

Science as a hobby: how and why I came to study the social life of an Indian primitively eusocial wasp*

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For the most part, my research career has involved prying into the life of the locally abundant primitively eusocial paper wasp, *Ropalidia marginata*, with the aim of understanding the origin and evolution of social life in insects. My interest in this wasp species began as a hobby, but I was privileged to soon convert my hobby into my profession. Here I describe how this conversion came about, what it meant to pursue my hobby as a full-time activity, describe some examples from my research and end with some reflections about the process of doing modern science, especially in India.

Keywords: Animal behaviour, organismal biology, primitively eusocial wasp, *Ropalidia cyathiformis*, *Ropalidia marginata*, sociobiology.

'... der Mensch spielt nur, wo er in voller Bedeutung des Worts Mensch ist, und er ist nur da ganz Mensch, wo er spielt.' (...man only plays when he is in the fullest sense of the word a human being, and he is only fully a human being when he plays.)

Johann Christoph Friedrich von Schiller,
German Poet, Philosopher,
Historian and Playwright 1759–1805

I have had the great fortune of pursuing my science as a hobby or, more precisely, of pursuing my hobby as my profession. Here I will attempt to recapitulate the circumstances that made this possible, cite three examples from my work, not only to describe the science, but also to set the stage for some reflections on the pursuit of science, especially in India. In my undergraduate days 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*¹ by Nobel Laureate James D. Watson. This book was inspir-

ing 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. Those days it was almost entirely prokaryotic molecular genetics but 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 of 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 mesmerizing 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 curriculum and none of my teachers and very few of my student friends were interested in or capable of discussing these matters.

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*² by Konrad Lorenz, not yet a Nobel laureate but soon to become one, at the time I read him. The study of animal behaviour so charmingly and unforgettably described by Konrad Lorenz 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 the latter was a play being enacted in heaven. Ethology, the study

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of animal behaviour, 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 birds³, that it was Karl von Frisch rather than I who deciphered the honey bee dance language⁴, that it was Douglas Spalding and not I that had put little hoods on newborn chicks and showed that their pecking behaviour was instinctive⁵, 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 nests⁶. In the field of animal behaviour, I not only read anything I could lay my hands on but also began to make my own naïve observations, on insects and frogs, birds and monkeys and indeed on my fellow human beings. Like molecular biology, animal behaviour was also entirely outside the sphere of interest and competence of most of my teachers and friends and ironically enough, I suspect that this was an important enabling factor in my intellectual development.

Armed with Bachelor's and Master's degrees in zoology from the Bangalore University, then called Central College, I applied for a Ph D programme at the Indian Institute of Science, arguably the best place in India to do modern scientific research and situated only a kilometre from where I grew up. Here there was no Ph D programme in animal behaviour, but there was a newly initiated Ph D programme in what was then called interdisciplinary molecular biology. I was told that a small group of professors from the departments of biochemistry, microbiology and cell biology, biophysics and organic chemistry had just set up a new inter-departmental programme in molecular biology. There was just one position in it that year for a Ph D student and when it was offered to me I grabbed it with both hands. And yes, I was instantly transported to heaven. The lysogenic bacteriophage lambda had been one of my favourite plays while watching the theatre in heaven and with great excitement I chose to work on a similar lysogenic mycobacteriophage, I3, isolated a few years before at this very Institute⁷. I had actually visited the Microbiology and Pharmacology Laboratory as an undergraduate student to meet and shake hands with Dr Sunder Raj who had isolated this mycobacteriophage. Although I did relatively unsophisticated experiments compared to those done in heaven, I truly lived in heaven during the next five years. Those years of excitement, with access to much more literature and especially the long and heated discussions I used to have with my lab mate and kindred soul Rasika Harshey, are forever etched in my mind. But luckily, I did not give up my interest in animal behaviour or my habit of reading indiscriminately. In addition to reading, now I pursued fairly serious, publication-quality research in animal behaviour, on the side, as a hobby. This was made possible

by a serendipitous event. On my first day at the Microbiology and Pharmacology Laboratory, I was assigned a desk on which its previous occupant had left a copy of India's premier science magazine, *Current Science*, dated 5 August 1974. Opening it casually, I saw an article entitled 'Caste differentiation in the paper wasp *Ropalidia marginata*' by M. Gadgil and A. Mahabal⁸. Perhaps the only reason I read this paper was that the first author was familiar to me. After reading the paper, I suspected that the wasps I had watched with fascination on the windows of the Zoology Department of Central College were *R. marginata*. Gadgil was at the Centre for Theoretical Studies at the Institute and I had met him briefly during the previous year's meeting of the Ethological Society of India. I sought him out to tell him that I knew of what I thought was a large population of the paper wasps that he was studying. We went to Central College on his scooter early the next Sunday morning, and he confirmed that the wasps were indeed *R. marginata*, but disappointed me by saying that he no longer studied them. But then he said, 'if you are interested, however, I can help you study them'. Thus I began to study *R. marginata* (Figure 1), by observing the colonies at Central College and also by rearing some colonies in the Microbiology and Pharmacology Laboratory. Most of my wasp watching had to be done on weekends but it opened up a whole new world to me. Now I added behavioural ecology, sociobiology and evolutionary biology to my reading territory and W. D. Hamilton, E. O. Wilson, Richard Dawkins, John Krebs, Nick Davies, Robert Trivers, Madhav Gadgil and the likes to my list of heroes.

At the end of my Ph D⁹ I had a rather difficult decision to make. I could easily have chosen to spend the rest of my life doing molecular biology. Indeed, it was impossible to imagine that I could ever stop handling my favourite



Figure 1. A typical nest of the primitively eusocial wasp, *Ropalidia marginata*, showing the paper comb with hexagonal cells, many adult wasps and some capped brood (pupae). (Photograph: Thresiamma Varghese.)

petri plates, pipettes, nutrient agar and soft agar, minimal and nutrient broth. I was not only captivated by the intellectual challenges offered by molecular biology but had actually become addicted to the daily routine of experimental work at the bench. And yet the prospect of working full-time on *R. marginata*, of starting an altogether new research programme on its natural history, aimed at understanding the organization and evolution of a tropical insect society, was equally enticing. I made my decision in the following way. If I were to build a career in experimental molecular biology I would much rather do so in a place like the USA where I would be much less limited by access to sophisticated laboratories and modern technology compared to the situation in India. If I chose to initiate a career in animal behaviour and evolutionary biology on the other hand, then it must certainly be in India where I would have access to far richer natural laboratory and be relatively independent of expensive instrumentation and technology. As I was equally interested in both options, the choice really boiled down to living in the USA versus living in India. I was beginning to lean toward the option of India when I sensed an enormous pressure from my professors and my peers against the decision of skipping the mandatory post-doc stint abroad, especially since I only had a domestic Ph D. I was told that it would be suicidal to stay back home and even more so because I was venturing into a new area of work – training abroad was all the more essential they argued. Besides, who would give me a job with neither a Ph D nor post-doc training from abroad? In retrospect I am grateful for this pressure because it hardened my stand. I might easily have taken the USA-Molecular Biology option both because of my passion for the subject and because I so much like to travel and experience different cultures. But the implication that I could not succeed as a scientist without training abroad was impossible not to challenge. I chose to stay back and work in India and have never had cause to regret my decision. True I had been told that I would get an assistant professorship at IISc after a mere two years of post-doc work abroad and I had to wait five years before I was appointed a lecturer. But these things do not really matter in the long run. The novel research programme I initiated and have been able to continue uninterrupted for over 30 years, more than compensates for any such minor disadvantages. I may have lagged behind my peers in position and salary but I believe that I was able to leapfrog ahead of many of them in my research career.

My students and I have had great fun uncovering one mystery after another as we have stayed steadfast in our extreme bias in favour of *R. marginata* as opposed to any number of other fascinating species all around our backyard, begging for attention. As promised, I will now cite three examples from our research before I offer some reflections on the pursuit of science. Each of our research projects begins with a question and I have picked three

out of many fascinating questions we have so far attempted to answer.

Question one: What do the wasps do and why?

I had made rather few observations on the behaviour of *R. marginata* nests during my hobby phase; I had mostly focused on population counts of nests, brood and adults. The world of behaviour was opened up to me by Mary Jane West-Eberhard, who while visiting India, showed me that one could easily mark the wasps with little spots of coloured paint. Watching a nest first without marking the wasps and then after marking them for individual identification is a real revelation. All wasps seem to be pretty much the same when they are anonymous but their individual personalities are starkly revealed when you have them individually marked. To this day, marking the wasps is the first step in our research. And we always use Testor's enamel hobby paint available from the Testor's Corporation, Illinois, USA. Having been made with all the care necessary for use by children and thus being non-toxic, non-smelling and quick drying, these paints come in a variety of colours and constitute perhaps the only item for our research that we must import from overseas. Unfortunately Testor's enamel paint cannot be ordered from the company; they will not send it to India as it is considered inflammable and hence cannot be airlifted. I have had to bring it myself and so I often readily seized upon opportunities to go to the USA, in the past. Now I have many former students visiting India from time to time and I request them to bring me Testor's enamel paints rather than chocolates!

My first challenge was to present objective proof for the existence of the wasp personalities so that even those not watching the wasps and merely reading my accounts are convinced of my claim. I first made a list in plain English of everything that the wasps did – sit, sit with raised antennae, sit with raised wings, walk, inspect cells, feed larvae, be absent from the nest, bring food, bring pulp, bring water, attack another wasp, chase another wasp and so on, the list went up almost to a hundred distinct behaviours. This process is called discretization of behaviour and one can be as fine-grained or as coarse-grained as desirable. Many hours of observation showed that nearly all the wasps performed nearly all the behaviours at one time or another. So the reason why I saw their distinct personalities must be because of quantitative differences in the propensities of different wasps to perform different behaviours. So I read Jeanne Altmann's bible on quantitative methods for sampling animal behaviour¹⁰ and standardized a suite of methods for measuring the proportion of time spent by each wasp in each behaviour (for the relatively longer duration behaviours) and the rates of performance of different behaviours (for the relatively short duration behaviours). In making these

measurements I deliberately ignored the fact that in each colony one wasp was a queen and the others were workers; I treated all wasps as equals. If there were consistent behavioural differences between queens and workers such differences should emerge from the data and their analysis rather than by my imputing these differences beforehand. I took one more precaution of avoiding bias. Which of the 100 behaviours should I focus on? Which behaviours were biologically more important? I decided that I should make no such decisions and let the wasps speak for themselves and tell me which wasp was more important for the colony and which behaviours were more important for me to understand the workings of their society. These decisions were on the one hand easy for me to make, as I was new to the field and naïve on account of my complete lack of training. But on the other hand, it was not easy to publish my work as referees generally discourage new approaches by naïve authors. I ignored the referees, stuck to my decisions and was content to publish wherever I could. This philosophy has helped me throughout my career as I have learnt to take from referees what I think is useful advice, and there often is useful advice, but to firmly reject what I consider bad advice, and I am afraid there is often bad advice too. And it is possible to reject bad advice only if you jettison another piece of bad advice that is often given to you implicitly or explicitly, namely that where you publish your paper is more important than what is in it!

So how do you make the wasps speak for themselves? I measured the proportions of time spent in different behaviours for all members of several colonies, and computed a time-activity budget for an average wasp. Using this mean time-activity budget, I arranged all the behaviours in descending order of time spent in them. To my great surprise it turned out that over 95% of the time of an average wasp is spent in performing only six of the hundred or so behaviours. I chose these six 'top' behaviours for the first step in my analysis. In other words I gave more importance to those behaviours in which the wasps spent more time. If the wasps spent more time in some behaviours then these behaviours must be more important for them. Such a measure of importance was one relatively objective way to choosing behaviours for my analysis—one way of letting the wasps speak for themselves. But the identity of the six top behaviours was, I must say, not very inspiring. The six behaviours which got the top position were sit and groom oneself, sit with raised antennae, sit with raise wings, walk, inspect cells and being absent from the nest. It seemed unreasonable to imagine that the time spent on these six behaviours, of no apparent biological or social significance, can tell us anything important about social organization of the wasp colony. Many people told me as much. That nobody had done anything like this before made it seem more unreasonable than I might care to admit now. Nevertheless, I resisted the temptation and advice to abandon this

'objective' approach. I used data on time-activity budgets for these six behaviours for all the members of two colonies and analysed the patterns of intra-individual variation in time-activity budgets. A visual inspection of the animal \times behaviour matrix showed quite clearly that while all individuals spent about 95% of their time in the same six behaviours the manner in which they allocated their time between the six behaviours was highly variable, reinforcing my suspicion that the differences between the wasps were quantitative rather than qualitative and that the wasps indeed had different personalities (Figure 2).

Yes, there was quantitative variation in the time-budgets of the different wasps in a colony but was this just random noise or was there a biologically meaningful pattern to this variability? Multivariate statistics was clearly needed to discern any pattern in this animal \times behaviour numerical data matrix. I have often found that reading outside one's field is helpful as is speaking to colleagues who study very different subjects; they make you see your own subject in a whole new light and one that is likely to have been overlooked by you and your colleagues in your own discipline. In this instance, I made friends with Sulochana Gadgil and Niranjana Joshi who were developing computer programs to use principal components analysis to discern patterns in and make meteorological sense of analogous quantitative variation in rainfall in India¹¹. I applied their method and found to my surprise that the wasps in a colony can be neatly classified into three distinct behavioural clusters (Figure 3). Yes, there was a nice pattern but did it make biological sense? At first I had no idea. Accepting the classification provided by the computer, I went back to the raw data and examined the behavioural profiles of the three clusters discerned by the principal components analysis. The

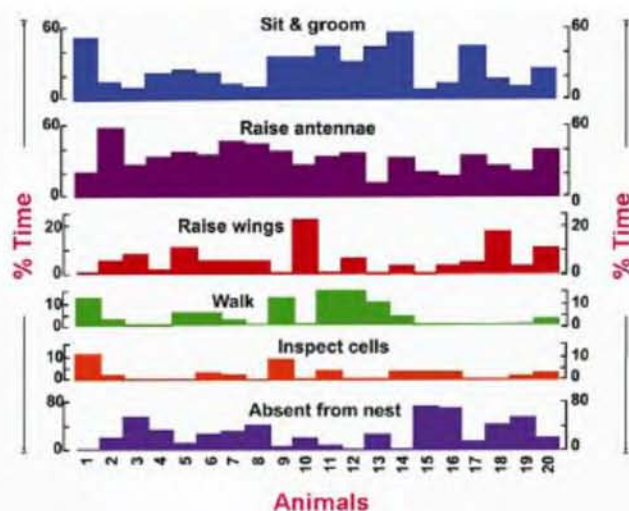


Figure 2. Time-activity budgets of 20 individually identified wasps drawn from two colonies of *R. marginata*. All wasps spend 85–100% (mean \pm SD = 95.9 ± 0.4) of their time in the six behaviours shown. Note, however, that how the wasps allocate their time among these six behaviours is highly variable (redrawn with permission from ref. 12).

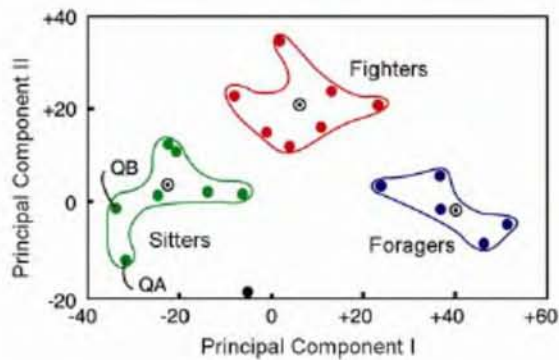


Figure 3. Behavioural castes of *R. marginata*. Twenty wasps are shown as points in the coordinate space of the amplitudes associated with the first two principal components. The points fall into three clusters (or castes) by the criterion of nearest centroid. Circled dot = centroid. Q = queen (redrawn with permission from ref. 12).

result was even more fascinating than the emergence of the clusters. Wasps in one of the clusters spent a great deal of their time (and much more than those of other clusters) simply sitting and grooming themselves, apparently doing nothing of 'importance'. I promptly called them sitters. Wasps in another cluster were similarly characterized by spending time away from the nest. Since wasps typically go out for foraging, I labelled them foragers. Wasps in the third cluster appeared to focus on sitting with raised antennae and I did not quite know what to call them. Then I examined data beyond the time budgets, such as the frequency of performance of relatively rare and short duration behaviours. It turned out that these wasps showed significantly more acts of aggression toward other colony members. I had already seen that wasps sitting with raised antennae were especially alert and able to respond quickly to any disturbance from within or without the colony. Taken together the character of being alert and being aggressive suggested the obvious name of fighters for this cluster. In advanced insect societies, workers that are morphologically specialized to perform different tasks are referred to as castes. I therefore proposed the name behavioural castes for the sitters, fighters and foragers in *R. marginata*¹². It seemed to make sense that the colonies have a forager caste to satisfy their requirements of food and fibre (for nest construction) and perhaps even a fighter caste to deal with internal and external disturbances, a sort of police and army combined. The significance of the sitter caste was less clear. It could contain reserves, yet to be differentiated young individuals or may have some other significance. Be that as it may, there was a much bigger paradox awaiting me.

The reason for deliberately refusing to recognize *a priori* that the queen is a separate or special individual was of course that one could now explore the role of the queen in this system of behavioural caste differentiation, *a posteriori*. Queens in primitively eusocial species are

expected to be the most active, interactive and aggressive members of their colonies because they are known to use physical aggression and harassment, both to suppress worker reproduction and to ensure that workers indeed work for their colonies. Since *R. marginata* can be classified as a primitively eusocial species, on account of the lack of queen-worker morphological caste differentiation and its small colony size, I certainly expected the queens to belong to the fighter caste. Contrary to this expectation, queens in nearly all colonies studied turned out to be sitters. How do these sitter queens get accepted as queens in the first place? How do they suppress worker reproduction and ensure that workers work, without using physical aggression? How and when is behavioural caste differentiation achieved in the life of an individual? How does the colony achieve the right proportions of sitters, fighters and foragers? Why has *R. marginata* evolved such behavioural caste differentiation while other primitively eusocial species do not seem to have done so? These and dozens of similar questions that did not even exist earlier have kept us busy for decades now and answering them has led to a much deeper understanding of the organization and evolution of insect societies. I have recently summarized many of these questions and the answers we have found so far, in my inaugural article invited by the National Academy of Sciences, USA on the occasion of my election as Foreign Associate^{13,14}. It is not my intention to repeat those details here. Instead I will turn to my next illustrative example.

Question two: Why do the wasps in a colony cooperate with each other?

I am often asked why I study social insects and *R. marginata* in particular. There are at least two kinds of motivations for my study. One has to do with the fact that I am an evolutionary biologist. Like all evolutionary biologists I am captivated by Theodosius Dobzansky's claim that 'Nothing in biology makes sense except in the light of evolution'. And like all evolutionary biologists I find good reasons to believe that Darwinian natural selection has been the predominant force that has shaped the evolution of life on earth. I am therefore ever alert to facts and phenomena that seem apparently paradoxical in the framework of Darwinian evolution. It turns out that social insects constitute one such paradox, one that troubled Darwin himself as he described some aspects of them as 'one special difficulty, which first appeared to me insuperable, and actually fatal to my whole theory'. There are several difficulties posed by social insects to Darwinian natural selection but the one we are concerned with here is their instinct of cooperation and altruism. Social insects typically organize themselves into colonies headed by one or a small number of fertile queens while the rest of the (female) colony members function as sterile slaves

devoting their lives for the welfare of the colony, i.e. in service of the queen's reproduction. How does natural selection favour the evolution and persistence of such altruism on the part of the workers? Should altruistic workers who live and die for their queen not disappear and be replaced by selfish individuals who maximize their own survival and reproduction? W. D. Hamilton provided a powerful modification of Darwin's theory of natural selection that potentially solves this problem. Hamilton argued that begetting offspring is not the only way of contributing one's genes to future generations; aiding the survival and reproduction of genetic relatives, with whom one shares genes by descent, can be just as effective or even more effective, depending on the circumstances. Hamilton thus introduced the concept of inclusive fitness that comprises both the direct contribution of genes to future generations by producing offspring as well as the indirect contribution through aiding genetic relatives. When each offspring or relative is devalued by the proportion of genes shared with it, all such contributions become conveniently additive. Hamilton went further and laid down rules for the evolution of altruism. Thus, Hamilton's rule simply states that an altruistic trait will spread in the population if the benefit of altruism is greater than its cost, except that here the benefit should be devalued by the proportion of genes shared between the altruist and the recipient of the altruism. For example if an individual has the option of either being fertile and producing one offspring or being sterile and taking care of two sisters, the latter option would yield more inclusive fitness, thus making possible for natural selection to promote the evolution of sterility. This was perhaps the most important insight since the original formulation of the theory of natural selection by Darwin¹⁵. But Hamilton went even further and realized that the evolution of altruistic sterility so common in ants, bees and wasps is especially facilitated in these very groups of organisms. Ants, bees and wasps belong to the insect order Hymenoptera in which males are haploid and females are diploid. Since males are haploid, they lack meiosis and the associated reduction division and thus produce identical (clonal) sperms by mitosis. Consequently, two full sisters share 0.75 rather than the usual 0.50 of their genes. Hence taking care of even one sister yields more inclusive fitness than producing one offspring. Altruistic sterility was then known to have evolved only once in the entire diploid world while it has evolved at least a dozen times in the Hymenoptera which constitute only about 2% of animal species. This so-called haplodiploidy hypothesis seemed to solve the paradox of altruism in one master stroke.

Hamilton's calculations were based on the assumption that queens in social insect colonies mate with a single male. However if the queens mated multiply and produced daughters who are half-sisters of each other then the argument would break down. Half-sisters, on account

of having different fathers would only share 0.25 of their genes with each other making it necessary to rear at least three sisters to get more inclusive fitness than can be got by producing one offspring. Thus the haplodiploidy hypothesis would break down if the workers in social insect colonies were not full sisters and this could happen if they had different fathers or different mothers. Here then was a simple way to test the haplodiploidy hypothesis. Relatedness among the workers of colonies of *R. marginata* could potentially drop below the theoretically expected 0.75 for at least two different reasons. First, queens might mate multiply and mix sperm from different males to sire daughters (a phenomenon that is labelled polyandry). Second, queens might be replaced from time to time so that coexisting workers might have been produced by different queens (a phenomenon that is labelled serial polygyny). We were able to check out both possibilities. The detection of multiple mating could only be accomplished with the help of biochemical/molecular markers as there are no useful morphological markers to tell apart daughters sired by different fathers. Hence I somewhat reluctantly went the high-tech route. Ironically, neither my training in biochemistry and molecular biology nor my friends and colleagues in these disciplines were of much help in this endeavour. I now recognize in retrospect that this was because biochemists and molecular biologists tend to be too narrowly focused on the problem at hand and often do not cultivate the habit of imagination and painting on a large canvas. One of my distinguished mentors had a large sign in his office which sternly read 'Don't think, experiment!' On the other hand, evolutionary biologists of course take special pride and pleasure in imagination and in addressing larger-than-life questions. It was very revealing that while my colleagues who did gel electrophoresis for a living had never heard of it, evolutionary biologists such as Madhav Gadgil, with absolutely no experience in biochemistry, raved about Hubby and Lewontin's 1966 breakthrough in using gel electrophoresis to make the first quantitative measurements of genetic variability in natural populations of *Drosophila*. I read the twin papers by Hubby and Lewontin^{16,17} and later Lewontin's masterly monograph¹⁸ with great excitement but did not know how to begin to do the required experiments. Another of my mentors H. Sharat Chandra presented me a large bottle of starch which he had brought from the USA in the hope of setting up gel electrophoresis in his kitchen. I played around with this and a locally fabricated electrophoresis unit but made little progress. I eventually succeeded by enlisting the help of my friend and colleague M. S. Shaila of the Department of Microbiology and Cell Biology and by hiring a post-doc K. Muralidharan and an able technician Priti Roy. We used the simplest possible methodology and genotyped wasp mothers and their daughters at a few non-specific esterase loci. Because males are haploid, genotyping mothers and daughters allowed us to reasonably

infer the genotypes of fathers or at least estimated the minimum numbers of fathers needed to sire the observed daughters. We found that *R. marginata* queens mate with 1–3 different males and the average relatedness among their daughters thus drops from the theoretically expected 0.75 to about 0.50, thus entirely negating the advantage of haplodiploidy for social evolution, as predicted by Hamilton¹⁹.

With regard to serial polygyny, I was happily able to go back to simple observational methods, with a large team of students and assistants including K. Chandrasekara, Swarnalatha Chandran and Seetha Bhagavan. All we had to do was to keep meticulous records of all eggs, larvae, pupae and adults and note the identity of the egg layers at all times. This was easily accomplished by making a map of the nest and noting the position of each item of brood in different cells, marking all eclosing adults and making brief daily observations to know whether the old queen continued or had been replaced. With this we obtained information on the genealogical relationships of all eggs, larvae, pupae and adults. From these data, we constructed pedigrees for queens, the first royal pedigrees for an invertebrate, as I like to say (Figure 4). To our surprise, we found that queens can be replaced quite frequently and not only by their daughters but also by their sisters, nieces and their cousins. Even more surprising was the observation that a queen replacement had little impact on the workers; workers who were daughters of an overthrown queen simply continued to work for her successor as if nothing had happened. Although queens live on average much longer (about 80 days) than the workers (about 30 days), the range of their respective life spans is

quite similar with some queens living only for a week and some workers living for several months. Thus there was considerable overlap between workers and brood of different matrilineal lines so that the brood turned out to be the brothers, sisters, nieces and nephews, cousins, cousins' offspring, mother's cousins, mother's cousins' offspring, and even mother's cousins grand-offspring, of the workers who cared for them. I must confess that we were totally unprepared to find that *R. marginata* colonies would have such a complicated family genetic structure; I am very fond of saying that *R. marginata* would put any Indian joint family to shame! Taking into account the combined effects of polyandry and serial polygyny, we computed worker-brood genetic relatedness in *R. marginata* which ranged from a maximum of 0.75 to a minimum of 0.0165 and when averaged over the entire period of study it ranged, from 0.22 to 0.44, for the four colonies. These results completely took the sail out of the haplodiploidy hypothesis. Considering only genetic relatedness, it was indeed more profitable for the workers to leave their natal colonies and raise their own offspring rather than to stay back and work for their queens. But perhaps the low mean intra-colony genetic relatedness caused by polyandry and serial polygyny does not matter because workers may possess well developed intra-colony kin recognition abilities and each worker may be able to identify her full sisters ($r = 0.75$) and dispense altruism selectively, i.e. nepotistically, and thus regain the advantage of genetic asymmetries created by haplodiploidy. To check out this possibility we embarked on a major research programme to study nestmate and kin recognition in *R. marginata*. To make a long story short we found no evidence for intra-colony kin recognition and therefore the haplodiploidy hypothesis could not be rescued.

The demise of the haplodiploidy hypothesis left an uncomfortable vacuum. Although several other factors that might promote altruistic behaviour could possibly be thought of, it was hard to imagine a factor as unique to the Hymenoptera as haplodiploidy that it can also explain the multiple origins of eusociality in that insect order alone. The factors that came closest to meeting this requirement were demographic ones such as long and variable life spans, highly variable fertilities, prolonged dependence of offspring on parental care and long and variable delays in achieving reproductive maturity. These are factors that can make altruistic sterility accompanied by dependence on indirect fitness, an attractive option. More importantly, these are factors that make it profitable for some individuals to focus on direct fitness and others to focus on indirect fitness creating just the kind of intra-species variability that is the hallmark of social insect species. Not surprisingly, other researchers were also thinking along similar lines. I have long had the habit of sending hard copies of my publications, about once a year, to friends, family and colleagues – it's my way of



Figure 4. A pedigree of queens in a colony of the social wasp *R. marginata*. This means that Q2, Q3 and Q4 were daughters of Q1, and Q5, Q6 and Q7 were daughters of Q3 and so on. The question mark indicates that the relationship of Q2 alone to Q1 was somewhat doubtful. Of the two numbers in parentheses the first one indicates the tenure in days of each queen and the second one indicates the number of offspring she produced during her tenure (reprinted with permission from ref. 15).

greeting them and staying in touch. Fortunately most people on my mailing list do not mind this unsolicited mail and many even write and thank me and some reciprocate with reprints of their own publications. As part of this ritual I once received in 1988, a bunch of reprints from my friend and colleague Joan Strassmann of Rice University in Texas, USA. In addition to several of her own interesting papers she had taken the trouble of including a manuscript 'in press' with *PNAS*, entitled 'The evolution of eusociality: reproductive head-starts of workers' and authored by her husband and colleague, David Queller. In this paper, Queller had argued that a worker has a 'reproductive head-start' because 'there are already young of various ages present on her natal nest' so that 'her efforts can immediately result in some of these reaching the age of independence'. Casting this idea into a simple mathematical model, Queller had calculated that his head-start hypothesis was an even more powerful force than the genetic asymmetries created by haplodiploidy in promoting the evolution of altruistic sterility in workers of social wasp species belonging to the genera *Polistes* and *Mischocyttarus*. He had shown that workers would break even with their solitary counterparts in spite of rearing brood with an average genetic relatedness to themselves as low as 0.03–0.09 or even if solitary foundresses are 6–17.4 times more efficient at rearing brood than the workers. Given my experience with *R. marginata*, which suggested repeatedly that I should look beyond the role of haplodiploidy, I was immediately attracted to Queller's head-start hypothesis. Such a powerful force favouring sociality as Queller's head-start hypothesis had seldom been suggested. And it was obvious that head-start hypothesis was ideal for a comprehensive exploration of the role of demography in the evolution of eusociality.

But it was equally obvious that there was a serious problem with the head-start hypothesis as formulated by Queller. The problem was that because 'there are already young of various ages present' on the natal nest of a worker, Queller gave full credit for the rearing of an offspring from egg to adulthood to a worker that may have eclosed only one or a few days before the completion of development of the offspring and thus cared for it only for that one or those few days. This assumption overlooks the fact the queen or other workers performed all the duties of rearing that offspring from egg to the stage at which the worker in question found it. The credit (contribution to fitness) for this part of the work should go to the queen or the other workers, not to the worker that eclosed later. The contribution to any worker's fitness should clearly be in proportion to her contribution to the rearing of each offspring; otherwise the full fitness benefit for rearing a given larva gets assigned to several workers. It was obvious to me that the magnitude of the advantage provided by Queller's head-start hypothesis is partly due to the unfair advantage that he gave to workers in formulating his model.

However, it was also obvious to me that there must be an advantage that a worker has over a solitary nest foundress, on account of having access to offspring that have already completed part of their development, leaving somewhat less work for her to do. I argued that if any worker works for part of the developmental period of the brood and dies before bringing the brood to independence, and if other workers can continue the work left unfinished, the dead worker should be assigned credit (fitness) for her fractional contribution to the survival and growth of the brood, irrespective of whether she cared for the brood at the beginning, middle, or later part of the brood developmental period. In other words, a solitary foundress has no assured returns for her labour and she must necessarily survive for the entire brood developmental time to get any fitness at all. But a worker in a multi-female nest has relatively more assured returns for her labour as she can get partial fitness even if she survives for a part of the brood developmental time. Hence I called this idea the advantage of 'assured fitness returns'²⁰. Computing the fitness of workers in proportion to their duration of survival, I computed that in *R. marginata*, owing to the advantage of assured fitness returns, workers would break even with solitary foundresses in spite of rearing brood related to them by a mere 0.14 or in spite of solitary foundresses being able to perform 3.6 times more work per unit of time. The more realistic assured fitness returns model is less powerful than Queller's unrealistic head-start hypothesis but powerful nevertheless; it is 2.4 times more effective than the haplodiploidy hypothesis in driving the evolution of altruistic workers. In a subsequent publication, Queller reformulated his model following my ideas but it amuses me to see that, with the exception of a single publication²¹ most researchers cite papers containing Queller's original, uncorrected head-start hypothesis and my assured fitness returns, side by side, without taking the trouble of understanding the difference between the two and taking a stand one way or another. My already low esteem for citation data has hit rock bottom! Be that as it may, the assured fitness returns model gave me much satisfaction as it provided at least a partial solution to a major unsolved problem in evolutionary biology.

I have since been able to incorporate various other factors along with assured fitness returns into a unified model for the evolution of eusociality. The days during the development of this unified model were heady times for me. The idea of a unified model that simultaneously considers various factors that might act in concert in promoting the evolution of altruistic workers is without doubt most attractive. But what exactly do we want the unified model to do for us? We already know that altruistic workers have evolved and also that selfish, solitary nesting also remains. Ideally we would like to predict, for each eclosing female wasp whether she should leave her mother's nest and opt for a selfish solitary nesting

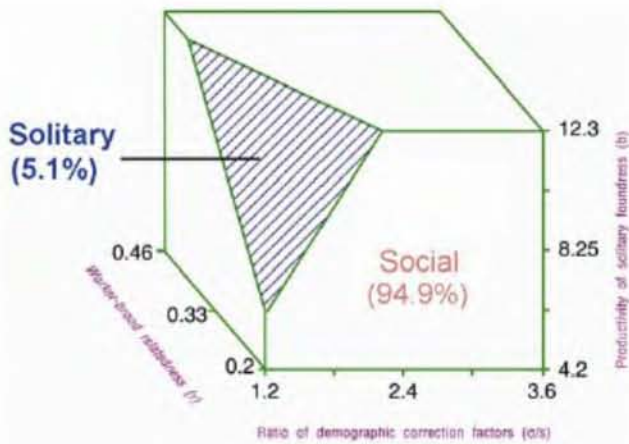


Figure 5. A graphic illustration of the unified model showing the parameter space where worker behaviour is selected (unshaded) and the missing chip of the block where solitary nesting behaviour is favoured (reprinted with permission from ref. 13).

strategy or whether she should stay back in her mother's nest and spend the rest of her life assisting her in producing more offspring. Although we now know that *R. marginata* females differ in their ability to succeed in these different strategies and that they seem to 'know' this and that they choose their strategies based on their abilities²², I think we are very far indeed from devising such a model and making empirically testable predictions for each wasp. So I became a bit less ambitious and attempted to construct a unified model that makes empirically testable predictions about what proportion of the population of eclosing wasps should opt for a selfish solitary nesting strategy and what proportion should opt for an altruistic worker strategy. At this I was surprisingly successful – I built a unified model that predicted that only about 5% of the population should opt for a selfish solitary nesting strategy while about 95% of the wasps should opt for the altruistic worker strategy (Figure 5). The empirical data on this matter are remarkably in close agreement¹³.

Question three: How do the wasps choose their queens?

Concerning any animal behaviour, we can and should, ask at least two kinds of questions. Why has natural selection favoured the evolution of that behaviour, and how do the animals manage to perform that behaviour, i.e. what proximate factors make that behaviour possible. In the context of the evolution of insect societies, we should not only ask how natural selection has promoted the evolution of a sterile worker caste and efficient division of labour, but we must also ask what proximate physiological and other mechanisms permit these insects to accomplish their division of labour and other remarkable feats. In the past there was a tendency to de-link the

investigation of these two kinds of questions but today we realize that the two kinds of questions go hand in hand and need to be investigated in parallel. In this spirit I try to divide my time fairly between the so called 'How' and 'Why' questions. Thus I will turn next to a set of 'How' questions, in my third and final example.

R. marginata colonies are headed by a single queen who monopolizes all reproduction as long as she is the queen. However the queen appears to be under constant scrutiny and is periodically replaced by one of the workers. The identity of the queen's successor and the mechanism by which she is chosen are of obvious interest and have therefore been the focus of our attention. Because natural queen replacements are rare and unpredictable, we have designed experiments to simulate queen replacements. Typically we study a natural undisturbed colony with its original queen, experimentally remove the queen and then study the queen-less or orphaned colony. We are also able to replace the original queen and study the same colony, a third time. The behaviour of the wasps upon queen removal and queen replacement is most unexpected and interesting. Within minutes after queen removal, the reasonably peaceful *R. marginata* colony is transformed into a highly aggressive society. The queen-less colony shows a several fold increase in aggression (we refer to this aggression as dominance behaviour) compared to that of the queen-right colony. Even more striking, dominance behaviour returns nearly to the original level once the queen is returned. More surprisingly, all the increased dominance behaviour in the absence of the queen is shown by a single worker who steps up her levels of dominance behaviour some ten-fold relative to her own levels on day one and brings down her aggression after the queen is returned. In experiments in which we did not return the queen, the worker who stepped up her aggression as soon as the queen was removed, gradually brought down her aggression, developed her ovaries and went on to become the next queen of the colony. We therefore call this hyper-aggressive worker as the potential queen (PQ) until she lays her first egg when she would of course qualify for the title queen. So how is the PQ chosen and when is she chosen? This is the question one of my graduate students Sujata Kardile (now Sujata Deshpande) set out to answer. In spite of her many experiments explicitly designed for the purpose, we have so far failed to predict the identity of PQ as long as the original queen is still present on the nest. After making detailed observations on queen-right colonies, Sujata removed the queen, identified PQ and then went back to data collected prior to queen removal in an attempt to understand what was unique about PQ. There appears to be nothing unique about PQ. She is not unique in her behaviour, in her dominance rank, in her body size, in her age and not even in the state of her ovarian development. Thus the verdict so far is that we cannot predict the queen's successor before removing the queen.

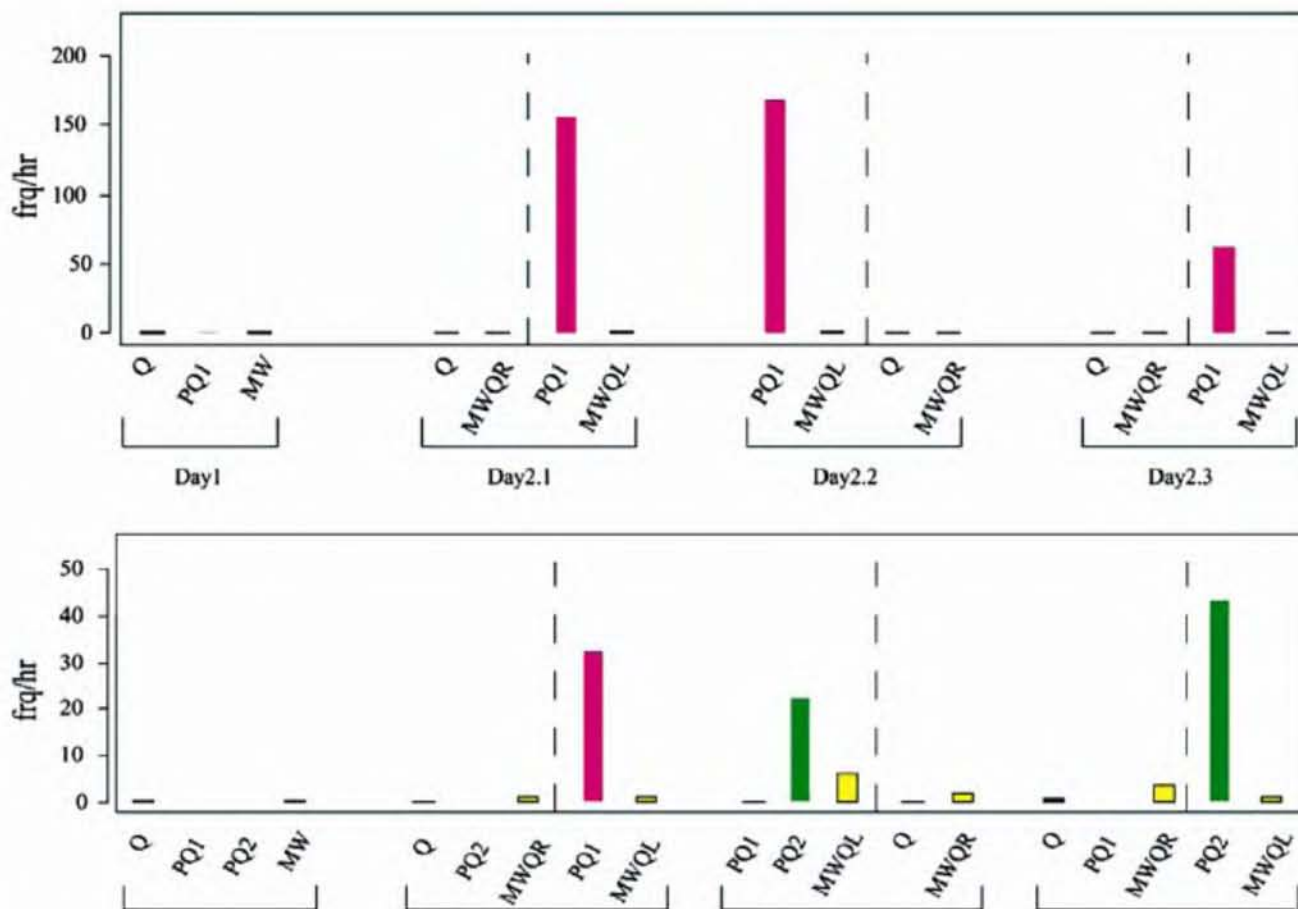


Figure 6. The Q-PQ exchange experiment designed to check if the wasps know the identity of the queen's successor. Upper panel: A typical experiment in which the PQ1 was the cryptic successor. The frequency per hour of dominance behaviour exhibited by the queen, PQ1 and max worker (defined as the worker showing maximum aggression) on day one in the normal colony, and on the queen-right and queen-less fragments in the three sessions on day two are shown. Lower panel: A typical experiment in which the PQ2 was the cryptic successor. The frequency per hour of dominance behaviour exhibited by the queen, PQ1, PQ2 and max worker on day one in the normal colony, and on the queen-right and queen-less fragments in the three sessions on day two are shown. See text for details (reprinted with permission from ref. 23).

Although we cannot predict the identity of the potential queen, the facts that (i) only one individual steps up her aggression after queen removal, (ii) the swiftness with which she does so and (iii) she is unanimously accepted by the rest of the workers, led us to suspect that, just as in other primitively eusocial species, there may also be a designated successor to the queen in *R. marginata*, although she may be 'cryptic' to us in the presence of the queen. We therefore designed another experiment to test such a 'cryptic successor hypothesis'. In this experiment, we cut a nest in half and separated the two halves by a wire mesh partition and randomly introduced half the workers on one side and the remaining half on the other side. The queen was introduced randomly to one of the sides. In such an experiment the workers on the queen-less side fail to perceive the queen across the wire mesh so that they behave like a queen-less colony. This means that one of the workers on the queen-less side becomes a hyper-aggressive PQ and eventually a queen. But as soon as a PQ became evident on the queen-less

side of the wire mesh, we exchanged PQ and the queen from side-to-side, leaving the workers undisturbed. The logic of this 'Q-PQ exchange experiment' is as follows. Since the workers are randomly distributed between the two sides, the cryptic successor, if there is indeed one, has a 50% chance of being on either the queen-right (QR) or the queen-less (QL) side. In those experiments where the cryptic successor happened to be in the QL side, she would become a PQ (we call her PQ1) and, being the true successor, she should be acceptable to the workers on both sides even when she is moved from side-to-side. And in those experiments where the cryptic successor happened to be on the queen-right side just by chance, a different individual should become PQ1 on the queen-less side due to the absence of the legitimate successor on her side. Hence, this PQ1 should be unacceptable to the workers on the opposite side when she is moved to that side. Instead, the real cryptic successor should now become the new PQ (we call her PQ2). Finally, PQ2 should remain unchallenged on both sides if

she is moved from side-to-side. Thus PQ1 should be acceptable to the workers on both sides in about half the experiments and PQ2 but not PQ1 should be acceptable to workers on both sides in the remaining half of the experiments.

This is a difficult experiment to perform but my graduate student Anindita Bhadra has managed to perform it eight times. In three of these experiments, the first PQ was accepted on both sides and in the remaining five experiments, the second PQ was accepted on both sides (Figure 6). We therefore concluded that there is indeed a designated successor to the queen in *R. marginata*, as in other species. But we refer to her as a cryptic successor because we cannot identify her in the presence of the queen by the same criteria that are adequate to identify PQ in other primitively eusocial species. An important feature of our results was that neither PQ1 nor PQ2 ever received a single act of aggression from any individual, although they themselves showed high levels of aggression. Thus when we say PQ1 was unacceptable when we moved her to the opposite side, we simply mean that she, on her own, stopped being aggressive and went back to work even though she was never challenged by anybody, not even by PQ2. Hence we argue that the cryptic successor is 'known' to the wasps even though we cannot identify her in the presence of the original queen²³. In summary, we do not know the identity of the queen's successor but we know that the wasps know who she is! In many ways I find the fact that the wasps know something we do not, rather charming and I dare say even satisfying. That's what happens to you if you are truly in love with (and in awe of) your study animal. I think I have been extremely fortunate in having had such an intimate and life-long relationship with my study animal – for over four decades. Not many are so blessed especially among those that choose to study an insect or a worm.

Some reflections on the pursuit of science, especially in India

My research strategy

Like most people I have over the years, developed my own strategy for doing scientific research. Most of us are somewhat embarrassed to articulate our research strategy in public. But I have found that such abstinence from public articulation is also a license to avoid even private introspection and hence this unabashed discussion of the principles that govern my research strategy. A key rationale of my research strategy is the realization and acceptance of the fact that making a significant new discovery in science is extremely difficult. It must be extremely difficult as it must be extremely rare and that must in turn be true, otherwise it would not be a significant new discovery in the first place. It follows then that business as usual

will not do; one cannot merely work very hard and hope for the best. One has to consciously and continuously position oneself so as to be poised to make new discoveries, or at least to stay ahead of your competitors. I believe that there are at least four ingredients needed to so poise yourself. One is to avoid fashions and crowds and choose a research problem that is obscure today but will become well known tomorrow, and if that transition from obscurity to fame is on account of you, so much the better. If you can't find a totally uninhabited territory, try to bring a novel perspective to the problem and one way to do so is to read outside your field and approach the problem from a new angle. Third, capitalize on your advantages and avoid your disadvantages as far as possible. And finally, I have always found it very helpful to continuously justify, at least to myself, the choice of research problem from first principles. I will illustrate this below in some detail.

Why I am an organismal biologist?

Biology today is an incredibly rich and complex discipline and can be practised in many different ways. Life processes are organized in many different hierarchical levels. At one level we have ecosystems, forests, populations and then the individual organism, which can be studied in its own right. At the other extreme, if you go deep inside an individual, you have cells, tissues, organs, organelles and finally, molecules. The ways of doing biology at these different levels of organization can be so different that they can be mutually incompatible and often mutually incomprehensible. While it is obvious that studying life processes at all possible levels of organization is necessary and interesting, this needs different classes of biologists trained in rather different methodologies, and driven by quite different philosophical orientations. All this makes it almost impossible to maintain a reasonable balance between the different kinds of biologies. This is true at the national and even international level, not to speak of the impossibility of maintaining a balance within an institution or department of biology.

For simplicity I will broadly classify biology into sub-organismal biology which includes cellular and molecular biology, and organismal biology which includes population biology, behaviour, ecology and evolutionary biology. Evolution should of course cut across these barriers but even today evolution is more often practised as a discipline among organismal biologists than among cell and molecular biologists, although this situation is gradually changing. Once dichotomized in this way, we find a major practical difference in pursuing sub-organismal and organismal biology. Practising cell and molecular biology almost always requires significant technological augmentation of our own sensory capabilities – we need fine

chemicals and instruments to isolate the components we wish to study, centrifuges, chromatographs and the like for their separation and microscopes, spectrometers and the like to visualize them. This inevitably makes the pursuit of sub-organismal biology a technology intensive and financially expensive proposition, leaving little scope for the amateur or layman to participate. Relatively speaking, organismal biology deals with structures and phenomena that are within the perception range of our own sensory capabilities. There is a great deal we can do without special isolation, separation and visualization, and therefore without the need for sophisticated technology and large research grants, indeed often without the need for laboratory experimentation, leaving ample scope for laymen and amateurs to make significant contributions – just think of the life time's work of Charles Darwin. There are two additional features of organismal biology that I am yet to mention. These are that it is facilitated by access to a rich biodiversity and is very labour intensive. The things that organismal biology is independent of (technology, fine chemicals, money) and those that it is dependent on (biodiversity, manpower) together make it just the right choice for someone like me in a developing country, in my attempt to stay at the cutting edge of international science. This I believe is a necessary and sufficient explanation for why I am an organismal biologist²⁴.

Why do I study the evolution of social behaviour?

I want to take this justification a little further. Following Theodosius Dobzansky I truly believe that 'nothing in biology makes sense except in the light of evolution'. It is also easy to argue that ecology is the foundation of organismal biology. Thus I am in search of a problem at the interface of ecology and evolution. Ecology is the study of the interaction between living organisms and their environment. The environment of all living organisms consists of both non-living components as well as living components. It turns out that there is a rather striking difference between the kinds of interactions that take place with the living and the non-living components of the environment. When organisms interact with their non-living environment, there may be some feedback from the environment but certainly there is no such thing as arms race. But organisms interact with the living component of their environment, there is considerably more feedback between the environment and the organism and often there is an arms race. In my opinion this feedback and arms race makes the study of the interaction between living organisms and the living component of their environment much more complex and therefore much more interesting.

Similarly, interactions of living organisms with the living component of their environment, i.e. interactions between different organisms can be broadly classified

into interaction of an organism with members of its own species and with members of other species. And again there are interesting differences. When organisms interact with other members of their own species, we can witness, at least in principle, all possible kinds of interactions, viz. selfish, cooperative, altruistic and spiteful. But when an organism interacts with members of other species, we can only witness selfishness and occasional cooperation, not altruism and probably no spite. Charles Darwin famously said, 'If it could be proved that any part of the structure of any one species had been formed for the exclusive good of another species, it would annihilate my theory'. Therefore, I would like to study the much richer and more complex patterns of intra-species interactions. Now, once I decide to study intra-species interactions, I can study selfishness, cooperation, altruism and spite. And how do I choose among these four? Selfishness is common and most easily explained in the framework of Darwinian natural selection. Cooperation is also common and also rather easily explained as both parties benefit. Altruism on the other hand is reasonably common but rather difficult to explain. Indeed altruism is considered to be of the two major unsolved paradoxes in evolutionary biology (the other being sex). Altruistic individuals, on account of their lower rates of survival and reproduction as compared to selfish individuals, should be eliminated by natural selection but sometimes they persist and that is the paradox. And finally spite, which is also difficult to explain, is however extremely rare. Indeed, its very existence in non-human animals is disputed. Now if I have to choose among these four, I will of course choose altruism, because it is common, which means I can study it easily and it is difficult to explain, which means there is a challenge to be met. And that's what I study, the evolution of altruism, more broadly speaking, the evolution of social behaviour. So this is my justification for what I do. And I think it is very useful to constantly make this justification. It is also good to make it in private, but that is not sufficient. I think it is necessary to make it in public, convince someone else because convincing oneself is rather easy while convincing others is much harder.

*Why do I study *Ropalidia marginata*?*

While interviewing potential candidates for our departmental Ph D programme every year, I usually ask the candidates what they would like to work on if they had complete freedom in the matter. Some years ago, an unusually determined student gave me a firm answer: he wished to work on lesser cats, asking whatever questions he might be able to and using whatever methods that might work. I tried to argue with him, reminding him that lesser cats were extremely hard to study – they were nocturnal, shy and difficult to locate, let alone observe and obtain quantitative data. Why not work on an easier animal

with which you can ask more sophisticated questions, I pleaded. No, he was adamant – lesser cats it would be, if he had any choice at all. His determination has stayed in my memory ever since. Other students have given me other kinds of answers, though I can recall none as determined as the young man in love with lesser cats. Some students gave primacy to the research field or question and were quite flexible about the study animal and methods to be employed. Others were sold on a method such as computer simulations or field biology, but were quite catholic about the exact questions or of the model organism.

No one has taught me more than students and their various answers have given me much food for thought concerning the sociology of science. How *do* people choose what topic to study, what animal to use and what methods to employ, and how *should* they choose? As a result of much brooding spurred by the responses of students that I interview every year, I have crystallized my personal prejudice as follows: 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 be slaves at the service of the question and the animal rather be the masters that dictate what we do. Nevertheless, I must confess that the research question and the model animal are hard to prioritize. I think this is primarily because, though of greater importance, the question is abstract and kind of ‘dead’ but the study animal is alive and often rather cute. It is hard not to fall in love with your study animal. But is that a bad thing? I do not know, but I have been in love with my study animal for over 30 years and no harm seems to have come of it so far.

I have already described the circumstances under which I chose to study the tropical, old world, primitively eusocial wasp, *R. marginata*. Having begun to study it I must confess I have fell absolutely in love with it. I have been stung dozens of times but never complained. I guess that is what love does to you. I once had a letter from a fellow wasp researcher who said that he had just arrived in the Philippines and had the great pleasure of being stung by *Ropalidia*, for the first time in his life! But of course the real beauty of *R. marginata* comes from its utility as a study organism. The genus *Ropalidia* itself is unique and remarkable, comprising both primitively eusocial and highly eusocial species with unparalleled diversity in colony sizes, and social biology. There are two quite different ways of utilizing the power of the genus *Ropalidia* to unravel the mysteries of social evolution. One is to capitalize on the diversity within the genus and undertake comparative studies of different species, a method of great power in modern evolutionary biology. The other is to concentrate on a single species and conduct detailed analyses of cooperation and conflict and assess the costs and benefits of social life. I have chosen the latter option which is of course an important reason for my developing such an affinity for my study animal.

Indeed, *R. marginata* has turned out to be a providential choice. Its small colony size, absence of morphological caste differentiation, coexistence of single and multiple foundress associations, multiple behavioural options available to eclosing females, all make *R. marginata* ideally suited for investigating the evolutionary forces that promote social life. But what makes *R. marginata* even more special is that its tropical address makes possible a perennial indeterminate nesting cycle with mortal wasps forming potentially immortal colonies with frequent turnover of workers and occasional turnover of queens, providing a perpetual stage for these wasps to play out their games of war and peace. If *R. marginata* has been priceless in investigating the evolutionary causes of sociality, it has been even more crucial in understanding the proximate mechanisms that make social life possible as will be evident in the few examples from my research that I have described above. Answers to many more fascinating questions are being constantly revealed as I now have a team of students similarly smitten by the beauty of *R. marginata*.

While these answers come in unabated, it seems most unreasonable for me to switch to the study of any other species, in spite of the lure of many fascinating species that my surroundings are endowed with. It is unlikely that I will ever find any reason to abandon my first love. I must confess that we do turn from time to time to the congeneric *Ropalidia cyathiformis* but I keep emphasizing, much to the chagrin of my students who devote themselves to the latter, that my only interest in *R. cyathiformis* is in using it to better understand *R. marginata*! This claim is easy to substantiate. *R. cyathiformis* turns out to be a typical text-book example of a primitively eusocial species. Its queens are impressively aggressive, occupying the alpha position in the colony’s peck order and appear to suppress worker reproduction through physical aggression and regulate worker foraging through centralized top-down control. The more we show the world that *R. cyathiformis* behaves like a typical primitively eusocial species even in our own hands, the more credible our unusual claims about *R. marginata* will be, thus justifying my slogan ‘*R. cyathiformis* in the service of *R. marginata*’²⁵.

I expect *R. marginata* in turn to be on duty for a long time in the service of science and as the object of my hobby.

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Is spatial occurrence of microsatellites in the genome a determinant of their function and dynamics contributing to genome evolution?

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The non-random distribution of microsatellites in the genome has been implicated in a number of cellular and evolutionary activities. Recently, microsatellites have gained much attention due to their suggested association with cancers, ageing and various other metabolic disorders. Microsatellites are thought to have evolved mainly through polymerase slippage with variable mutation rates. It is difficult to develop a typical molecular evolutionary model that may describe genomic dynamics of these sequence elements. Microsatellites may also be the accidental sites of action by selection forces in events like genome divergence and speciation. Substantial evidence is available to describe various life stages of microsatellite evolution. This review addresses the current state of knowledge suggesting interrelationships between genomic locations, functions and evolutionary dynamics of microsatellites, and their subsequent implications in genome evolution.

Keywords: Genome evolution, life-cycle concept, microsatellites, microsatellite instabilities, mutation models.

AVAILABILITY of whole genome sequences and a wealth of published literature reporting the analysis of genomic sequences facilitate studies that aim at understanding various aspects of genome organization and evolution in different forms of life. The present day eukaryotic genomes pack a bulk of non-coding DNA embedded with protein coding regions. A part of this non-coding DNA plays a regulatory role, whereas the other part simply provides structural stability to the chromosomes.

Repetitiveness of nucleotide sequences is an important feature of all genomes, however, the extent to which it occurs within genomes varies greatly. The only consensus reached so far is that the amount of repetitiveness exceeds the expected values of repetitiveness¹. Repetitive sequences are now known to play important roles in a cell, define genome structure and drive the adaptive evolution of an organism²⁻⁴. These sequences are broadly classified into interspersed repeats and tandem repeats,

and may constitute a significant proportion of some genomes⁵⁻⁷. Tandem repeats are broadly classified into satellites, minisatellites and microsatellites, and mainly distinguished on the basis of the length of the repeating unit. Here, we critically overview the genesis and propagation of microsatellites and how the evolutionary events involving microsatellites affect the genomic transitions leading to major changes including genetic drift and speciation over long periods.

Microsatellites are remarkably constituted of small repeating units, 1–6 bp in length. Such a unit formation is structurally simple and therefore these repeats are also called as simple sequence repeats. These sequences constitute hypervariable regions of the genome and undergo structural changes through addition or removal of repeat units or through point mutations therein^{8,9}. The latter event can cause imperfections in these arrays, thus leading to the formation of perfect and imperfect microsatellites¹⁰. The idea whether microsatellites are evolutionary junks, or useful sequences that are repeated throughout the genome has been a topic of debate in the scientific community. Evidences are being gathered in favour of the hypothesis that the simplicity of these sequences in itself is a useful attribute of the genome³ and also that they are strategically placed in the genomes. However, a recent study by Buschiazzo and Gemmell¹¹ indicates that for most of the microsatellites, survival in mammalian genomes is only by chance and there are no evolutionary designs behind their conservation over long evolutionary periods.

Genomic location, functions and hypervariability

Considering that not all of the microsatellite motifs have uniform distribution in the genomes¹²⁻¹⁶, they are likely to be involved in different genomic activities with defined biological roles. Whether such roles are dictated by genomic location and motif characteristics of the microsatellites or by the specific genomic requirements⁴ is not precisely clear. Based on their genomic locations, we have grouped microsatellites as gene-associated, mobile element-associated, telomere-associated, centromere-associated, and microsatellites present elsewhere in the

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