests as compared to LW individuals, suggesting that when both had the same recent experience, a winning penultimate experience gave the fish an advantage over another who had a penultimate losing experience (see the first pair of bars in *Figure 11.5*).

Next, they conducted 31 trials with LL vs WL individuals. Notice that once again both contestants had a similar recent experience, albeit a losing one, and opposite penultimate experiences. This time, WL individuals won significantly more contests as compared to LL individuals showing once again that when the recent experience is the same, the penultimate experience gives an edge to an individual over another who had a penultimate losing experience (see the second pair of bars in *Figure 11.5*). Taken together, these results show that the winner and loser-effects not only last for two days but are not overturned by a more recent experience of the opposite kind.

Finally, they conducted 38 trials with LW vs WL individuals. Here the individuals differed from each other in both their penultimate and recent experiences and permit us to ask which is more important, the penultimate experience or the recent experience. As it turned out, and not so surprisingly, LW individuals had a significantly higher probability of winning the contest as compared to WL, clearly indicating that when the penultimate and recent experiences are of the opposite kind, the recent experience trumps the penultimate experience (see the third pair of bars in *Figure 11.5*).

Summary

In the mangrove killifish *Rivulus marmoratus*, experience gained from at least two prior fighting experiences influence future fighting success. When two fish have

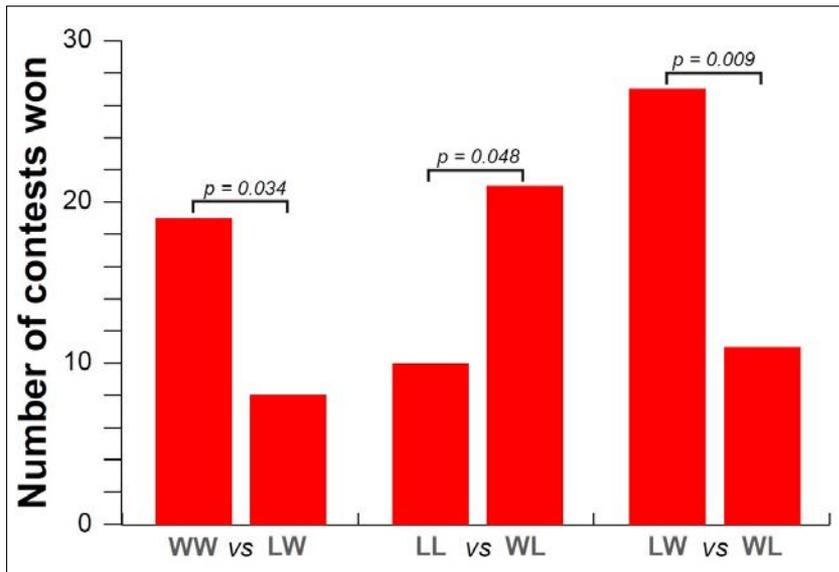


Figure 11.5: Numbers of contests won by different individuals in contests between individuals having different penultimate and recent winning and losing experiences. In the letters below the bars, W = winning experience and L = losing experience, while the first letter refers to the penultimate experience and the second letter refers to the recent experience. See text for details. (Figure drawn by the author using numerical data from Y Hsu and L L Wolf. The winner and loser effect: integrating multiple experiences, *Animal Behaviour*, Vol.57, pp.903–910, 1999).

identical penultimate winning or losing experiences but have differing recent experiences, the recent experience influences future fighting success. Conversely, when two fish have similar recent experiences but different penultimate experiences, their penultimate experience influences future fighting success.

11.5 Green Swordtail Fish, *Xiphophorus helleri*

The next two studies, answering two different questions, have used the green swordtail fish *Xiphophorus helleri*. This is a brackish water, live-bearing fish, native to North and Central America. It has been introduced in many places and has become an invasive species and quite a nuisance, causing ecological damage, but it's also very popular aquarium fish (Figure 11.6).



Figure 11.6: Green Swordtail *Xiphophorus helleri*—Male aquarium fish, used by Beaugrand, Goulet and Payette (1991) to study if body size and winning/losing experience cancel each other and by Dugatkin and Druen (2004) to examine the social implications of winner-loser-effects. (Image Source: Shutterstock.com)

11.6 Can Body Size and Experience Cancel Each Other?

Background

Early studies of winner- and loser-effects often lamented that most studies of fighting and aggression focused only on the intrinsic fighting abilities and the resulting resource holding capacities of animals, and neglected to consider extrinsic factors such as the role of experience e.g., winner- loser-effects. Of course, both intrinsic and extrinsic factors are important. Once winner/loser effects were demonstrated in many species, people have begun to consider the possible interaction between intrinsic and extrinsic factors. In a set of simple experiments, Jacques Beaugrand, Claude Goulet and Daniel Payette, of the Department of Psychology at the University of Québec in Montréal, Canada, have simultaneously considered the effects of body size and prior winning or losing experience, on the success or failure during future contests [4]. Previous research on this species had already shown the effect of body size as well as prior experience, but these factors had only be considered separately. With the knowledge that both body size and prior experience are important, these researchers were able to design simple experiments to study the possible interaction of these factors. The fish they used, and indeed, many such common laboratory animals, are often available for purchase from animal

breeders. As the author says, stocks of these fish were always available in their laboratory for various experiments, maintained in mixed groups of 100–150 adult and immature males and females in large tanks ($90 \times 50 \times 40$ cm).

Experimental Design

When required for the experiments, adult male fish from these tanks were captured ‘as randomly as possible’ and the experimental tests staged in different glass tanks measuring $30 \times 15 \times 15$ cm. Possibly because winner and loser effects had already been demonstrated in this species, they simply used the method of self-selection. Introducing two randomly chosen fish which were rather similar in body size into such tanks, they determined who was the winner and who was the loser, at the end of 12 hours. Let us label them as winners and losers, in keeping with the language we have been using in this chapter, although the authors themselves label them as dominant and subordinate. At the end of this, they separated the fish. On the following day, they staged a second contest using the following combinations of fish: (1) winners and losers of equal size, let us label them as EW and EL, (2) large winners versus small losers (LW vs SL), (3) small winners versus large losers (SW vs LL), (4) large winners versus small winners (LW vs SW) and (5) small losers versus large losers (SL vs LL). In these contests, they considered a fish as having won the contest if it successfully chased its opponent at least six times without being threatened, attacked or bitten by the opponent.

Results

Because these authors use the method of self-selection, they should have used the null hypothesis of 0.67 to check their results, as recommended and proved mathematically by the paper discussed above [1]. For reasons that are not entirely clear, the present authors nevertheless used 0.5 as their null hypothesis [4]. I will say more about this soon, but for the present, I will describe the results as described by the authors themselves using the null hypothesis of 0.5. In the 1st set (EW vs EL), when both fish had similar body size, the winner of the 1st contest clearly won the second contest too, and the loser of the 1st contest lost the 2nd contest too. We can, therefore, conclude that when body sizes are similar, prior experience of winning or losing in the first contest alone will determine the outcome of the second contest. Does this prove the existence of a winner-effect, a loser-effect, or both? This is an interesting question. The answer is that it either proves the existence of a

winner-effect or the existence of a loser-effect but not necessarily both. If there is a winner-effect, a winner is expected to win again even against a randomly chosen individual. In other words, a winner will win again even if there is only a winner effect and no loser effect. Similarly, if there is a loser effect, a loser is expected to lose even against a randomly chosen individual. In other words, a loser will lose again even if there is only a loser-effect and no winner-effect. Thus, for this result to be obtained, there must be at least one of the two, a winner-effect or a loser-effect but not necessarily both. There may be both winner- and loser-effects, as we know from previous work, but it is important to realise that this particular experiment can only be taken to conclude the presence either of a winner-effect or of a loser-effect but not of both, and we cannot tell which one it is. In the second set when a large winner was pitted against a small loser (LW vs SL), the large winner won again. This could be a combination of the advantage of body size as well as the winner effect. In the third set, when a small winner was pitted against a large loser (SW vs LL), the large loser won showing that a large body size can overcome the ill effects of the loser effect. In the 4th set, when a large winner was pitted against a small winner (LW vs SW), the larger fish won, showing the pure effect of body size. In the 5th set, when a small loser was pitted against a large loser (SL vs LL), the large loser won, showing again, the pure effect of body size.

These cleverly designed sets of experiments demonstrate the existence of either a winner- or a loser-effect, the advantage of body size and that body size differences can override winner-loser effects. To be more precise, these experiments allow us to conclude that body size can counteract either the winner-effect or the loser-effect, but for similar reasons as discussed above, we cannot be sure that body size can overcome both winner-and loser-effects, but only that it can overcome at least one of them. I would like to encourage readers to think of how to design experiments (using self-selection), that will help us to demonstrate both winner-effects and loser-effects, rather than just one of them. Similarly, how to design experiments that check whether a body size can overcome both winner-effects and loser-effects, rather than just one of them.

The results of all the five sets of experiments described above yielded statistically significant results, considering a null hypothesis of 0.5. But we have seen in the beginning of this chapter that a null hypothesis of 0.5 is not adequate to demonstrate winner- or loser-effects when the method of self-selection is being used. The relevant set here is the first set namely, EW vs EL, from which we concluded that there is either a winner-effect or a loser-effect. On the face of it, this conclusion is not valid because it used a null hypothesis of 0.5. I, therefore,

recalculated the statistical significance of the first set using the null hypothesis of 0.67 and the difference is still significant! Luckily, we can still infer that there is either a winner-effect or a loser-effect. But this is just a matter of luck. We, therefore, have to be careful to use the correct null hypothesis. The conclusions of this experiment remain valid even with the null hypothesis of 0.67, for the 1st, 3rd and 4th sets. It falls below the required level of significance ($P < 0.05$) for the 2nd and 5th sets. Luckily, we are not inferring winner-or loser-effects from these sets but only the effect of body size for which a null hypothesis of 0.5 is adequate. Thanks to luck, all the three conclusions of these experiments namely, (i) there is a winner-or a loser-effect, (ii) there is an effect of body size and, (iii) body size can overcome either the winner-effect or the loser-effect, are valid.

When we say that body size can overcome winner- and/or loser-effects, how much difference in body sizes is required for this to happen? To answer this question, the authors of the study repeated the 3rd set from the previous experiment, pitting a small winner with a large loser (SW vs LL) several times with a range of differences in body size between winners and losers of the 1st contest. Body size may be tricky to measure in fishes, because as you might imagine, much depends on the shape of the body. In this case, it is known that the lateral surface area (LSA) is a good measure of body size especially in deciding the outcome of dyadic dominance interactions. For each fish in the experiment, they measured three parameters namely, (1) the total length (L, measured from the snout to the end of the caudal fin); (2) the flank height (H, measured from the base of the dorsal fin to the origin of the gonopodium), and (3) the sword length (S, measured from the end of the middle rays of the caudal fin to the tip of the sword). They measured these parameters with minimum disturbance to the fish, using a wire mesh partition in the tank with which they could gently nudge the fish towards one side of the tank and mark its measurements on the glass wall of the tank with a felt-tip-pen. It is important to mention these little details so that others can use them or improve upon them. The lateral surface area was then computed as:

$$LSA = (L \times H) + S.$$

Next they computed the size difference between the members of a pair as:

$$d = \frac{LSA \text{ of the large fish} - LSA \text{ of the small fish}}{LSA \text{ of the small fish}} \times 100.$$

This means they calculated the extent to which the large fish were larger than the small fish as a percentage of the body size of the small fish. In the experiments

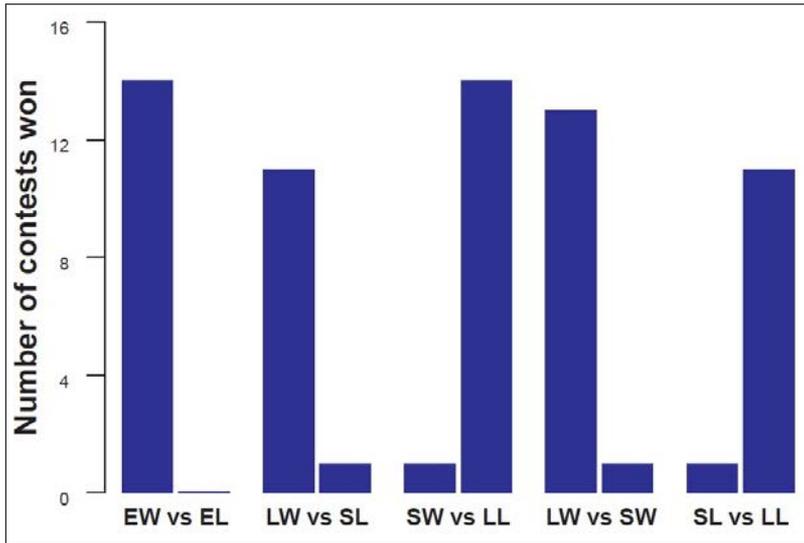


Figure 11.7: Effects of prior winning and losing experiences and of body size, in influencing the outcome of fights in the green swordtail fish, *Xiphophorus helleri*. In the X-axis labels, the first letters E (equal), L (large) and S (small) refer to the body size of the fish and the second letters W (winner) and L (loser) refers to winning and losing experiences respectively in the previous encounter that these fish participated in. The left-most pair, bars labelled as EW vs EL refers to a contest between equal sized winners and losers, the second pair of bars refer to contests between large winners and small losers, and so on. See text for details. (Figure drawn by the author using numerical data from J Beaugrand, C Goulet and D Payette, Outcome of dyadic conflict in male green swordtail fish, *Xiphophorus helleri*: Effects of body size and prior dominance, *Animal Behaviour*, Vol.41, pp.417–424, 1991.)

pitting small winners versus large losers, to see if a large body size can overcome the negative effects of the loser-effect or whether a small body size can negate the advantage of a winning effect, they paired the fish such that the large losers were from 0% to about 75% larger than the small winners. Is there a relation between the percentage size difference and the probability that the larger loser will defeat the smaller winner? Can we, therefore, predict the probability of success of the larger loser given the body size difference between it and the smaller winner? To answer these questions, they used a special kind of regression analysis known as the ‘logistic regression’. This is required when the dependent variable (probability of success, in our case) is not a continuous variable but a binary one, such that an individual may either win or lose with nothing in between. In this case, using

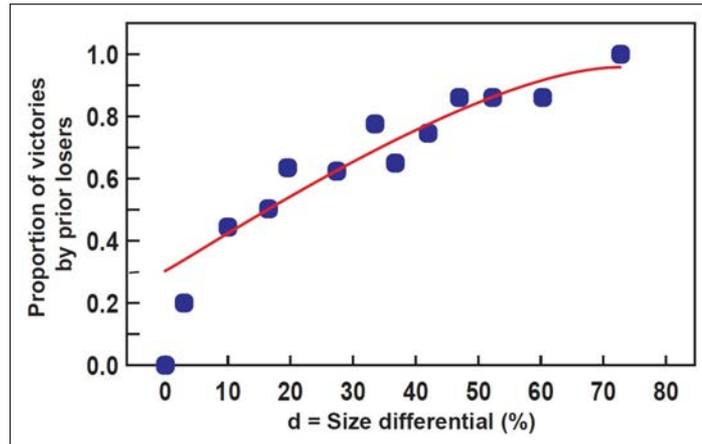


Figure 11.8: The relationship between the size differential and proportion of victories of the loser as given by the logistic function (red line). The points represent the proportion of conflicts won by the prior loser at successive 5% d-intervals. See text for details. (Reprinted with permission from J Beaugrand, C Goulet and D Payette, Outcome of dyadic conflict in male green swordtail fish, *Xiphophorus helleri*: Effects of body size and prior dominance, *Animal Behaviour*, Vol.41, pp:417–424, 1991.)

the body size differential as the continuous independent variable, and the probability of victory for the loser as the binary dependent variable, they performed a logistic regression analysis which turned out to be highly significant ($P < 0.001$), meaning that the size differential is a good predictor of the outcome of these fights (Figure 11.8). We can see from the figure that when the size difference is small, there is not much chance that the previous losers will now win but as the size differential increases, there is a significant increase in the probability that the larger losers will win against the smaller winners. The effect of size differential on the probability of winning is gradual and linear and there does not appear to be any particular threshold difference required for the larger losers to defeat the smaller winners. As always, the ‘winner’ and ‘loser’ refers to winning and losing experiences respectively in the previous contest in which these individuals took part.

Summary

Based on this and previous studies we can conclude that in green swordtail fish, *Xiphophorus helleri*, fighting success is influenced both by body size as well as by previous winning and losing experiences and that these two effects can cancel each other out.

11.7 Do Winner- and Loser-effects Affect Hierarchy Formation?

Background

As we have seen, all studies demonstrating winner- loser-effects conduct experiments with two individuals at a time. This is a bit artificial because animals in nature do not merely indulge in dyadic interactions. In many species, individuals organise themselves in dominance hierarchies and subsequent interactions depending on the position of respective individuals in the hierarchy. Using green swordtail fish, *Xiphophorus helleri* again, Dugatkin and Druen (2004) [5], therefore, investigated whether the winner- and loser-effects evident in dyadic interactions influence the position in the hierarchy that winners and losers occupy. If not, the winner-and loser-effects that we detect in dyadic interactions will have no relevance in the natural lives of these species.

Experimental Design

Dugatkin and Druen used a simple experimental design. In the first part of the experiment, they prepared winners, losers and neutral fish, using the method of random-selection. As in previous experiments, a randomly chosen fish was made a winner by pairing it with a fish sufficiently smaller than itself. Conversely, a randomly chosen fish was made a loser by pairing it with a fish larger than itself. Individuals were labelled as winners if they made ten consecutive acts of aggression on their opponents without being attacked in turn by their opponents. Neutrals were randomly chosen fish that did not interact with any other fish. In the second part of the experiment, they brought together, in a single fish tank, a randomly chosen winner, a randomly chosen loser and a randomly chosen neutral, in order to observe the formation of a dominance hierarchy. The observed hierarchy was considered stable if the relative positions of the three individuals did not change over a period of three days. The question they were interested in concerned the relative positions of the winners, losers and neutrals in the hierarchy of three individuals.

Results

Repeating the experiment 20 times, they successfully observed stable hierarchy formation in 18 trials. In hierarchies of 3 fish of three types (winner, loser and neutral), we expect six types of hierarchies depending on the relative positions

of the three kinds of fish in the three possible positions namely, top, middle and bottom of the hierarchy. These would be:

W-L-N, W-N-L, N-L-W, N-W-L, L-W-N, and L-N-W,

where, W = winner, L = loser and N = neutral and the positions from left to right would be top, middle and bottom of the hierarchy respectively. If there is no effect of the winner- and loser-effect on the positions occupied by the various fish in the hierarchy, then we would expect each of the six types of hierarchies at an equal frequency of 3/18. If on the other hand, being a winner, loser or neutral influenced a fish's position in the hierarchy, certain kinds of hierarchies should be seen more often than expected by chance alone, and others should be seen less often than expected by chance alone. Dugatkin and Druen found that the observed frequencies of the six different types of hierarchies differed significantly from 3/18, expected by chance alone. If both winner- and loser-effects have a strong influence on the positions of the fish in the dominance hierarchy then we would expect the winner in the dyadic interactions should be at the top of the hierarchy and the loser in the dyadic interactions should be at the bottom of the hierarchy while the neutral, i.e., the fish with no fighting experience should be in the middle of the hierarchy. Indeed, Dugatkin and Druen found that only the W-N-L type of hierarchies were significantly over-represented, with a frequency of 11/18 as compared to the chance expectation of 3/18. This was significant at $P < 0.001$ by a standard χ^2 test with 5 degrees of freedom (*Figure 11.9*). Looked at in another way, winners were the top-ranked individuals in 13/18 of the hierarchies and losers were the bottom-ranked individuals, also in 13/18 hierarchies. These numbers were significantly greater than the chance expectation of 6/18 at $P < 0.05$ as judged by Fisher's exact test [5] (*Figure 11.9*). Notice that the chance expectation here is 6/18 because, in the absence of any effect of previous experience, each type of fish (winner, loser or neutral) can be in each of the three positions (top, middle or bottom) in six of the 18 hierarchies. Clearly, the winner- and loser- effects had a significant influence in hierarchy formation showing that winner- and loser-effects that we detect in 'artificial' dyadic interactions have real-life consequences for the individuals involved. We can speculate that stable dominance hierarchies can be more easily established if there are strong winner- loser-effects so that individuals can sort themselves out without unnecessary, continued fighting.

Summary

Winner-and loser-effects do affect hierarchy formation.

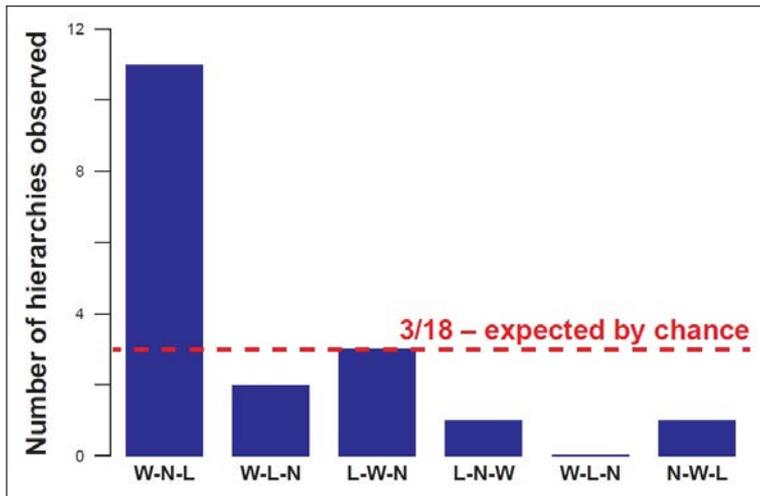


Figure 11.9: Frequencies of each of the six possible types of hierarchies observed (blue bars). The horizontal red line indicates the frequency for each type that would be expected by chance, i.e., if winner- loser-effects have no influence on hierarchy formation. Compared to this chance expectation, only the W-N-L hierarchies were over-represented. W = winner, L = loser and N = neutral, i.e., the fish had no prior experience of either winning or losing. W-N-L means the winner fish occupied the top position in the hierarchy, the neutral fish occupied the intermediate position in the hierarchy and the loser fish occupied the bottom position in the hierarchy, and so on. See text for details. (Figure drawn by the author using numerical data from L A Dugatkin, M Druen, The social implications of winner and loser effects, *Proceedings of the Royal Society of London Series B (Suppl.)*, Vol.271, S488–S489, 2004.)

11.8 Reflections

All the experiments described in this chapter are not very hard to perform and do not require very much by way of facilities or expense, other than the ability to rear the animals in the laboratory or home. How many millions of people must be keeping fish in fish tanks as a hobby or for simple pleasure! No wonder fish have been one of the most favourite animal groups for studying winner- and loser-effects. It is also telling that so many studies, even by scientists, have erred in choosing the correct design of the experiments (self-selection vs random selection) and in inferring the correct predictions against which to test the results (0.5 vs 0.67, as the null hypothesis). We do need a book on ‘how to design experiments’, don’t we?

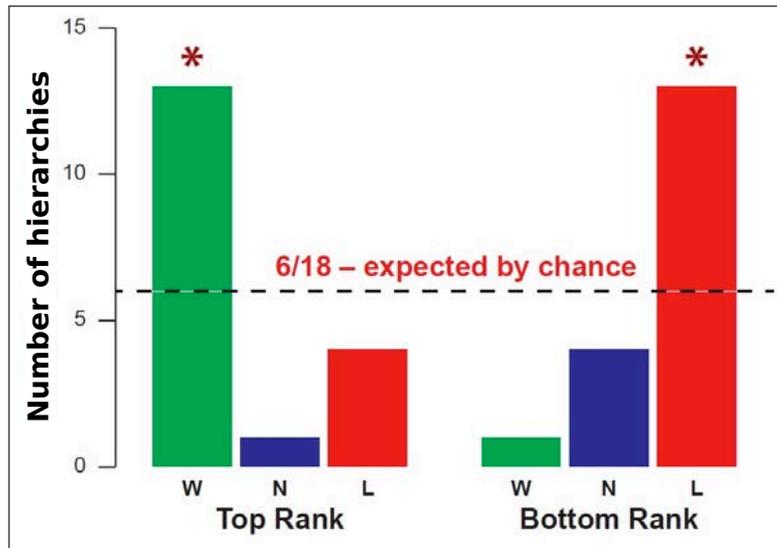


Figure 11.10: The observed frequencies of winner, neutral and loser fish at the top and bottom positions in the hierarchies (bars). The horizontal line represents the frequencies expected by chance alone, if winner- loser-effects had no influence on which position the fish occupied in the hierarchy. See text for details. (Figure drawn by the author using numerical data from L A Dugatkin, M Druen, The social implications of winner and loser effects, *Proceedings of the Royal Society of London Series B (Suppl.)*, Vol.271, S488–S489, 2004.)

In spite of the relative ease of conducting experiments to determine the presence or absence of winner- and loser-effects, and their ramifications and consequences for animals, this field is very much in its infancy. But the little that has been done shows that it is an extremely promising field. In a review of the literature conducted in 2006 Hsu *et.al.*, [6] discovered that winner-and/or loser-effects have been studied only in about 48 species of animals, including 6 insects, 3 arachnids, 3 crustaceans, 23 species of fish, one reptile, 5 birds and 7 mammals. I do not know how many such studies have been conducted since the publication of this review 14 years ago, but I am tempted to mention that one of my students Alok Bang, whom we met in the 8th chapter, and I wondered why no one had looked for winner- and loser-effects in any social insect. We found this gap in the literature particularly surprising because social insects might be especially expected to benefit from having winner- and loser-effects because they live in colonies and need to interact with each other repeatedly. We remedied this

situation by demonstrating that both winner- and loser-effects exist in the Indian paper wasp *Ropalidia marginata*. I must also add that this gives us a rather unique opportunity to study fighting by females while most of the other studies concern fighting by males only [7].

Imagine the opportunities available to study these interesting phenomena in many animal species from different branches of the evolutionary tree? When and why do winner- and loser-effects exist and when not and why not? Can only one of them, winner- or loser-effect exist without the other, and if so, what does that mean? How long do these effects persist and why? What intrinsic or other extrinsic factors can complement or counter-act winner and loser-effects? What is the consequence of winner- and loser-effects on the participation of individuals in their social life? Do winners and losers fight more and less, or do they simply win more and less? What effect does winning and losing have on their subsequent behaviour? How is the apparent increase or decrease in confidence manifested in their behaviour? How and when are multiple experiences integrated? How many prior experiences matter? We need a great deal of data to answer these questions in many different species to be able to take this field to the next step namely to understand the effects of these behavioural outcomes on the physiology of the animals. Even without studying physiology, there is a great opportunity to develop this into a field of comparative ethology if we study diverse taxa and take an evolutionary approach.

Consider the very large numbers of students and early career scientists who can exploit this relatively unpopulated field of research and conduct cutting-edge research at a trifling cost, using their favourite animal species. Here is a gold mine but in order to exploit it, we require a fundamental change in the behaviour of researchers. We need to stop jumping on the bandwagon of fashionable areas of research and have the courage to identify as yet unfashionable areas of research that have the potential to be made fashionable in the near future. This will require a different mindset, a different system of education, a different set of values, and a different system of evaluation, rewards and incentives. Are we up to the challenge?

Suggested Reading

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- [2] I D Chase, C Bartolomeo, and L A Dugatkin, Aggressive interactions and inter-contest interval: How long do winners keep winning? *Animal Behaviour*, Vol.48, pp.393–400, 1994.
- [3] Y Hsu and L L Wolf, The winner and loser effect: Integrating multiple experiences, *Animal Behaviour*, Vol.57, pp.903–910, 1999.
- [4] J Beaugrand, C Goulet and D Payette. Outcome of dyadic conflict in male green swordtail fish, *Xiphophorus helleri*: Effects of body size and prior dominance, *Animal Behaviour*, Vol.41, pp.417–424, 1991.
- [5] L A Dugatkin, M Druen, The social implications of winner and loser effects, *Proceedings of the Royal Society of London Series B (Suppl.)*, Vol.271, S488–S489, 2004.
- [6] Y Hsu, R L Earley and L L Wolf, Modulation of aggressive behaviour by fighting experience: Mechanisms and contest outcomes, *Biol. Rev.*, Vol.81, pp.33–74, 2006. doi: 10.1017/S146479310500686X
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12

Male Frogs Sing for Sex but Why Don't They Do Their Best?

Charles Darwin proposed a separate theory of sexual selection, as distinct from his theory of natural selection, to account for adaptations that confer success in finding a mate, which may sometimes be quite the opposite of what is best for survival. Darwin's proposal that females have a sense of beauty and choose mating partners that appear beautiful to them was met with much scepticism. But today we have a rather detailed understanding of what animals find beautiful and why. In this chapter, I will describe a few very simple experiments performed by Michael J Ryan, in collaboration with A Stanley Rand, herpetologist extraordinaire and Merlin D Tuttle of the Bat Conservation International fame, that laid the foundation for our current understanding of the meaning and evolution of beauty. Studying the túngara frog on Barro Colorado Island, a research station of the Smithsonian Tropical Research Institute in Panama, they showed that (1) male túngara frogs can produce both simple calls, consisting of just a whine, or complex calls in which one or more chucks are added to the whine, (2) female túngara frogs have a decided preference to mate with males giving complex calls, (3) males are nevertheless reluctant to add chucks to their calls and generally do so only when they hear other males calling, and (4) the local predatory fringe-lipped bat also has a decided preference to eat males giving complex calls.

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Male túngara frogs thus face a trade-off between sex and survival. These experiments not only answered the question of why males don't do their best when it comes to singing, but they also set the stage for many more sophisticated investigations that have led to an understanding of how and why natural selection has favoured this particular sexual aesthetic in the frogs and this particular culinary aesthetic in the bats.

12.1 The Cost of Sex

Nearly all multicellular organisms practice sexual reproduction—the union of male and female haploid gametes to form a diploid zygote in every generation. Very few multicellular species routinely use the ancient practice of asexual reproduction, although many have retained it for use under certain conditions. This is something of an evolutionary paradox because sexual reproduction is costly—it requires two parents to produce offspring. Unlike in most sexual species where about half the population consists of males, in an asexual population all individuals are identical (not differentiated into males and females, but traditionally referred to as females because they give birth to offspring) and reproduce all by themselves. This two-fold cost of sex is the subject of much research and many theories. That apart, there is also the problem in sexually reproducing species that males and females have to find each other in every single generation. Every time I see an obscure insect I can't help marvelling at the fact that at least some members of that species must have found sexual partners in every generation without fail, for the millions of years that the species has been around. Not surprisingly, sexually reproducing organisms go to a great deal of trouble to ensure the union of males and females.

It is a curious fact of the biology of sexual reproduction that the burden of finding a sexual partner falls more heavily on males than on females. Because females generally produce relatively small numbers of costly eggs and devote relatively more time and effort in caring for their offspring, they are the limiting resource. Males on the other hand, generally produce large numbers of cheap sperm, spend relatively less time and effort caring for their offspring and can, therefore, mate with many more partners than is possible for a typical female. The mass of a nutrient-filled human egg, for instance, is about a million times the mass of a human sperm, which contains little more than a haploid set of chromosomes! In this asymmetric situation, success in finding a sexual partner is relatively more uniformly distributed among the females but is highly skewed among the males—

while most females will find a sexual partner, some males will find many partners and many males will inevitably be left without any. Males, therefore, have to work much harder to find a mate—they either have to fight with other males in order to win in the male-male competition and/or they have to advertise themselves to the females and emerge successfully through the filter of female choice. Darwin realised that what it takes to succeed in the sexual market is not always the same that is required to succeed in the struggle for survival. He, therefore, proposed a separate theory of Sexual Selection, to account for adaptations that confer success in finding a mate, which may sometimes be quite the opposite of what is best for survival.

Perhaps not so surprisingly, male-male competition for females has been much better studied while female choice has been rather neglected, at least until recently. Darwin's proposal that females have a sense of beauty and choose mating partners that appear beautiful to them was met with much skepticism, including by Alfred Russel Wallace, the co-discoverer of the principle of natural selection. That females can indeed choose, and have a sense of beauty is the theme of this chapter, but of course, we will mainly use it as a context in which to continue to explore the practice of science and illustrate more ways of designing experiments and doing low-cost research. In particular, we will dwell on how male frogs advertise their beauty and what female frogs find beautiful.

Frogs may seem an unlikely subject to explore beauty. Our common perception of frogs is perhaps not one they would be proud of! There are over 7000 species of frogs, and a great deal is known about them—at least biologists have found them worthy of attention. In 2007 Professor Kentwood D Wells of the University of Connecticut in the USA published a 1400-page book entitled *The Ecology & Behaviour of Amphibians* [1], which has been described as “the definitive one-volume work on the world's amphibians”. Professor Wells begins his book with an amazing quote by Carl von Linnæus, the father of taxonomy: “These foul and loathsome animals... are abhorrent because of their cold body, pale colour, cartilaginous skeleton, filthy skin, fierce aspect, calculating eye, offensive smell, harsh voice, squalid habitation, and terrible venom; and so their creator has not exerted His powers to make many of them.”

This reputation of amphibians apparently did not deter Professor Michael J Ryan (*Figure 12.1*), one of the protagonists of this chapter, to choose a frog to explore beauty. Clearly, he made a wise choice as his 40-year research [2] on the Túngara Frog has led recently to the publication of his fascinating book, *A Taste for the Beautiful: The Evolution of Attraction* [3].

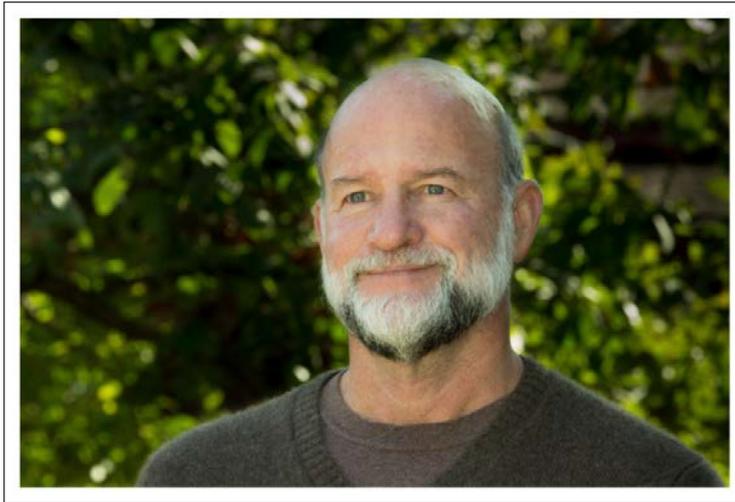


Figure 12.1: Michael J Ryan (1953–). Born to a truck driver in Bronx, New York as the oldest of eleven siblings, Michael J Ryan rose to become the Clark Hubbs Regents Professor in Zoology at the University of Texas, Austin, USA. Michael Ryan has spent a lifetime studying the túngara frog in an attempt to understand what is beauty and how and why some traits come to be regarded as beautiful while others not. His research, culminating in the theory of sensory exploitation, has shown that while males have exploited the sensory biases of females over evolutionary time, at the present time females are the biological puppeteers, making the males sing exactly what their brains desire—a paradigm shift in the study of sexual selection. The latter part of *A Taste for the Beautiful: The Evolution of Attraction* [3] was written after Mike suffered a serious accident which has left him in a wheelchair with a spinal cord injury.

12.2 The Túngara Frog

Physalaemus pustulosus is a small, brownish frog, about 3 cm in length (length of frogs is usually measured as the distance between the tips of their snouts to the end of their vents, i.e. not measuring the legs) (*Figure 12.2*). Its pustular skin, which gave it the specific name *pustulosus*, and the bumps on its skin make it look more like an ‘ugly’ toad than a ‘beautiful’ frog. In fact, Ryan went to Panama to study the much more beautiful red-eyed tree frog *Agalychnis callidryas* but gave it up in favour of the fairly non-descript túngara frog that the Panamanians consider a toad, rather than a frog, but more about that later.

Frogs and toads belong to the order Anura, in the vertebrate class Amphibia. Anura is divided into some 55 families, one of which is Leptodactylidae with 57



Figure 12.2 : A calling male túngara frog, *Physalaemus pustulosus*. Notice the large vocal sacs. (Photo: Ryan Taylor).

genera and some 650 species, including *P. pustulosus*. The genus *Physalaemus* is distributed throughout most of middle and south America and few additional places. Michael Ryan studied *P. pustulosus* in Barro Colorado Island (BCI) in the Isthmus of Panama. BCI is an island of about 15 km² in the man-made Gatun Lake in the Panama canal. Preserved as a nature reserve and administered by the Smithsonian Institution, it is the main research station of the Smithsonian Tropical Research Institute (STRI), making it one of the best-studied tropical forests in the world, and not surprisingly, a mecca for tropical biologists. During my three-month stay on BCI in 1980-81, I had the pleasure of meeting, in addition to the large number of outstanding staff scientists (including Stanley Rand, another protagonist of this chapter), many famous visiting scientists including John Maynard Smith and Richard Dawkins and of course, Michael Ryan.

On BCI and elsewhere, túngara frogs breed in temporary water bodies where the males advertise themselves to the females by calling thousands of times from dusk to midnight. Females arrive only when they are ready to mate, so ready that all their eggs will ooze out of their bodies and go to waste if they do not mate within the next few hours. In spite of being in a hurry to mate, females are choosy, and they have plenty of males to choose from. To quote from Michael Ryan's *A Taste for the Beautiful*, "A female gives her choice of a mate some serious thought. She will sit in front of one male for a time, often move on to others, and sometimes return to a male she has already sampled. She checks out the males by listening to what they have to say, that is, their whining and chucking. When a female

decides to mate, she slowly moves to the male. He clasps her from the top. They are now mating, although the mechanics are a bit different from those to which we are accustomed.” When the eggs are extruded by the female the male catches them in his hind feet, fertilizes them and makes a foam nest for them. Michael Ryan found the sex life of the túngara frog so fascinating that he watched them for “186 consecutive nights. . . watching everything [they] did from sundown to sunup—more than one thousand of them, all individually marked so [he] could tell them apart. . .” He had one simple question—how do the females choose their mates, what is their standard of beauty?

How can we answer this question? As Michael Ryan says, a few well-designed behavioural experiments “can have the precision of a surgeon’s scalpel”. So, let us now study some of these well-designed experiments that he conducted.

12.3 What Does a Túngara Frog Call Sound Like?

One way to answer this question is to listen to the frogs themselves, and you can do so here [<https://www.youtube.com/watch?v=UoUL-jGgU1I>]. While it is easy to hear sounds, enjoy them, and even classify them, it is often difficult to describe them in words. Apparently, some people have attempted to do so by creating words whose pronunciations imitate the very sound that they are supposed to describe. This exercise even has a name—onoma- topoeia, and common examples are cuckoo, sizzle, splash, crack and bang. In his earlier monograph *The Túngara Frog* [4], Michael Ryan claims that the word túngara sounds remarkably like the call of the frog itself, if the first syllable is stressed and the second syllable is uttered very rapidly. I am not convinced, but of course, you should check it out for yourself. I am quite comfortable with his verbal description, “The. . . call has two components, a “whine” and a ‘chuck’; the call resembles the sounds produced by some “star wars” video games. The call always contains a whine and can contain from 0 to 6 chucks. . .”. My dictionary defines a ‘whine’ as “a long, high-pitched complaining cry” which sounds about right to me, and the chuck, I would agree, is onomatopoeic.

But scientists need something much more precise and objective. Recall the words of Dr Louise Banks in the film *Arrival* (2016) (based on the short story entitled *Story of Your Life* by the inimitable American science fiction writer Ted Chiang; I cannot resist the temptation to say that Ted Chiang’s short story is infinitely more profound than the movie): “Maybe we’ll be able to hear the difference between alien phonemes, given enough practice, but it’s possible our ears simply



Figure 12.3: A Stanley Rand—herpetologist extraordinaire (1932–2005). A world expert on lizards, iguanas, crocodiles and frogs, Stan, as he was affectionately known, spent most of his career at STRI in Panama. When Stan died in 2005, Michael Ryan wrote “[except] perhaps for BCI, Stan was STRI’s most valuable resource. . . . When I informed by mass e-mail numerous colleagues of Stan’s death, I received a plethora of responses in which the word “love” was used much more than one might associate with “macho” (and “macha”) field biologists.”.

can’t recognize the distinctions they consider meaningful. In that case, we’d need a sound spectrograph to know what an alien is saying.” Even though we can hear the calls, in the interests of precision and objectivity, A Stanley Rand, staff scientist at STRI and herpetologist extraordinaire (*Figure 12.3*) [5] and Michael Ryan began by recording the túngara frog calls and analysing them, just as Dr Louise Banks does for the sounds produced by the (alien) *heptapods*. In such analyses, the whines and chucks can be unambiguously distinguished and the numbers of chucks following each whine can be accurately determined. See a graphic representation of four kinds of calls, whine only and whine with 1, 2 or 3 chucks in *Figure 12.4*. Oscillograms plot the energy contained in the calls on the Y-axis and time on the X-axis, while the sonograms (‘sound spectrographs’ in the terminology of Dr Louise Banks) graph the frequency of the oscillations on the Y-axis and time on the X-axis. They then labelled the call with only a whine and zero chucks as a ‘simple call’ and a call with whines followed by one or more chucks as a ‘complex call’.

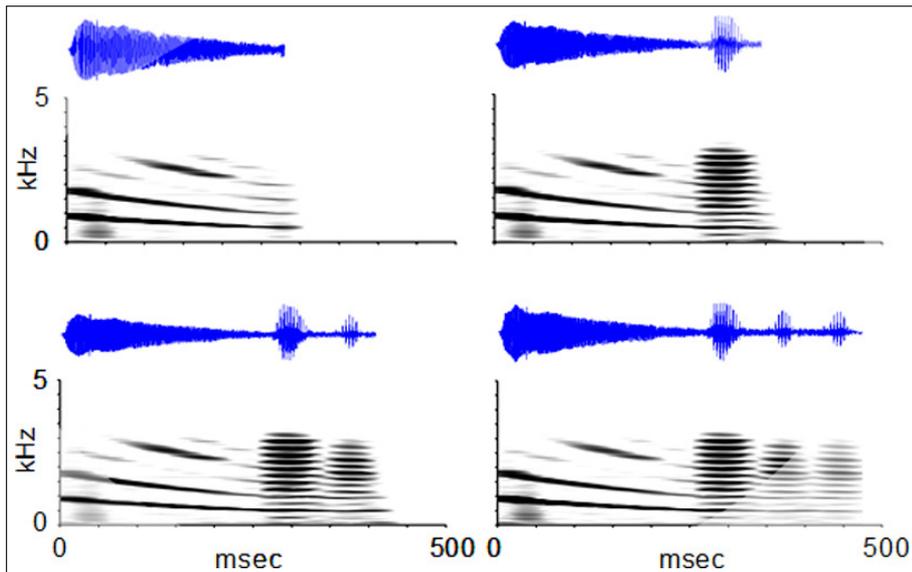


Figure 12.4: Oscillograms (in blue) and Sonograms of the calls of the male túngara frog, *Physalaemus pustulosus* of increasing complexity (top left: whine only, top right, bottom left and bottom right: whine with 1, 2 and 3 chucks, respectively). (Courtesy: Michael J Ryan).

12.4 What Kind of Calls Does the Female Frog Like?

Well, we should ask her, shouldn't we? More generally, such 'asking' is called a bioassay. David John Finney, a famous British statistician and a student of the even more famous statistician Ronald A Fisher, and who was one-time President of the Royal Statistical Society, and who lived to be 101 (in 2018), defined bioassay as "the measurement of the potency of any stimulus, physical, chemical or biological, physiological or psychological, by means of the reactions which it produces in living matter". While bioassays are most commonly used to determine the concentration or potency of a substance by its effect on living cells or tissues, the most interesting bioassays, I think, are those that involve whole animals and the effect being sought is in terms of their behaviour. The first bioassay I ever witnessed was so impressive that the experience is etched in my memory.

I was visiting Delhi University in 1970 as an undergraduate Summer Research Fellow and one Professor Kailash Narain Saxena kindly and most enthusiastically showed me around his laboratory. He was studying the sex pheromones of some

agricultural pest insects in the hope of using the sex pheromones to trap the insects in the agricultural fields, before they did much damage. To identify and isolate the sex pheromones, he would make a crude extract of some glands of female insects (which produce the sex pheromone) and separate their constituents by means of a marvellous (now old) technique called paper chromatography. The crude extract would be applied to one end of a large sheet of filter paper and that end would be dipped into a tray containing an organic solvent. The paper would absorb the solvent, and the solvent would move across the paper carrying the molecules in the crude extract with it. The interesting thing is that different molecules in the extract move to different distances along with the solvent based on how soluble they were in that solvent. At the end of their journey, different molecules would make distinct spots on the filter paper. Generally, the molecules would have some colour, and the spots could thus be located. The next step would normally be to re-extract the compounds from all the different spots and test each of them to see which ones would be attractive to the male insects. This would be the usual bioassay. But what impressed me was how Professor Saxena used a clever shortcut to save himself a lot of time and money. He would simply release the male insects onto the filter paper, and they would promptly go and sit on the spot that they found most attractive—pheromone located. Talk of low-cost research.

Now, just as Professor Saxena got his male insects to tell him which of the many compounds that the female insects produced, they liked best, Professor Ryan had to get his female frogs to tell him which of the two kinds of calls his male frogs produced (simple or complex), that they liked best. And he did, with equal success. Stanley Rand and Michael Ryan recorded the calls of male frogs, both simple and complex, using a tape recorder. Soon, they also learned to artificially synthesise the calls so that they could playback either the naturally recorded calls or the synthesised calls, with the whine only or with the whine and the desired number of chucks, at will.

Next, they placed a female túngara frog in a small cage in the middle of an octagonal arena. Now they played back the male túngara frog calls, either natural or synthesized, from two speakers placed on either side of the female at 75 cm. One speaker played the simple call while the other played the complex call, alternately. At this point, they removed the cage enclosing the female so that she was free to hop towards whichever speaker (call) she liked, and they recorded her choice she thus revealed. After testing 15 females the result was clear—in 14 out of 15 cases the females clearly preferred the complex call (*Figure 12.5*). A simple bioassay yielded a clear result. Technically, the movement of the frog towards

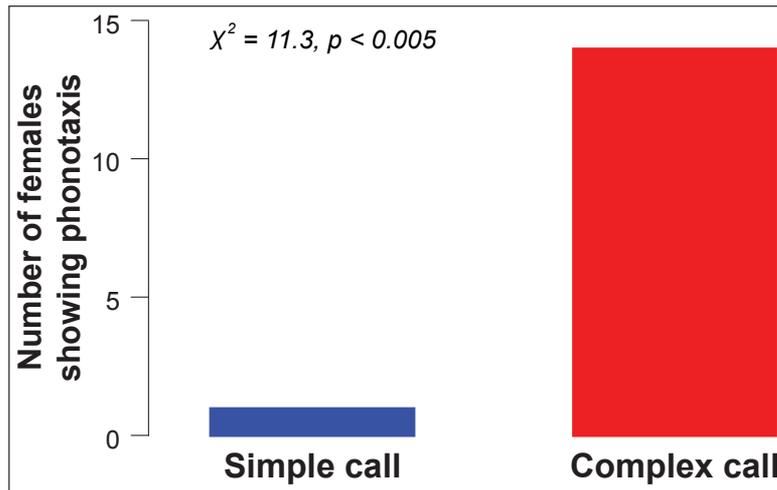


Figure 12.5: Female túngara frogs prefer males who give complex (whines followed by chucks) rather than simple (whines only) calls. Figure drawn by the author, based on data in A S Rand, and M J Ryan. The adaptive significance of a complex vocal repertoire in a Neotropical frog, *Z. Tierpsychol.*, 57:209-14, 1981. [6]

the sound she preferred is called ‘phonotaxis’. As you can imagine, such movement of animals in response to external stimuli, phonotaxis (sound), phototaxis (light) or chemotaxis (chemical) can be conveniently used to perform bioassays with whole animals. Coming back to the frogs, it is clear that female frogs like complex calls, with not just whines but with added chucks [6].

12.5 What Kind of Calls Do the Males Like to Produce?

Stanley Rand who had worked with túngara frogs on BCI and other places had observed that solo frogs generally preferred to produce the simplest call (whine only) but tended to increase the complexity of their calls by adding one or more chucks when in the company of other calling conspecific males. Stanley Rand and Michael Ryan now set out to use their bioassay to carefully test the hypothesis that males increase the complexity of their calls as the size of the chorus of calling frogs increases. This time their bioassay needed no phonotaxis and simply involved counting the number of chucks that the male frogs added to their whines in response to the calls of other frogs. The calls of test male frogs were recorded without any playback or after playing back the calls of other males, varying the

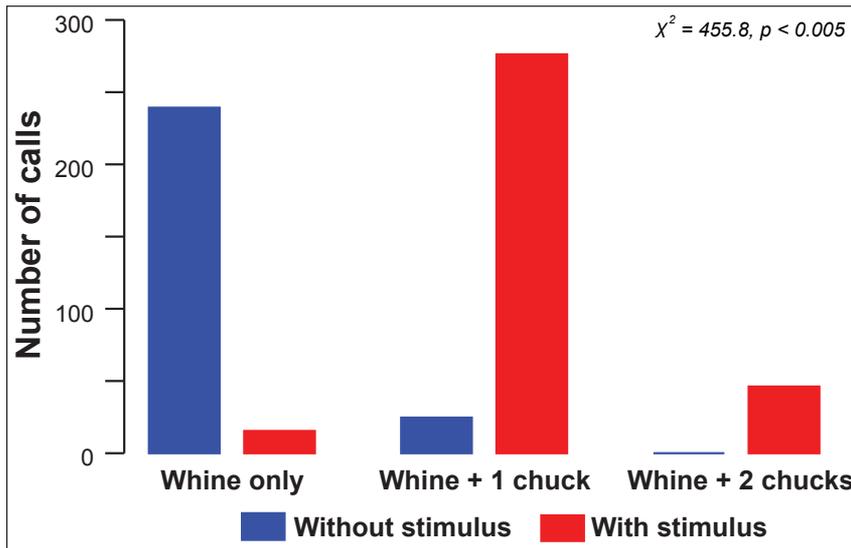


Figure 12.6: Males túngara frogs give simple calls (mostly whines without chucks and very few calls containing both whines and chucks) when they do not hear other males calling (no stimulus) but switch to more complex calls (adding one or two chucks to their whines) when they hear other males calling (with stimulus). Figure drawn by the author, based on data in A S Rand, and M J Ryan. The adaptive significance of a complex vocal repertoire in a Neotropical frog, *Z. Tierpsychol.*, 57:209-14, 1981 [6].

complexity of the playback calls and their loudness (by placing the speakers at different distances from the test frog). In each case, they counted the number of chucks in the calls of the test frog. I have graphed a sample of their results in *Figure 12.6* which clearly support the hypothesis that male frogs prefer to give simple calls when no one else is calling but make their calls more complex if they hear the calls of other males [6].

12.6 Why Don't Males Do Their Best?

Although the results of these two experiments are very clear, they create a paradox. If female frogs prefer males who give complex calls why don't male frogs always do so? Why are they so reluctant to add chucks to their whines and appear to do so only when faced with competition from other males? In attempting to answer such why questions, we must always remember that animal behaviour evolves in

nature and not in the laboratory. We must also remember that natural selection is expected to maximise lifetime reproductive success and not short-term gains. Michael Ryan and his colleagues, therefore, wondered if there is a cost to giving complex calls that would actually serve to reduce the lifetime fitness of the male frogs even though such calls are attractive to the female frogs. They did not consider it likely that the energetic cost of the complex call was the problem. Calls are energetically expensive, but the frogs seem to spend most of the energy in producing a whine and adding one or two additional chucks seems to cost very little extra energy, as we can also see from the upper part of *Figure 12.4*.

So, the culprit must lie outside the frog rather than inside. That immediately suggests that there must be something in the environment of the frogs that renders complex calls costly. If we now think of the male frogs' calls as signals directed towards the female frogs, we can point a finger at potential eavesdroppers. But why should someone eavesdrop on the male frog calls, and only on the complex calls, and why should that reduce the fitness of the calling frogs? Michael Ryan writes "There is another cost of chucks, one that remained hidden to me for more than a year but that had been influencing the evolution of sexual beauty in túngara frogs for millennia: the cost of eavesdroppers."

But who is this elusive eavesdropper? Enter Merlin Tuttle (*Figure 12.7*), a world-famous bat researcher and even more famous bat conservationist. Every bat researcher and every conservation biologist, passionate about bats or some other animal, knows about Tuttle's *Bat Conservation International*. Given STRI's attractiveness to scientists, it is perhaps not surprising that Merlin Tuttle was visiting. But, surprisingly, he had photographed a local fringe-lipped bat *Trachops cirrhosus* with a túngara frog in its mouth (*Figure 12.8*). Surprising because up until this time, a frog was considered a most unlikely item in a bat's diet. Moreover, at that time it seemed most unlikely that the bat could hear the frogs and use their calls to locate them. This is because the well-known echolocation of bats works in the ultrasonic region (50,000 to 100,000 Hz) while the frog calls (700 to 2200 Hz) are well within our hearing range. Merlin Tuttle was eager to know how the fringe-lipped bats on BCI were able to catch túngara frogs, and indeed, whether they were doing so routinely. Michael Ryan and Stanley Rand were equally eager to know if the threat of predation by the fringe-lipped bat was the reason why túngara frog males were so reluctant to add chucks to their calls.

Hot on the trail of the culprit, the three scientists joined forces and conducted new experiments involving the frogs as well as the bats. First they simply set up nocturnal observation stations and, to their surprise, they saw that the fringe-



Figure 12.7: Merlin D Tuttle (1941–). With over 60 years of in-depth knowledge and experience as a renowned bat expert, educator and wildlife photographer, Merlin Tuttle founded Bat Conservation International (BCI) and Merlin Tuttle Bat Conservation (MTBC), to teach the world how to understand and appreciate the vital contributions bats make to human beings and the world we live in (www.MerlinTuttle.org). His unique vision of winning friends instead of battles has led to amazing success in addressing one of the world's greatest conservation challenges. He has turned countless bat haters into protectors by diplomatically showing them how helping bats help people. His classic research on population ecology and behaviour of bats has been published in leading journals, including a cover story in *Science*. And his conservation work has been featured in five National Geographic articles, as well as in *The New Yorker*, *The Wall Street Journal*, and numerous other leading publications worldwide.

lipped bats were catching six túngara frogs per hour of the night. Were the bats catching the frogs by actually listening to them rather than by some other means? An obvious possibility was that the bats were using their well-known echolocation to bounce ultrasound off the frog's bodies, rather than listening to the frog calls. However, preliminary experiments playing back túngara frog calls from speakers



Figure 12.8: A fringe-lipped bat *Trachops cirrhosus* that has just captured its favourite prey, the túngara frog *Physalaemus pustulosus*. (Photo: Merlin Tuttle).

indicated that the bats were indeed attracted to the speakers. But as Michael Ryan says, the acid test was to repeat the experiment that he and Stanley Rand had performed to discover that female túngara frogs preferred the complex rather than the simple calls of the male frogs. They did this in two different ways.

First, they caught some fringe-lipped bats and offered them two speakers, one playing the túngara frog simple calls and the other the complex calls, in their flight cages. It is thrilling to imagine the experiment in progress—the observer in one corner, the bat in the opposite corner and the two speakers in the two other corners. The bats significantly preferred to fly toward or land near the speaker emitting the complex calls (*Figure 12.9a*). In a second set of experiments, speakers playing the simple or complex calls were installed simultaneously in the field, at five different sites. Once again, the bats flew past the speakers playing the complex call significantly more often than they did for the speakers playing the simple calls (*Figure 12.9b*) (see the figure legends and suggested reading [7] for more details). You will have to agree that Michael Ryan was justified in proclaiming “paradox resolved!”, or more poetically that “The males are at the tipping point between sex and survival: more chucks tilt the balance one way; fewer chucks, the other.” So, yes, male frogs sing for sex, but they cannot do their best. To be more pre-

cise, they cannot always sing their best as far as the females are concerned, but they achieve the best possible compromise as far as their lifetime reproductive success is concerned. Recall that males do produce complex calls when they face competition from other calling males. [6, 7].

12.7 What is Beauty?

During the next four decades, Michael Ryan enlisted the collaboration of scores of students, postdocs, technicians, and colleagues to cross the t's and dot the i's as it were, and gave us the most complete understanding yet, of how the (female túngara frog's) brain perceives beauty (in the male túngara frog's call). We now know why the female túngara frog likes calls with chucks and why the fringe-lipped bat also prefers calls with chucks, and also how frog-eating bats have special adaptations in their inner ears to permit them to hear frog calls even while remaining

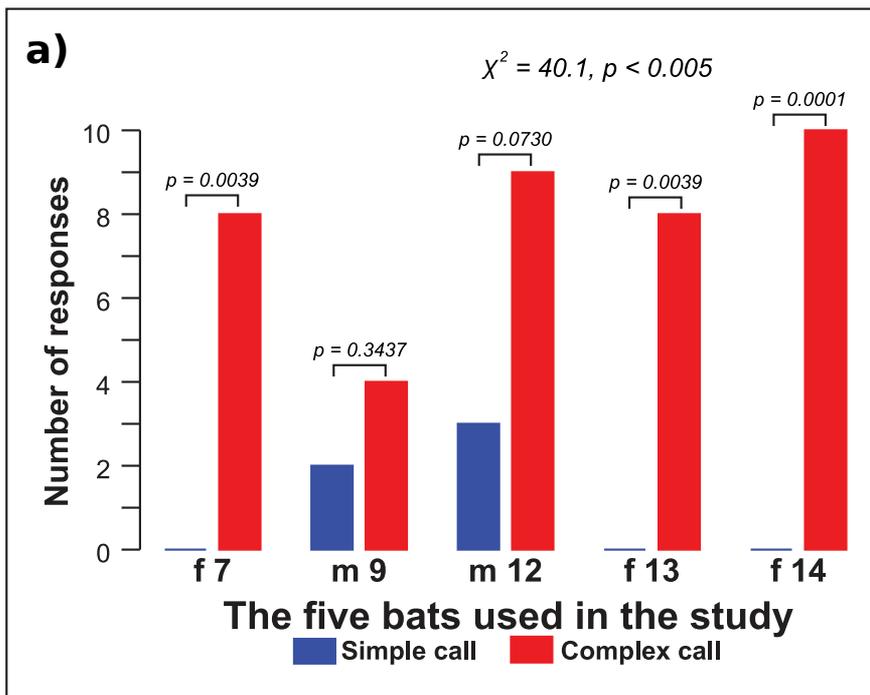


Figure 12.9 continued...

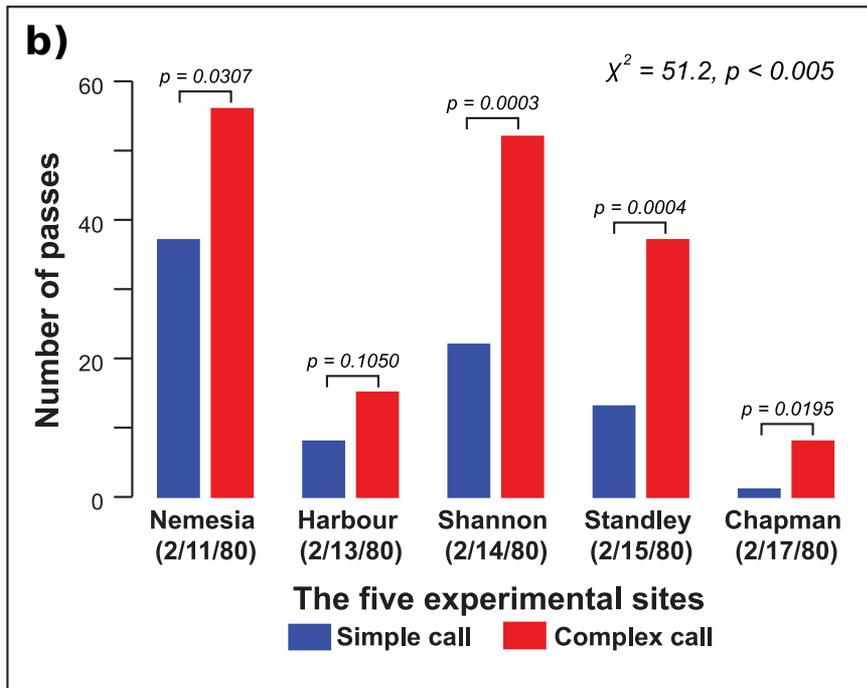


Figure 12.9: The predatory bat *Trachops cirrhosus* is more attracted to frogs giving complex calls (whines followed by chucks) rather than simple calls (whines only). Three out of five bats tested (a) and at four out of five sites tested (b) bats made significantly more passes (responses) at speakers playing complex calls as compared to passes made at speakers playing simple calls. Because sample sizes of the number of trials for different bats were not equal and because the numbers of bats making passes in the field experiments were not known, the null hypothesis of no preference was tested and was rejected in both cases ((a): $\chi^2 = 40.1, P < 0.005$; (b): $\chi^2 = 51.2, P < 0.005$). *Figure* drawn by the author, based on data in M J Ryan, M D Tuttle, and A S Rand. Bat predation and sexual advertisement in a Neotropical frog, *Amer. Natur.*, 119: pp.136–39, 1982 [7].

sensitive to ultrasonics they use for echolocation. All this required that he studied the anatomy of frog and bat brains, their larynges and ears, electrical activity and gene expression in their brains in response to various stimuli. The big picture concerning why natural selection has favoured this particular sexual aesthetic in the frogs, and this particular culinary aesthetic in the bats make a fascinating story [3].

Biologists are well aware that animals may use not just vision, but many other sensory modalities including smell, sound, touch and substrate vibration to per-

ceive their world. We, therefore, rewrite the poet's claim that 'beauty is in the eyes of the beholder' and proclaim, in the words of Michael Ryan, that 'beauty is in the brain of the beholder'. But, as scientists, we do not stop further inquiry, assuming that 'love is blind'. We are interested in and confident about understanding how and why certain sensory inputs but not others make the brain of the receiver perceive beauty. As Professor Michael Ryan's life-time research, summarized so beautifully in *A Taste for the Beautiful: The Evolution of Attraction* [3] shows, such inquiry and understanding do not take the beauty out of beauty—they take the mystery out of beauty and thus make it more beautiful. Indeed, that is how the scientist's brain perceives beauty.

12.8 Reflections

As the readers of this book would be familiar by now, it is time to reflect on these experiments, to glean more general lessons for the inclusive, democratic pursuit of science. The experiments I have described in this chapter abundantly illustrate all the qualities of simplicity, curiosity, and unsophisticated, low-cost research that are the hallmarks of the experiments running throughout this book. Nevertheless, there are at least three rather unique points to which I would like to draw the attention of my readers.

The first point to reflect upon is that the experiments described in this chapter were indeed very simple and required rather little by way of sophistication. Notwithstanding the need to record, analyse and playback frog calls, I can easily see high school kids, undergraduate students, and amateur lay persons performing these experiments with no difficulty. This is largely true of most of the experiments described in all the chapters in this book. What is perhaps unique however is that these simple experiments laid the foundation of knowledge that permitted the very same Michael Ryan to go on to perform many more complicated, sophisticated and expensive experiments to complete the story, and provide a fairly complete understanding of how female choice works, what the females find beautiful and why all males cannot always be as 'beautiful' as the female would like. These sophisticated experiments included the study of frog and bat anatomy, recording electrical activity from the frog brains, measuring gene expression in brain cells, constructing neural network models and designing robots to mimic the frogs. But, these more sophisticated experiments would not have been thinkable, nor would they have been meaningful without the foundational knowledge that (1) males can make simple and complex calls, (2) females prefer complex calls, (3) males are

reluctant to make complex calls and (4) a local predatory bat also prefers males that make complex calls. By first performing simple, unsophisticated and low-cost experiments, Michael Ryan was able to produce the foundational knowledge that was so essential for all his subsequent work.

The lesson I draw from this is that research enterprises should start simple and unsophisticated, and if the researcher is asking the right questions and doing clever-enough experiments, much more is likely to follow. In many cases, as was true in the case of Michael Ryan, the same researcher who started small would have made a name for herself good enough to attract the funding and facilities needed for the next steps. I worry that there is a greater danger of not starting research due to lack of money or, of having too much money in the beginning and willy-nilly neglecting natural history and basic biology, and thus building a grand castle on loose sand. But if a particular researcher is indeed unlucky for some reason, someone else can take the next steps, and the credit for producing the foundational knowledge still stays with the original researcher who will forever be recognized for this.

The second point worth reflecting upon is Michael Ryan's decision to work on the túngara frog [2–4]. Studying bullfrogs in the 1970s, the young Michael Ryan “became interested in the mechanisms by which females selected mates and the factors that influenced the evolution of this behaviour.” In the hope of studying these phenomena, he began looking for a suitable frog species that had a long breeding season to enable long-term observations and the gathering of large amounts of data, and in which there was not the confounding effect of males defending territories. As it happens far too often in biology, even to this day, inadequate natural history knowledge makes it difficult to identify species suitable for answering specific questions. Based on other people's inadequate natural history observations, Michael Ryan had to initially content himself in choosing the red-eyed treefrog *Aglychnis callidryas* as his model organism (Figure 12.10).

Arriving in person on Barro Colorado Island and pursuing his own natural history studies, he soon discovered the disadvantages for his purpose, of this “incredibly beautiful species”—males called infrequently and usually high up in the canopy, coming down only after having secured a mate for which visual cues were likely to have been more important. In a telling comment, Michael Ryan writes, “As I would gaze into the night time canopy, trying to discern the form of one of the treefrogs, there was always a great commotion at my feet. It was the cacophony of a túngara frog chorus.” The obvious thing to do was to ditch the beautiful treefrog and embrace the ugly little ‘toad’, and so he did. To quote him again, “To study



Figure 12.10: The red-eyed treefrog *Agalychnis callidryas*. Yes, frogs can be beautiful, and yet they may be unsuitable to answer certain kinds of scientific questions—a pity!

Source: https://commons.wikimedia.org/wiki/File:Red_eyed_tree_frog_edit2.jpg

animal behaviour in the field, one needs much fortitude. . . . Extreme fortitude and patience may produce great benefits later on. However, a time may come when the researcher must decide that for conducting a particular study, a certain species is not appropriate, after all. Science tends to popularise the Jane Goodall studies, but probably more numerous cases exist in which dedicated and intense research paid few or no dividends because of an initial error in choosing a species to study.”

As researchers, we do not always pay adequate attention to deliberately and consciously choose our research questions, our study species and our research methods. If we honestly introspect on the choices we have made in the past, we will find that chance, arbitrariness, and external constraints have played a more important role than we would like to admit. Even worse, the desire and presumed safety in choosing all three—question, species and method—from among the most common and fashionable ones around, play a no small part. My prejudice is that 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. It is true that the research question and the model

animal are hard to prioritise. I think this is primarily because, though of greater importance, the question is abstract, but the study animal is alive and often rather cute. It is hard not to fall in love with your study animal [8]. My solution to this problem is that we should avoid love at first sight! Leonardo da Vinci said, “Love of anything is the offspring of knowledge; the more certain the knowledge, the more fervent the love”. We should, therefore, look around, indulge in a bit of trial and error, and gradually fall in love with the species as it begins to prove more and more suitable for the questions we want to answer. Instead of love at first sight, we should let love for our study animal grow with time and success, and it certainly will, if we have made the right choice for the questions that we are passionate about. Ideally, such trial and error and gradual falling in love should apply to our questions, species as well as methods.

Reviewing a PhD thesis recently, I wrote that: “First, I must commend the choice of the study animal. Ethologists and behavioural ecologists often tend to choose unusual, endangered or otherwise glamorous animals for their study, even at the cost of many constraints in the availability of samples for study as well as the feasibility of observation and experimentation. Free-ranging dogs, especially in India, are abundantly available, easy to observe and experiment and also of great practical importance to society. Dogs are thus ideally suited both from the point of view of basic research in ethology and behavioural ecology as well as producing knowledge relevant to society, especially in the context of human-animal conflict”. On a personal note, I myself began my research career with bacteria, switched to bacteriophages and then to geckos before falling permanently in love with the evolution of social behaviour and with the Indian paper wasp *Ropalidia marginata*.

The final point I want to reflect on concerns scientific etiquette, cooperation and generosity. When Michael Ryan was thinking of abandoning the red-eyed treefrog in the canopy and succumbing to the túngara frog chorus at his feet, Stanley Rand, who was studying the túngara frog on Barro Colorado Island, not only encouraged Michael Ryan to use it for his studies on sexual selection but gave him the unpublished manuscript that he had written in the late 1960s and early 1970s and in which he had described their simple and complex calls, “a manuscript... filled with incredibly interesting and detailed natural history as well as experimental studies of female phonotaxis” [5]. On the other hand, we have all seen far too often that scientists are resentful of colleagues encroaching on their ‘territories’ of species, study sites and equipment. It is now history that Michael Ryan and Stanley Rand became lifelong friends and collaborators (until Stan’s

death in 2005), prompting Michael Ryan to write that “Stan was always very generous with his time, his ideas, his equipment, and his immense knowledge of tropical biology” dedicate his *A Taste for the Beautiful* to Stan with the words “In memory of Stan Rand, fellow traveller” and begin his obituary of Stan with the words “There is a hole in my chest where my heart used to be and a chasm in tropical biology the size of the Panama Canal”.

Suggested Reading

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- [2] M J Ryan, An improbable path. In Drickamer, L, and Dewsbury, D, eds., *Leaders in Animal Behavior: The Second Generation*, pp.465–96, Cambridge: Cambridge University Press, 2010.
- [3] M J Ryan, *A Taste for the Beautiful: The Evolution of Attraction*, Princeton University Press, Princeton and Oxford, 2018.
- [4] M J Ryan, *The Túngara Frog – A Study in Sexual Selection and Communication*, University of Chicago Press, Chicago and London, 1985.
- [5] M J Ryan, *Profile: A Stanley Rand*, (1932–2005), *Iguana* 13: pp.43–46, 2006.
- [6] A S Rand and M J Ryan, The Adaptive Significance of a Complex Vocal Repertoire in a Neotropical Frog, *Z. Tierpsychol*, Vol.57, pp.209–14, 1981.
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- [8] R Gadagkar, In Love with *Ropalidia marginata*: 34 years, and Still Going Strong, In: *Social Behaviour: Genes, Ecology and Evolution*, (Eds.) T Szekely, A J Moore and J Komdeur, Cambridge University Press, Cambridge, pp.85–87, 2010.

13

Harmless Snakes Mimic Venomous Snakes to Avoid Predation, But Why Don't They Do Their Best?

There are many examples of perfectly palatable animals resembling related unpalatable species and, thereby, avoiding attack by predators who have learnt or evolved to avoid the unpalatable species. To facilitate recognition by predators, unpalatable species often have warning colourations, which is what is mimicked by the palatable species. This form of mimicry is known as Batesian mimicry. While there are many well-documented examples of Batesian mimicry among butterflies and other arthropods, there are somewhat fewer examples amongst vertebrates, and even these examples are often debated. The coral snake mimicry system in North America, where non-venomous kingsnakes and milksnakes mimic venomous coral snakes, is one of the best-studied vertebrate examples of Batesian mimicry. However, it has also been debated for over a century. In this chapter, I will describe three experiments using plasticine replicas of the mimics designed to understand the effectiveness of their mimicry. These field experiments were performed in the natural habitats of the mimics, the models and their predators, by David W Pfennig and his students and collaborators, in the states of Florida, North

Carolina, South Carolina, and Arizona in the USA. The simple, clever, and low-cost experiments have significantly strengthened the hypothesis of Batesian mimicry in this system. They have also provided an unexpected new understanding of how mimics might evolve from cryptic ancestors through a process of gradual natural selection.

13.1 Mimicry

Plants use solar energy to make biomolecules such as proteins, carbohydrates, and lipids. Plants, therefore, provide an attractive option for herbivores to derive all their nutrition by eating the plants and not bother with photosynthesis. Not surprisingly, plants have evolved adaptations to prevent, or make it difficult, for herbivores to consume them, and herbivores have evolved adaptations to improve their chances of finding, eating, and digesting the plants. In this evolutionary arms-race, plants and herbivores are continuously chasing each other to win the battle for survival. But the herbivores (prey) themselves are an attractive option for carnivores (predators) to derive their nutrition by eating them. And of course, prey and predators have also evolved adaptations and counter-adaptations in their mutual battle for survival. The evolutionary arms race between prey and predators becomes more exciting because the predators can literally chase their prey and catch them. Prey species have two diametrically opposite strategies to defeat their predators. They can be cryptic and avoid detection or recognition. A variety of fascinating mechanisms including background matching, disruptive camouflage, and countershading, have evolved in prey species to remain hidden from their predators [1].

A diametrically opposite strategy is for the prey to become super-conspicuous. But how can this help? The secret is that this is only the second part of a dual strategy. The first part is to become unpalatable, toxic, or develop ways of biting or stinging, to injure or inject venom. Being unpalatable alone is insufficient because it usually comes too late in the sequence of events—the prey has already been captured, injured, or eaten. To make unpalatability profitable, predators have to be given an opportunity to recognize dangerous prey and avoid messing with them. This can be accomplished by being super-conspicuous. The super-conspicuousness is most often achieved through the evolution of aposematic (warning) colouration. Aposematic colouration is well known in many prey species. There is plenty of evidence that predators come to avoid aposematic prey

either by a process of learning in their lifetimes or by the evolution of an instinct to avoid them.

My alert readers would have already recognised that there is a potential problem in explaining the initial evolution of aposematism. The first individuals to develop warning colouration must have had to face a double whammy. They would have been more conspicuous than their wild-type, cryptic, counterparts, and their predators would not yet have acquired the necessary behavioural or innate adaptations to avoid eating them. It is of great historical interest that as early as 1958, Ronald A Fisher suggested a solution to this problem which, we see in retrospect, is the forerunner of the modern theory of kin selection [2]. Fisher argued that if distasteful prey are gregarious larvae, for example, one may be eaten but the rest will be spared. And if the distasteful larvae are a large group of siblings, as it is not unlikely, then they all stand to benefit from the sacrifice of the one eaten. There is evidence for Fisher's idea, especially in the form of correlations between the evolution of chemical defences and the evolution of gregariousness.

Such co-evolution of chemical defences and warning colouration in some species opens up an evolutionary opportunity for other species to cheat—to evolve the relatively inexpensive warning colouration without backing it up with the more expensive chemical defences. Thus, palatable species come to superficially resemble unpalatable species, a phenomenon that is known as Batesian mimicry, named after the English naturalist Henry Walter Bates (1825–1892). Bates discovered many examples of mimicry and proposed the hypothesis mentioned above for their evolution. There is also the related phenomenon of Müllerian mimicry, named after the German zoologist and naturalist Johann Friedrich Müller (1821–1897). Müller proposed the idea that multiple unpalatable species may evolve similar warning colouration (mimic each other) and thus reinforce the benefits of being avoided by their predators. It is noteworthy that Müller provided a mathematical model, one of the first mathematical models in biology, to show how Müllerian mimicry might work. There are many examples of both Batesian and Müllerian mimicry, especially among insects, and some model species may have both Batesian and Müllerian mimics resembling them. Indeed, butterflies provide a Klondike of examples for both Batesian and Müllerian mimicry [3]. In addition to butterflies, examples of mimicry, either as models or as mimics have been found, perhaps we should say hypothesised, in a large number of animal and even plant taxa. These include millipedes, moths, beetles, ants, bees, wasps, mantids, hoverflies, crabs, cuttlefish, octopuses, spiders, fish, toads, lizards, snakes, birds and mammals, and also orchids and some other angiosperm families [4, 5].

13.2 Coral Snakes

Compared to the arthropods, mimicry has been less well studied and is considerably more controversial, among the vertebrates. Here, we will focus on coral snakes, a group in which mimicry has been extensively studied and hotly debated for over a century. Coral snakes belong to the family Elapidae (the same family as such deadly species as cobras, kraits, and sea snakes). Coral snakes are believed to have originated in the Old World, with about 16 extant species present today, and diversified in the New World with about 65 extant species today. In North America, coral snakes belonging to the genera *Micrurus* and *Micruroides* are strikingly conspicuous with their bright, red, yellow/white and black bands and are also highly venomous. Their venom contains powerful neurotoxins. These snakes are hypothesised to serve as models for several co-occurring non-venomous snakes such as kingsnakes and milksnakes (genus *Lampropeltis*), which resemble the venomous coral snakes to varying degrees (Figure 13.1). The resemblance is not always perfect, and one can tell them apart with some effort, at least when not confronted by a live specimen in the wild! People in the USA, where the native coral snakes have red and yellow/white bands that touch, but where the native kingsnakes and milksnakes have red and yellow/white bands that are separated by black bands, have invented mnemonics to aid in their distinction such as, “Red touches yellow, you’re a dead fellow; Red touches black, you’re okay Jack”. However, these mnemonics do not work in Central and South America, where coral snakes (and their mimics) can exhibit either pattern, so it is best to avoid both. Avoiding both is exactly what their predators also seem to do. Therein lies the advantage for the harmless mimics, who gain the benefit of being avoided by predators, without paying the cost of producing venom or developing the fangs and other paraphernalia needed to inject venom. Kingsnakes and coral snakes are, therefore, a textbook example of Batesian mimicry [6].

13.3 Is Coral Snake Mimicry a Just-So-Story?

It has sometimes been suggested that the non-venomous, brightly coloured shield-tail snakes (family: Uropeltidae) in India are Batesian mimics of the local coral snakes. On closer examination, it appears very unlikely that uropeltid snakes in India are mimics of our coral snakes. Their resemblance is very poor, their range overlap is not extensive, and where they overlap, the coral snakes are very rare. Moreover, recent fascinating work by Ullasa Kodandaramaiah and his student, at

The Models	The Mimics
<p data-bbox="194 291 600 382"><i>Micrurus fulvius</i> (Eastern coral snake, photo: Wayne Van Devender)</p> 	<p data-bbox="675 291 1042 382"><i>Lampropeltis elapsoides</i> (Scarlet kingsnake, photo: David W. Pfennig)</p> 
<p data-bbox="200 687 595 778"><i>Micruroides euryxanthus</i> (Sonoran coral snake, photo: David W. Pfennig)</p> 	<p data-bbox="639 687 1081 778"><i>Lampropeltis pyromelana</i> (Sonoran mountain kingsnake, photo: David W. Pfennig)</p> 

Figure 13.1 : Photos of two venomous coral snakes that serve as models (left) and their two corresponding non-venomous mimics (right).

the Indian Institute of Science Education and Research, Thiruvananthapuram has shown instead that the bright colours of shieldtail snakes are their own warning signals to predators not to mess with them—even though they are not venomous, they are very difficult to capture and require long handling times to process and eat them. Thus, unprofitability due to other causes, such as the need for long handling times, can also be signalled by the conspicuous colouration of prey which the predators learn to avoid [7, 8].

It is, therefore, essential to treat every example of mimicry as a hypothesis waiting to be tested. To claim Batesian mimicry involving specific model and mimic species is not easy. In most cases, the resemblance between the mimic and

the model is based on human perception and not that of the relevant predators. We seldom know the evolutionary history of the model and mimic species and for how long they have overlapped in their distribution. We do not usually know the strength of the selective pressure exerted by the predators in the absence of the mimicry and the extent to which it is relieved by the adaptation of mimicry. We rarely know the relative frequencies with which the models and mimics should co-exist for there to be a significant advantage to the mimic without the predators learning (or evolving to “know”) that they are being cheated. An even more complicated problem is whether there would be selective pressure on the model to subsequently evolve to become different from the mimic so that its defence against predation is not diluted. This plethora of unknowns makes testing the hypothesis of mimicry, especially Batesian mimicry, a challenge worthy of the most creative and skilled researchers.

The hypothesis that kingsnakes and milksnakes are Batesian mimics of coral snakes, in North America, has been held up to rigorous scrutiny by both its proponents and its opponents for well over a century. The main contention of the critics has been that the proposed hypothesis of coral snake mimicry violates two theoretical predictions; predictions derived mainly from the study of mimicry in butterflies. These predictions are that mimics should not be found outside the geographical range of the models and that the models should be much more common than the mimics. On the face of it, these theoretical predictions sound entirely reasonable and are largely supported by the data on mimicry in butterflies. So, the question arises if the coral snake mimicry story is a just-so-story. Proponents of the coral snake mimicry hypothesis have taken up these challenges in good spirit and have now studied coral snake mimicry in North America extensively over many decades [9]. And that is what makes the story of coral snake mimicry so rich and interesting to survey in retrospect.

Here, I will describe three experiments that have played a significant role in generating confidence in the validity of the coral snake mimicry hypothesis in North America. As the readers of this book will understand, I have chosen these experiments primarily because of their simplicity, cleverness and low-cost. David Pfennig performed these experiments along with his students and collaborators. David Pfennig is now Professor of Biology at the University of North Carolina, Chapel Hill in the USA. He began his research career investigating nestmate discrimination in paper wasps, and now he mainly focusses his research on the role of developmental plasticity in evolution, the role of competition in generating and maintaining biodiversity, and Batesian mimicry in snakes. He recently told me



Figure 13.2: David W Pfennig (left) holding a scarlet kingsnake, William Harcombe (middle) and Karin S Pfennig (right) holding a Texas horned lizard (*Phrynosoma coronatum*) near Portal, Arizona, where they all conducted some of their field experiments.

that “The mimicry stuff was one of my ‘side projects’ that I just did with some undergrads and former grad students for the fun of it, so I’m always happy to talk about these studies. These were indeed simple and inexpensive experiments that anyone can perform.”

13.4 Does Protection for the Mimics Depend on the Presence of the Models in the Vicinity?

If the hypothesis that the non-venomous kingsnakes are Batesian mimics of the venomous coral snakes is valid, then the extent of protection that the mimics get from predator attacks must depend, at least to some extent, on the presence of the model species in their vicinity. David Pfennig (*Figure 13.2*, left), his student William Harcombe (*Figure 13.2*, middle), and his wife and collaborator Karin Pfennig (*Figure 13.2*, right) have put this prediction to the test using simple and inexpensive field experiments. At the time of the study, William Harcombe was an undergraduate doing his honours thesis (he is now Associate Professor in the College of Biological Sciences, the University of Minnesota in midwestern United States, currently studying the evolutionary ecology of microbial communities). Karin Pfennig is also a Professor of Biology at the University of North Carolina, Chapel Hill, and she studies the role of behaviour in the origin, maintenance and distribution of biodiversity, including the role of mate choice in the formation and maintenance of species boundaries.



Figure 13.3 : A plasticine replica, *in situ* in North Carolina, of a scarlet kingsnake, a Batesian mimic of the eastern coral snake. (Photo: David W Pfennig)

In North and South Carolina, the trio studied the mimicry system of the scarlet kingsnake *Lampropeltis elapsoides* (Figure 13.1, top right), which resembles the eastern coral snake *Micrurus fulvius* (Figure 13.1, top left). In Arizona, they studied the mimicry system of the Sonoran mountain kingsnake *Lampropeltis pyromelana* (Figure 13.1, bottom right), which resembles the Sonoran coral snake *Micruroides euryxanthus* (Figure 13.1, bottom left). Their experimental strategy was to present naturally occurring, free-ranging predators (mostly carnivore mammals, such as grey foxes, racoons, coyotes, and black bears) with artificial replicas of snakes that either did or did not resemble the relevant mimic, and see whether the predators preferentially avoided those replicas that resembled the mimics. They did so by constructing thousands of these replicas and placing them in natural areas. These snake replicas were in the form of 1.5 cm × 18 cm cylinders made of pre-coloured, non-toxic plasticine, which was threaded onto S-shaped wires (Figure 13.3). The replicas were either made to resemble the mimic species (referred to as ringed replicas) in the locality of study (scarlet kingsnake or Sonoran kingsnake, as the case may be) or not to resemble the mimics. The latter, which served as controls, were of two kinds; one had stripes with identical colours and proportions as the mimics (but not the same patterns), and the other was plain

brown. The former control served to test whether predators avoided any brightly coloured snake, whereas the latter control served to test whether predators avoided any snake (regardless of the colour pattern).

A triplet of three replicas, one resembling the mimic and one of each of the two control types, were placed 2 m apart from each other in the natural habitat of the predators. At each study site, they placed 10 triplets, 75 m apart, along a transect. We must realise that the predators may or may not consider it worthwhile to attack plasticine replicas; they may realise that it is not a snake at all. The absolute number of attacks on the plasticine replicas is, therefore, not very informative. However, the proportion of attacks on the replicas resembling the mimics (out of the total number of attacks on all the replicas, including the controls) would be informative. If this proportion is low, then it means that the predators are avoiding the replicas that resemble the non-venomous mimic species, which in turn resemble the venomous model species. At the end of the experiment, the replicas were collected, brought to the lab, and a person who was unaware of the location of the replicas checked them for signs of predation by noting impressions of teeth, claw, or beak marks on them.

In North/South Carolina, they conducted the experiment at eight sites in which the model species, namely the eastern coral snake *Micrurus fulvius* occurs (sympatric sites), and eight sites in which it does not occur (allopatric sites), using a total of 420 replicas. The study sites were 16–420 km apart, and the allopatric sites were all more than 80 km outside the range of the venomous model species. Here, sympatry and allopatry refer to whether or not the distribution of the mimic overlaps (sympatry) or does not overlap (allopatry) with the distribution of the model species. At the end of 4 weeks, 25 (6.0%) of the replicas had been attacked. Although this absolute number is very small, a significantly greater proportion of ringed replicas (those that resembled the mimics) were attacked in the allopatric sites (mean \pm s.e.m. = 0.654 ± 0.107) compared to the corresponding proportion in the sympatric sites (mean \pm s.e.m. = 0.083 ± 0.116) ($P < 0.009$, 2-tailed Wilcoxon two-group test).

In Arizona, they conducted the experiment at 24 sites along an elevational gradient (from 1,204 m to 2,866 m), 3–100 km apart from each other, using a total of 720 replicas. Since the distribution of the venomous Sonoran coral snake *Micruroides euryxanthus* is restricted to elevations below 1,770 m, they thus had 14 sympatric and 10 allopatric sites. At the end of two weeks, 49 (6.8%) of the replicas were attacked. Again, although the absolute numbers were small, a significantly greater proportion of ringed replicas were attacked in allopatric sites

(0.496 ± 0.078) compared to the proportion in the sympatric sites (0.138 ± 0.060) ($P < 0.006$, 2-tailed Wilcoxon two-group test). With the Arizona data, they were also able to compare the rates of attacks on the ringed replicas with what is expected by chance. Their data showed that the proportion of ringed replicas attacked in the sympatric sites was significantly less than expected by chance ($P = 0.010$) whereas the proportion of ringed replicas attacked in the allopatric sites was not different from the chance expectation ($P = 0.188$) (2-tailed Wilcoxon signed-rank test). Notice that since there were two control replicas (striped and plain brown) for every ringed replica, the chance expectation for attacks on ringed replicas is 0.33. Looking at their data in another way they plotted the proportion of ringed replicas attacked as a function of the latitude of the study site in North/South Carolina and elevation of the study site in Florida and found a significantly positive slope in both cases. Coral snakes decrease in abundance with increasing latitude in the Carolinas and they decrease with increasing elevation in Florida (*Figure 13.4*).

Taken together, these results show that the ringed replicas were attacked less by predators in localities where the model snakes are present or abundant, and attacked more in localities where the model species are absent or rare. We can infer from this that non-venomous mimics are likely to get protection from predators due to their resemblance to the venomous model species. The results of this experiment take us a step further in validating the hypothesis that scarlet kingsnakes and Sonoran mountain kingsnakes are Batesian mimics of the Eastern and Sonoran coral snakes, respectively, and give us confidence that it is not a just-so-story [10].

But the question of why the mimics have not been completely eliminated in localities where their venomous models are absent or rare remains unanswered. I encourage my readers to come up with plausible hypotheses to answer this question. If, after doing so, you read up the more recent research on coral snake mimicry, you will see that several of your hypotheses have been put to the test, and some have been vindicated [11, 12]. I assure you that it will be much more fun to read the literature after you have come up with your own hypotheses than do so without putting your own ideas at stake.

13.5 Why Aren't Mimics Perfect?

We might think that natural selection would act to make mimics resemble their models as closely as possible. Indeed, we might think that imperfect resemblance is evidence against the hypothesis that the mimic species we are studying is a

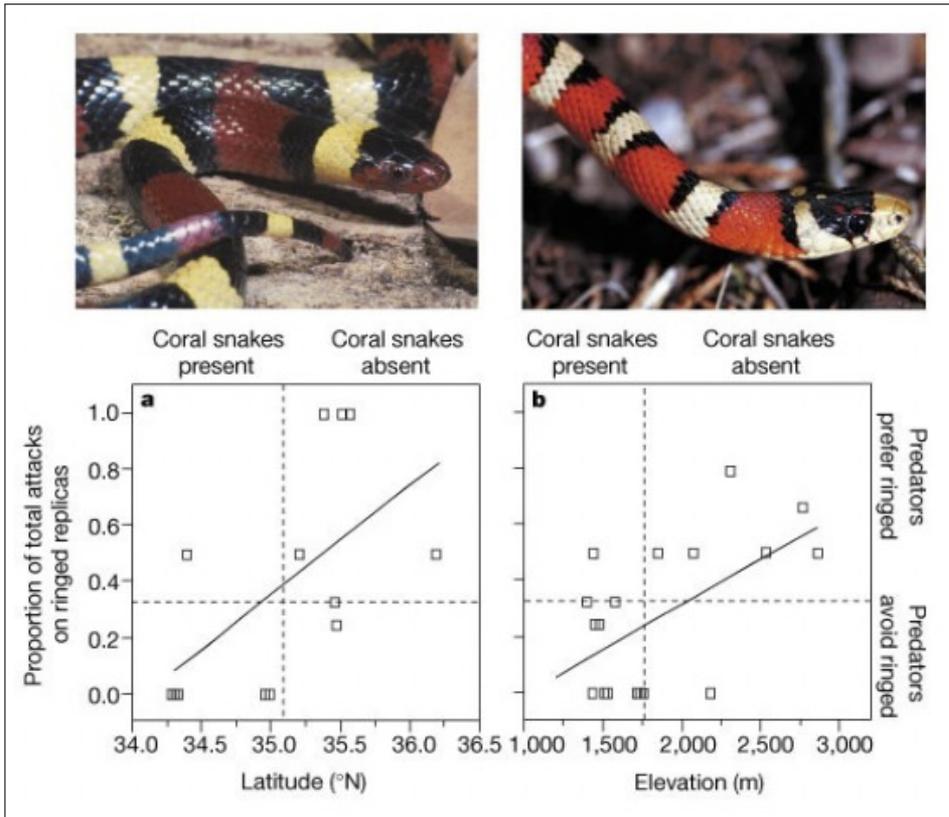


Figure 13.4: Frequency-dependent mimicry. The proportion of carnivore attacks on ringed replicas of scarlet kingsnakes (top left; a mimic of eastern coral snakes) and Sonoran mountain kingsnakes (top right; a mimic of Sonoran coral snakes) increased with (a) latitude ($y = -13.314 + 0.391x$, $P < 0.035$, $R^2 = 0.345$) and (b) elevation ($y = 0.329 + 0.00032x$, $P < 0.014$, $R^2 = 0.310$). The horizontal dashed line indicates the proportion of attacks on ringed replicas expected under randomness. The vertical dashed line indicates the maximum latitude and elevation for coral snakes in North Carolina and Arizona, respectively. [Reprinted with permission from: D W Pfennig, W R Harcombe and K S Pfennig, Frequency-dependent Batesian mimicry: Predators avoid look-alikes of venomous snakes only when the real thing is around, *Nature*, 410, 323, 2001]

Batesian mimic of the proposed model species. Such reasoning ignores other factors which may be more important for the survival of the mimic. Besides, why should we imagine that a perfect mimic always survives better than an imperfect mimic? If we think of mimicry as an evolutionary game between the mimic, the

model and the predator, we might expect much more complicated dynamics and many final outcomes. Thus, imperfect mimicry may not always be maladaptive or evidence against the hypothesis of mimicry. In this way of thinking, we can begin to hypothesize potential selective reasons for imperfect mimicry.

For example, we may propose the hypothesis that in places where the venomous model is very common, the predators are selected to be extremely careful and hence avoid approaching even something that looks vaguely like the model. In other words, imperfect mimics may get protection from predators in spite of their imperfection. On the other hand, where the model is quite rare, predators may not be strongly selected to be very careful because the chances of encountering the venomous snake are quite small. In such a situation, we may expect that imperfect mimics may be eaten and only the perfect mimics avoided by the predators. This line of argument, if realistic, would suggest a very counter-intuitive outcome. Where the models are common, mimicry should be imperfect, and where the models are rare, mimicry should be perfect. This, of course, is just arm-chair theorizing. There is no guarantee that it is realistic. But the fact that this hypothesis makes a rather counter-intuitive prediction makes it a powerful hypothesis, meaning that it is really worth testing. If its counter-intuitive prediction is upheld, we may have somewhat greater confidence in our hypothesis because the prediction was borne out in spite of the fact that there are many reasons why it should not—that is why it was counter-intuitive in the first place.

David Pfennig, along with his graduate student George Harper (*Figure 13.5*) decided to test the counter-intuitive prediction of this interesting hypothesis. They used the venomous eastern coral snake *Micrurus fulvius* (*Figure 13.1*, top left) and its model, the scarlet kingsnake *Lampropeltis triangulum elapsoides* (*Figure 13.1*, top right) mimicry system in Eastern North America. The model species is distributed in Florida and southern parts of Mississippi, Alabama, Georgia, South Carolina and North Carolina. The mimic species has a much wider distribution, including Florida, almost the entire region of all the other above-mentioned states as well as Tennessee and parts of Kentucky and Virginia (*Figure 13.6*). The experiments described in the previous section of this chapter have already shown that the mimics get little or no protection from predators when they are outside the range of the model (allopatric). And, it is expected that the model species would decrease in abundance at the edges of its distribution. Harper and Pfennig, therefore, decided to set up some of their study sites in Florida, which is deep inside the distributional range of the model, and other study site in North Carolina, which is at the very edge of its distribution. The mimic species occur through-



Figure 13.5: George Harper in the field in North Carolina while he was a graduate student with David Pfennig at the University of North Carolina, Chapel Hill. He is now an Associate Professor in Hendrix College, in Arkansas, USA. (Photo: David Pfennig)

out, and it may be said to show deep sympatry with the model in Florida and edge sympatry in North Carolina. Based on the counter-intuitive prediction of the hypothesis outlined in the previous paragraph, Harper and Pfennig had three questions to answer: (1) Is the mimic relatively imperfect or more variable in Florida compared to North Carolina? (2) Is the model indeed more abundant in Florida than in North Carolina? (3) Is the predation pressure on good and poor mimics different in Florida and North Carolina?

Is the mimic relatively imperfect or more variable in Florida compared to North Carolina?

As you can imagine, we cannot rely only on our perception of what is a good and what is a poor mimic. Moreover, the closeness in resemblance of the mimic

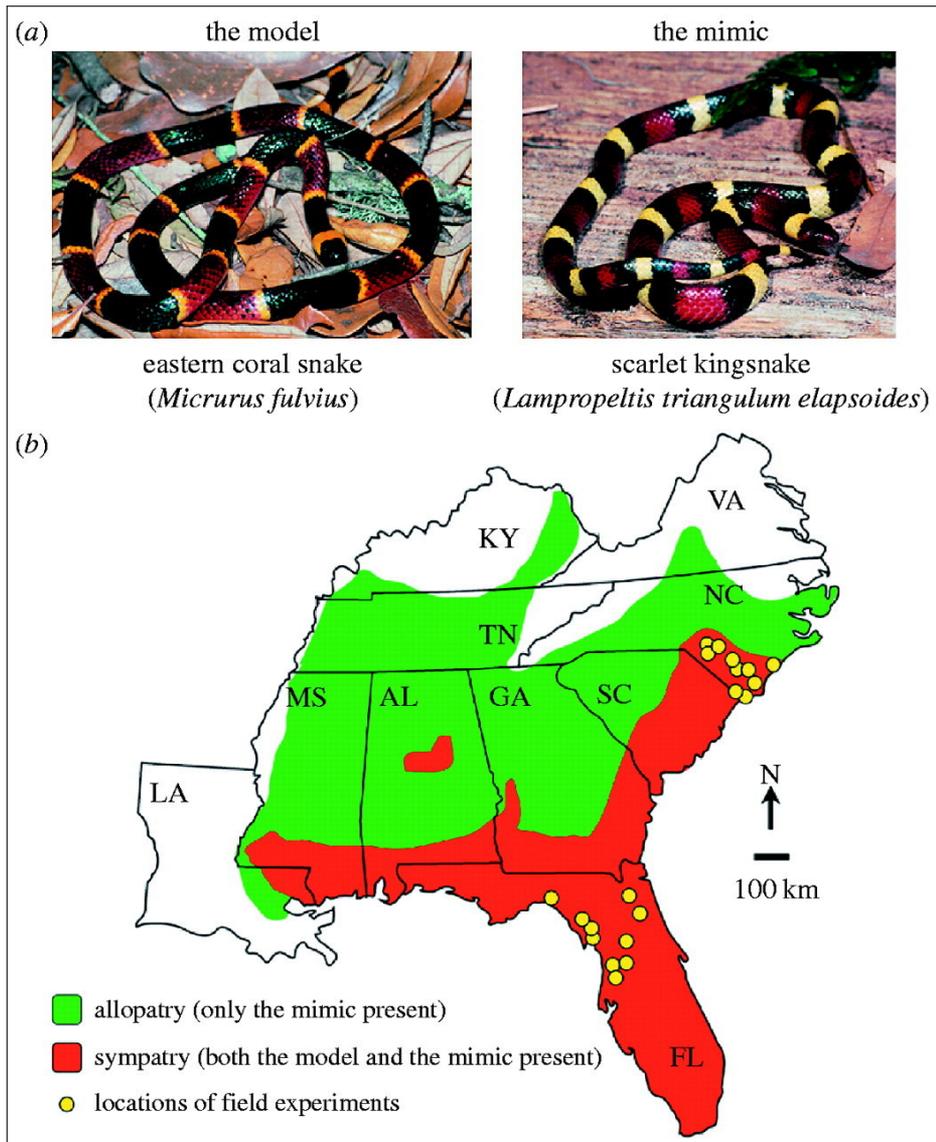


Figure 13.6: (a) The non-venomous scarlet kingsnake (*L. t. elapsoides*) mimics the highly venomous eastern coral snake (*M. fulvius*). (b) The geographical range of *L. t. elapsoides* (the mimic) greatly exceeds that of its model. [Reprinted with permission from: G R Harper Jr. and D W Pfennig, Mimicry on the edge: why do mimics vary in resemblance to their model in different parts of their geographical range? *Proc. R. Soc. Lond. B*, Vol.274, pp.1955–1961, 2007.]

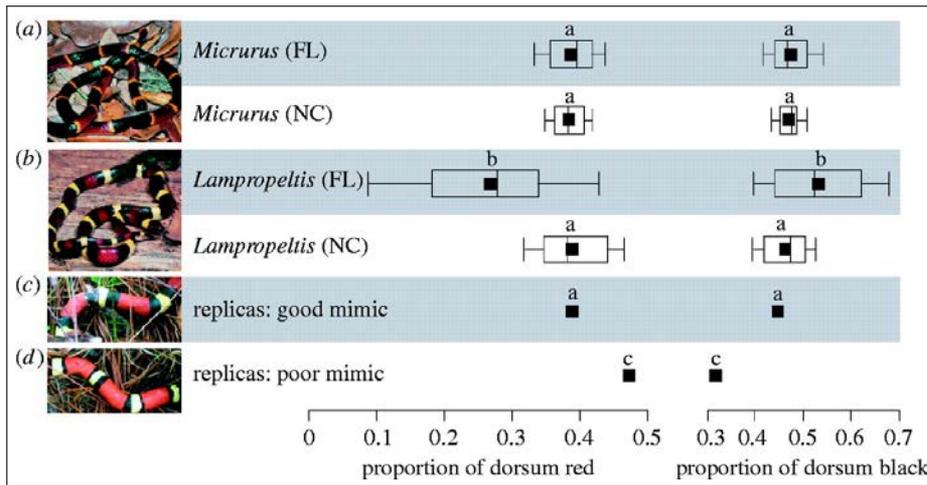


Figure 13.7: Comparison of two diagnostic traits for (a) the model (*Micrurus*) and (b) the mimic (*Lampropeltis*) from Florida (FL, deep sympatry) and North Carolina (NC, edge sympatry) and for (c) the good and (d) poor mimic replicas. Black squares show means. Box plots show 10th, 25th, 50th (median), 75th and 90th percentiles. Means with different superscripts are significantly different ($P < 0.05$; Tukey–Kramer HSD). [Reprinted with permission from: G R Harper Jr. and D W Pfennig, Mimicry on the edge: Why do mimics vary in resemblance to their model in different parts of their geographical range? *Proc. R. Soc. Lond. B*, Vol.274, pp.1955–1961, 2007].

should be in relation to the appearance of the model species in that particular area. Hence, Harper and Pfennig conducted a detailed morphometric analysis (a study of the measures of external body parts) of both the model and the mimic. First, they photographed both species by placing them on a similar background. As part of his PhD thesis, George Harper had previously determined that two characteristics helped to differentiate between good and poor mimics. These were: (i) the proportion of the snake’s mid-dorsum that is black, and (ii) the proportion of the snake’s mid-dorsum that is red. Incidentally, he had come to this conclusion because these are the two patterns that break down in allopatry [11]; i.e., where the model is not present, and the predators attack the mimic. So, for each species, from each locality, they now calculated the proportions of red and black on the mid-dorsum. The results of their study can be seen in (Figure 13.7). For both traits, the model species did not differ between Florida and North Carolina. The mimics, however, differed between the two locations. In North Carolina (edge sympatry), the model and the mimic did not differ from each other, meaning that

the mimicry was good. In Florida (deep sympatry), however, the mimic species was much more variable and significantly different from the model, meaning that the mimicry was not good. In other words, the answer to the first question is that mimicry is good in edge sympatry (where the model is rare) but poor in deep sympatry (where the model is common), just as predicted.

Is the model more abundant in Florida than in North Carolina?

To answer this rather difficult question, Harper and Pfennig counted the number of specimens of the model and mimic species in various museums. They also used some data which were available for North Carolina. Harper and Pfennig are conscious that this method of estimating abundance is not likely to be very precise in terms of absolute numbers of each species. Nevertheless, this method should give an unbiased estimate of the relative abundance of each species, which is what they were after. As it turns out, their numbers confirmed the common knowledge that the venomous coral snake is much more abundant in Florida than in North Carolina. They found that the ratios of the model to the mimic (more important than absolute numbers) differed significantly between Florida and North Carolina. Models outnumbered mimics in Florida (median of one model per mimic). By contrast, in North Carolina, mimics outnumbered the models (median of zero models per mimic). Thus, the second question was also answered in the affirmative.

Is the predation pressure on good and poor mimics different in Florida and North Carolina?

To answer this question, Harper and Pfennig prepared plasticine replicas of the naturally occurring good and poor mimics. To construct their good mimics, they ensured that the proportions of black and red in the replica were not significantly different from the model and the mimic in North Carolina (where mimicry is more precise; see the first question above). Their replicas of the poor mimic were significantly different from (i) the models in both localities, (ii) their own plasticine replicas of the good mimics, (iii) the good mimics in North Carolina, but, as it happens, they were also different from the poor mimics in Florida. They nevertheless used their replicas of poor mimics for experimentation in both the localities because previous research had shown that the phenotype corresponding to these replicas could be regarded as a poor mimic.

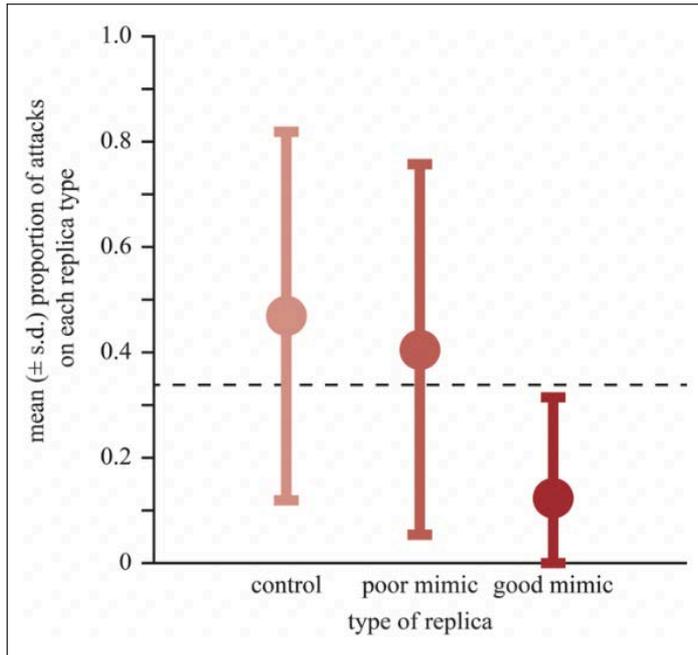


Figure 13.8 : Mean (s.d.) proportion of total attacks on each replica type in edge sympatry (North Carolina). The observed proportion of attacks only on replicas of good mimics was significantly ($p < 0.05$) less than 0.33 (dashed horizontal line), the value expected if predation attempts were random with respect to colour pattern. [Reprinted with permission from: G R Harper Jr. and D W Pfennig, Mimicry on the edge: why do mimics vary in resemblance to their model in different parts of their geographical range? *Proc. R. Soc. Lond. B*, Vol.274, pp.1955–1961, 2007.]

Choosing ten sites each in Florida (deep sympatry) and North Carolina (edge sympatry), they arranged triplets of replicas (with replicas of one good mimic, one poor mimic, and one control) at ten different points along the transect in each site. As before, a person without knowledge of the location of the replicas scored for predator attack marks (made by carnivores such as black bear, bobcat, coyote, fox or racoon) on all the replicas at the end of the experiment. They found that in North Carolina (edge sympatry), only the good mimics were attacked significantly less often than expected by chance (*Figure 13.8*). In Florida (deep sympatry), however, good and poor mimics were attacked equally frequently (data not shown). On the whole, it may be said that the predators distinguished between the good and poor mimics in North Carolina, but not in Florida.

Taken together, the three results show that the model is more abundant in Florida than in North Carolina, the mimics are more imperfect in Florida than in North Carolina, and that the predator did not discriminate between good and poor mimics in Florida but did so in North Carolina. The counter-intuitive prediction is thus upheld. There indeed is a stronger selection to be a good mimic where the model is rare (North Carolina) than where the model is common (Florida). This counter-intuitive result should change how we imagine that the evolution of mimicry would work. As noted above, this result makes sense when we take into consideration all three actors: the model, the mimic, and the predator. Mimicry is a dynamic game played with three players over evolutionary time. Thus, mimics can be imperfect when selection for being perfect is relaxed in the presence of large numbers of the model species, which in turn makes predators very careful. And here, by very careful means predators will avoid anything that even vaguely looks like the venomous model species. So, why don't mimics do their best? Because it is not really necessary for their survival when the models are common [13].

13.6 How Do Mimics Evolve from Non-Mimics?

The counter-intuitive result obtained above that mimics tend to be much more precise when the model species are rare, but they tend to be imprecise when the model species is common, potentially solves another major evolutionary problem. This concerns how mimics can evolve from their cryptic ancestral species through natural selection. When any phenotype evolves by natural selection, this process is usually assumed to occur through a gradual step-by-step process involving many intermediate stages of the phenotype. The unsolved problem concerns how the intermediate phenotypes in such a gradual evolutionary process might survive in the context of mimicry. The intermediate stages in the evolution of mimicry are neither expected to have the benefit of crypsis (which they have given up) nor the benefit of perfect mimicry (which they have not yet attained). Therefore, predators should preferentially attack the intermediate forms so that evolution should not proceed any further. However, based on the result in the previous section, the hypothesis can now be proposed that intermediate forms survive and, therefore, can evolve further into better mimics in areas where their model species is abundant.

In technical language, the evolution of mimicry can be visualized on what is called 'the adaptive landscape', in which high points (or 'peaks') are phenotypes associated with high fitness and low points (or 'valleys') are phenotypes associ-

ated with low fitness. These concepts of adaptive valleys and adaptive peaks were introduced by Sewall Wright, one of the three major architects of modern evolutionary theory, in his famous Shifting Balance Theory of Evolution [14]. If there is an adaptive valley between two adaptive peaks, then evolution by natural selection cannot easily cross the valley; to do so would require an individual to produce offspring with phenotypes that are associated with lower fitness than the parents, which is not favored by natural selection. Essentially, the problem with the evolution of mimicry is that crypsis on the one hand and mimicry on the other can each be thought of residing on different peaks on the adaptive landscape. How, then, can mimicry evolve from crypsis (which is generally assumed to be the ancestral condition) through the traditional process involving the accumulation of small genetic changes? After all, this process would require that a population must first cross a fitness valley associated with intermediate forms (for instance, an imprecise mimic). Such imprecise mimics should be poorly adapted and, therefore, not be able to cross the valley and reach the next adaptive peak (the one associated with more precise mimicry) where they would be well adapted to survive. Theoreticians generally overcome this problem by postulating so-called ‘supergenes’ which are supposed to bring about a major phenotypic change in one shot and allow their bearers to jump over the valley. This is exactly what theoretical models for the evolution of Batesian mimicry have proposed. While there is evidence for the role of such supergenes in the evolution of Batesian mimicry in butterflies [3], this need not be the case in all instances of mimicry. In the present context, the new hypothesis is that areas of high model abundance present a scenario where there is no adaptive valley between the adaptive peak of crypsis and the adaptive peak of perfect mimicry. If this hypothesis is correct, there is no need to postulate supergenes.

David Pfennig and another of his graduate students, David Kikuchi, set out to test this hypothesis using the coral snake mimicry system of the Eastern coral snake *Micrurus fulvius* and its mimic *Lampropeltis elapsoides*, both in Florida where the model species is abundant and in North Carolina where the model species is rare. The hypothesis predicted equal predation rates on cryptic species, poor mimics, and good mimics in Florida where the model is common. David Kikuchi (*Figure 13.9*) is now a Post-doctoral Research Fellow at the University of Bielefeld in Germany, and I had the pleasure of meeting him when he spent the academic year 2019–2020 at the Wissenschaftskolleg, Institute for Advanced Study in Berlin. First, they confirmed that the scarlet kingsnake is indeed likely to have evolved from cryptic ancestors. For this, they performed a test known as



Figure 13.9: David Kikuchi on field work as Karin Pfennig (whom we met in the first experiment) and the Pfennig children, Katrina and Elsa, look on—what a great experience for the children and adults alike!

ancestral character-state reconstruction. This means they examined a recently published phylogeny of the scarlet kingsnake and its relatives, 31 species belonging to the tribe Lampropeltini. They determined the colour patterns of all the snakes represented in the phylogeny and found evidence that all species that had a strong mimetic resemblance to coral snakes were located in a single clade which in turn was derived from clades containing cryptic species [see *Figure 2* in Suggested Reading 15]

Next, the two Davids made plasticine replicas of three different phenotypes of the mimic, a cryptic phenotype which resembled the Eastern milksnake *Lampropeltis triangulum*, an intermediate phenotype which resembled the coastal plains milksnake *Lampropeltis temporalis*, and the mimetic phenotype which resembled the scarlet kingsnake *Lampropeltis elapsoides* (*Figure 13.10*). Perhaps I should mention a technical detail about the preparation of the replicas. As part of David Kikuchi's PhD thesis, he had previously gathered extensive morphometric data on the Eastern milksnake, *L. triangulum*. George Harper and David Pfennig (whose

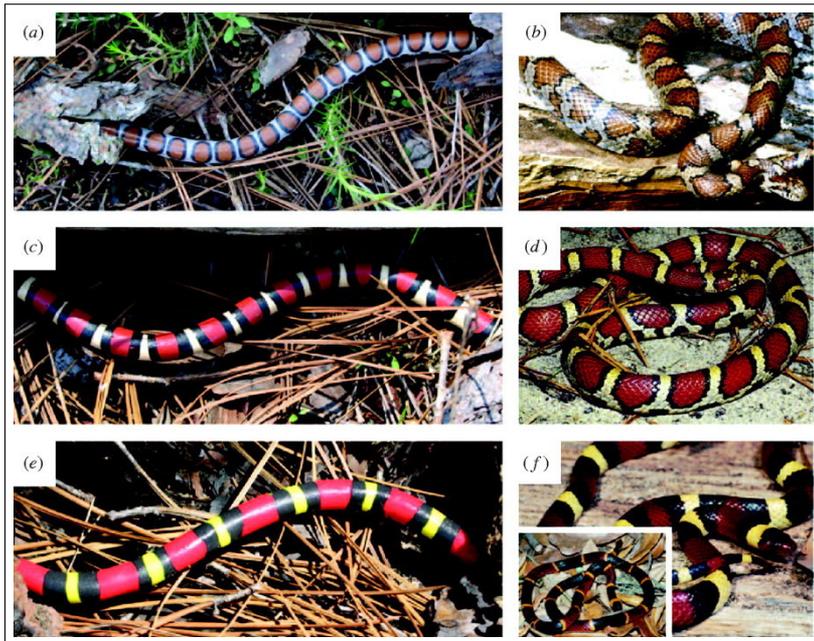


Figure 13.10: Replicas of different snake phenotypes used to measure predation in the field, along with the species or subspecies of snake that each most closely resembled. (a) Cryptic phenotype, which resembled (b) the eastern milksnake, *L. t. triangulum* (photo by Roger W Barbour); (c) intermediate phenotype, which resembled (d) the coastal plains milksnake, *L. t. temporalis* (photo by Richard D Bartlett); (e) mimetic phenotype, which resembled (f) the scarlet kingsnake, *L. elapsoides* (inset: the eastern coral snake, *M. fulvius*, which *L. elapsoides* mimics; photos by Wayne van Deventer). [Reprinted with permission from: D W Kikuchi and D W Pfennig, High-model abundance may permit the gradual evolution of Batesian mimicry: An experimental test, *Proc. R. Soc. Lond. B*, Vol.277, pp.1041–1048, 2010.]

experiments we saw in the previous section) had obtained similar morphometric data on the mimetic kingsnake *L. elapsoides*. There is good reason to believe that the Eastern milksnake *L. triangulum* is a good proxy for the cryptic ancestral phenotype. They used these morphometric data to make their plasticine replicas of the cryptic and mimetic phenotypes. To make the replicas of the intermediate phenotype, however, they did not have morphometric data on the coastal plains milksnake *L. temporalis*. They, therefore, used the average values of the cryptic and mimetic phenotypes in each of the 12 morphometric dimensions to create the intermediate phenotype, which luckily resembled the coastal plains milksnake.

To test the probabilities of predator attacks on the three phenotypes, they tied together sets of three replicas, with one of each kind (cryptic, intermediate, and mimetic), so that there was a distance of one meter between each of them. Ten such triads were placed along a transect at a distance of about 75 meters from each other, in the natural habitats of the snakes and their predators. They repeated this experiment with 13 such transects each in Florida (where the model abundance is high) and in North Carolina (where the model abundance is low). All replicas were collected 30–36 days later and, as before, they were scored for predator marks on them by a person unaware of the locations of the replicas.

The results of these experiments were clear-cut. Recall that their hypothesis predicted that in Florida, where the model species is very common, imperfect mimics should be protected even though they are imperfect. As expected, they found that in Florida, the replicas resembling the intermediate phenotype were not attacked any more than the replicas resembling the cryptic and mimetic phenotypes (*Figure 13.11*, upper panel). Conversely, their hypothesis predicted that in North Carolina, the imperfect mimic should not be protected. As expected again, they found that in North Carolina, the replicas resembling the intermediate phenotype were attacked more than the corresponding rates of attacks on the replicas resembling the cryptic and mimetic phenotypes. In other words, while there indeed was an adaptive valley difficult to cross for the intermediate phenotype in North Carolina, there was no such adaptive valley in Florida. Extending the metaphor of valleys and peaks, we can say that there was a smooth evolutionary landscape in Florida, where the mimic could evolve from being cryptic to be a perfect mimic in spite of having to go through intermediate imperfect phenotypes, perhaps a series of successively improved mimics. Because the intermediate phenotype is protected in Florida, there was no need for one large jump in phenotypic space of the kind facilitated by supergenes; gradual changes in phenotype in small steps can lead from crypsis to mimicry. This simple experiment provided a powerful solution to a long-standing problem regarding the evolution of Batesian mimicry by gradual evolution [15]. As early as 1958, Ronald Fisher had considered aposematism and mimicry as ‘the greatest post-Darwinian application of natural selection but it is only really now, after the possibility of the gradual evolution of Batesian mimicry has been confirmed, that we can reaffirm Fisher’s claim.

In summary, the three simple experiments described in this chapter have added much confidence in the hypothesis that the non-venomous kingsnakes are Batesian mimics of the venomous coral snake in North America. In addition, the third experiment added further confidence in the hypothesis of Batesian mimicry by

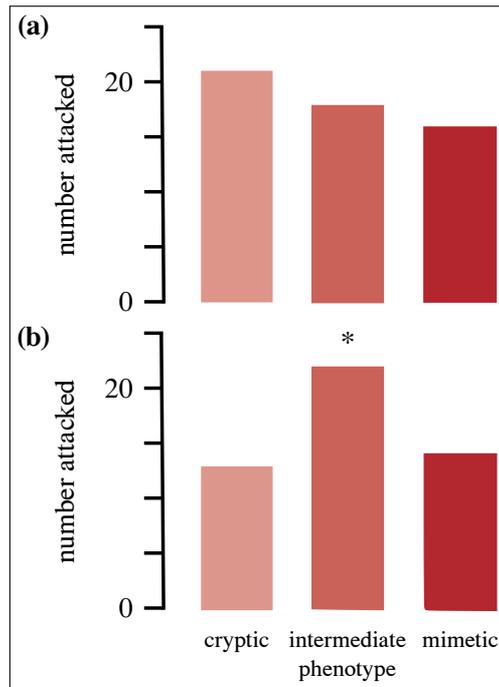


Figure 13.11 : Two alternative adaptive landscapes observed in a coral snake Batesian mimicry complex. (a) No adaptive valley in Florida where coral snakes are highly abundant models. An apriori contrast showed no difference between the attack rate on the intermediate phenotype versus the attack rate on cryptic and mimetic phenotypes ($Z = 0.01$, $P > 0.9$, $n = 389$). (b) Selection against intermediate phenotypes around southern North Carolina where coral snakes are rare. The intermediate phenotype is attacked at a higher rate than cryptic and mimetic phenotypes ($Z = 1.95$, $p = 0.05$, $n = 359$), indicating the presence of an adaptive valley in North Carolina. Asterisk indicates statistical significance. [Reprinted with permission from: D W Kikuchi and D W Pfennig, High-model abundance may permit the gradual evolution of Batesian mimicry: An experimental test, *Proc. R. Soc. Lond. B*, Vol.277, pp.1041–1048, 2010.]

showing that perfect mimics can evolve from cryptic ancestors through a process of gradual natural selection. Unlike the existing theory, these experiments, rather than showing how the mimics could jump over the adaptive valley, showed that there was no valley in the first place, at least in some localities. More generally speaking, these experiments overcome the objection that coral snake mimicry violates some theoretical predictions of Batesian mimicry. But they do so not so

much by showing that coral snakes do not violate the predictions, but by showing that some predictions based on mimicry in butterflies are not really valid for coral snakes. Thus, the results of these experiments argue strongly that Batesian mimicry in coral snakes is not a just-so-story.

13.7 Imperfect Mimicry and the Limits of Natural Selection

More recent research has uncovered many interesting facts about the evolution of Batesian mimicry in the coral snake mimicry complex. After a long period of high abundance of models and the consequent adaptation of the predators to avoid them, if the models go extinct, there would be continued selection on the mimics to remain close to the appearance of the models at least for a time. This is because the predators who know to avoid the models and those that look like them, are still around. Such selection on the mimics could continue much longer if the ability of the predators to avoid the models has become instinctive. There could even be further selection on the mimics to attain improved resemblance to the now-extinct models because the major attention of the predators would now be turned to the mimics (with the models gone), especially the imperfect mimics [16]. The co-evolution of the models, the mimics and the predators, under different combinations and densities, and under sympatry and allopatry, constitute a rich, and dynamic system for further theoretical and experimental investigations. Evidence is also accumulating that, unlike some of the butterfly and other arthropod examples, coral snake mimicry may constitute a much more dynamic, back and forth between mimetic warning colouration and crypsis, rather than a stable evolutionary endpoint [17–19].

13.8 Reflections

The experiments described in this chapter score very high marks on all the criteria that I am trying to espouse and eulogise in this book—simple and clever experiments, the thrill of fieldwork, attention to ecology, a side-project becoming larger than life, working with family and former students, asking fundamental questions and coming up with substantial new findings and solving long-standing problems—and all this, I must emphasise, at a trifling cost. Here I would like to especially dwell on two features of these experiments.

13.9 “A Different Kind of Modelling”

The first point concerns the clever use of plasticine models in all the experiments described. This turns out to be such a simple and clever technique, and I would love to see it used even more often. The charming subtitle of this section is borrowed from the title of a fascinating review article entitled “A different kind of ecological modelling: the use of clay model organisms to explore predator-prey interactions in vertebrates” that I have recently come across [20]. In this review article P W Bateman and A K Wolf from Curtin University and P A Fleming from Murdoch University, both in Perth, Australia, have reviewed 143 studies from 1984 to 2015, all using clay or plasticine models. The studies investigated prey morphology, habitat fragmentation, predator behaviour and social interactions, using artificial models of frogs, salamanders, snakes, lizards, birds (including bird eggs) and mice, to study predation by various species of birds, reptiles and mammals.

The review article shows how much we can do in the field using artificial models of animals, encourages others to do so, and suggests ways of improving the technique. For example, we can add smell to the models, or we can test whether predators can call the experimenters’ bluff with repeated exposure. It is not at all obvious beforehand whether predators would learn to ignore the models. There are many examples of super-normal stimuli in the ethological literature showing that animals can easily be fooled into preferring biologically inappropriate objects with exaggerated stimuli over biologically appropriate objects with normal stimuli. Here, therefore, is a rich area for experiments that would not only tell us about the limits of the clay models but even more about the relative roles of different stimuli in the perception of the world by different animals in different contexts. And this can lead to further inquiry about why natural selection has made them so. Opportunities for creating significant new knowledge even with low-cost research are limited only by our imagination.

13.10 Empirically Uninformed Theory versus Field Biology Sans Theory

My second point concerns the healthy interaction between theory and data. Notice that in the previous chapter we asked why male frogs do not do their best when singing to attract mates and, in this chapter, we asked why non-venomous snakes do not always do their best in mimicking venomous snakes. The recurring theme is that natural selection does not always produce what we may naïvely consider

as a perfect adaptation. The fault is ours, not that of natural selection. How then do we correctly frame our expectations regarding adaptations of animals and plants in nature? Clearly, we need both theory and empirical research, the latter preferably under natural or at least well-defined laboratory conditions. We have to steer between the devil of empirically uninformed theory on the one hand, and the deep sea of fieldwork without a sound theoretical foundation on the other. The experiments described here illustrate this particularly well. As you would have noticed, every experiment was carefully designed to test the prediction of a well thought out hypothesis stemming from the theory of Batesian mimicry. And yet, the results of previous experiments influenced the theoretical predictions and the design of the subsequent experiments.

Nowhere is this better illustrated than in the case with the counter-intuitive result that there was a stronger selection to be a good mimic where the model was rare (in North Carolina) but not where the model was common (in Florida). This result led to the abandoning of the theoretical idea that mimicry cannot evolve through a process of gradual natural selection because the putative mimic will encounter and be lost in an adaptive valley when it is midway between being cryptic and mimetic. The counter-intuitive result suggested that there is no need to postulate a super-gene which lets the putative mimic jump from crypsis to mimicry. Instead, it suggested the hypothesis that there may be no adaptive valley in the first place. The next experiment vindicated this hypothesis by showing that predators do not discriminate against imperfect mimics in the high model density areas of Florida. Florida thus provides them with a smooth evolutionary landscape to gradually evolve from cryptic forms to mimetic form via the intermediate stages of imperfect mimicry. More generally speaking, the experiments described here and in the previous chapter have shown interesting new light on ‘imperfections’ produced by natural selection, be they less than the most attractive songs or less than the most perfect mimicry. The bottom line, of course, is that these are deemed to be imperfections only because we are ignorant of the underlying ecology during the operation of natural selection.

How do we facilitate a healthy interplay between theory and empirical research? Educating students in both theory and experiment from a very early stage is, of course, very useful and, one might say, even essential. But lessons learned from the best education are often laid to waste by the periodic eruption of fashions that promote an unbalanced approach in the pursuit of science. Fashions will inevitably rise and fall, but the problem is compounded by the institutional promotion of one fashion or another in the form of biases in funding, hiring and

promotion practices. To overcome this tendency, science managers and policy-makers need to display a reasonable amount of modesty and a better appreciation of the history of science.

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14

Cuckoos Lay Their Eggs in Others' Nests, But Why Do the Hosts Get Fooled?

That the cuckoo lays its eggs in the nests of other species and does not build its own nest or raise its own offspring, is one of the oldest known facts about Natural History and has been abundantly and eloquently immortalised in myths and stories, art and literature, music and poetry, philosophy and morals. Attempts to understand this curious phenomenon in any rational way began just about 100 years ago. With a landmark study consisting of a few simple and elegant experiments that needed no laboratory or funding, Nick Davies and Michael Brooke at Cambridge University in the UK ushered in its modern scientific study as recently as 1988. In this chapter, I will describe their experiments and their results and conclusions, accompanied by a running commentary relating their work to the theme of this book and end with some more general reflections on the pursuit of the science of animal behaviour.

Exploring examples of simple, clever and inexpensive experiments in animal behaviour in this book, we have been encountering diverse animal taxa and at the same time, understanding fundamental principles in the proximate and ultimate causation of behaviour. Not surprisingly, evolution by natural selection has been a recurring theme. Because natural selection promotes the survival of the fittest, here and now, we often see the unexpected. We saw in chapters 12 and 13 that

natural selection can promote cheating if that's what it takes to survive, and since those cheated are also subject to natural selection, we witness arms races between the cheaters and the cheated that can reach a variety of outcomes. The ongoing arms races make it appear to us as if evolution has perfected neither party, and that is how it should be if both parties are to co-exist. In the 12th chapter, we saw that male frogs sing to attract mates but cannot do their best because of lurking predators that eavesdrop. And we saw in the 13th chapter that while non-venomous snakes mimic venomous neighbours to escape predation, their likeness to their models is only as much as is necessary for their survival and not more. Considering only one of the interacting parties shows the limitations of natural selection but considering all parties, including others in the environment, can help us make more sense of the patterns observed in nature.

14.1 The Cuckoo

Having studied fishes, frogs and snakes in the previous three chapters, here we will study some excellent examples from among the many elegant experiments done on birds. Birds are arguably the best-known animals to scientists and amateurs alike. Birds have provided endless opportunities for people of all walks of life to observe, study and experiment, to shoot, cage and eat, to admire, eulogise and write poetry on. Their flight, nests, eggs, family life, pair-bonding, songs and migrations capture the imagination of young and old alike. Their occasional departures from what we might consider an honest and harmonious family life are also equally well known. Perhaps the foremost example of the latter is the cuckoo's habit of laying its eggs in the nests of other species and avoiding the chores of building a nest and caring for its young, a fact that has been known at least since the time of Aristotle and has given us the word cuckold in the English language. Cuckoos belong to the family Cuculidae along with koels, malkohas, coucals and anis. Only some species of cuckoos are brood parasites while the rest rear their own young. This is convenient because it allows us to understand the gradual evolution of brood parasitism derived from closely related nest building and brood rearing species (*Figure 14.1*).

The cuckoo has had a very prominent place in the mythology, art and literature of many human cultures including Greek, Indian and Japanese, but this is not so much on account of the 'aberrant' behaviour of the female cuckoo. Rather, it is on account of the real and imagined nature of the male cuckoo, not the least of which is his song. The cuckoo has been depicted as the symbol of love and desire and



Figure 14.1 : Common cuckoo, *Cuculus canorus*, adult female, photographed at Bhondsi, near Gurugram, Haryana, India, by Satyajit Ganguly. Reproduced with permission.

as the harbinger of spring. But for the evolutionary biologist, it is the ‘cheating’ female cuckoo that is of greater interest. The female cuckoo’s unusual behaviour of laying all her eggs in the nest of other species and never bothering to build her own nests and exercise her own maternal instincts, is a striking evolutionary curiosity and one that did not escape Darwin’s notice (*Figure 14.2*). The cuckoo not only attracted Darwin’s notice but also drew out of him his speculation of the evolutionary sequence by which such a behaviour could evolve and be perfected. And yet, the proximate and ultimate causation of the cuckoo’s unusual behaviour were not really understood until the 1980s. This is all the more surprising because what it finally took to bring about this understanding was no more than a set of simple, low-cost experiments of the kind we have been celebrating in this book.

14.2 Brood Parasitism

The habit of laying eggs in the nests of other birds is not restricted to the cuckoo. It is practiced by many species, some laying their eggs in the nests of other members of their own species (conspecific brood parasitism) while others laying some or all their eggs in the nests of other species (interspecific brood parasitism). Con-



Figure 14.2: **Top:** Three reed warbler eggs and one cuckoo egg (slightly larger) in a reed warbler nest. **Bottom:** A 14-day old cuckoo chick in a reed warbler nest. Notice that the cuckoo chick has managed to evict all host eggs and/or chicks and occupy the whole nest for itself, into which it barely fits; its most conspicuous and most important part is the open gape designed to receive food and to induce the host to feed and to receive food. *Photos courtesy:* Nick Davies. Reproduced with permission.

specific brood parasitism has been observed in over 250 species while interspecific brood parasitism is practised by over 100 species of birds. This means that there are over 350 species offering us opportunities to investigate this fascinating 'aberrant' behaviour. Although there is now a growing literature on the ecology, behaviour, evolution and coevolution of avian brood parasitism, very few species have been studied in much detail (reviewed in [1]), and unfortunately, almost none in India so far. This is an embarrassing contrast to the fact that the complete genomes of more than 100 species of birds have been sequenced, annotated and published, suggesting to my mind that while scientists who have access to expensive laboratories and large funds have been busy fulfilling their responsibilities, those of us who need relatively few facilities and modest budgets for our research, have come up somewhat short. This is the irony that I hope to mitigate with this book on the charms of low-cost research in animal behaviour. There are many interesting species and many more passionate bird watchers, and there is much to be learned by simple field experiments preceded, and followed, by careful observations. Although some of the pioneering work on brood parasitism by cuckoos was done by the Englishman Stuart Baker in India [2, 3], almost nothing has been published from India barring one recent report by another Englishman Gaston [4]. There is, fortunately, some new work emerging from Bangladesh on the Asian cuckoos [5] and the Asian Koel [6], and also on the common cuckoo from China [7].

The perfect exemplar for our exploration of what we can learn about brood parasitism from simple experiments is the classic study of the common cuckoo *Cuculus canorus* parasitizing the reed warbler *Acrocephalus scirpaceus* in Cambridge, England by Nick Davies and Mike Brooke, in the 1980s [8] (*Figure 14.3*). Nicholas Barry Davies, now Professor of Behavioural Ecology at the University of Cambridge, and Fellow of Pembroke College, and Michael Brooke, now Strickland Curator of Ornithology, University Museum of Zoology, University of Cambridge, then recent PhD-buddies from Oxford, and both stricken by a passion for birds, teamed up to study cuckoos and reed warblers in Wicken Fen, a patch of old fenland 15 Km from Cambridge city. Wicken Fen is a 250-hectare nature reserve protected as a wetland site of international importance and yet open to the public, not to mention young naturalists, year-round. By all accounts, Davies and Brooke had great fun, two old friends, riding their bicycles to locate and monitor nests of reed warblers and, as we shall soon see, playing cuckoo, by parasitizing reed warbler nests with artificial cuckoo eggs and watching the fun. The title of their paper 'Cuckoos versus reed warblers: adaptations and counteradaptations' promises a thriller.



Figure 14.3 : **Top:** Nicholas Barry Davies, now Professor of Behavioural Ecology at the University of Cambridge, and Fellow of Pembroke College. **Bottom:** Michael Brooke, now Strickland Curator of Ornithology, University Museum of Zoology, University of Cambridge. *Photo copyright:* Michael Brooke.

14.3 The Behaviour of Cuckoos and Their Hosts

Like so many before them, Nick Davies and Michael Brooke were struck by the contradictions in the host's behaviour (reed warblers, in this case). On the one hand, the reed warblers seemed so well adapted to their lifestyle, navigating by stars from Europe to sub-Saharan Africa in the winter and returning to their own specific territories in spring, building exquisite nests and foraging with care and diligence to feed their chicks. And yet, they seemed utterly stupid to be fooled

into blindly feeding a grotesquely large cuckoo chick (five times their own size) if their nest happened to be parasitized. How could this be? There were some clues. The cuckoos did not always succeed. Of the 142 reed warbler nests they monitored in 1985, only 32 were parasitized by cuckoos; and in 1986 only 12 out of 132 nests were parasitized. Of these 44 parasitized nests, two were destroyed by predation, and of the remaining 42, the reed warblers rejected the cuckoo eggs in eight nests, either by deserting the nest or by ejecting the cuckoo egg. So, the reed warblers were doing something right and/or the cuckoos were doing something wrong. Successful detective work required that they carefully study the behaviour of both parties.

There are some striking features in the way cuckoos lay their eggs in the nests of their hosts. There are several genetically distinct “races” of the common cuckoo, each specializing on different hosts such as reed warblers, pied wagtails, redstarts and meadow pipits. Now each host lays somewhat different looking eggs; for example, redstarts lay plain blue eggs, meadow pipits lay brown eggs with spots while reed warblers lay greenish eggs with spots. Each race of cuckoos produce eggs that closely mimic the eggs of their hosts in their colour and markings. Davies and Brooke studied the race of the common cuckoo that specializes on reed warblers and lay eggs that mimic reed warbler eggs. The common cuckoo lays eggs that are very small for a cuckoo, as compared to non-parasitic cuckoos, for example. Not only are the eggs thus well adapted in the service of parasitism, but so is the egg-laying behaviour of the cuckoos. Cuckoos appear to patiently and deliberately plan their egg-laying, marking out particular nests for their use and waiting until the hosts themselves have begun to lay their own clutch of eggs. The common cuckoo lays just one egg in each host nest, and before doing so, she removes and promptly eats one of the host’s eggs. While the hosts do their egg-laying in the mornings, the cuckoo does so in the afternoons. Perhaps most striking of all, the cuckoo accomplishes her clandestine act of parasitism so quickly and stealthily that there was previously a long-standing debate about how the cuckoo eggs ever came to be in the host nests. Finally, the cuckoo hatchlings eject, from their foster nests, any remaining host eggs or chicks by a complex set of manoeuvres [9, 10].

14.4 Speculation versus Proof

Based on what we have seen of the behaviour of the reed warblers and the cuckoos and of the rates and outcome of brood parasitism, we can make a reasonable

argument that the reed warblers and cuckoos are locked in an evolutionary arms race, making it difficult for either party to declare total victory. Though the argument sounds reasonable, it is nevertheless speculation. Nick Davies and Michael Brooke designed experiments to put this speculation to the test by questioning the adaptive significance of each step in the cuckoo's behaviour. And they did so by 'playing cuckoo'. In order to play cuckoo, they needed to produce eggs, and this they did by making model eggs. In his recent book *Cuckoo: Cheating by Nature* that indeed reads like a thriller, [9] Nick Davies writes "I have a recipe for making cuckoo eggs. I borrow a cuckoo egg from a museum; make a mould around it, in two halves; pour resin into the two halves; stick them together and wait for it to harden; open the two halves of the mould—and there is a model cuckoo egg, of exactly the same dimension and weight as a real cuckoo egg. With acrylic paints, I now paint them various colours to represent the different races of cuckoos: green and speckled for reed warbler specialist cuckoos, brown and speckled for meadow pipit specialist cuckoos, greyish-white and speckled for pied wagtail cuckoos, and immaculate plain blue for redstart specialist cuckoos. I have a hundred of these model cuckoo eggs on my desk. Now I am ready to become a cuckoo myself." And "The model eggs looked realistic to our eyes, and they warmed up when the warblers sat on them, just like real eggs. We were delighted when our friend Bruce Campbell, one of Britain's most experienced ornithologists at the time, came across one of our experimental nests and noted it as parasitised by a real cuckoo." Armed with these model cuckoo eggs and a spirit of adventure, Davies and Brooke asked a series of questions about the adaptive significance of the cuckoos' behaviour. They located a number of reed warbler nests at the appropriate stage in their egg-laying and, in each, replaced one of the host's eggs with one of their model eggs (*Figure 14.4*).

Question 1. Why are cuckoo eggs mimetic?

Cuckoos lay eggs that mimic the eggs of their hosts, and different races of cuckoos specialize in mimicking the eggs of the particular hosts that they specialize in parasitizing; all this has been known since the 19th century. Yes, it is obvious that the egg mimicry is meant to fool the hosts into accepting the cuckoo eggs, but nothing that is merely obvious should be accepted as a proven fact. Thus, Davies and Brooke "parasitized" 83 reed warbler nests with model eggs bearing varying degrees of resemblance to real eggs laid by cuckoos in reed warbler nests. In 16 reed warbler nests they replaced a host egg with a pied wagtail type cuckoo



Figure 14.4: **Left:** Reed warbler nest with one of its eggs replaced with a mimetic model cuckoo egg. **Centre:** Reed warbler nest with one of its eggs replaced with a non-mimetic model egg. **Right:** several model eggs ready to be sneaked into reed warbler nests; the large model egg at the bottom is about the size that a non-parasitic cuckoo of the same body size is expected to lay. *Photo courtesy:* Nick Davies. Reproduced with permission.

model egg (i.e., models resembling eggs laid by cuckoos parasitizing pied wagtail nests), in 14 nests they introduced redstart type cuckoo eggs and in nine nests they introduced meadow pipit type cuckoo eggs. These were all non-reed warbler type models, i.e., the models resembled eggs laid by cuckoos parasitizing species other than reed warblers. They also used reed warbler type eggs, but some of them were painted differently. In 12 nests they placed reed warbler type models eggs but painted with rufous spots, in 13 nests they placed model eggs painted with brown spots, and in 17 nests they placed model eggs painted with the “correct” green spots.

Their results were clear-cut. Acceptance or rejection of the model cuckoo eggs depended significantly on the appearance of the egg. The non-reed warbler type of model cuckoo eggs were rejected more often than the reed warbler type model cuckoo eggs and even among the reed warbler type, eggs that more closely mimicked real cuckoo eggs laid by cuckoos in reed warbler nests (i.e., with the correct green spots) were significantly more likely to be accepted (17 out of 17 cases, in this experiment) (*Figure 14.5*).

That 17 out of 17 model eggs resembling the eggs laid by cuckoos in reed warbler nest were accepted, is not however adequate to argue that the acceptance is due to egg mimicry. The model eggs may simply have been accepted because

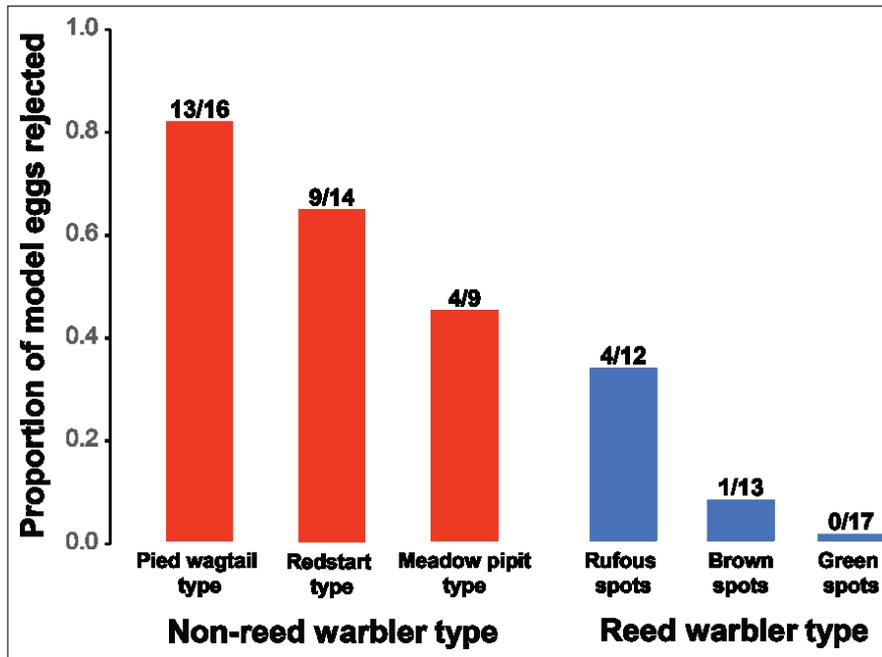


Figure 14.5 : Responses of reed warblers to the different type of model eggs sneaked in by Davies and Brooke, shown as number rejected/total number introduced. There was a significant difference in the rates at which the reed warblers rejected the six types of model eggs ($\chi^2 = 32.32, df = 5, P < 0.001$). Within the reed warbler type models, green spots were more likely to be accepted than rufous spots or brown spots. Only the most mimetic eggs, those of the type that cuckoos themselves lay in reed warbler nests, were accepted in all trials (17/17). [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

the reed warbler considered them as harmless objects. It is the combined facts that model eggs resembling those laid by cuckoos in reed warbler nests were accepted, and that model eggs deviating from the ‘correct’ patterns were rejected, that is much more convincing. The model eggs of the non-reed warbler type and the reed warbler types that were painted differently, acted as controls to strengthen the conclusion to be drawn from the acceptance of the mimicking eggs. It is also a way of ruling out other possible explanations for the observed result of 100% acceptance rate of the mimicking eggs.

That the reed warblers might have accepted the mimicking model eggs because they considered them harmless, is not the only alternative explanation that needs to be ruled out. Alfred Russel Wallace, the co-discoverer of the principle of natural selection, had long suggested that egg mimicry by cuckoos may be selected because by achieving the same level of camouflage as the host eggs they might escape the attention of predators. Davies and Brooke had seen avian predators such as crows, magpies and jays and mammalian predators such as the mink. They were able to distinguish such predation from egg rejection by the reed warblers most of the time because predation usually resulted in the loss of the whole clutch or in the whole nest being damaged. Moreover, egg rejection by the reed warblers often accompanied pecking at the model eggs and in the discovery of the rejected model eggs nearby. Is egg mimicry favoured by natural selection because it fools the hosts or because it fools the predators? It is possible to distinguish between these two possibilities and test Wallace's hypothesis. Davies and Brooke examined Wallace's hypothesis in three different ways. First, they looked for heterogeneity in the probability of being lost to predation among the nests receiving the different types of model eggs and found none (*Figure 14.6*). Second, they compared predation rates between nests receiving non-mimetic model eggs and mimetic model eggs and found no significant difference (*Figure 14.7*, upper panel). Third, they compared daily rates of predation between nests with mimetic and non-mimetic eggs and found no significant difference (*Figure 14.7*, lower panel). Thus they were able to rule out Wallace's hypothesis.

We can see that there is a successive refinement of the analysis going from *Figure 14.6*, to *Figure 14.7* upper panel and finally to *Figure 14.7* lower panel. The analysis in *Figure 14.6* merely tells us that the kind of model egg contained in the nest does not influence its probability of predation. But this could simply be because of a high level of variation in predation rates. The analysis in *Figure 14.7* upper panel makes a more specific comparison of the nest with mimetic and non-mimetic eggs. This answers a more direct question and also benefits from the sample sizes obtained by pooling all nests with different kinds of non-mimetic eggs and similarly pooling all nests with different kinds of mimetic eggs. Moreover, considering only the reed warbler type eggs painted with brown spots and green spots as mimetic and leaving out the reed warbler type eggs painted with rufous spots should increase the probability of detection of any existing difference in predation rates on account of the type of eggs. This is because the reed warbler type eggs painted with rufous spots appeared to be intermediate in their level of resemblance to the reed warbler's own eggs; reed warblers rejected them more

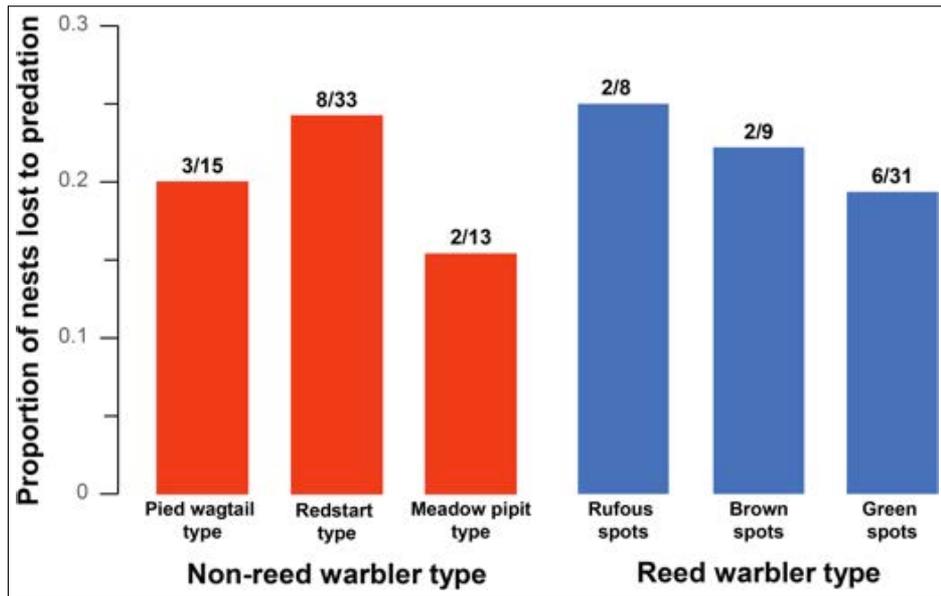


Figure 14.6: Reed warbler nests receiving different types of model eggs did not differ significantly in their probability of being lost to predation. [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

often than the reed warbler type models eggs painted with brown or green spots but less often than model eggs resembling the non-reed warbler type eggs. And yet, they found no effect of egg type on nest predation.

Finally, comparing daily rates of predation (in *Figure 14.7* lower panel) is even more powerful because in the previous two kinds of analyses predation rates of nests containing non-mimetic eggs may have been underestimated, making it less likely to detect a difference between nests bearing mimetic and non-mimetic eggs. This is because reed warblers harbouring non-mimetic eggs are more likely to reject those eggs, thus giving less time for predators to find and destroy the nests on account of being given away by the non-mimetic eggs. The analysis of daily rates of predation corrects for this bias by only considering the days when the model egg was present in the nest. And yet, no effect of egg type on predation rates was seen. All this makes the conclusion more robust that egg mimicry is unlikely to be selected by the ability of predators to detect nests with non-mimetic eggs,

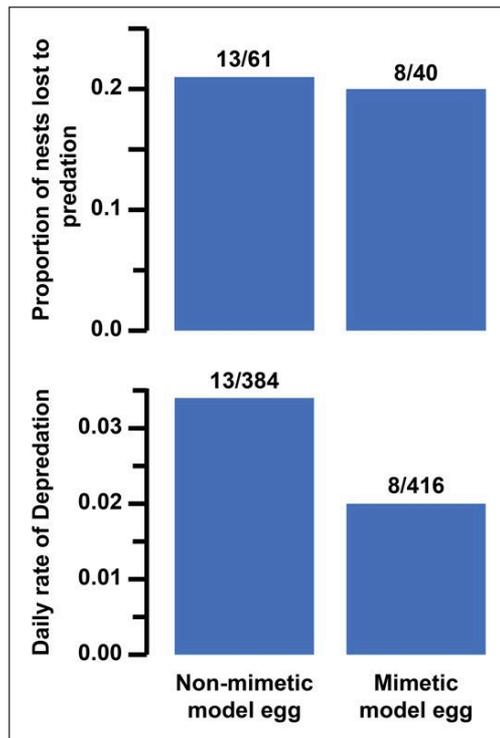


Figure 14.7: No effect of model egg type on rates of predation. **Upper panel:** No significant difference in predation probability between nests receiving non-mimetic versus mimetic model eggs ($\chi^2 = 0.008, df = 1, NS$). The pied wagtail, redstart and meadow pipit type model cuckoo eggs are taken as non-mimetic and the reed warbler type cuckoo model eggs with brown and green spots are considered as mimetic; the reed warbler type painted with rufous spots are ignored in the calculations because they are intermediate. **Lower panel:** No significant difference when daily rates of predation, either ($\chi^2 = 1.205, df = 1, NS$). 13 nests receiving the non-mimetic eggs and 8 nests receiving the mimetic eggs were lost to predation. In the upper panel these predation events are compared by dividing each number by the total number of nests receiving the non-mimetic and mimetic eggs respectively, while in the lower panel, the same numbers are compared by dividing by the numbers of days for which the model eggs remained in the nests and hence are available for predation. [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

as proposed by Wallace and, by implication, more likely to have been selected because of the ability of the reed warblers to detect non-mimetic eggs.

Field experiments of the kind described here are very tedious to perform and involve considerable disturbance to the animals involved, making it prudent to limit the total number of experiments, and squeeze as much juice out of the data as possible by clever and imaginative data analysis of the kind witnessed here. Field biologists have to sharpen their skills at planning and executing clever, well-designed experiments and imaginative data analysis for they seldom have the luxury of growing more experimental animals in the lab and repeating their experiments again and again, or simply performing new experiments for answering each question. They must perfect the art of combining rigour with economy, but that's the charm of field ethology. It's time to recall the words "attention, patience and heightened awareness" in the definition of ethology given by Peter and Jean Medawar [11].

Yet another hypothesis Davies and Brooke considered is that egg mimicry is favoured because it protects a mimetic egg from discovery by a second cuckoo who might try to lay her egg in an already parasitized reed warbler nest. Cuckoos are known to do so; of the 142 reed warbler nests monitored by Davies and Brooke in 1985, 32 were parasitized by cuckoos and six of these were subsequently parasitized by a second cuckoo. Since cuckoos always remove one existing egg before they lay their one and since only one cuckoo chick can survive in one reed warbler nest, it would make sense for the second cuckoo to remove any pre-existing cuckoo egg rather than a host egg before laying her own. So, the hypothesis that egg mimicry may reduce the chances of the second cuckoo removing the egg of the first cuckoo is plausible. However, they found little evidence in support of this hypothesis; second cuckoos were not significantly more likely to remove a non-mimetic model egg than a mimetic one. In fact, they seemed to remove one egg more or less randomly; even if there was a slight preference for removing the cuckoo egg, it was not statistically significant. The inability of the cuckoos to remove a non-mimetic egg selectively is somewhat surprising—if the reed warblers can do it why not the cuckoo? Davies offers an interesting explanation. Cuckoos lay their eggs quickly and stealthily (see below) and fly away without looking back. And since they never build a nest and incubate their eggs, they have probably never seen a cuckoo egg! In any case, the data here are scanty and one cannot be sure that they do not preferentially remove the previous cuckoo's egg; we can only say that there is no conclusive evidence that they do preferentially remove cuckoo eggs rather than reed warbler eggs. Moreover, as Davies and Brooke point

out, even if they do discriminate and remove cuckoo eggs, the selection pressure from host discrimination is likely to be much greater than from discrimination by the second cuckoo.

Hence, we are moving closer to the conclusion that egg mimicry is an adaptation to avoid detection and rejection by the hosts. It is an impressive adaptation because the same species of cuckoos parasitize several host species whose eggs are quite different in appearance—one size doesn't fit all. The common cuckoo species consists of several races (called 'gentes' or 'gens' in singular) which specialize in producing eggs resembling the eggs of the targeted host species. Even if we were to imagine that such gradual perfection of mimicry can be brought about by natural selection over evolutionary time, there is the interesting problem that the different races of cuckoos should breed true at least in their egg types and in their preference for certain host species. How is this 'purity' of racial egg type and host preference maintained within a race? One possibility is that egg type, and host preference is genetically passed down only through the female line, from mother to daughter to granddaughter. This is possible in principle because in birds females are the heterogametic sex with ZW chromosomes and males with a ZZ configuration (mirror image of the more familiar XX females and XY males in humans and most other organisms). The other possibility is that the various races are indeed reproductively isolated, with females of one race mating only with males of that race. The answer to this question is not yet clear. Be that as it may, since reed warblers remove non-mimetic or imperfectly mimetic model eggs, such as eggs of the wrong race of cuckoos, it is clear that there is an ongoing arms race between host and parasite. Even more suggestive of an ongoing process of adaptation and counter-adaptation (over evolutionary time, of course) is the observation that reed warblers reject some, but not all, non-mimetic eggs. Besides, egg mimicry is not the only weapon in the cuckoo's arsenal. There are several more, and Davies and Brooke test their efficiency too, as we will see below.

Question 2. Why are cuckoo eggs so small?

In order to masquerade their eggs as though they belong to the hosts themselves, cuckoos not only have to mimic host egg colour and pattern of spots but also have to make their eggs rather small in size. Parasitic cuckoos do lay eggs that are really small for a cuckoo. Since there are many non-parasitic cuckoos, it is possible to make a comparison to what the size of their eggs might have been if they were not selected for a brood-parasitic way of life. Non-parasitic cuckoos

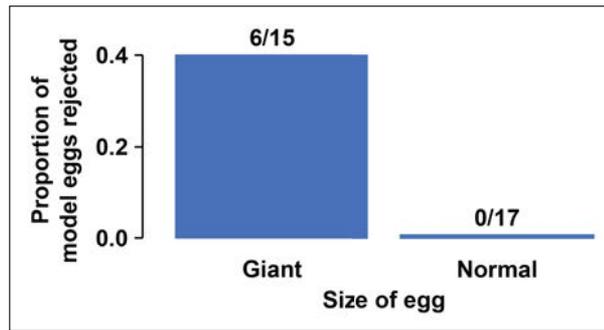


Figure 14.8: Reed warblers reject the giant model eggs about the size of eggs laid by non-parasitic cuckoos more often than the model eggs resembling common cuckoo eggs ($\chi^2 = 5.949, df = 1, P < 0.02$). [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

of comparable adult body size lay eggs weighing about 10 g while the common cuckoo parasitizing reed warblers lays eggs that weigh a mere 3.4 g. And yet, their chicks are enormous compared to reed warbler chicks. The physiological and developmental processes that take part in orchestrating a phenotype that can stay in the (arms) race between host and parasite remain to be understood in detail. But does the reduction in egg size really help the cuckoo, and if so how? One possibility, and by no means the only one, is that, like the appearance of the egg, it helps fool the host into confusing the identity of the cuckoo egg and accepting it as its own, as first suggested by Darwin. This is the possibility that Davies and Brooke put to test. In addition to the model eggs mimicking the cuckoo eggs that in turn mimic reed warbler eggs, they used model ‘giant’ eggs that were about the size expected to be laid by non-parasitic cuckoos.

Their giant model eggs weighed between 9.3 and 10.1 g and were 1.3 times the length and 2.8 times the weight of the eggs laid by the common cuckoo. As Darwin might have predicted, reed warblers rejected the large model eggs in six out of 15 nests, significantly more often than they rejected the normal cuckoo eggs (*Figure 14.8*). This suggests that one of the factors that select for small eggs laid by the common cuckoo is the ability of the reed warblers to detect and reject eggs that are oversized even though they may be similar to their own eggs in colour and pattern of spots. Notice that this experiment does not rule out other reasons that might also select for small eggs, such as Darwin’s other suggestion

that small eggs may be at an advantage because they may hatch within a shorter period of time. Nevertheless, the experiment does show that the small-sized egg is a second item in the cuckoo's bag of tricks. The fact that reed warblers rejected six giant model eggs and accepted 9, reminds us that neither party wins in the arms race. In addition to modifying their eggs to resemble those of their hosts in size and appearance, cuckoos employ a number of behavioural tricks to fool their hosts, and these can also be tested for their significance and efficiency in similar experiments.

Question 3. Why do cuckoos wait until the hosts start laying their own clutch?

A striking feature of the cuckoo's strategy is the timing of its parasitism. Male cuckoos have no role in facilitating brood parasitism by the females. While male cuckoos occupy and defend territories to keep out competition from other males, the female cuckoos occupy and defend territories to keep out competition from other females for access to reed warbler nests suitable for parasitism. They patrol the chosen reed warbler nests surreptitiously and bide their time. They have to not only avoid detection by the reed warbler parents but have to time their egg-laying such that they can sneak in one of their own eggs sometime after the reed warbler female has begun but not completed her egg-laying. Why do the female cuckoos have to time their act of parasitism in this way and indeed, how precisely do they have to time it? Does such timing increase the chance of the cuckoo egg being accepted by the host? Davies and Brooke set out to answer these questions by timing their own acts of parasitism either by being as precise as the cuckoo or less precise or more precise.

In one experiment, they placed their mimetic model eggs (the kind that were accepted 17 out of 17 times) in six reed warbler nests even before the host had commenced its own egg laying. All six were rejected—a far cry from all 17 accepted, when they did what the cuckoos did! (*Figure 14.9*). So it certainly pays for the cuckoos to be precise. It is probably not surprising that the reed warbler is suspicious about a mysterious egg when she has not laid any of her own, which of course means that she is somehow 'aware' of whether or not she has begun egg laying. But how precise should the cuckoos be in their timing? Does it matter how many eggs the host has already laid? To answer this question, Davies and Brooke first examined the stage of host egg-laying at which the 17 accepted mimetic cuckoo eggs had been introduced; six when the hosts had laid one egg, six when the host had laid two eggs, one when the host had laid three

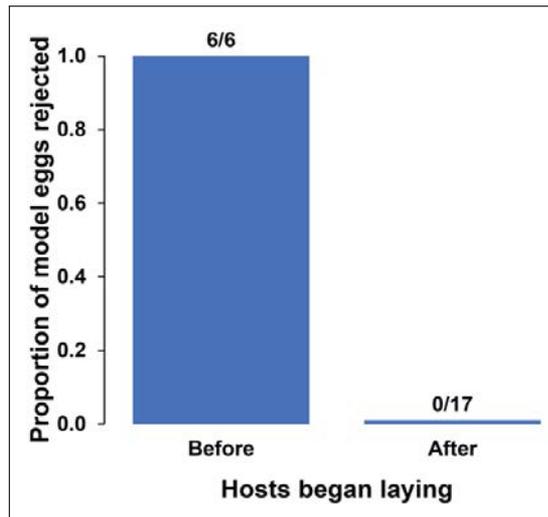


Figure 14.9: Six out of six model eggs are rejected if introduced before the hosts began laying their own clutch while 17 out of 17 model eggs are accepted when introduced after the hosts had begun egg laying. Although the sample sizes are small, these rejection rates are statistically significantly different ($\chi^2 = 18.106, df = 1, P < 0.001$). [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

eggs, and four when the hosts had laid four eggs. These numbers suggest that the stage of egg-laying does not matter as long as the host has started laying. But it's not good enough to look only at the accepted cases. What about rejections? Are the rejections stage dependent? To answer this question, they also introduced non-mimetic eggs, both the pied wagtail type model cuckoo eggs as well as the reed warbler type non-mimetic eggs (i.e., painted with rufous spots or brown spots) at various stages during the host laying period. The results of these experiments also showed that rejection rates (when some model eggs were indeed rejected) did not depend on the number of eggs already laid by the host (*Figure 14.10*).

Considering that all model eggs introduced before the commencement of egg-laying by the host were rejected, and that mimetic egg models were accepted at all stages, and non-mimetic model eggs were rejected at the same rates at all stages of egg-laying, we can conclude that the cuckoos need to be precise enough to time their egg-laying so as not to begin until the hosts have commenced egg-laying but need to be no more precise as to the number of eggs laid by the host at the time

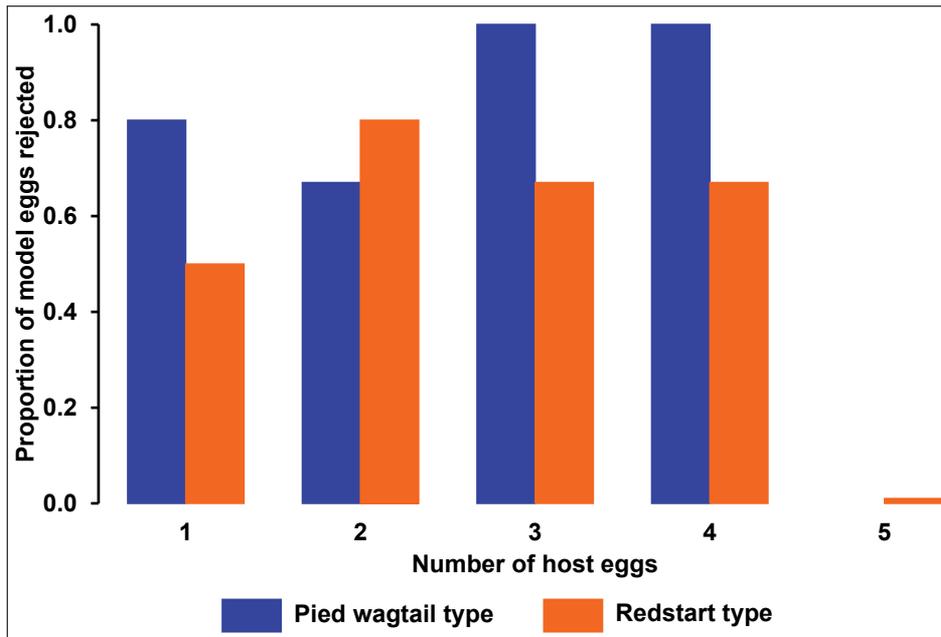


Figure 14.10: Rejection rate for non-mimetic eggs does not depend on the stage of egg laying by the host ($\chi^2 = 0.975, df = 3, P > 0.05$). [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

of parasitism. But there may be another reason for the cuckoos to lay their egg as soon after the host has started laying as possible. This is not because the hosts care any more about the stage of their own laying (as we have seen) but because the sooner they lay their egg, the sooner it will hatch and get a head-start, ahead of the host chicks, for it will have to eject the host eggs and chicks and gain monopoly over the nest and the host parents' attention. But of course, the more mimetic the eggs, the better for acceptance, whenever they are laid. While cuckoos thus use a mixture of strategies to fool their hosts, the hosts use information about whether or not they have started laying eggs to augment their ability to detect foreign eggs. As we will see below, this is not merely because their ability to detect foreign eggs may be limited but because they cannot afford to be too finicky, lest they reject some of their own eggs that might look a little different by chance. Not only is there an arms race between host and parasite, but there are also trade-offs between

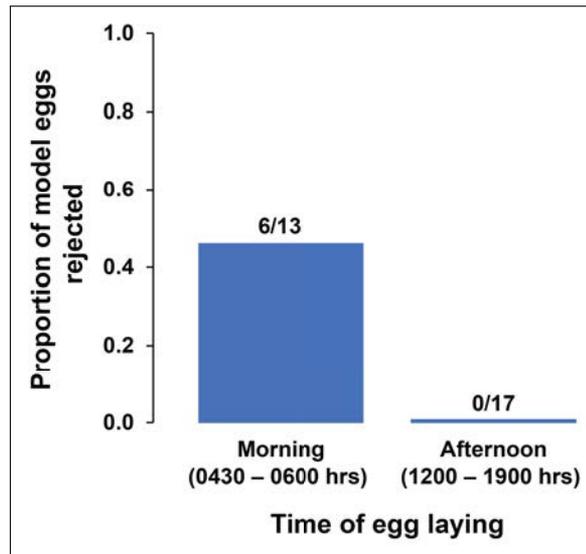


Figure 14.11 : Model eggs introduced in the mornings (0430–0600) rather than in the afternoons (1200–1900) (as the cuckoos themselves do) were rejected by reed warblers significantly more often than when the model eggs were introduced in the afternoons ($\chi^2 = 7.135, df = 1, P < 0.01$). [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

different strategies used by each party. This is the kind of fascinating complexity we should expect when natural selection is simultaneously perfecting two or more parties attempting to undermine each other’s survival. But there is more to come.

Question 4. Why do cuckoos lay their eggs in the afternoon?

A passionate egg collector by the name of Edgar Chance (more about him later) had discovered through a considerable amount of detective work of his own in 1920, that cuckoos always lay their eggs in the afternoon, although most of their hosts are known to lay their eggs early in the morning. Is this timing adaptive, meaning does it increase the chance of acceptance of the cuckoo egg by the host? The answer seems to be yes. When Davies and Brooke place their model eggs in the morning, a significantly larger fraction was rejected (*Figure 14.11*). It turns out that we do not quite know why afternoon laying is better for acceptance. Are

the hosts otherwise busy in the afternoons, do they only inspect the contents of their nests in the mornings, immediately after they lay their egg?

If any reader was beginning to get the feeling that Davies and Brooke have discovered everything that can be discovered, this is just one of the numerous examples of unanswered questions. There is much more for all of us to do, there will always be. It is usually the case that answering one question opens up at least one (usually more) unanswered question/s. It would not be so unreasonable, I think, to judge the success of a scientific investigation by how many additional questions it has opened up, instead of how many previously known questions it has answered.

Question 5. Why do cuckoos remove a host egg before laying their own?

Cuckoos always remove one (and rarely two) of the host eggs before laying their own single egg. Why should they do so? Perhaps the host remembers how many eggs she has laid and will be suspicious of an additional egg. If this conjecture is correct, then model eggs introduced without removing a host egg should be rejected more often than when they were introduced after removing a host egg. But that is not what happened. Davies and Brooke found no significant difference between the rates of rejection of model eggs when they removed one or more host eggs, and when they did not (*Figure 14.12*). Then why do the cuckoos bother? Spending more time at the host nest to remove an egg might increase the cuckoo's risk of being detected and jeopardise its effort. Another possibility is that cuckoos may remove a host egg before laying one of their own because, if the host accepts the cuckoo egg and also lays her full clutch then there would be one egg too many in the nest and the incubation efforts of the little reed warbler may be inadequate to hatch all the eggs efficiently.

Perhaps it is no longer necessary, but I will pause here to remind my readers that when we make statements of the kind I have just made, we are of course not pretending that the cuckoo makes any conscious decision about what is good for her eggs. We are merely using a convenient shorthand to replace the following more precise but extremely cumbersome language. If there are two different genotypes of cuckoos, one producing a phenotype that removes a host egg before it lays its own and another that lays its eggs without removing a host egg, and if the eggs laid without removing a host egg have a lower probability of hatching, then the genotype which produces an egg-removing phenotype will have higher reproductive success and genes for removing a host egg may come to be disproportionately

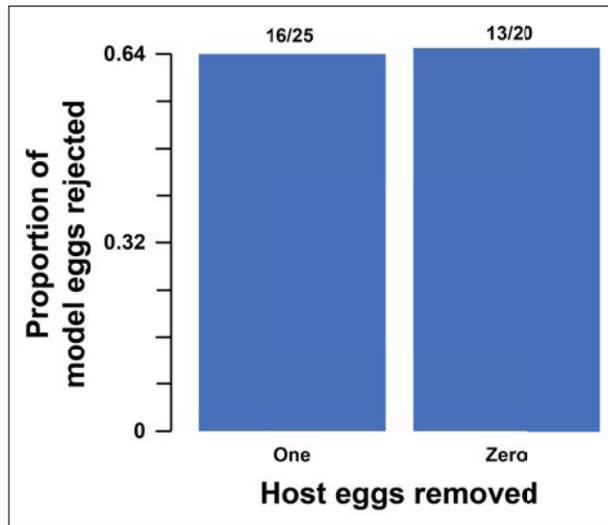


Figure 14.12 : No difference in the rates of rejection of model eggs whether host eggs were removed or not removed before introducing the model egg ($\chi^2 = 0.059, df = 4, P > 0.05$). [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

over-represented in future generations, leading eventually to the situation where all or most cuckoos remove a host egg before laying their own.

Stated clumsily or not, the hypothesis is the very same one, and a very eminently testable one. Assuming that the detrimental effects of an extra egg for the host's incubation efforts will be evenly distributed across all eggs, host and cuckoo, Davies and Brooke performed three kinds of experiments to test this hypothesis. In one experiment, they removed a host egg before introducing the model cuckoo egg. In the second experiment, they again removed a host egg before introducing the model egg, but in this case, they replaced the removed egg back into the nest after the host had completed laying her clutch of eggs. In the third experiment, they introduced the cuckoo model egg without removing the host egg at all. If the above-mentioned hypothesis is valid, then the number of eggs that should hatch successfully at the end of the incubation period should be higher in experiment one where they removed a host egg as compared to experiment three where they did not remove the host egg. The second experiment, where they removed a host egg but replaced it later, serves as a control to see if the

disturbance caused by the act of removing the egg itself affects incubation success rather than the number of eggs. It is very important, but not always easy, to imagine alternate explanations and introduce appropriate controls. This aspect of designing experiments, especially in animal behaviour, is, I would say, as much an art as science, depending much more on imagination than on knowledge. I am, therefore, always surprised at how little importance we give to encouraging imagination in our education. Worse, we actively curb the ability of students to imagine, imploring them to stick to facts. Even worse, this is what I have sometimes been told even by the reviewers of my papers.

Luckily, Davies and Brooke had apparently escaped such suppression of their imagination and performed all three experiments and were rewarded handsomely. The results of the three experiments combined did not support the hypothesis that removing an egg facilitates better incubation (*Figure 14.13*). If we look closely at the data in *Figure 14.13*, we get the impression that there are somewhat more nests with two or three unhatched eggs in the third experiment where no host eggs were removed. This seems to support the hypothesis being tested. However, such apparent trends can be misleading, and we should go only by the results of rigorous statistical tests. The statistical tests specifically check whether the observed trends could have been obtained by chance alone. Nevertheless, this is a tricky business, especially when we have a fondness for a particular hypothesis or expect it to be right or wrong based on previous knowledge. It is only human to have such expectations and biases, and it is not possible to sanitise ourselves completely from them because then we will hardly have the imagination to design good experiments. My solution to this conundrum is to neither accept them nor to ignore them totally but to pay close attention to non-statistically significant trends and use them to generate further hypotheses and design new experiments. So, in this case, at least for the time being, we have to look for another possible explanation for the cuckoos' behaviour of taking the trouble of removing a host egg before laying her own.

Let us look at the behaviour of the cuckoo more carefully. What does she do with the egg she removes? Well, she eats it. It could of course be that she removes it for a different reason and, having removed it, why not eat it? Or, could she be removing it for the very purpose of eating it and getting much-needed nutrition and getting a head-start on producing her next egg to parasitize the next nest? It is not so easy to test this hypothesis directly, at any rate, not by the technique of introducing model eggs into host nests. It would require forcing some cuckoos not to eat the egg they have removed and following the future egg production by

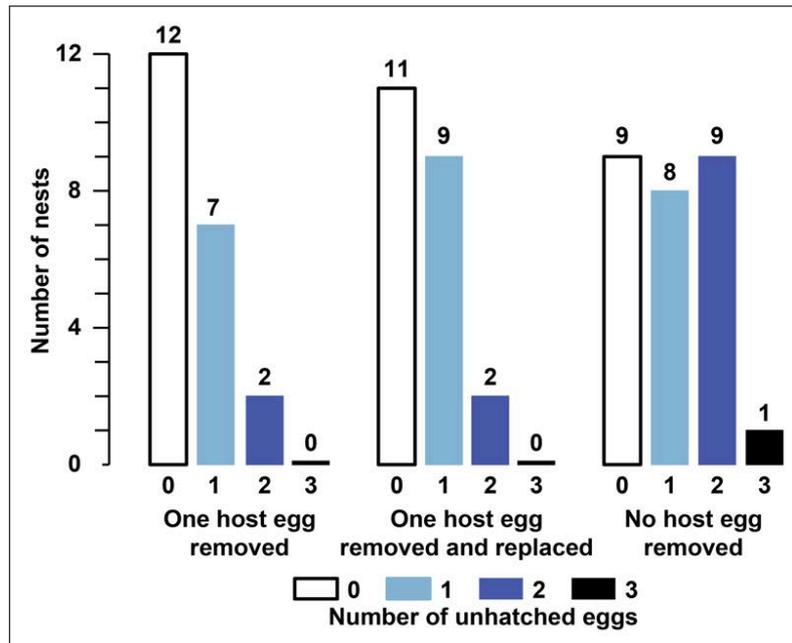


Figure 14.13: The numbers of nests with different numbers of unhatched eggs are not significantly different whether a host egg was removed, removed and replaced or not removed at all ($\chi^2 = 8.216, df = 4, P > 0.05$). [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

cuckoos who had the chance to eat the egg they have removed and those that were prevented from doing so. But even in the present situation, one can go at least a step further. If the purpose of removing the egg is to eat it, why do cuckoos remove just one, or at most two eggs, and not help themselves to even more? We have already seen that the hosts don't seem to count the number of eggs because they don't seem to care whether or not one of their eggs is removed before a cuckoo lays her egg. One conclusion from this line of reasoning is that eating may not be the primary purpose of removing a host egg but another possible conclusion is that while removing and eating one egg is okay, being too greedy and eating more eggs may somehow reduce the chances of success for their own eggs. This, last mentioned idea suggests a new kind of experiment within the paradigm of parasitizing reed warbler nests with model cuckoo eggs. As you might expect,

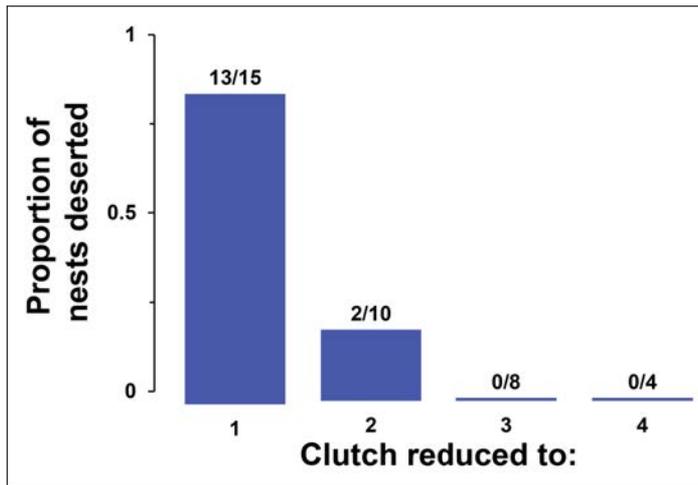


Figure 14.14: As more eggs are removed, the greater is the chance that reed warblers will desert their nests altogether. [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

this is what Davies and Brooke did next. They introduced model cuckoo eggs into reed warbler nests after removing different numbers of host eggs, reducing the remaining clutch size to one, two, three or four eggs. They found that the smaller the remaining clutch size, the greater was the chance that the hosts would abandon their nest altogether (*Figure 14.14*). Although not conclusive, especially in the absence of a rigorous statistical test, these data suggest a reason why cuckoos do not remove too many eggs. The implication is that the hypothesis that they remove the eggs in order to eat them may still be valid because the objection as to why they don't remove and eat more may not be valid. In summary, we do not quite know why the cuckoos remove an egg before laying their own, but it could well be to gain some nutrition without jeopardising the chances of success for the egg she has herself laid.

Question 6. Why do cuckoos lay their eggs so quickly and stealthily?

Another striking feature of the cuckoo's behaviour is that she lays her eggs ever so quickly and stealthily; cuckoos remove a host egg and lay their own, in an

incredible, 10 seconds or less, as compared to about 20 minutes that other birds typically take to lay their eggs and that too without the additional task of having to remove any eggs! No wonder there is a long and interesting history about the mystery of how the cuckoo's egg ever got into the host nest. You can read an enchanting account of how Edgar Chance, Director of a glass manufacturing company and passionate egg collector finally discovered how the cuckoo lays its eggs, in the book *Cuckoo: Cheating by Nature* by Nick Davies [9], and if you are lucky to find them, in Edgar Chance's own words in his two books *The Cuckoo's Secret* [12] and *The Truth about the Cuckoo* [13], and with no need to depend on luck (just on YouTube), you can watch him in the act of discovering these thousand-year-old secrets in a charming black and white, silent film he made in 1921 [14].

How the cuckoo manages this incredible feat is a most interesting question, but here we are concerned with why she even bothers. Several obvious hypotheses suggested themselves. Maybe, she is trying to avoid drawing attention to the nest in which she lays her egg to other cuckoos or other predators. Maybe, she is worried about being attacked and chased by the hosts. Or perhaps she is actually increasing the chances of the host accepting her egg by this stealth. Apparently, there is not much evidence to support the first two hypotheses, but the third hypothesis is more interesting precisely because it is not so obvious. And this is the hypothesis that Davies and Brooke put to the test with an interesting twist to their strategy of playing cuckoo. Before introducing the model cuckoo egg, they placed a stuffed adult cuckoo on the nest of reed warbler and allowed the hosts to watch this for five minutes. The question they were asking is whether the sight of the adult cuckoo on their nest will increase the chances of their rejecting the cuckoo egg. And the answer is yes. Significantly more model eggs were rejected by reed warblers when a stuffed adult cuckoo was placed on the nest compared to when it was not (*Figure 14.15*). It appears that the reed warblers check much more carefully for possible cuckoo eggs if they have seen a cuckoo on their nest and are somewhat more relaxed if they have no *prima facie* reason to worry about a cuckoo having laid an egg in their nest.

14.5 Tug of War

In summary, cuckoos employ a variety of strategies to maximize their chances of sneaking in one of their own eggs into reed warbler nests and minimize the chances that the host will detect and reject their egg. They make their eggs resem-

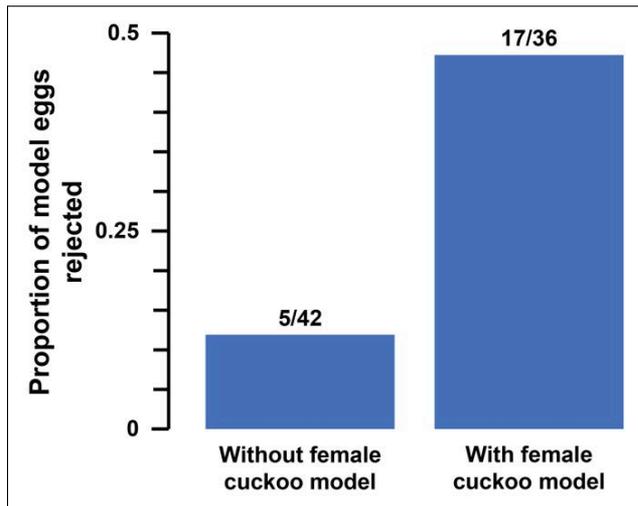


Figure 14.15: Rejection of model cuckoo eggs is significantly higher if a stuffed adult cuckoo is placed on the reed warbler nest before the model egg is introduced (G test: $G = 12.04$, $df = 1$, $P < 0.001$). [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

ble the host eggs as closely as possible in appearance and size; they wait until the hosts have started laying their own clutch, and then lay their single egg in the afternoon, after removing and eating one of the host eggs, and they do so incredibly quickly and stealthily in less than 10 seconds. The series of experiments described above demonstrate that each of these strategies of the cuckoo helps in maximising the acceptance of cuckoo eggs by the hosts. All of these strategies must have been favoured by natural selection because they maximize the cuckoo's reproductive success. But the cuckoos don't always succeed. It is easy to see why. Although they would not be expected to drive the hosts to extinction leaving no nests for them to parasitize, clearly there is room for them to be at least somewhat more successful than they seem to be. We have seen that in the study site where Davies and Brooke worked, cuckoos managed to parasitize only 44 out of 272 (16%) of the available nests. The reason for this, as we have mentioned before, is because natural selection is also acting on the reed warblers to minimize the chances of being parasitized and therefore maximize their own reproductive success. This is what leads to an arms race between the hosts and the parasites. Reed warblers

have evolved their own counter-strategies. They reject the eggs that do not sufficiently mimic their own in appearance and size; they reject eggs laid before they have started laying any of their own; they reject eggs laid at the wrong time of the day, and they are more likely to reject eggs found in their nests if they have seen a cuckoo around their nests. Moreover, many reed warblers nest away from bushes from where the cuckoos can easily spy on them. It is also true that there are many fewer cuckoos than reed warblers; one reason for the low (and now further declining) populations of cuckoos, may be because they are not more successful at parasitizing host nests, but there may be other reasons as well, including more ecological reasons such as high mortality during migration.

If the reed warblers prevent the cuckoos from doing much better at cheating, what prevents the reed warblers from doing much better at detection? It is not merely a push back from the cuckoos. There is a curious asymmetry in this arms race. The reed warblers, unlike the cuckoos, must face a push back also from themselves. If they become too finicky, they might end up rejecting their own eggs instead of the cuckoo eggs. If there is a cost to accepting cuckoo eggs, there is also the cost (danger) of rejecting their own eggs. Reed warblers must therefore balance these two costs; it might well be that accepting some cuckoo eggs may ultimately be better than losing their own eggs.

Question 7. Is there a cost that hosts pay when rejecting cuckoo eggs?

By observing how often and when reed warblers mistakenly reject their own eggs instead of the model cuckoo egg, Davies and Brooke have demonstrated that there indeed is a cost of rejection. It turns out that when the model egg is a perfect mimic, the reed warblers accept the model egg and never reject their own. But if the model egg is not a good mimic, then there is scope for confusion and the reed warblers will have to decide whether to reject the egg in their nest that looks the most alien. Interestingly, it is in this situation when the model egg is not a perfect make that reed warblers make mistakes and reject eggs of their own, significantly more often than when the model is a perfect mimic (*Figure 14.16*). If they accept, it may be a cuckoo egg, and if they reject it may be their own—it's a tough life for the reed warblers!

But reed warblers and other birds parasitized by cuckoos or other brood parasitic species seem to have managed to survive over many millions of years, occasionally rearing cuckoo chicks but usually their own. What I find even more remarkable is the fact that parasitic cuckoos have managed to maintain their lifestyle

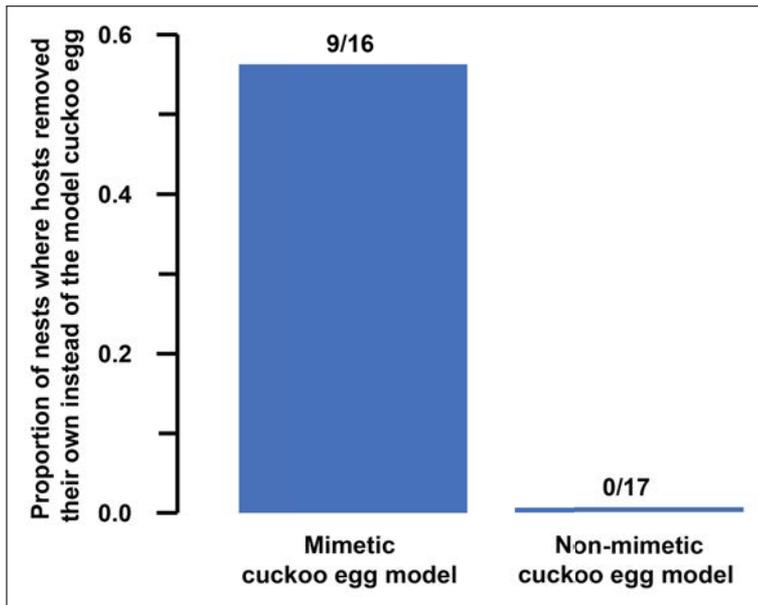


Figure 14.16: Reed warblers are significantly more likely to reject one of their own eggs instead of the model eggs when the model eggs were not perfect mimics of their own eggs ($\chi^2 = 4.369, df = 1, P < 0.05$). [Redrawn by the author based on data from N B Davies and M de L Brooke, Cuckoos versus reed warblers: Adaptations and counteradaptations, *Animal Behaviour*, Vol.36, No.1, pp.262–284, 1988, doi: 10.1016/S0003-3472(88)80269-0].

as obligate brood parasites for millions of years, never failing to fool at least some hosts of some species in every generation—as good an illustration of the balance of nature as we can think of. Little wonder then that Richard Dawkins says “If I were asked to nominate my personal epitome of Darwinian adaptation, the *ne plus ultra* of natural selection in all its merciless glory, I might hesitate. . . But I think I’d finally come down on the side of a parasite manipulating the behaviour of its host—subverting it to the benefit of the parasite in ways that arouse admiration for the subtlety, and horror at the ruthlessness, in equal measure” [15].

14.6 Reflections

As will be familiar to readers by now, my aim in writing this series of chapters is to show that almost anyone can perform cutting- edge research and create signifi-

cant new knowledge while also having a great deal of fun, even without access to any sophisticated laboratory or equipment and virtually no special funding. The set of experiments by Davies and Brooke that I have described in this 14th chapter in the book, eminently illustrate every aspect of this theme. Luckily, we can hear confirmation from the horses' own mouth. Nick Davies recalls in his *Cuckoo: Cheating by Nature*: "When I was a student, one of my tutors warned me: 'The days when you can go out into the countryside with binoculars and notebook and discover something interesting are long gone.' By this he was implying that scientific progress often depends on new techniques. For example, since the 1980s new and powerful methods of DNA profiling have been developed which allow us to determine paternity and maternity in wild populations. These have revolutionised studies of animal mating systems, revealing that socially monogamous birds are not the models of fidelity that we had once assumed. But sometimes progress is made not through new techniques but through new ideas, or simply by asking new questions. Darwin's idea that cuckoos are exploiting the 'mistaken instincts' of their hosts immediately raises new questions. How do hosts recognise their own eggs and chicks? What is it about cuckoo eggs and chicks that leads to mistaken acceptance? Patient observations with binoculars and notebook can still provide a fresh look at the natural world and lead to new discoveries, provided that the new questions are interesting. I felt sure that cuckoo–host interactions would be a fascinating corner of Darwin's 'entangled bank', and one that could be untangled by simple field experiments." [9].

We have seen plenty of evidence that Davies and Brooke had a great deal of fun performing their experiments and needed no sophisticated laboratory or other facilities, and there is no evidence of any great deal of money that was required for this research. All they probably needed were bicycles, some resin, paint and brush, some rubber boots maybe, perhaps a pair of binoculars, a cuckoo egg and a stuffed cuckoo, both of which could be borrowed, and lots of passion. But what about creating significant new knowledge? In an appraisal of this 1988 paper 25 years later, Mary Stoddard and Rebecca Kilner [16] write: "With their landmark publication... Davies & Brooke ushered in a new era of research on avian brood parasitism. Building on centuries of rich natural history and detailed observation of common cuckoos,... [they] performed a set of simple but powerful experiments to understand the adaptive value of a female cuckoo's behaviour as she parasitizes a host nest. In this essay,... we evaluate four conceptual innovations made by [them] involving rejection costs, egg mimicry, frontline defences and chick discrimination, and we show how these advances have shaped research in

the last 25 years. Davies & Brooke... paved the way for diverse and dynamic research on avian brood parasites...”. Need I say more?

If this paper were sent for publication today, I can easily imagine some hypercritical reviewers complaining about the statistics, especially about the small and non-uniform sample sizes and the lack of statistically independent data sets. For example, they might complain that the same data set of 17 out of 17 mimetic model eggs being accepted, were used to compare with many other data sets to derive many different conclusions (see *Figures* 14.5, 14.8, 14.9, 14.11 and 14.16). Is this a valid criticism? Should Davies and Brooke have done, and should we do today, five different sets of experiments with mimetic eggs to compare with each data set where the model eggs were sometimes rejected, to understand why they were rejected? This is a complex question, and we have to have the wisdom to consider the requirements of rigorous statistics in context and decide what is possible, and indeed, what is desirable. The problem is especially acute and tricky in the context of field experiments with wild animals. Whether we like to admit it or not, every experiment, every intervention, might negatively impact the animals and their environment. Of course, the benefits of the knowledge gained may compensate for the small damage we cause, and may even help mitigate damage caused by humans while not in the pursuit of knowledge. Nevertheless, it is our moral imperative to gain the maximum possible knowledge with the minimum possible damage. Here is another interesting trade-off and one which we will have to negotiate on behalf of both parties, because the animals are merely silent spectators at best or mute sufferers at worst.

And the problem is not restricted to experiments in the natural environment alone. There is a growing realization of the need to minimise the use of animals even in laboratory experiments, even for experiments that may claim to test new drugs and vaccines and make a difference between life and death for humans. There is nothing wrong with some of us seeking knowledge for its own sake, but it behoves us to show even greater responsibility. You can easily get much expert advice on the ethical use of animals in research [17], in estimating the required sample sizes for a given experiment [18] and about how to reduce the number of animals used [19]. Some years ago I met Bernhard Voelkl of the Division of Animal Welfare at the University of Bern, in Switzerland, who told me about his fascinating work showing that while “Single-laboratory studies generally failed to predict effect size accurately, and larger sample sizes rendered effect size estimates even less accurate... multi-laboratory designs including as few as 2 to 4 laboratories increased coverage probability by up to 42 percentage points without

a need for larger sample sizes.” [20]. I think there are lessons to be learned here even for those of us who do field experiments. Surely, we would like to reduce the number of animals used in research and, in addition, if we can distribute even the small disturbance we might cause among different localities, that might be even better. For example, several educational institutions such as The Indian Institute(s) of Science Education and Research (IISER’s) might coordinate their field projects for undergraduates and get their students to do the same experiment with small sample sizes each, at different localities and pool their data. They will have an excellent opportunity to produce publication quality research. The training and experience the students will get in collaborating, standardising methods, pooling data, maintaining uniform ethical and intellectual standards, co-publishing, agreeing on the order of authorship. . . are not merely collateral benefits but, I would say, the essence of education and training.

Just as Davies’ tutor told him in the 1980s that ‘The days when you can go out into the countryside with binoculars and notebook and discover something interesting are long gone.’, there will be plenty of tutors telling you the same thing today with renewed conviction. But, nothing could be further from the truth. Taking just the example of avian brood parasitism, decades of research have shown that there are so many species exhibiting brood parasitism, that species can be quite different from each other and that there is so much more to be discovered using simple natural history experiments, not to mention the inevitable discovery of more examples of conspecific and interspecific brood parasitism. Consider the claims of just a few recent papers: ‘Learning to recognize nestlings is maladaptive for cuckoo hosts’ [21], ‘Constraints on egg discrimination and cuckoo–host co-evolution’ [22], ‘How to learn to recognize conspecific brood parasitic offspring’ [23], ‘Communal breeding: Clever defense against cheats’ [24], ‘Resistance is futile: Prohibitive costs of egg ejection in an obligate avian brood parasite host’ [25], “. . . Isolated host nests are more vulnerable to cuckoo parasitism,” [26], “egg recognition ability of chestnut thrushes was likely a retained anti-parasitic strategy because of being parasitized by cuckoos in the past” [27], “Son or daughter, it does not matter: brood parasites do not adjust offspring sex based on their own or host quality” [28], “. . . brood parasitism as a driver of phenotypic diversity in birds” [29], “Cuckoos use host egg number to choose host nests for parasitism” [7]. For modellers and the theoretically minded, there are rich opportunities to explore the evolution of animal recognition systems, more generally [30]. There is much to learn and it will cost very little—this topic is tailor-made, and will remain so for a long time, for cutting edge research at trifling cost.

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15

Why Do Parents and Offspring Quarrel?

15.1 Parental Care

Since all of us have been children at some time in our lives, and many of us have also been parents at other times, we are well aware of the psychological and physiological importance of parental care, both for the offspring as well as for the parents. But at the same time, we are also painfully aware that there is often some conflict between parents and their offspring about the amount of parental investment that should flow from parents to offspring. As evolutionary biologists, however, we must pause and ask: why the conflict? According to the theory of natural selection, living organisms are expected to maximize their Darwinian fitness. Since offspring are the currency of Darwinian fitness, should not 'parent-offspring conflict' be an oxymoron? Why should there be a conflict between parents and offspring? Offspring should be selected to survive and grow, and have offspring of their own, and parents should be selected to do everything in their power to help the offspring to achieve their goals. Indeed, there is no parent-offspring conflict in some species, notably, in those species that produce a single offspring or produce all their offspring in one go, and die. There is nothing more important for parents of such species than the welfare of their offspring, leaving no scope for parent-offspring conflict.



Figure 15.1 : (Left) The French-American artist Louise Joséphine Bourgeois (1911–2010) (Portrait by Subhankar Biswas) and (Right) her famous installation *Maman* (1990) in Ottawa, Canada, a sculpture of a spider, symbolising the ultimate in motherhood. (“Maman-Spider Sculpture-National Gallery of Canada” by Arch_Sam is licensed under CC BY 2.0)

The honour of being the paragon of parenthood—motherhood really—seems to have been bestowed on the spider by both artists and scientists. The French-American artist Louise Joséphine Bourgeois (1911–2010) celebrated the motherhood of the spider by erecting a mammoth 30 feet tall metal statue of a spider and christening it *Maman* (meaning ‘mother’ in French) (Figure 15.1). *Maman* (1990) has been described as “her... deeply personal... explicit reference to painful childhood memories of an unfaithful father and a loving but complicit mother” [1]. Bourgeois said that “The Spider is an ode to my mother. She was my best friend. Like a spider, my mother was a weaver... Like spiders, my mother was very clever... spiders are helpful and protective, just like my mother” [2].

Unfortunately, Louis Bourgeois appears not to have been aware of the enormity of the spider’s motherhood. In some species of spiders, especially those that produce their lifetime’s reproductive output in one installment (technically called semelparous) the mother offers herself as food to her offspring. The survival and growth of her offspring are significantly enhanced by this source of nutrition, in the absence of which the offspring often cannibalize each other. And it’s not as if the offspring aggressively or callously eat up their mother when she is too tired to resist. It is known that in some species, spider mothers have special adaptations to facilitate such ‘matriphagy’ by their offspring. In the Australian social spider

Diaea ergandros, mothers, after laying viable eggs to produce offspring, produce non-viable, un-layable trophic eggs by sequestering nutrition into them. The offspring then obtain these nutrients by eating these trophic eggs while eating up all or part of their mothers. As expected, there is a positive correlation between maternal weight loss (due to matrophagy) and offspring survival [3]. Another social spider *Stegodyphus lineatus* exhibits what is perhaps an even more drastic adaptation for suicidal matrophagy. The mother feeds her offspring by regurgitation of nutrients, but not just the nutrients she has in the mouth and stomach. Her entire body is gradually degenerated and liquified so that her body becomes a sack of liquid nutrients which the offspring access by piercing her abdomen, leading to the consumption of her complete soma, first by regurgitation and finally by direct matrophagy [4].

This ultimate sacrifice by the spider mother is of course in the service of her own Darwinian fitness—the mother’s interests are completely aligned with those of her offspring. Fortunately or unfortunately (depending on your point of view) these examples are more the exception than the rule. The rule instead is that parent-offspring conflict is widespread. This is because most parents are not semelparous; they produce offspring repeatedly and are selected to maximise their lifetime reproductive success; not to sacrifice their lives for their current offspring. Parents should therefore be selected to invest some amount in their current offspring and withhold the rest for the future, both to keep themselves in good shape to be able to produce more offspring and to nourish and care for them. But how much should they give and how much should they withhold? And what should the offspring do? Should they not be selected to refrain from demanding so much as to jeopardize the fitness of their future siblings? Can we really hope to answer these questions, in precise, quantitative terms?

15.2 Parent-Offspring Conflict

In 1974, Robert Trivers (*Figure 15.2*) showed that there is a surprisingly simple way to answer these questions and answer them precisely [5]. Trivers has been described as “one of the most important evolutionary theorists since Charles Darwin”. As Sherlock Holmes often said, solutions to seemingly complex problems appear deceptively simple when once explained. Trivers’ answer fits this description well. It is reasonable to think that the benefit of parental investment (to parent and to offspring) will first increase with the amount of investment but will eventually taper off as the offspring gets all it needs (*Figure 15.3*, blue line). But of



Figure 15.2: A recent photograph of Robert Trivers (1943–). See [21] for a one-stop source for everything you ever wanted to know about Robert Trivers. (Photo courtesy: Robert Trivers).

course, there would be a cost associated with the investment. First, let us consider the cost to the parent (*Figure 15.3*, red line). The cost to the parent will be on account of the fact that any investment in the present offspring will reduce the potential to invest in future offspring. At some intermediate level of investment, the net benefit (benefit minus cost) will be the greatest, and this is the optimum level of investment that the parent should be selected to provide.

Now, what about the offspring? Hamilton's inclusive fitness theory (see [6]) tells us that the offspring should also care about the welfare of their future siblings as they would be genetically related to them. So the offspring should also experience a cost of the parental investment in terms of a possible reduction in their inclusive fitness. However, and here's the crucial difference, while the parents are equally related to all their offspring, the offspring are more related to themselves than to their siblings. Considering a diploid outbred population, parents would have an expected coefficient of genetic relatedness to their offspring of 0.5, the offspring would also have a coefficient of relatedness to their siblings of 0.5, but they would be related to themselves by 1.0. This means the cost of parental investment that the offspring incur will be exactly half of what the parents incur (*Figure 15.3*, grey line). The offspring will also have an optimal level of investment that they would be selected to seek, but their optimum would be higher than the optimum that the parents will be selected to give. Hence the conflict.

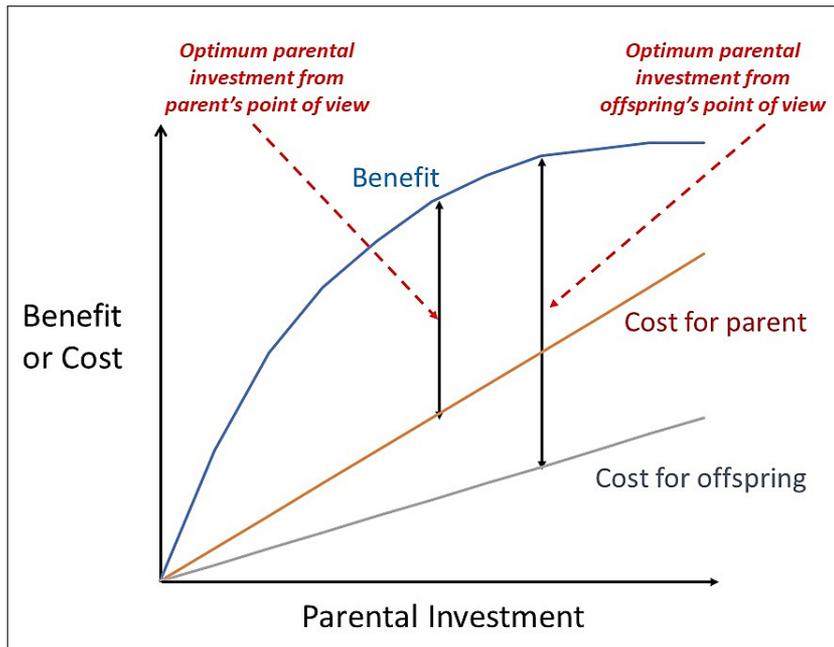


Figure 15.3: A graphical representation of the benefit and costs of parental investment and illustration of the different optima (benefit minus cost) for parents and offspring (see text for details). [Redrawn with permission from R L Trivers, Parent-offspring conflict, *American Zoologist*, Vol.14, pp.249–264, 1974].

Although the conflict itself has of course been known forever, the parent-offspring conflict theory (hereafter, POC theory) proposed by Trivers ushered in a new way of understanding parental investment and explaining the conflict between parents and offspring. Stated in this form, however, the theory is hard to test quantitatively. If we can empirically measure and plot the curves describing the relationships between the benefit and the costs to the parent and offspring, we could, in principle, determine the optimal parental investments from the perspectives of both parties. But this is not usually possible. A more serious problem is that even if we empirically generate the graphs in *Figure 15.3*, how do we test whether parents and offspring obey the theory? How do we test whether the two parties exhibit behaviours suggesting conflict only when the investment being made is sub-optimal from their point of view? It is also not so easy to alter the levels of investment as precisely as may be necessary.

Trivers plotted the same graphs in a different way that is much more promising for the empiricist. If we plot the ratio of benefit to cost (let us say from the parent's point of view) as a function of time, we may expect to see that the benefit/cost, B/C will decline with time (*Figure 15.4*). As the offspring grows and begins to become independent of the parent, the benefit of further investment will diminish. By the same logic that was used to identify the optimum investment from the parent's point of view, we can now predict that the parent will no longer be selected to invest when B/C becomes less than 1.0 (benefit is less than the cost). Now, since the offspring cost is half that of the parent, it should be selected to keep demanding investment until its B/C becomes less than 1.0, which is the same as the parent's B/C becoming less than 0.5. The region (shown in red lines in *Figure 15.4*) between the parent's B/C being 1.0 and 0.5 is thus the zone of conflict. But both parents and offspring should agree on continuing further investment in the region before B/C becomes 1.0, and should agree on discontinuing investment after B/C becomes lower than 0.5. Since the X -axis in *Figure 15.4* is time, the three zones are identified in real-time. When the offspring are very young, both parties should concur on investment, and when the offspring are beyond a certain age, both parties should concur on discontinuing investment. But there should be a time period in the growth of the offspring when parents and offspring should disagree and display conflict. If the actual slopes of the cost and benefit curves can be determined empirically, specific predictions can be made about the timing (the beginning and the end) of parent-offspring conflict. Even if this is not possible, and it usually is not possible, at least one can say that there should first be agreement, then disagreement and agreement again, in that sequence. There is plenty of qualitative evidence for such a pattern in the time course of parent-offspring conflict. The best known example, and one that Trivers used to great advantage in making his argument, is weaning conflict in a variety of mammals, including humans. Mothers usually wish to wean their offspring away from suckling some time before the offspring themselves wish to stop suckling. Moreover, as we will see below, there are other tricks one can use to try to test the predictions of the POC theory.

15.3 Testing the Predictions of POC Theory

In recent years, Anindita Bhadra (*Figure 15.5*) and her students at the Dog Lab in the Indian Institute of Science Education and Research (IISER-K) Kolkata, have subjected the predictions of the POC theory to a series of tests using free-ranging



Figure 15.5: Anindita Bhadra, the leader of the Dog Lab at IISER-K [<https://sites.google.com/view/doglabiiserkolkata/home>].

15.4 Weaning Conflict

For one study of suckling behaviour and possible weaning conflict, Anindita Bhadra and her student Manabi Paul studied 15 dog groups during the period when the pups were 3 to 17 weeks of age [7]. Pups cannot be observed easily when they are less than three weeks old because they remain in the den and are closely guarded at this time; many of us are familiar from our childhood experiences that mother dogs can be very aggressive towards us when they have young pups, so we have learnt not to mess with them at this time. The groups contained various numbers of adults and pups of both sexes. With the enviable luxury that mammal researchers often have, they individually identified all the adults and pups based on their coat colour patterns and patches and gave them names. Some groups had more than one lactating female, and in all, they could observe 22 mothers and 78 pups over a period of 15 weeks. Let us pay close attention to how they collected their quantitative data.

There were often many dogs in a group, so who do you observe and when? You cannot and should not try to observe all of them simultaneously. The fewer animals and fewer behaviours you need to record, the more accurate your data will be. When you try to observe too much at a time, your mind will subconsciously bias your attention to what appears more conspicuous or more unusual. Since their

main goal was to test the predictions of POC theory, they naturally focussed on the 22 mother-litter sub-groups and one lactating female who did not have surviving pups of her own, as their observational targets. This is sometimes called ‘focal group sampling’. They observed each group during two periods—three hours in the morning (0900–1200 hrs) and three hours in the afternoon/evening (1400–1700 hrs), once a week for a period of 15 weeks. Again, you cannot and should not observe for three hours continuously. So they followed a strict and pre-planned regimen of observation. They used two methods to sample behaviour. One is called instantaneous scans or scan sampling. This involves noting the behaviour of each individual at a chosen instant of time. Another involves recording all occurrences of chosen behaviours in a chosen, small interval of time, abbreviated as AOS. For practical convenience, they chose one minute as an ‘instant’ for each scan and five minutes as the period for each AOS. They took the precaution of randomly intermingling the scans and AOS sessions so that the chosen instants and periods of time were neither periodic nor predictable.

In all, they were thus able to perform 540 (18 scans/AOS per session \times 2 sessions per week \times 15 weeks) scans and an equal number of AOS for each of the 23 lactating females and the pups they were suckling, giving them a very large observational data set. During these observations they recorded many behaviours, but of interest to us here are the durations of suckling bouts and the identity (mother or pup) of the initiator and terminator of each suckling bout. During the suckling bouts, the pups drink milk produced by the lactating female. From the point of view of the pups, this is called suckling, and from the point of view of the lactating female, this may be referred to as nursing, but of course, it’s the same thing. For convenience and consistency, I will refer to it as suckling irrespective of whose point of view we are referring to and irrespective of who initiated the behaviour. Stray dogs are ideal for such observation and the gathering of large data sets, because they are commonly found, easily observed and not too shy about being observed (*Figure 15.6*). Notice that Suggested Reading [7] and some of the other papers suggested here are freely available online publications where you can read the original paper, access the raw data as well as watch video clippings of the dogs and pups in action.

During the period of observation, all 23 lactating females suckled one or more pups, resulting in 1378 bouts of mothers suckling their own pups and 594 bouts of females suckling pups that were not their own; the latter is called allonursing, or more generally, allomothering. As expected, suckling decreased as the pups grew older. The researchers inferred this in two different ways. First, when the



Figure 15.6: Suckling behaviour. Images of stray dogs suckling their and other's pups, and sometimes refusing to be suckled, as in the bottom right panel (Photos: Manabi Paul).

pups were 3 weeks old, they found that their mothers spent about $18 \pm 9\%$ of their total time suckling their pups and this time decreased significantly with the age of the pups, reaching a value of zero by the 13th week. Second, they measured the duration of each suckling bout and plotted them as a function of pup age (Figure 15.7). These days, all of us use computer software to plot these kinds of graphs, and we seldom pay adequate attention to exactly what is being plotted and what it all means. We are quickly satisfied with the final verbal conclusion. This is a great pity for we fail to appreciate the richness in the data and its full import. More tragically, we fall easy prey to incorrect verbal conclusions and perpetuate them. This is mainly because in our system of education we teach students to consume knowledge while accepting it uncritically, rather than to be sceptical and examine everything they read with a critical eye; this, unfortunately, makes the idea of becoming producers of knowledge too remote to contemplate.

95.45% of the data lie between mean ± 2 s.d, 99.73% of the data points lie between the mean ± 3 s.d. and so on. This knowledge allows computing the probability with which any data point belongs to the distribution knowing only its mean and s.d, and this is the basis of the so-called parametric statistical tests.

When data is skewed, as our behavioural data usually are, these luxuries don't exist. The mean is no longer a useful measure of central tendency and the s.d. is no longer a valid measure of dispersion. Instead, we calculate a different measure of central tendency, namely the median. The median is by definition the midpoint of the distribution with half the data points above it and half below. To get a handle at the dispersion, we use the quartiles, which divide the numbers of data points into four quartiles. One fourth of the data points lie below the 1st quartile, one fourth between the 1st quartile and the median, one fourth between the median and the 3rd quartile and the final one fourth above the 3rd quartile. All this information is visually depicted using the rather clever 'box and whiskers' plots. The box is the rectangle showing the location in the data range of the 1st and 3rd quartiles, with the median indicated inside the box (not necessarily at its centre, of course). The whiskers are meant to indicate the region between the first quartile and the minimum value on one side and the region between the third quartile and the maximum value on the other side. But this is not quite how we usually draw the whiskers.

When our data sets are small, we can expect a great deal of random variation so that we may have some really extreme values, further distorting the distribution. In such a case, we don't stretch the whiskers until the observed minimum and maximum values. Instead, we stretch the whiskers only up to the lowest or highest value that lies within 1.5 times the interquartile range on either side. The data points that lie outside these whiskers are shown separately as outliers, so as not to forget about them altogether. We thus see that the box and whiskers plot reveals a great deal about the distribution of the data, without having to plot all the raw data points.

Paul and Bhadra subjected these data on the duration of suckling bouts to a generalised linear mixed model (GLMM) with suckling duration as the response variable and the age of the pups as the predictor variable. GLMMs have rapidly become the favourite method for answering these kinds of questions because they are better at handling data that may not be normally distributed. They are also conveniently designed to simultaneously consider both fixed effects (variables we are interested in or those we deliberately vary) and random effects (variables that we cannot control) in a regression model. Paul and Bhadra showed that suckling

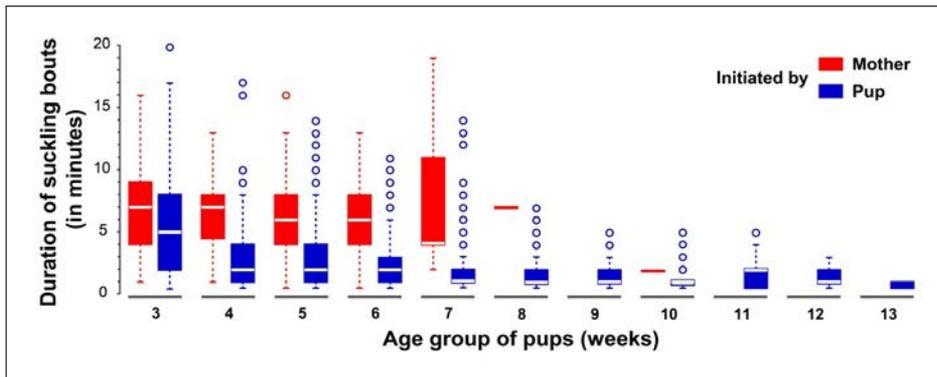


Figure 15.8 : The duration of suckling bouts initiated by the mother declined more sharply with the age of the pups than did the duration of suckling bouts initiated by the pups themselves, suggesting a conflict between the mother and offspring about when the suckling should stop, as the pups age. [Redrawn from M Paul and A Bhadra, *Selfish Pups: Weaning Conflict and Milk Theft in Free-Ranging Dogs, PLoS ONE*, Vol.12, p.e0170590, 2017].

duration decreased significantly with the age of the pups. Thus the total time spent by the mothers in suckling, as well as the duration of the suckling bouts, decreased significantly with the age of the pups. This is of course expected and by itself does not provide evidence for the predictions of POC theory. Nevertheless, it is good to know and forms the necessary basis of further analysis of the data in search of POC and any efforts to test the predictions of the POC theory. Towards these goals, they analysed their data in two additional ways. First, they broke up the data on suckling duration (for a subset of the lactating female-pup pairs) into those suckling bouts that were initiated by the mother and those that were initiated by the pups. If the mother offered to suckle by presenting herself either in the standing or lying down position, they considered the suckling bout as mother-initiated. If suckling was a result of solicitations by the pups, they considered that bout as pup-initiated.

Another generalised linear mixed model (GLMM) showed that the identity of the initiator of the suckling bout, the age of the pups and the litter size, all significantly influenced the suckling bout duration. These results are quite revealing. The duration of suckling bouts initiated by the mother decreased more rapidly than those initiated by the pups themselves (*Figure 15.8*). This suggests a divergence between the interests of the mother and the pups—parent-offspring

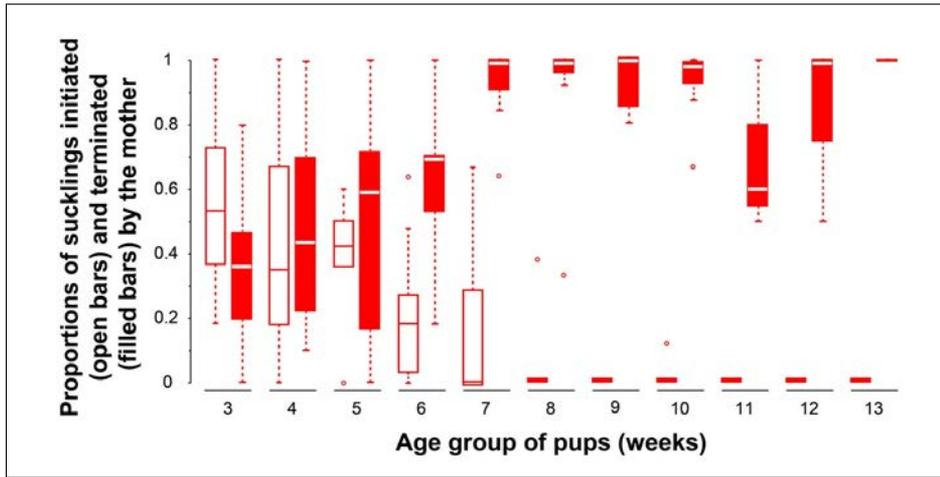


Figure 15.9: The proportions of suckling bouts initiated by the mother (as opposed to initiated by the pups) declined with the age of the pups while the proportions of suckling bouts terminated by the mothers increased with the age of the pups, providing an even more clear indication of conflict between the mother and her pups over how long the suckling bouts should last. [Redrawn from M Paul and A Bhadra, *Selfish Pups: Weaning Conflict and Milk Theft in Free-Ranging Dogs*, *PLoS ONE*, Vol.12, p.e0170590, 2017].

conflict. Even more revealing is the comparison between the proportion of suckling bouts initiated and terminated by the mother as opposed to the pups, and how these proportions change with the age of the pups. Termination of a suckling bout by the pup simply means that it stops suckling while termination by the mother happens when she walks away and thus prevents the pups from suckling her any more. The data clearly show that the proportions of suckling bouts initiated by the mother decline sharply with the age of the pups, while the proportions of suckling bouts terminated by the mother sharply increase with the age of the pups (*Figure 15.9*). This is an even clearer indication of parent-offspring conflict, or what is traditionally called weaning conflict. It is a clearer indication of conflict because the mothers not only stop initiating suckling bouts but terminate suckling bouts initiated by the pups. If we only knew that mothers stopped initiating suckling, it might simply mean that they know that the pups have learned to initiate suckling whenever they want, so why bother to initiate. From the 8th week to the 13th week, mothers only terminate suckling bouts and do not initiate any; conversely, pups only initiate suckling bouts but do not terminate any—this is the period of conflict.

All this provides clear evidence for POC, for a weaning conflict between the mother and her offspring. Indeed, the period between the 7th week to the 13th week can be identified as the period of conflict. Before the 7th week, the mother initiates many of the suckling bouts, terminates very few, and the mother-initiated suckling bouts are quite long, suggesting little conflict. After the 13th week and up to the 17th week at least (which is how far their observations went) neither the mothers nor the pups initiated suckling bouts. In the language that we used above to describe the predictions of the POC theory, both the mother and the pups favoured parental investment up to the 6th week, during the 7th to the 13th week the mother was reluctant to invest in her current offspring while the offspring favoured more investment in them; after the 13th week, neither the mother nor the pups favoured continuation of parental investment, at least in the form of suckling.

There are very few data of this kind from studies on mammalian species in nature. For this reason, Trivers's parent-offspring conflict theory, elegant though it is, has been thought of being contentious and not amenable to empirical testing in nature. Choosing an appropriate animal model is very crucial for success in testing predictions of evolutionary theories, especially in nature. This does not mean that the theories apply only to a few species; rather it implies that only a very few species permit the rigorous experimental and observational intervention necessary to test the predictions. The study by Manabi Paul and Anindita Bhadra described above shows that stray dogs are indeed one such model system that can be deployed in service of field testing of POC theory. But the best is yet to come.

15.5 Post-Weaning Conflict

Weaning conflict that we saw above is only the first line of evidence that Anindita Bhadra and her students have brought to bear on the POC theory. To provide a second line of evidence, they have cleverly capitalized on the fact that stray dogs live in large communal groups and depend a great deal on food provided by humans. This creates opportunities for the dogs, including mothers and their offspring, to continue to cooperate or compete as appropriate. Here is, therefore, another context to look for evidence for the predictions of the parent-offspring conflict theory, during the post-weaning period [8]. To this end, Manabi Paul and Anindita Bhadra designed extremely simple experiments, not so different in principle, from what we have all done playfully as children and often as adults—they fed dogs bread and biscuits and made careful observation of the drama that ensued. Stray dogs are quite used to receiving items of food such as bread and biscuits from people, or

scavenging these by themselves, making the experiment quite a natural situation for the dogs. They offered a chosen group of dogs one piece of bread or biscuit as per the pre-determined preference of the group and waited until that item was completely consumed before offering the next piece and continued to do so until they offered as many pieces of food as there were individual dogs or pups in the group. The experiment was video recorded for later extraction and analysis of quantitative data.

They conducted these experiments on 15 groups of mothers and their pups, when the pups were between 8 and 11 weeks of age, over a two-year period on the campus of their institute. They offered bread or biscuits to each group between 1000 and 1230 hrs and again during 1530 to 1700 hrs for three consecutive days in a week for four to six weeks and thereby obtained data during 430 such feeding sessions. It is important not to interpret working with easily accessible animals and performing experiments using commonplace techniques, as a casual approach to research. Anindita Bhadra and her students played like children but with the rigour and precision befitting serious scientists. First, they offered their dogs bread and biscuits to see if they had any marked preference for one or the other; if they did then they were given their choice during the experiment—you can't measure conflict over food if the food is a non-preferred item. Their preliminary observation revealed that there was not much variation between sessions in the response of the pups to the offered food, but the mothers showed considerable variation. Hence they focussed on the mothers' behaviours to try to detect signs of parent-offspring conflict.

Behaviour is in reality a continuous stream of actions and reactions, including, of course, the absence of action and reaction. If we are to do quantitative ethology, we must convert behaviour into numbers and an important early step is to delineate well-defined behaviours with clear starting and ending points so that we can count how often a particular behaviour is repeated in different contexts. This process is called discretisation of behaviour; we can use as fine-grained or as coarse-grained a sieve to separate one behaviour from another. Here they identified seven distinct behaviours of the mothers to being offered food in the presence of their pups. These are (1) showing disinterest (and doing nothing), (2) allowing the pup to take the food, (3) actively taking and offering the food to the pups, (4) taking the food and sharing it with the pups, (5) competing for the food and accepting the principle of first-come-first-served, without any aggression, (6) competing with aggression and grabbing the food, and (7) aggressively snatching the food from the pups even when they got hold of it first. Every time they offered

an item of food, they recorded the responses of the mothers as belonging to one of these categories. Next, they computed the proportion of items in the consumption of which the mothers showed cooperation or conflict. They lumped all the instances of disinterest, allow, offer and share into the composite category of cooperation. Conversely, they lumped all instances of compete, compete aggressively and snatch into the composite category of conflict. From the videos, they also computed, for each item of food, the time taken to approach the food item offered, the time taken to consume it and the amount eaten by the mother and the pups.

They found that the proportion of times that the mothers showed conflict rather than cooperation depended significantly on the age of the pups. Mothers showed conflict only in about 30% of the cases if the pups were eight weeks old and in about 80% if the pups were 15 weeks old (*Figure 15.10*, upper panel). Considering all food items in the consumption of which there was conflict, they found that such contested food was equally divided between the mother and her pups. This fact, they convincingly argue, suggests genuine conflict. But does this conflict help the mother in any way? Only then would we be justified in concluding that the parent-current-offspring conflict is in aid of future offspring.

To address this question, they monitored the body condition of the mothers throughout the course of the experiment. From the videos they gave scores for body size (small, medium, large), condition of fur (poor, medium, good), nutritional status (poor, medium, good) and disease (present, absent), for each mother at the beginning of each week of the experiment. Converting these scores into simple numerical values such as 1 for poor nutritional status, 2 for medium nutritional status and 3 for good nutritional status, for example, they constructed an index of body condition for each mother for each week. Their analysis showed that while there was no significant difference between different mothers in any given week, the body condition of the mothers improved during the course of the experiment. At least one of the factors responsible for the improvement of their body condition might be the food they managed to eat by competing with their offspring (*Figure 15.10*, lower panel).

In summary, we can see that mothers displayed increased conflict with their offspring in the context of food sharing, as the pups grew older. To appreciate the significance of these trends in increased conflict with pup age, we must reflect on the fact that the same mothers showed much less conflict with the same offspring, when the offspring were young. It is the modulation of levels of conflict with time, such that the mothers contribute more to the growth and survival of their offspring up to a point, and then begin to contribute increasingly to their own

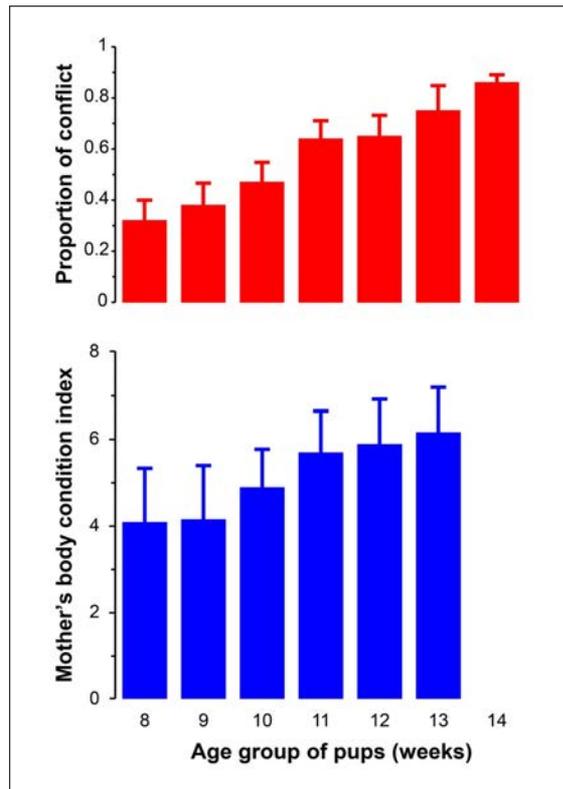


Figure 15.10: Top: The proportion of food items in the consumption of which there was conflict between the mother and her offspring increased with the age of the pups as revealed by a GLMM ($p = 0.0004$). Bottom: Mean and standard deviation of the body condition index (BCI) of the mothers ($N = 15$) at different weeks of pup age, estimated from the videos during the entire span of the experiment for each group. There was significant improvement in BCI over time for individual mothers (repeated measures ANOVA, Wilks' $\lambda = 0.023$, $F_{6,9} = 65.151$, $p < 0.0001$). [Redrawn with permission from M Paul, S S Majumder, and A Bhadra, Selfish mothers? An empirical test of parent-offspring conflict over extended parental care, *Behavioural Processes*, Vol.103, pp.17–22, 2014.]

health and survival that is a key prediction of POC theory. And that is because, from an evolutionary point of view, their own improved health and survival are entirely at the service of their future offspring. Hence, the pattern of post-weaning conflict that Paul and Bhadra have documented is a second line of evidence for the prediction of the POC theory. And there is even more to come.

15.6 Effect of Resource Quality on POC

Anindita Bhadra and her students have provided a third line of evidence in support of the POC theory that is not only entirely novel but what we might call clinching evidence. All the arguments so far, both in terms of the theory and the empirical verification of the theory's predictions have focussed on a predicted increase in conflict with offspring age and the predicted existence of a zone of conflict where there should be disagreement between parents and offspring. In their next study, however, they make a novel argument and a novel prediction. The argument is that the level of parent-offspring conflict, and indeed, the slope of its increase with age should be a function of the quality of resources available for sharing between the parents and their offspring. The argument goes as follows. How should mothers behave in resource-poor and resource-rich environments? Since mothers are selected to maximize their lifetime reproductive success, and not focus only on their current offspring, they should allocate a fixed amount of resources to their current offspring and allocate what is left for themselves, meaning for their future offspring. In resource-limited environments, such a strategy would make them suffer because there may not be much leftover after allocating a fixed amount to their current offspring. But that may be OK because they may not even produce another litter in a resource-poor environment. This strategy would at least maximise the chances of the survival of their current offspring. Nothing is gained by providing sub-optimal resources for their current offspring and saving another sub-optimal amount for the future.

In a resource-rich environment, however, such a strategy would mean that they allocate about the same resources to their current offspring as they did in the resource-poor environment, and keep the bonanza for themselves. This leads to the somewhat counter-intuitive prediction that in resource-rich environments, mothers should show more conflict with their offspring even when the offspring are young, as compared to the situation in a resource-limited environment. As a shorthand, let us call mothers who behave in this fashion in resource-rich environments as 'selfish mothers'; notice that POC theory predicts such selfish mothers. On the other hand, if mothers are merely maximising the probability of survival of their current offspring and making no allocation for future offspring, then they should allocate their resources very differently in resource-rich environments. They should allocate a bare minimum for themselves, enough to survive and care for the current offspring and allocate any excess to their current offspring even if they are in a zone of diminishing returns. If they will have no future offspring, diminishing returns to their current offspring are better than no returns to them-



Figure 15.11 : Anjan Nandi (left) with the author at Grünewald train station in Berlin in 2011
(Photo: Geetha Gadagkar).

selves. Let us call such mothers as ‘altruistic mothers’. In summary, the theory of parent-offspring conflict predicts selfish mothers, and not altruistic mothers, in the sense defined above.

Appealing as they are, verbal arguments of the kind made above can be deceptive. While making verbal arguments we often focus only on what interests us at the moment and are prone to ignore other factors that may come into play and change the outcome. A mathematical model that clearly specifies all the assumptions of our argument and objectively calculates the outcome of the processes we postulate is very helpful, and often necessary. Anindita Bhadra, therefore, did well to enlist the collaboration of a smart modeller Anjan Nandi (I know because he modelled some phenomena in our wasp work) to confirm the verbal arguments made above (*Figure 15.11*). The results of the model can be seen in (*Figure 15.12*). Recruiting an additional student, Sreejani Sen Majumder, they now performed a

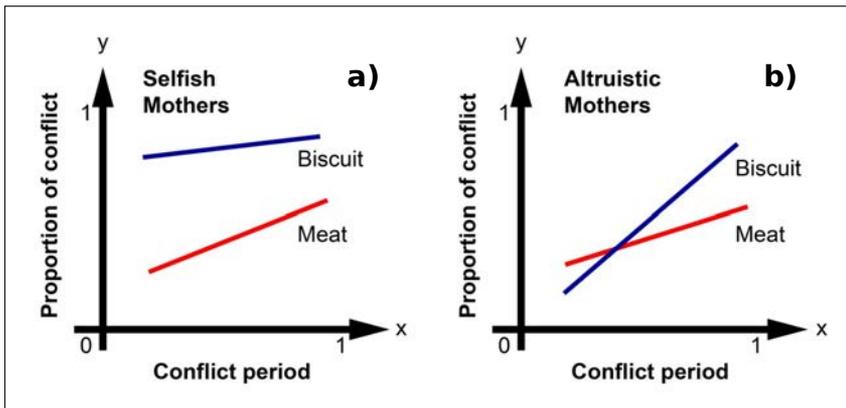


Figure 15.12: Graphical representations of the models for mothers' strategies under low (biscuit) and high (meat) resource conditions. **(a)** Selfish mothers reserve a fixed amount of food for their offspring and allocate any extra food for themselves. **(b)** The altruistic mothers reserve a fixed amount of food for themselves and allow the pups to have the rest. These selfish and altruistic maternal strategies lead to different slopes for how conflict should increase over time. [Redrawn from M Paul, S S Majumder, A K Nandi, and A Bhadra, Selfish mothers indeed! Resource-dependent conflict over extended parental care in free-ranging dogs, *R. Soc. Open sci.*, Vol.2, p.150580, 2015.]

clever experiment to test these predictions [9]. They gave some dog groups biscuits as before, and they gave other dog groups meat instead (pieces of chicken). The biscuits, containing mainly carbohydrates and starch, represent a resource-poor environment as compared to protein-rich chicken which represents a relatively resource-rich environment. Their predictions were confirmed in two ways. First, they found that, as predicted, there was a much greater conflict in sharing meat than in sharing biscuits. Mothers took significantly more meat than the pups, while the pups were allowed to take significantly more biscuits than the mothers. Also, mothers took more meat than they took biscuits while pups got more biscuits than they got meat (*Figure 15.13*).

The modelling had made more detailed predictions regarding the change of levels of conflict with the age of pups, with meat versus biscuits, as we saw in *Figure 15.12*. This detailed prediction was also clearly borne out. We can see from *Figure 15.14* that, as predicted, the conflict over meat was always high with only a small increase with age while the conflict over biscuits rose sharply with age, starting at a low level of conflict when the pups were young and reaching a relatively high level as the pups grew old. This third line of evidence for the

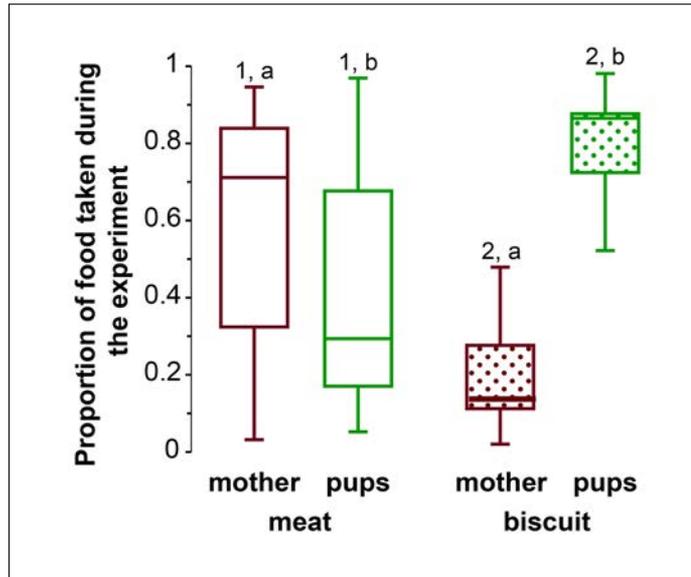


Figure 15.13 : Box and whisker plots for the patterns of food distribution between the mother and her pups when given biscuits (low quality food) or meat (high quality food). The different letters above the bars show a significant difference between mother and pup, in each kind of food. Mothers take significantly more meat than the pups while pups get significantly more biscuits than the mother (χ^2 -test, $p < 0.05$). Different numbers above the bars show the difference between biscuit and meat for each category of individual (mother and pup). Mothers take more meat than they take biscuits while pups get more biscuits than they get meat (Mann–Whitney U-test, $p < 0.05$). [Redrawn from M Paul, S S Majumder, A K Nandi, and A Bhadra, Selfish mothers indeed! Resource-dependent conflict over extended parental care in free-ranging dogs, *R. Soc. Open sci.*, Vol.2, p.150580, 2015].

theory of parent-offspring conflict is the strongest yet, both in Anindita Bhadra's work and, I suspect more generally. Besides, it is a new line of evidence which should increase our confidence in the theory. But there is even more to come!

15.7 Grandmotherly Care

Many of us have employed the strategy of running to our grandparents when in conflict with our parents. Stray dogs seem to be no different. Manabi Paul and Anindita Bhadra have documented grandmotherly care in stray dogs. In a particularly detailed case of one grandmother, they have documented the patterns of motherly care and grandmotherly care by the same individual and the receipt

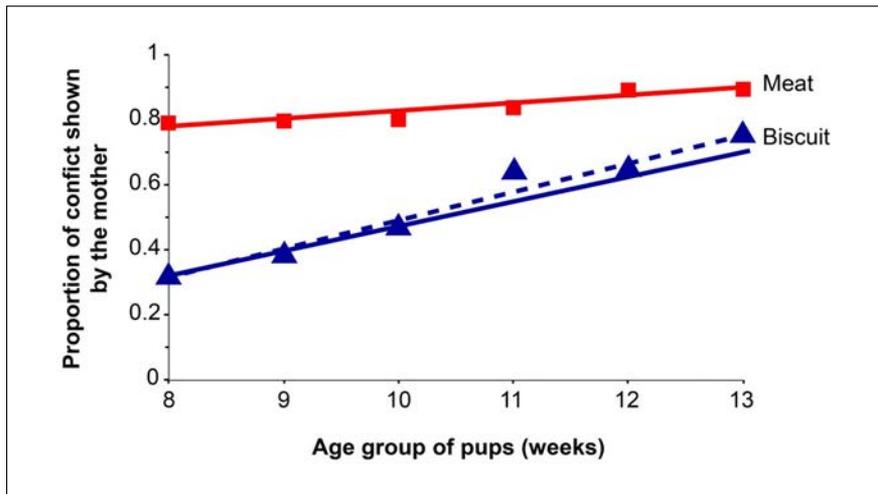


Figure 15.14: The proportion of food items in the consumption of which there was conflict between the mother and her offspring increased with the age of the pups as in *Figure 15.10*. But the slopes with which they increased were different for a biscuit diet and a meat diet, as predicted by the model for parent-offspring conflict under resource-rich and resource-poor conditions. The experiments were done with 16 mother-pup pairs. The solid lines are the model predictions and the dotted lines are the fitted regression lines for the experimental data (the dotted line is not visible for meat as it completely overlaps with the model prediction). [Redrawn from M Paul, S S Majumder, A K Nandi, and A Bhadra, Selfish mothers indeed! Resource-dependent conflict over extended parental care in free-ranging dogs, *R. Soc. Open sci.*, Vol.2, p.150580, 2015].

of motherly care and grandmotherly care by the same pups [10]. This creates a unique opportunity to make a controlled comparison of motherly care and grandmotherly care, which sheds unexpected new light on parent-offspring conflict theory. The grandmother in question was an individual they call ML who herself was litter-less when her daughter called PW gave birth to two pups. ML, now spent considerable time caring for her two grandchildren, the offspring of her daughter PW. Although the grandmother could not nurse her grandchildren because she was not lactating, she showed other behaviours that help care for pups, such as allogrooming, cleaning the den by eating the pups' faecal matter, offering solid food as well as regurgitated food, piling up with the pups for warmth and protection and playing with the pups; all these are classified as active care, as they require contact with the pups and expenditure of energy specifically for the pups.

The grandmother also showed passive care toward her grandchildren, such as tolerating the pups near her when she was looking for food, feeding, drinking and grooming herself—this offers protection and education for the pups.

It is of course most interesting that a grandmother cared for her grandchildren but do the data throw any light on the theory of parent-offspring conflict? A typical grandmother is generally older than a typical mother, and she is, therefore, expected to have a lower future reproductive success compared to a mother. Generally speaking, the constraints of not being able to invest everything in the current offspring and having to save for future offspring is less applicable to grandmothers than it would be to mothers. The increase in conflict as a function of pup age can therefore be predicted to be less pronounced in the case of a grandmother as compared to that of a mother. In support of this prediction, Paul and Bhadra found no significant change in grandmotherly care as a function of pup age (*Figure 15.15*, upper panel). Could this lack of increase in conflict as a function of pup age be on account of something peculiar about these pups that ML was caring for as their grandmother? Apparently not, because the pups' mother, PW, at the same time increased her conflict with the same pups during the same period (*Figure 15.15*, middle panel). Could the unusual pattern of pup age-independent care be on account of something unusual about ML, who perhaps did not know how to or was incapable of escalating conflict with aging pups? Apparently not, because the same ML, behaved exactly as predicted by the POC theory when she herself was caring for PW and her sibling, in the role of mother (*Figure 15.15*, lower panel). In other words, ML behaved very differently in her role as a mother as compared to her role as a grandmother. Her contradictory behaviours in the two roles are both consistent with the POC theory. We may, therefore, consider this as the fourth line of evidence in support of the POC theory.

Grandmotherly care is of course a form of altruism, but it is no longer paradoxical since the advent of inclusive fitness theory (see [6]). Of course, grandmothers in most species still have a finite chance of future reproduction, but its diminishing prospects requires them to hedge their bets between current grandchildren and future children (and future grandchildren), just as mothers have to hedge their bets between their current children and future children. Mothers are equally related to their current and future children, and the dynamics of their parent-offspring conflict will be largely influenced by the difference between the relative certainty of current offspring and the relative uncertainty of future children. Grandmothers are related to their grandchildren only by 0.25 and to their future children by 0.5, tilting the balance in favour of future children. But the difference between the rel-

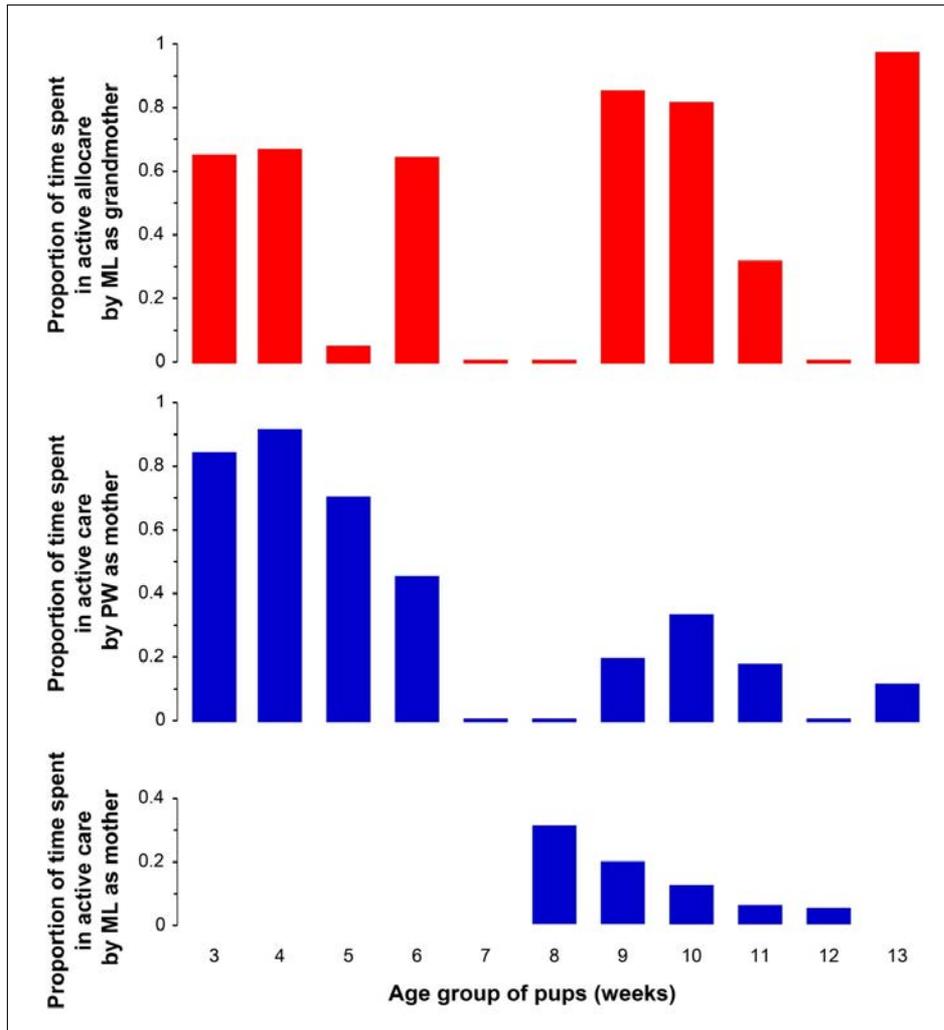


Figure 15.15: Proportions of time spent by a grandmother (ML) in caring for her grandchildren (upper panel), by a mother PW towards the same pups, who are her offspring (middle panel) and by the ML towards her offspring when she was a mother. The proportion of time spent caring for pups decreased significantly with the age of the pups for the two mothers but not for the grandmother. Both patterns are consistent with POC theory, see text for a detailed explanation. [Redrawn with permission from M Paul, S S Majumder, and A Bhadra, Grandmotherly care: a case study in Indian free-ranging dogs, *J Ethol.*, Vol.32, pp.75–82, 2014].

ative certainty of current grandchildren and the significantly higher uncertainty of future children can sometimes tilt the balance in favour of current grandchildren, more strongly than it might for mothers.

Only in two groups of mammals, namely humans and whales has natural selection valued indirect fitness from current grandchildren so much that it has led to the evolution of menopause [11]. In these two groups, females have significant life expectancies beyond their reproductive age so that fitness from future children during menopause is zero. Indirect fitness from grandchildren is the most likely evolutionary explanation for the phenomenon of menopause. Before the advent of inclusive fitness theory, menopause would have been paradoxical. But now, why menopause is so rare is a mystery. The evolution of menopause is likely to depend on the importance of communal lifestyle and alloparental care for the survival of offspring. It is being now revealed that ecological knowledge and leadership of grandmothers may be the key to the evolution of menopause, not only in humans but also in killer whales [12].

15.8 Is There Scope for a Compromise?

Parent-offspring conflict seems such a pity, not only from an anthropomorphic and emotional point of view but even from a biological and evolutionary point of view. Parents have to show aggression to their own offspring and withhold care and food from begging children for the sake of the uncertain prospects of future offspring. Yes, natural selection may be expected to have set the optimal levels of conflict factoring in the uncertainty of the future. Nevertheless, parents should be selected to do everything they possibly can to enhance the welfare of their current offspring without jeopardising the welfare of their future offspring. Even from proximate, physiological considerations, parental care being known to be modulated by hormones such as oxytocin, it should be difficult for mothers to abandon their current offspring so easily. From all points of view, therefore, one would expect any compromise between parents and offspring, any possible win-win situation to be explored and exploited. Such is indeed the case, at least in stray dogs. In a beautiful study with the title 'Clever mothers' Anindita Bhadra and her students produce some remarkable evidence of compromise [13]. They demonstrate a striking contrast between the patterns with which active parental care (which require effort and energy and are more likely to diminish the fitness of future offspring), and passive parental care (which require relatively less effort and energy and are less likely to diminish the fitness of future offspring), change

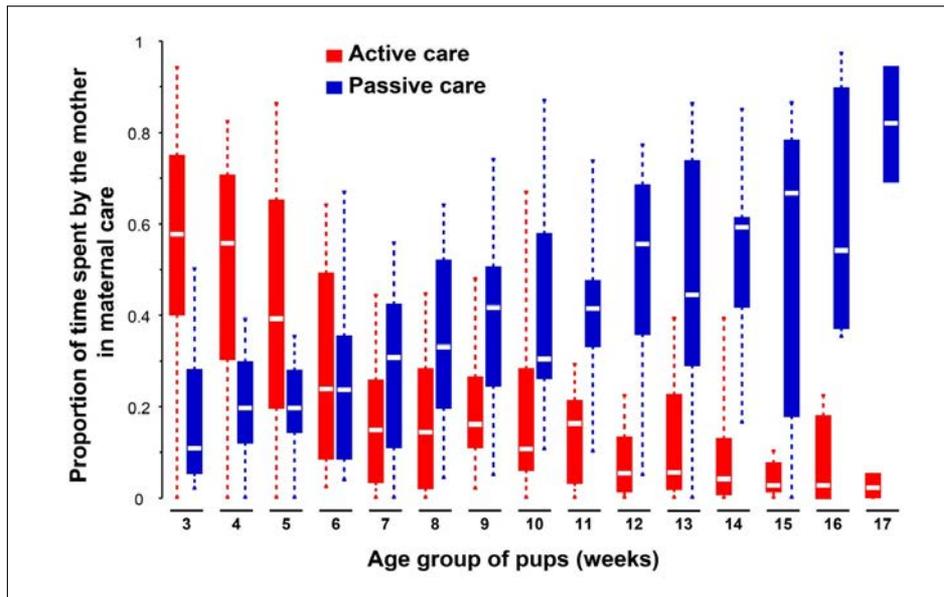


Figure 15.16: As the pups grow mothers decrease the proportion of time they spend giving them active care but increase the proportion of time they spend in giving them passive care. See text for details. [Redrawn from M Paul, S Sau, A K Nandi, and A Bhadra, Clever mothers balance time and effort in parental care: a study on free-ranging dogs, *R. Soc. Open sci.*, Vol.4, p.160583, 2017].

with the age of the pups. Their slopes are just the opposite of each other. They show that as active care declines, passive care increases with the age of the pups (Figure 15.16).

15.9 It's a Harsh World Out There

The dog *Canis lupus familiaris* may have bought a ticket to long-term survival more than 15,000 years ago by being domesticated [14, 15], or as is more likely, by self-domestication. But the species eke out a treacherous existence in the harsh world of human companionship. Only 20–25% of the world's dogs are kept as pets and often bred for grotesquely enhancing one or the other of their many features, such as aggressiveness, hunting ability, retrieving ability or simply cuddliness. The remaining 75–80% of the world's dogs hang around human habitations and, while deriving some benefit by this proximity, they also suffer a great deal from

humans. Research from Anindita Bhadra's Dog Lab has shown that they experience over 80% early life mortality, more than 60% of which is caused by humans. Dogs have evolved a number of adaptations to deal with their plight. They live and breed communally. This means that several but not necessarily all individuals reproduce, multiple females littering simultaneously in some groups. An important adaptation for survival in these difficult conditions is the fine balance between cooperation and conflict they are able to strike, both with other dogs and with humans. Allo-parental care appears to be crucial for their survival, especially for young pups. Various members of the group, including siblings, aunts, grandmothers and even fathers help out, though not without keeping their own interests also in focus. This can include all forms of active and passive care with the exception of nursing/suckling. Manabi Paul and Anindita Bhadra have documented these, at once, illuminating and moving facts in a recent paper with the charming title "The great Indian joint families of free-ranging dogs" [16]. They have documented and analysed different forms of care that pups of different ages receive from their mothers, allomothers and (putative) fathers (*Figure 15.17*). We can imagine how these additional helplines for the pups permit the mothers to manage their parent-offspring conflict and set their optimum maternal care a little bit lower and allocate a little bit more for future offspring and thus achieve a little bit more lifetime reproductive success.

15.10 Reflections

The studies described in this chapter score very high on all the features that this book espouses—using clever, simple experiments that can be performed by almost anyone without the need for access to a sophisticated laboratory, as long as they have a child-like curiosity and playfulness and a passion to unravel the mysteries of nature. But there is one feature that I wish to dwell on in some detail, and that concerns the choice of the study animal—stray dogs (*Figure 15.18*). Ethologists and behavioural ecologists tend to choose unusual, endangered or otherwise glamorous animals for their study, often at the cost of many constraints in the availability of samples for study as well as feasibility of observation and experimentation. Free-ranging dogs, especially in India, are abundantly available, easy to observe and experiment on, and also of great practical importance to society. Dogs are thus ideally suited both from the point of view of basic research in ethology and behavioural ecology as well as producing knowledge relevant to society, especially in the context of human-animal conflict (*Figure 15.19*). And

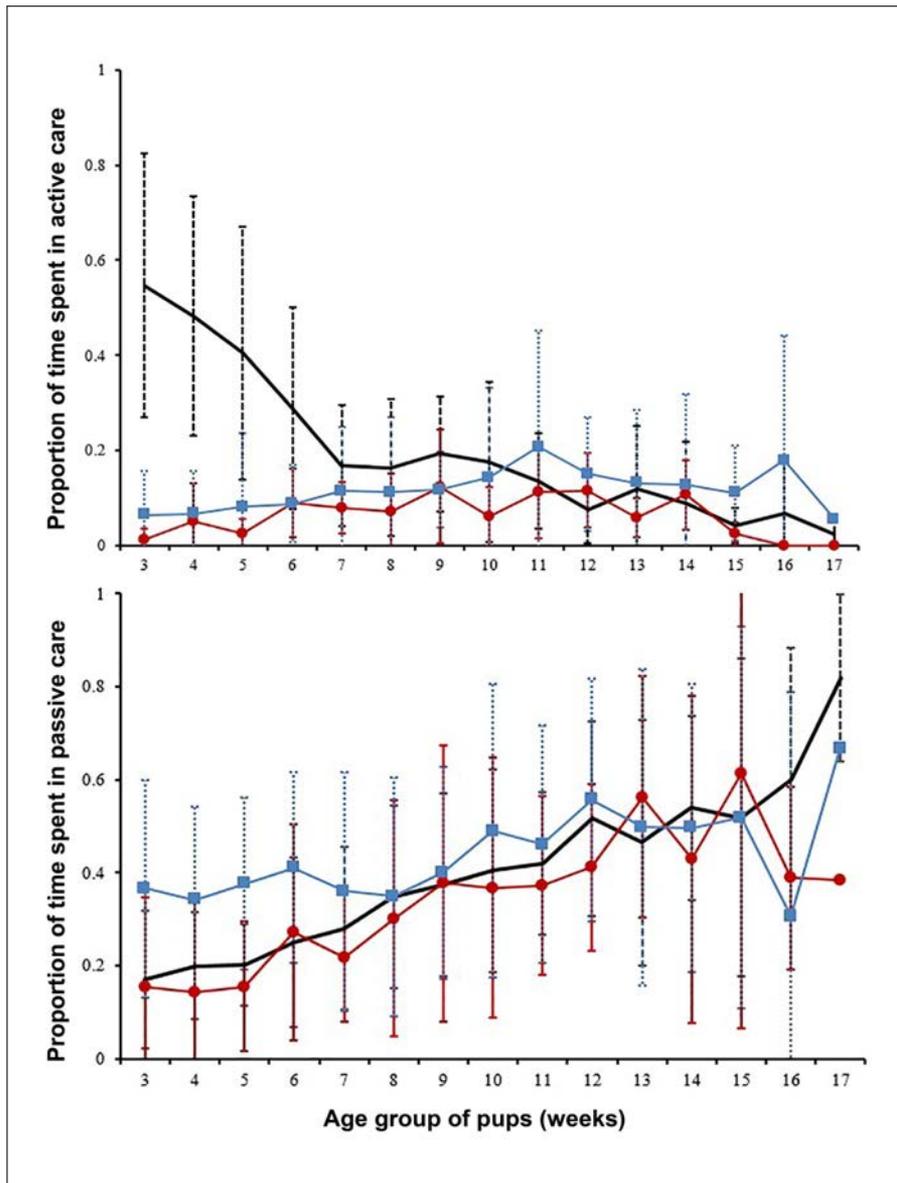


Figure 15.17: Mean and s.d of the proportion of time spent by mothers ($n = 22$, black line), putative father ($n = 14$, blue lines) and allomothers ($n = 14$, red lines) in giving active (upper panel) and passive (lower panel) care to growing pups. [Redrawn from M Paul and A Bhadra, The great Indian joint families of free-ranging dogs, *PLoS ONE*, Vol.13, No.5, p.e0197328, 2018].



Figure 15.18 : Dogs are everywhere and easy to observe. **(Left)** A group of dogs at a meat shop; **(Right top)** A group with females, males and two litters; **(Right middle)** Female with her own and others' pups; **(Right bottom)** A mother and her pup (Photos: Manabi Paul).

yet, there are hardly any studies of this obvious animal model. Why should this be so? I don't think it is unreasonable on my part to expect that dozens of Indian researchers from dozens of zoology departments in many Indian Universities should be making world-class contributions to ethology, behavioural ecology, sociobiology, evolutionary biology and conservation biology. But what is preventing this dream from being realized? We should reflect on this.

I do not have an exhaustive list of possible reasons for this regrettable paucity of research on stray dogs. But I can think of several. Especially in India, we have very narrowly defined the limits of respectable science, indeed, of science itself. Students of Anindita Bhadra who have had the courage and wisdom to work on dogs will tell you that they have often been told by learned experts sitting on selection committees that what they are doing is not science. This attitude is not just



Figure 15.19: Anindita Bhadra's family and students experimenting with dogs. **(Top)** POC over meat, experiment being performed by Ujaan Banerjee (*Photo: Manabi Paul*); **Middle** A choice test with pups being performed by Rohan Sarkar (*Photo: Shubhra Sau*); **(Bottom)** An experiment to test if dogs can count, performed by Arunita Banerjee (*Photo: Arunita Banerjee*).

regrettable, it is suicidal for Indian science. We should all carefully select areas of research where we have a unique advantage in becoming world-leaders, either because of the easy availability of study species, or because of the possibility of doing cutting-edge research with little money or funding. Instead, we tend to de-



Figure 15.20 : Yes, you can get a PhD working on stray dogs—Manabi Paul (the first author of all the six dog papers discussed in detail in this chapter) during her convocation, with Mentor Anindita Bhadra (June 2018).

clare these very areas as unfashionable and unrespectable. Conversely, we tend to place on a high pedestal of respectability precisely those areas of research where we are in a losing competition with the much better-endowed parts of the world. Suicidal indeed!

It is time to change this. I am very pleased that Anindita Bhadra has chosen to devote her career to the study of dogs. To gain more respectability, Anindita Bhadra refers to the stray dogs she studies as ‘free-ranging’ dogs but I have often deliberately and defiantly used the more common, and no less respectable term ‘stray dogs’. Here I have only described her experiments concerning the parent-offspring conflict. But her Dog Lab has begun to teach us so many remarkable facts about these most common and familiar animal companions. They have studied the time-activity budgets of dogs with surprising results [17], foraging behaviour with new insights [18], dog-human interactions with worrisome results [19] and their intelligence and cognitive abilities with even more surprising results [20]. In the process, Anindita Bhadra has trained passionate and curious students to conduct rigorous experiments with the dogs they love (*Figure 15.20*).

Suggested Reading

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16

Cutting-Edge Research at Trifling Cost

I have had multiple aims in writing this book. My primary aim has been to show how simple and innovative experiments can be performed at almost no cost, by nearly anyone, to create significant new knowledge. The history of science shows that this is true in most areas of scientific research, albeit to varying degrees. I have focussed on the field of animal behaviour both because I am more familiar with this field than others, but also because, the field of animal behaviour is especially well-suited for such low-cost research. It has also been my aim, of course, to discuss the principles of ethology (the scientific study of animal behaviour), through the medium of these experiments. My motivation in writing this book is to bring social prestige to low-cost research, make the practice of science more inclusive and democratic, and empower large numbers of people to become knowledge producers rather than merely remain knowledge consumers. The people I especially have in mind are, less-endowed sections of society, including, but not restricted to, underdeveloped countries, marginalised institutions and individuals, students, the general public, amateurs, and all those with little or no access to large research grants and sophisticated laboratory facilities, for whatever reason.

Note: Some passages in this chapter are reprinted from Suggested Readings [4, 5, 15 and 16].

16.1 Animal Behaviour: An Especially Ideal Subject for Low-cost Research

In the fifteen chapters that preceded this one, and were published in *Resonance – journal of science education* between August 2018 and November 2020, I have illustrated experiments meant to answer such questions as, how do wasps find their nests, do bees have colour vision, how do ants find the shortest path, how do bees estimate distance flown, how do ants estimate distance walked, why are male wasps lazy, how do wasps decide who would be their queen, why do wasps fight, does experience matter in fighting fish, why don't male frogs do their best when they sing to attract females, why is mimicry in snakes imperfect, why do hosts care for cuckoo eggs, and why do parents and offspring quarrel? Experiments attempting to answer each of these questions illustrate how it is possible to make significant new discoveries by conducting simple, low-cost experiments, both in the laboratory and in nature. Although they do not require any expensive or sophisticated facilities, the ability to succeed in conducting such experiments depends crucially on many other attributes and skills of the researchers. These include adequate knowledge of the empirical literature, an understanding of the theoretical foundations of the discipline, lasting passion and undying curiosity, a healthy disrespect for authority, confidence that there is much about the natural world waiting to be discovered, willingness to undertake labour-intensive manual work, identifying the appropriate study animal, asking the right questions, designing innovative experiments with adequate controls, foresight in framing expectations, caution in coming to conclusions, ruling out alternate explanations, recognising the level of precision that is necessary and adequate for the question at hand, conducting sound statistical analyses, respect for a negative result, and more. I have discussed many of these in some detail in the 'Reflections' section of each chapter.

In this final chapter, I will make some general remarks about the importance of low-cost research, for science and scientists, and for society.

16.2 The Importance of Low-cost Research for Scientists

It is not impossible to do first-rate research without applying for and obtaining financial grants, and I will return to the topic of grant-free research later in this chapter. But for the majority of professional scientists employed in research and educational institutions, it has become the norm to apply to various funding agencies, both government and non-government, for so-called 'grants', in order to

carry out research. It is almost a universal experience of such grant applicants that they are granted less money than they had requested. Conducting research with less money than one had budgeted for is, therefore, the norm. How do we deal with this situation? Do we simply downsize the quality and quantity of our research correspondingly and tell the funding agencies that they will get what they paid for? This, of course, makes no sense because we are not doing research for the sake of the funding agencies. The nearly universal practice of obtaining grants for doing research has the danger of creating a mindset that our research effort is a contract, with and for, the funding agencies. At least in most areas of basic science, we should be doing research because we are passionate about it.

How then do we ensure that the quality of our research does not simply scale with the quantum of money we can raise for the purpose? Maintaining the same high quality of research with a reduced budget requires a great deal of creativity and innovation. The quantum of money and the quality of facilities that researchers can muster for a given kind of research varies enormously depending on the researcher's standing in the field and his or her geographical location and institutional affiliation. And yet, I see surprisingly little discussion, let alone training, in how to get more and better research done with less money. This topic is almost taboo in the scientific community. Indeed, there is a positive selection for raising and spending more money, but I will come back to this below. There is an urgent need to change our mindset and initiate a discussion on how to do the best possible research with less money. Those who are well-endowed with big grants will not do this for us. Those of us who have less research money than we would like, whether this is because we are from the developing or underdeveloped world or because we are from underprivileged institutions or sections of the society, have to take the lead. Of course, our discussions and findings may help those with more money than us to do even better science than they might otherwise have done, and this will be our contribution to science as a whole.

Nevertheless, there is only so much we can do by trying to make the best of an inadequate grant received for research already planned and a proposal already submitted. A much more effective way of doing great science with less money is to choose an area of research that we can pursue with maximal efficiency with the quantum of funds that we are likely to be able to raise. There is wide variation in the cost of research, whether due to the number of personnel required, the costs of travel to research locations, or the nature of the sophisticated equipment and technology or due to the cost of chemicals or other consumable supplies. Some areas can be pursued with maximal efficiency at relatively small costs while others

may need orders of magnitude greater financial investments. It is a great mistake to think that areas of research that require less money are less important, less intellectually challenging or less interesting. There is little correlation between the cost of conducting research and its importance, or interest. If we give more importance to conducting first-rate scientific research rather than to the area of science we might work in, there is great scope to tailor our research to the funding likely to be available while keeping the bar on top-quality research consistently high. For convenience, I will refer to low-cost and high-cost research as if they are binaries, but of course, there is a continuum.

Funding situations are bound to vary, going both up and down, with time, with our (changing) geographical and institutional locations, our inevitably increasing age, our standing in the field, with the change of governments and their priorities, with changing fashions and needs of the society, not to mention wars and natural calamities. If we ignore these changes and inflexibly pursue the same kind of research at all times, then what will inevitably vary is the quality and quantity of our research. It follows then that if we want to keep the quality and quantity of our research approximately constant, through the changing fortunes in terms of grants and other facilities, we must adaptively alter our research areas to suit the times and the circumstances. This is not as impossible as it may first sound. Having very broad interests and being widely read and interested in many different areas of science and beyond, is necessary to do high-quality research even in a single area. Thus, being well equipped to undertake top-class research in any one area will automatically make it relatively easy to change our areas of research. Innate curiosity, pleasure in creative innovation and passion for the truth will let us slide easily across disciplines. These should, therefore, be the primary items in our tool-kit, rather than specialised knowledge of a narrow discipline or rare expertise in the use of some high technology. Interest in a broad array of questions rather than an infatuation with particular methods or techniques or even particular model systems is sure to facilitate mobility across areas of research.

I find it surprising, therefore, that we place so little emphasis on the problem of how to choose a research question. In few other areas of human enterprise do people embark on long-term, not to mention life-long plans with as little feasibility analysis, as scientists do in choosing the areas of their research. Historical contingency seems to explain nearly all variation in the choice of research areas among scientists. If we change this aspect of our scientific culture and training and begin to choose our areas of research more pragmatically, not only will we be able to do first-rate research with less money, but we will also be able to adapt to

changing fortunes in funding while maintaining the quality of our research. The bottom line is that we should be able to work as close to the limit of our intrinsic ability as possible, unconstrained by external limitations such as funding. I have seen both in myself and in others that it can become a habit to say that we did quite well given the constraints. But when the constraints are not inevitable, we should position ourselves in physical or disciplinary space so as to work at the limit of ability. One way to begin is to make it a habit to compare ourselves with others in absolute terms, not after factoring in our real and imagined constraints.

16.3 The Importance of Low-cost Research for Science

Somewhat distinct from its importance to individual scientists, low-cost research is also profoundly important for science as a collective human enterprise. If different areas of science require different amounts of money and sophisticated instrumentation, as I have argued above, it follows that we might neglect areas that need very little investment, if some of us do not diligently pursue low-cost research. If everyone is in the race to get large grants and pursue research questions that require such large grants, and worse still, if those who fail to get large enough grants do the best of a bad job in the same area, then surely many important areas will remain neglected. If the importance and intrinsic interest of scientific areas are uncorrelated with how expensive they are, it follows that many important and exciting areas will remain unresearched. Ironically, if everybody succeeds in getting large grants, then many areas will suffer—science as a whole will suffer! We usually further exaggerate the problem by spreading the total amount of money available too thinly so that nobody has enough money. Yet, everybody is trying to pursue expensive research. It would be prudent to allocate sufficiently large grants to some individuals to pursue research questions that are inevitably very expensive and encourage others to pursue research questions that do not need large investments. But this will only be possible if we do not treat those who get small grants as losers and deprive them of dignity and social prestige. I will have more to say about social prestige later. I often come across discussions about low-cost technology and low-cost technology substitutes but seldom about low-cost science.

I am less familiar with other fields, but at least in biology, the variation in the cost of doing research in different areas and the neglect of low-cost areas is glaring. The long-term negative consequences of such neglect are already being felt, and some would say the damage already caused is irreparable. At the most

fundamental level biology needs to find, identify, and classify the Earth's vast biodiversity. The fact that over 90% of the species remain uncatalogued and do not even have a name is a failure of monumental proportions. And this is usually one of the least expensive areas of biological research. To see this in perspective, imagine that 90% of the naturally occurring elements on Earth were yet to be discovered. Our woefully inadequate knowledge of the Earth's biodiversity and how this tragedy is twice compounded by the rapid and irreversible loss of species on the one hand, and equally rapid and irreversible loss of taxonomists, on the other hand, have been repeatedly lamented [1–3].

Natural history is slightly (but only slightly) more respectable but an equally low-cost enterprise that remains mostly neglected. However, it is evident that questions and hypotheses for subsequent research almost always stem from an exploration of the natural world that is open-ended, and fuelled by a passionate love of nature and a spirit of adventure. Somewhat higher up on the social prestige scale is what we might broadly call organismal biology, which, as we have seen repeatedly in this book provides abundant opportunities to answer important questions with clever and simple low-cost experiments. And yet, we are very far from utilising these opportunities on a large scale. The main drawback of all these kinds of research seems to be that they are low-cost and, therefore, not well respected.

We have to closely examine the growth of knowledge (or the lack thereof) in specific fields to understand how impoverished our future research will be if we do not pursue taxonomy, natural history and organismal biology, much more vigorously than we have been doing in recent times. To take an example closer to my area of expertise, while reviewing the state of our knowledge of the social wasp genus *Ropalidia*, and lamenting on our ignorance of swarm-founding species, I concluded that: “To understand the social dynamics of such societies with hundreds of queens and thousands of workers cooperating to build and repair the nest, deal with predators and parasites, self-organise division of labour to forage for and feed tens of thousands of larvae and stage periodic swarms to make new colonies, along the lines of similar knowledge of independent-founding *Ropalidia*, would be a naturalist's challenge even as it is a theoretician's dream. Be that as it may, there isn't even a single account of their swarming behaviour and colony foundation, which is their unique feature. Unless major corrective steps are taken, the prospects of improving our knowledge in the future will remain bleak, owing to large-scale habitat destruction and accompanying species loss, dwindling of the numbers of field naturalists and the nearly complete obsession of the community of social insect researchers, with understanding the genetic, developmental, and

molecular mechanisms of a small number of phenomena in fewer than a handful of model organisms” [4].

Similarly, when asked to comment on the opportunities for future research in insect social behaviour, I argued that “Studying the molecular mechanisms that make social behaviour possible requires access to well-equipped laboratories and significant infrastructure and funding. It is best done by a minority of the research community that can command such resources. The vast majority of researchers who cannot command the required resources should not be forced to do molecular biology at a suboptimal level but must be encouraged and empowered to do first-rate natural history and organismal biology. Researchers from economically backward but biodiversity-rich countries in Asia, Africa and Latin America are ideally placed to undertake first-rate natural history and discover new species and new phenomena and feed the molecular biologists with new research questions. It is sadly ironical that these researchers are often under pressure to use the meagre resources of their countries to enter into a losing competition with laboratories in advanced countries to study the molecular biology of social behaviour, instead of proudly studying the rich biodiversity in their backyard, at a fraction of the cost. The onus is on research policymakers in the developing countries to create an environment where their scientists can undertake with pride, the kind of research that they can do best” [5].

16.4 The Importance of Low-cost Research for Society

Quite apart from its importance for individual scientists and the healthy growth of science discussed in the two previous sections, low-cost research is crucially important for the society as a whole. The most obvious importance is the saving of money, but I will discuss this in a later section. Here I will focus on an even more important but less tangible gain to be had from promoting and pursuing low-cost research. Low-cost research is the single most important way to make the practice of science—the production of scientific knowledge—democratic and inclusive. Presently the opportunities to pursue scientific research are extremely unevenly distributed along numerous axes. This is so obvious and so well known that I will not belabour the point. But let us remind ourselves of some of the most common axes of inequality. The first and perhaps the most severe inequality is between rich and poor countries, between developed, developing and underdeveloped countries. Expenditure on science as a fraction of the GDP varies between countries by orders of magnitude. Tragically, the fraction of GDP is an inappropri-

ate measure for comparison because expensive research needs money irrespective of the GDP of the country conducting such research.

Given that the GDP itself varies enormously and that countries with high GDP generally spend a higher fraction of their GDP on science, the resulting inequality in money available for science is truly mind-boggling. If scientists in all countries follow the same model of doing science and attempt to work in the same areas using the same methodologies, the variation in the quality and quantity of scientific knowledge production will rival the inequality in money available, nay, it will be worse because developing countries have other disadvantages due to shortage of trained scientists and poor education. The importance of low-cost research, especially for developing countries is enormous, and only if we learn to adapt to this situation by learning to get more science for less money, can we hope that science can be democratised and become more inclusive.

Inequality is not just between countries. In every country, there is enormous inequality in the money available for research between different institutions. Some of this is due to their varying ability to use internal resources to support research, so-called intra-mural funding. There is also substantial inequality in the capacity of scientists in different types of institutions to raise external resources, so-called extramural funding, often unrelated to genuine variation in their ability to do research. This is true in all countries, including the richest ones that spend the most money on research. The starkest variation in India is between research institutes and traditional Universities, with undergraduate colleges being relegated to a distant third position, and high schools being pretty much barred from seeking such funding. Despite a growing effort worldwide, to reduce the disadvantages that early-career scientists are bound to face, there is great inequality between early-career scientists and established scientists, even after correcting for any possible differences in talent and competence. It follows that early-career scientists should be more interested (and should be allowed to be more interested) in exploring low-cost research to mitigate the consequences of their relatively low funding to compete with established scientists.

Much of modern science, especially when pursued in mainstream academia, is characterised by slow maturation of scientists, with long periods of apprenticeship and late transition to the status of independent researchers. Maturation here is not measured so much by age as by accomplishment and track record in producing scientific knowledge. Relatively low-cost research may therefore be an important option for fast track movement to the rank of an independent scientist. It can be especially attractive when mid-career movement between research questions is

possible and even appreciated, as it often is. What can be smarter than letting your research questions and strategies evolve to suit your changing funding fortunes while maintaining a consistently high quality of your research? If early-career scientists don't have a level playing field in acquiring funding and other facilities for doing science, students are in a worse situation. However bright their ideas and whatever be their level of competence, students need to work under a so-called Principal Investigator or PI (more on PI later) and seldom have the opportunity to be independent scientists.

To some extent, this may be because students may genuinely need more training, experience and maturity, but why should we let this be exacerbated by the dependence on a high level of funding? Students may sometimes face a trade-off between doing expensive research, as it already is being done under the banner of their mentors, or do more independent research with lower costs and alternate strategies. In such situations, enhancing the quality of their independent research by cleverly choosing their research area to be relatively unaffected by less money may come in very handy.

Even more stark inequalities in funding opportunities and even more uneven playing fields are indeed faced by amateurs, not to speak of the general public who may wish to and be quite capable of conducting scientific research. Should their research be of correspondingly lower quality? By paying attention to low-cost research, they can certainly get more for less money both by cleverly aligning their interests and inventing innovative alternatives to traditional methods and technologies employed by the privileged professional, who has less need to innovate to save costs. Indeed, they have the opportunity to show the way and put the professionals to shame. It is widely known, although not always admitted, that there is not a level playing field across other axes such as gender, race and ethnicity. Smartly employed low-cost research, maintaining high quality without lessening interest and importance has a useful role to play in many such situations.

By promoting low-cost research as a means to mitigate the ills of inequality, I am by no means justifying or condoning inequality. Access to funds, facilities, opportunities, recognition and appreciation are unequal across multiple axes and for numerous reasons, and their levels are unacceptable. We must continuously endeavour to create a level playing field. With the best of intentions and the brightest of ideas, this will take time. The question is, what do we do in the meanwhile? It is also almost always true that the power to change the situation lies more with the haves rather than with the have-nots. Low-cost research, therefore, has a special place for those who fail to get high levels of funding, for whatever reason. My

optimistic three-step dream is that (1) the privileged will do all they can to level the playing field, (2) the underprivileged will strategically use low-cost research and other methods to supplement the efforts of the privileged to level the playing field, and finally (3) the underprivileged will become the privileged and remember to continue efforts to see that all privileges reach all deserving people.

I will close this section with a brief discussion of the advantages of democratising science and making it all-inclusive. The advantages may seem obvious but let us state them explicitly. First, there is a moral imperative to provide equal opportunities for all people irrespective of nationality, wealth, age group, professional affiliations, race, caste and gender to pursue science and become knowledge producers. We frequently hail the importance of making access to knowledge universal, but I think equal opportunities to participate in knowledge production is an even more important prerequisite for people to have dignity and self-esteem.

But the imperative to democratise science goes well beyond the moral. It is a prerequisite for the healthy growth of science itself. Scientists are all too human, complete with social, political, religious and idiosyncratic prejudices. It is unreasonable to expect that all individual scientists are coldly objective truth seekers. This was memorably expressed by Richard C. Lewontin in his *The Genetic Basis of Evolutionary Change* (1974): “It is a common myth of science that scientists collect evidence about some issue and then by logic and ‘intuition’ form what seems to them the most reasonable interpretation of the facts. As more facts accumulate, the logic and ‘intuitive’ value of different interpretations change, and finally, a consensus is reached about the truth of the matter. But this textbook myth has no congruence with reality. Long before there is any direct evidence, scientific workers have brought to the issue deep-seated prejudices; the more important the issue and the more ambiguous the evidence, the more important are the prejudices, and the greater the likelihood that two diametrically opposed and irreconcilable schools will appear” [6].

I have, therefore, argued elsewhere that we should find ways of letting some scientists who satisfy certain high standards of competence and who are wedded to some pet hypothesis to pursue their passions so that the scientific community can wait and watch and see when and where their ideas fail. Only such scientists will be willing to risk their careers and reputations to take their hypotheses to their logical conclusions. The rest of us may rather timidly stop after early signs of failure, owing chiefly to the low esteem with which we hold negative results. While it is neither possible nor necessary for every individual scientist to be totally dispassionate, it is important and possible for the scientific community as a

whole to be objective [7]. The historian of science Naomi Oreskes has argued that society trusts (or should trust) science because scientific knowledge is based on agreement and verification by large groups of scientists. She persuasively argues that consensus is rather hollow unless scientific communities are not only inclusive and encompass geographical, national racial and gender diversity but also embrace traditional or civilisational knowledge including traditional knowledge of tribal people, farmers, fishermen, patients and midwives. To repeat her mesmeric metaphors, “diversity serves epistemic goals”, and “the non-expert world is not epistemically vacuous” [8, 9]. I would argue that low-cost research, which finds alternate ways of achieving high-quality research, would be a powerful ally in fostering such diversity in the scientific community.

16.5 Familiar Objections to Low-cost Research

I am amazed that when I espouse low-cost research, some people raise objections. The most common concern I hear is that such arguments in favour of low-cost research will reduce funding for science; politicians and funders will use the same arguments to cut back on funding. Well, even if there was any truth in this fear, we cannot, of course, be dishonest and inflate the cost of our research, nor can we morally justify spending more money than is required—after all it is somebody else’s money. But I think the fear itself is entirely unjustified. I am not arguing for less money but more efficient use of funds so that more people can do cutting-edge research for the same total amount of money. Needless to say I am of course not claiming that we cannot do great science by spending a lot of money, nor am I saying that some areas of science do not need a great deal of money. All I am saying is that a great deal of good science can be done with little or no money.

In *We Are All Stardust* (2015) [10] the German physicist, author and essayist, Stefan Klein says of VS Ramachandran, the Indian-American neuroscientist and author of *Phantoms in the Brain* (1998) [11], “While other neuroscientists spend millions on their experiments and perform expensive computed tomography scans on dozens of test subjects, he uses quite simple materials. Sometimes all he needs is a mirror, a wooden box, or a cotton swab in order to achieve spectacular results.” When asked whether he had anything against technology, VS Ramachandran replied, “I have nothing at all against fancy equipment. We need it and use it at times. But personally, I do research because I find it fun. And high-tech science seems less gratifying to me. The greater the distance between the raw data and the conclusion drawn from an experiment, the more boring it is.... Luckily, I studied

medicine in India. There you had to fall back on your intuition and very simple tests in order to make a diagnosis. And if that didn't work, we just had to come up with something."

The amount of money that we should spend on science, how we distribute that money and how we should spend money are all different and independent arguments. Consider the case of a country like India. Given our population and our proven ability to educate and train large numbers of scientists and the impressive track record of hundreds of our scientists, I think it is not unreasonable for us to aim for a 10-fold increase in our scientific output, say in the next decade. Needless to say, the chances of getting a 10-fold increase in funding is, of course, zero. However, I believe that a doubling of the budget and a five-fold increase in the overall efficiency of science per Rupee spent are well within the realms of possibility.

16.6 Barriers to Low-cost Research and How to Overcome Them

Apart from the relatively harmless casual and verbal objections to low-cost research that are usually made as counterpoints during a discussion which I have referred to above, I am afraid there are real and severe barriers to pursuing low-cost research. I will briefly mention three and add three partial remedies.

The first barrier is the universally witnessed sentiment, "money is power". Money is indeed power for scientists too. It is not merely the psychological feeling or illusion of power, but grant money brings real power because of the way academia is organised. In many institutions around the world, scientists with large grants are allowed to buy themselves out of many duties, especially teaching and administration, both of which take a great deal of time, but are also part of being responsible members of the academic community. In some extreme cases, this means that a Professor is on the rolls of an institution in name only. Not surprisingly, the temptation to have large grants, even if they are not really necessary for the best research outcome, is great.

Secondly, money brings prestige. And not just to the social standing of the winners of large grants but more detrimentally, also to their research. According to prestige to research proposals in direct proportion to the quantum of money requested begins with the receipt of the application at the grants offices. Small grants are sometimes much more casually dismissed, and very large requests result in a team of experts making a site visit to the applicant's institution and laboratory. Another kind of problem is the perception (justified in some cases) that

you get a certain fixed fraction of the money you ask, so that the more you ask, the more you get. I find it amazing that most applicants accept even very substantial cuts in the money asked for and neither complain nor report having to drastically alter their research plans, let alone decline the grant. Something is clearly wrong!

The third barrier to low-cost research comes from the practice in many institutions (thankfully not so common in India) to depend rather heavily on the overheads that extramural grants bring, to run the institutions, including paying salaries and constructing buildings. This practice incentivises getting large grants as its most benign effect and makes it impossible to do low-cost research as its most deleterious effect. Selection for faculty who will bring in large grants begins at the hiring stage and continues through cycles of assessment and promotion, not to mention awards and accolades. As we saw earlier, this results in the uneven growth of different areas of science and the neglect of important areas, their only crime being that they don't need large grant money. Surely, institutions should not be built on a business model that depends heavily on overhead grants from extramural funding for research.

Unfortunately, even institutions that are not built on this pernicious business model have made it a social norm to accord unnecessary prestige to faculty who bring in large grants. It is quite the norm to prominently display the list of grants earned on our CVs, often ahead of the list of our publications. All this is surely a great disservice to science and should be done away forthwith. At the very least, scientists and their work should be evaluated irrespective of the grants brought in. Ideally, the money spent on research should be in the denominator of the performance index. I have never heard a good argument for why evaluation should not be in terms of research output per Rupee or dollar spent. Perhaps it will be a bit unfair to those who pursue expensive research, but that is better than penalising those who do inexpensive research. Moreover, I think it is not unreasonable to put some pressure on those who spend a great deal of someone else's money, public or private, to perform well. Perhaps we should rename 'grants' as 'loans', to be repaid with commensurate scientific knowledge. Sometimes when I speak about doing good science with less money, I am amused to see my interlocutors deflect the argument and say let's just talk about how to do good science, why bring money into the picture? This is quite absurd. Doing good science with less money requires many more skills, and often more imagination and creativity, than just doing good science.

We can do even more to promote low-cost research. Some years ago, I was invited by the students of a prestigious research institute to speak about my life in

science. The students with expected creativity had christened the series of talks *The Life of PI*, a take on the popular novel by that name by Yann Martel [12], except that PI was meant to be 'Principal Investigator'. I began by praising their creative title for the lecture series and spent the next 15 minutes telling them why I hated their title, or more precisely why I hated the title, Principal Investigator. PI was unheard of when I was a graduate student or even when I was an early career scientist. It seems to have been invented in the last two or three decades and has gone to fixation driving to extinction all rivals including professor, scientist, mentor, faculty member, etc. As far as I know, PI was invented by granting agencies to know who is to be held responsible for the grant to be well spent. There is nothing more principal about the PI. People now ask me how many PIs are there in your Department and I say all 100.

Why should it be a foregone conclusion that the one who gets the grant is the principal contributor to the science that is being done? Is it not possible that a junior colleague, a student or even a technician plays the principal role in the research being done? Perhaps different actors may play principal roles in different parts of the research. I think it is a mistake to decree a fixed and pre-determined hierarchy in a research environment. Science is meant to be non-hierarchical, and we are unnecessarily creating a mindset and further empowering the already powerful. A lowly student at the bottom of the power hierarchy is further frightened into submission and told in no uncertain terms as to who is the boss. How can we expect students to question and challenge the PI, or do we not want them to do so? Besides, any good set of ethical guidelines will discourage grant of authorship to those who simply provide the money for the study. Thankfully my PhD and post-doc mentors were not called PI in those days, or I would have been deeply offended. In neither case did my mentors, wonderful as they were, play a principal part in my research. I find it pompous enough to be called group leader, but PI? Never. I feel honoured to be a mentor to my students and friend to my colleagues, and willing to be the PI responsible only for my funders and their financial auditors.

My quarrel is not just with 'PI' but with 'grant' itself. I am not saying that there should be no grants; all I am saying is we should provide space for 'grant-free' research; indeed, we should encourage grant-free research. Instead, it seems to go without saying that research begins with applying for a grant. How to choose your area of work, how to ask the right questions, how to design an experiment, how to collect data—all such questions seem to have been relegated to a lower priority. I have attended far too many workshops designed to mentor early-career

scientists where 'how to get big grants' was the question of paramount importance for both the mentors and the mentees. It seems to be the assumption that getting a big grant guarantees excellent research. In such workshops, I would instead like to see a discussion of all the cool research we can do in the grant-free mode.

16.7 A Personal Note

In my experience, a discussion of high-cost versus low-cost research often boils down to a discussion of molecular biology versus animal behaviour, ecology and evolution. But of course, this need not be the case. Such debate can involve almost any area of science, be it physics, chemistry or biology and also in virtually any areas of biology, including expensive versus inexpensive ecology and animal behaviour or even high-cost versus low-cost molecular biology. But there is a good reason why the discussion often boils down to molecular biology versus behaviour and ecology, in my case. This is because molecular biology and animal behaviour are the fields I am trained in, and the two areas closest to my heart. I will, therefore, end this chapter and indeed, this book on a personal note, reproduced here from [15].

“As an undergraduate, I read voraciously and indiscriminately, partly because there was little else to do. Of all that I read, two books completely blew my mind. One was *The Double Helix* [13] by the Nobel Laureate James D. Watson. This book was inspiring at many levels and instantly made me a life-long addict of molecular biology. I subsequently read every book and research paper in the field of molecular biology that I could lay my hands on. The discovery of DNA, its demonstration as the hereditary material, the elucidation of the double-helical structure of DNA, the proposal and subsequent proof of semi-conservative replication, the unravelling of the steps in the synthesis of proteins and the study of bacteria, bacteriophages and plasmids were all like an epic play being played out in the theatre of heaven where Gods like Watson and Crick, Luria and Delbruck, Messelson and Stahl, Ochoa and Kornberg, Nirenberg and Khorana lived and continuously scripted, directed and enacted various acts and scenes. And these ever novel and mesmerising scenes in the play came to me almost daily, in the form of research papers in various journals. The feeling that I was a lowly earthly being watching an epic play in heaven with awe and respect was enhanced by the fact that these topics were not part of our [study] curriculum.

But I also read well beyond molecular biology. The other book that I can easily single out for having made a life-long impact on me was *King Solomon's*

Ring (1952) by Konrad Lorenz [14], not yet a Nobel laureate but soon to become one, at the time I read him. The study of animal behaviour [on the other hand], was a complete contrast to the epic molecular play in heaven. It was an earthly matter. Charles Darwin, Konrad Lorenz, Niko Tinbergen, Karl von Frisch, Oskar Heinroth, Douglas Spalding, Jacob Von Uexküll, Ivan Pavlov, Desmond Morris were all earthly beings close to me and I admired them in a wholly different kind of way—not in awe but as a fellow compatriot. The reason for this was that they all did what I felt I could also do quite easily, at least in principle.

As an undergraduate student trapped in an environment without access to any well-equipped research laboratories, I perceived a massive, insurmountable technological chasm between molecular biology and me, and hence molecular biology was a play being enacted in heaven. Ethology, the study of animal behaviour on the other hand, was well within my capacity to pursue.

There was no reason for me to feel jealous of Watson and Crick for having discovered the structure of DNA—it was not something I could have done anyway. But I did feel a tinge of jealousy that it was Konrad Lorenz and not I who had discovered imprinting in birds, that it was Karl von Frisch rather than I who deciphered the honey bee dance language, that it was Douglas Spalding and not I that had put little hoods on new-born chicks and showed that their pecking behaviour was instinctive, that it was Niko Tinbergen and not I that had placed a ring of pine cones around the nest of wasps and discovered that the wasps use landmarks to locate their nests”.

“At the end of my PhD, I was in a serious dilemma, being equally in love with both Molecular Biology and Animal Behaviour. The difficulty, or should I say impossibility, of doing cutting-edge research in molecular biology under Indian conditions, was brought home painfully to me every day of my PhD. If I were to continue with molecular biology, it would have to be in the USA or some such developed country. But if I could swap animal behaviour into my profession and molecular biology into a hobby, then, of course, I could stay in India and spend the rest of my life doing low-cost research on the Indian paper wasp *R. marginata*. I chose the latter option...and I have never regretted my decision” [16].

Suggested Reading

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The Masterclass series of eBooks brings together pedagogical articles on single broad topics mostly taken from Resonance, the journal of science education that is published monthly by the Indian Academy of Sciences since January 1996. Primarily directed at students and teachers at the undergraduate level, the journal has brought out a wide spectrum of articles in a range of scientific disciplines. Articles in the journal are written in a style that makes them accessible to readers from diverse backgrounds, and in addition, they provide a useful source of instruction that is not always available in textbooks.

