More Fun Than Fun: Strife in the Harmonious World of Honey Bees, Part 2

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A portion of the cape honey bee (Apis melliferacapensis) nest showing eggs laid not by the queen but by parasitic workers. These eggs can develop into future queens. Photo: Benjamin Oldroyd

RAGHAVENDRA GADAKGAR

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

- *It is a striking irony that the study of strife in honey bee colonies, however rare, is helping us understand how strife is prevented under most conditions.*
- *It is often true in biology that we have to break the system or find a naturally occurring dysfunctional situation to understand the functional one.*
- *This underscores the need to pay attention to exceptions and results that seem to contradict the prevailing paradigm.*

Last month, we saw in part one of 'Strife in the Harmonious World of Honey Bees' that although honey bees are renowned for their harmonious cooperation and efficient colonial life, there is nevertheless an underlying scope for conflict. Such conflict takes the form of *disagreement* over who should produce the males of the colony – the queen or the workers. We saw that this conflict is all but suppressed by workers policing each other to prevent drone production and also by apparent self-restraint on the part of most workers.

We also saw that not all workers show adequate self-restraint and have some trick up their sleeves to evade policing by other workers. We saw that by a willingness to question the long-held assumption that workers produce much less than 1% of the drones, and by conducting a careful new study, Madeleine Beekman, Benjamin Oldroyd and their colleagues found that workers may produce as much as 4-6% of the drones.

The discovery of such 'cheating' by worker bees was the culmination of a suspicion that had been building up for a quarter of a century.

In science, undertaking research with the explicit goal of calling into question, even deliberately hoping to overturn the work of previous researchers, is not an act of unkindness or meanness. The essence of science is that everything should be constantly questioned and examined for its continued validity. Science is a work in progress and needs to update itself continually. For this reason, we honour and venerate scientists but not their theories.

Anarchy in the hive

On October 27, 1994, a group of scientists from the School of Genetics and Human Variation, La Trobe University in Australia, published a sensational paper entitled 'Anarchy in the beehive'. The group included Benjamin Oldroyd, whom we met in part 1 of this article, two other scientists, and the late Ross H. Crozier, well-known for his pioneering work on the evolutionary genetics of social behaviour. I vividly remember the excitement of reading this paper when it was first published. The manner in which they had discovered anarchic honey bees was as interesting as the phenomenon itself.

To detect whether workers cheat, i.e. whether they lay eggs even in the presence of a healthy queen, we need to determine whether a given egg was laid by the queen or the worker. While this can be done with fancy tools using molecular markers, where does one begin? Worker reproduction is expected to be quite rare, and only a very small percentage of the drone-producing eggs are expected to be laid by the workers.

Oldroyd and his colleagues devised a remarkably clever trick to solve this problem. At least for me, cleverness is significantly enhanced if the trick is simple and costs nothing – when it is based on thinking out of the box rather than acquiring some new expensive technology. Such, indeed, was their trick.

Beekeepers keep honey bees in wooden boxes. The bees they keep (*Apis mellifera* in Europe, America, Africa and Australia; *Apis cerana* in Asia) normally nest in cavities in trees or rocks, and they build several parallel wax combs with cells on both sides. Beekeepers mimic this situation by providing several wooden frames in their boxes. Although the combs are at only one level in the natural colonies, beekeepers place one or more additional boxes on top of the basal box and let the workers move freely between the upper and lower boxes.

The queen, however, is confined to the lower box by the simple trick of placing a queen-extruder between the lower and upper boxes: its holes are large enough to let the workers through but not the much larger queen. The frames in the upper box are used to extract honey. Because the queen cannot visit the upper box, she lays all her eggs, male-destined and female-destined, in the frames in the lower box. And because workers normally do not lay eggs, there would be no eggs above the queen-extruder.

Wooden hives used to house honey bees. Photo: osiristhe, CC BY-ND 2.0

Thus, the honey extracted is not mixed up with the eggs. This is, of course, very convenient because the honey is pure vegetarian!

It occurred to Oldroyd and colleagues that this separation of eggs and honey can come in handy to discriminate between queen-laid eggs and worker-laid eggs. They argued that if any eggs are found above the queen extruder, they are likely laid by workers. Brilliant!

But how many hives will you inspect to look for the improbable occurrence of worker-laid eggs above the queen extruder? The answer is crowdsourcing! Like all good honeybee researchers, Oldroyd and his colleagues frequently interact with beekeepers. The association between honeybee researchers and beekeepers is reciprocal; both parties learn from each other. Beekeepers often have much experience-based wisdom, which is helpful to the researchers, and researchers occasionally make a few discoveries that may be useful to beekeepers.

So, Oldroyd and colleagues put out an advertisement seeking to know if any beekeepers have noticed eggs above the queen extruders in their colonies.

Sure enough, they got a positive response. A beekeeper from Ipswich, Queensland, reported that his otherwise normal colony had more than 100 drone cells (drone cells are larger than those used for rearing workers or storing food) with brood in them, above the queen-extruder. This strongly indicated that workers may have laid the eggs that produced this brood.

Now, the researchers could focus on this single colony which was very promising for the possible discovery of anarchy in the hive. Using DNA-based markers, they genotyped the brood from above the queen-extruder, worker-destined brood from below the queen-extruder, and some adult workers.

The brood that shouldn't have been there

There were three possible hypotheses for the presence of a drone brood above the queen-extruder.

1. There may have been two queens in the colony, one trapped in the lower box and one in the upper box.

2. The workers may have carried the male-destined eggs laid by the queen in the lower box and placed them in the upper box.

3. Workers themselves may have laid the eggs that gave rise to the brood in the upper box.

Their results were clear. The colony had only one queen. Workers and not the queen sired all 49 pupae sampled from above the queen-extruder. Thus, hypotheses 1 and 2 were ruled out, and hypothesis 3 could be accepted. So they concluded that they had detected egg-laying by workers and dubbed this phenomenon 'anarchy in the hive'. The justification for this catchy label is that, normally, worker ovaries are suppressed by the queen pheromone, and workers refrain from laying eggs and spend their time working for the welfare of the colony. But egg-laying by workers disrupts colony harmony and potentially creates anarchy.

Workers are known to develop their ovaries in the absence of the queen, but that is another matter – easily understandable from the mechanistic (absence of queen pheromone) and evolutionary (no benefit of harmony) points of view.

Oldroyd and his colleagues had an even more exciting result. Honey bee queens mate with several males and simultaneously use sperm from different males so that the workers in a colony belong to many different patrilines. But they found that workers of a single patriline sired 48 of the 49 pupae located above the queen extruder, a worker of another patriline sired one pupa, and all the remaining patrilines were unrepresented.

One patriline's monopoly of anarchic behaviour suggested that anarchic behaviour has a genetic basis and that some males had genes that would make their daughters anarchic. It is easy to see that natural selection would favour such 'selfish' genes as long as they do not become too common.

Egg-laying by the anarchic workers is a different phenomenon, distinct from the small proportion of workers laid eggs that are quickly eaten by the police workers, which we saw in part 1 of this article. The eggs of anarchic workers are obviously not policed. Thus, anarchy is a complex phenotype requiring anarchic workers to evade the suppressing effect of the queen pheromone and develop their ovaries and lay eggs, which can go undetected by the police workers.

Benjamin Oldroyd examining a frame taken from the upper box for evidence of worker-laid eggs. Photo: Benjamin Oldroyd

Breeding for anarchy

The next obvious step in unravelling the genetic basis of anarchy (or any trait) would be to see if the incidence of anarchy in colonies can be increased by selective breeding. To this end, Oldroyd and Katherine E. Osborne artificially inseminated some queens with sperm from drones sired by anarchic workers. As a control, they inseminated other queens with different proportions of sperm from drones sired by wild-type (normal) workers and sperm from drones sired by anarchic workers.

They found that when the queens were inseminated by sperm from drones of anarchic workers, the colonies headed by such queens showed a higher incidence of workers having developed ovaries and increased survival of worker-laid eggs. These results reinforce the possibility of a genetic basis for anarchic behaviour, involving increased tolerance to the queen pheromone's inhibitory effects and increased ability to evade worker policing.

The colonies headed by queens inseminated with different mixtures of normal and anarchic sperm revealed a level of complexity that might have been missed were it not for these control colonies. The levels of anarchic behaviour seen in these colonies make it clear that the phenomenon of anarchy is the result of a complex interaction between the genotype of the queen, the genotypes of the different patrilines of workers, both anarchic and wild-type, and the external environment.

One never knows what a control experiment will reveal. It is a mistake to worry too much about whether a control experiment is really needed in view of our confidence that the main experiment is so clear-cut. Sometimes, it may be unclear what a control experiment will reveal and how it will help. That we do not know what the controlled experiment will reveal is itself an adequate justification for performing it.

In this case, it is not surprising, though in hindsight, that the expression of anarchy is so complicated. After all, the anarchic workers receive 50% of their genes from the queen and only 50% from their anarchic-gene-bearing fathers. Both sets of genes will influence their ability to resist the inhibitory effects of the queen pheromone and develop their ovaries.

Moreover, the survival of their eggs will depend on the policing efficiency of other workers in the colony belonging to different patrilines, based on their different abilities to sniff out their eggs.

Search for the 'anarchy gene'

Now the search is on for the 'anarchy' gene, which can confer these properties. An 'anarchy' gene is the opposite of a 'social' gene: the latter is expected to have the opposite effect, making the workers respond to the queen pheromone and refrain from developing their ovaries. If it is a gene that helps switch between developing and not developing worker ovaries, then we would have two birds in one shot – we will have a gene that will prevent anarchy in one configuration and cause anarchy in another configuration.

In an unpublished preprint deposited in the increasingly popular database called bioRxiv, Oldroyd and his many colleagues have now reported a gene (technically a non-coding RNA) that seems a very promising candidate. It causes cell death in normal worker ovaries and prevents their development.

It is a striking irony that the study of strife in honey bee colonies, however rare, is finally helping us understand how strife is prevented under most conditions and how social organisation and cooperation evolve. It is often true in biology that we have to break the system or find a naturally occurring dysfunctional situation to understand the functional one – by studying the abnormal, we understand the normal. This underscores the need to pay attention to exceptions and results that seem to contradict the prevailing paradigm.

A more serious threat to colony harmony

Dramatic as the story of anarchy in the hive is, the threat posed by anarchic workers to honey bee colonies is rather modest. Anarchic workers lay only male-destined eggs and therefore cannot produce future queens. Producing future queens remains the queen's prerogative, so there is no danger that queens will lose all their fitness.

The fact that worker anatomy has been sufficiently modified to prevent them from mating is a powerful safeguard against cheating. Thus, the queen continues propagating her genes by maintaining a reasonable degree of harmony in the colony because workers cannot produce diploid female-destined eggs without mating. We might therefore say that anarchic workers do not pose an existential threat to the queen.

But nature forever springs surprises at us. The southernmost part of South Africa has a truly remarkable subspecies of honey bees, appropriately called *Apis mellifera capensis.* In the cape honey bee, as this is often called, workers can indeed pose an existential threat to their queens and to queens of other capensis colonies as well as to queens of the sister subspecies *Apis mellifera scutellata* in the neighbourhood.

This is because capensis workers can lay both haploid and diploid eggs, and the latter develop into females, either into workers or queens, depending on how the larvae are fed. (A haploid cell contains one set of chromosomes and a diploid set, two sets.) Thus, capensis workers can produce new queens, usurping what is usually the exclusive prerogative of the queens in all other subspecies of honey bees.

The remarkable phenomenon of cape honey bee workers frequently developing their ovaries and laying male and female destined eggs was discovered by one George William Onions (1867-1941), a carpenter in South Africa with no specialised training but with beekeeping as a hobby. As is often the case, his discovery was met with much scepticism during his lifetime. But today, it is a well-established fact and the subject of great interest.

How do capensis workers manage to lay diploid eggs? Surely, they have not reversed their anatomical loss of the ability to mate. What they do is no less remarkable. I found it more extraordinary still that what they do was demonstrated by a relatively unknown Indian scientist, named Savitri Verma.

Savitri Verma, PhD (J.W. Goethe University Frankfurt Germany), retired in 2009 as senior professor and head, Department of Biosciences, University of Shimla, Himachal Pradesh. Photo: Dr Savitri Verma

All diploid organisms with two sets of chromosomes must reduce the diploid number by half to produce haploid gametes to restore the diploid number when sperm meets eggs to make the next generation. The reduction of the chromosome number happens during the process of cell division, known as meiosis.

Meiosis consists of two consecutive cell divisions, the first reductional and the second mitotic, i.e. equational, resulting in four haploid cells. In contrast, mitosis is the process by which somatic cells divide without reducing the number of chromosomes.

The question of interest is whether reduction of chromosome number never happens in capensis bees or whether it happens, and then two haploid cells fuse at the end of meiosis to restore diploidy. The latter idea is not as outrageous as it sounds because, after all, it is the destiny of the haploid products of meiosis to fuse with other haploid cells to restore diploidy, except that they usually fuse with haploid gametes from another individual of the opposite sex.

In this case, the fusion would be, if that is how it happens, among the haploid cells of the same individual and thus a form of parthenogenesis. This form of parthenogenesis, in which the diploid female bee produces diploid female offspring without mating, is called 'thelytoky'. It is distinct from 'arrhenotoky', the other (more usual) kind of parthenogenesis in which a diploid female bee produces haploid male offspring without mating. In arrhenotoky, the haploid products of meiosis directly develop into haploid adult males.

Savitri Verma worked with Friedrich Ruttner, the well-known honey bee biologist at the University of Frankfurt in Germany. They examined what is often called the dance of the chromosomes under the microscope to distinguish between the two hypotheses: no reduction in chromosome number or reduction followed by restoration of diploidy by cell fusion. She demonstrated clear evidence of reduction followed by fusion to restore diploidy.

Here, I want to pay tribute to Savitri Verma for her pioneering work of great significance, especially because she is all but unknown to the scientific community, even in India. As irony would have it, the paper that made her immortal is sometimes cited in high profile journals not correctly as 'Verma, S. and F. Ruttner (1983)' but incorrectly as 'Verma, L.R. and F. Ruttner (1983)'.

Verma L.R. was her better-known husband and also a honey bee biologist!

Apis mellifera capensis queen alongside three workers. Photo: Benjamin Oldroyd

Scientists are pretty lax about ensuring the accuracy of their citations, so they frequently copy the required citations second-hand from any paper that has already made the citation, often without accessing or reading the original paper being cited. This can lead to the perpetuation of inadvertent citation errors, especially if the wrong citation appears in a prominent paper likely to be used as the source for copying citations.

Reference lists in scientific papers are like silent genes that are free to mutate, unchecked by natural selection in the form of proofreading. It would make an interesting student project to determine the frequency of citation errors in scientific papers.

Savitri Verma obtained her PhD from the University of Frankfurt, Germany, and returned to India to pursue a distinguished teaching and research career in India in the fields of cytogenetics, molecular biology and human genetics. She retired in 2009 as senior professor and head at the University of Shimla in Himachal Pradesh.

The phenomenon of capensis workers laying female-destined eggs discovered by George William Onions a century ago and whose cytological mechanism was elucidated by Savitri Verma and Friedrich Ruttner some 40 years ago is now at the forefront of research in honey bee genetics and evolution, with considerable ramifications for beekeeping too.

The cape honey bee (*Apis mellifera capensis*) is turning out to be even [more remarkable] than was originally believed. Large numbers of capensis workers develop their ovaries and lay diploid eggs. These eggs are not policed, apparently because they are chemically indistinguishable from queen-laid eggs.

Interestingly, capensis workers do not police their sisters' thelytokous eggs even though they police worker-laid eggs introduced experimentally from the subspecies *scutellata*. Thus, in addition to being queen-like, capensis workers also lay eggs that are also queen-like.

Madeleine Beekman in a bee suit holding a frame with brood from different crosses between capensis and scutellata so see how genetics affects the amount of food received by larvae and its effect on reproductive traits.

Although capensis queens can mate and produce daughters sexually, utilising sperm from males, virgin queens can lay both arrhenotokous haploid male-producing eggs and thelytokous diploid female-producing eggs – suggesting that they can control which kind of meiosis their eggs go through even after they are laid.

Recently, a gene that controls the switch between thelytoky and arrhenotoky has been identified. The social disharmony-causing thelytoky in cape bees may help us understand the molecular basis of meiosis. Such are the ways of biology!

Not only do capensis workers lay eggs that can be reared as queens, they seem to have a competitive edge over their queens. In [one study,] 23 out of 39 queens produced were sired by workers. If [queens are experimentally removed,] capensis workers prefer to rear new queens from worker-laid thelytokous eggs ignoring queen-laid eggs that the experimenter may provide.

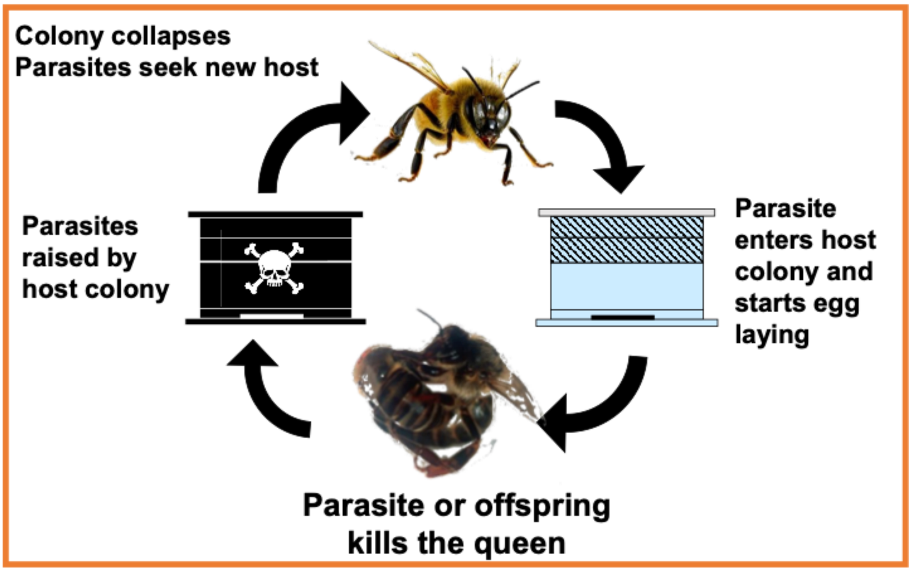
But queens have a different trick up their sleeve. They can pass on 100% of the genes to future queens by producing new queens, thelytokously avoid male genes altogether. Each one for herself – such a far cry from the harmonious cooperation and altruism that we expect from honey bees.

The cape bee's ability to create strife is not restricted to competition between workers and her queen within the colony. Much greater fitness payoffs await workers who enter and parasitise colonies of other honey bee subspecies. Capensis queens produce more pheromones to keep their workers in check.

So, a capensis worker entering the colony of another subspecies encounters less queen pheromone than she is used to. She, therefore, develops her ovaries even faster and lays potentially queen-destined eggs rapidly. Worse, she may kill the queen and put the host workers to work to rear her daughter queens.

Beekeeping practices further exacerbate the capensis workers' parasitic tendencies. Capensis bees can enter a commercial beehive and start a little nursery of their own daughters in the upper box to which the host queen has no access. Sometime later, the mother parasite and her daughters can go down and attack the host queen. They will have little interest in the welfare of the host colony. Once they utilise the resources of the host colony, they can quit and enter another healthy colony.

A little tweaking of the meiotic cell division has allowed the cape honey bee to utilise all the features meant to ensure harmonious social life into a nefarious antisocial lifestyle. Not surprisingly, this has dire consequences for the beekeeping industry. How can one practice beekeeping if your colonies parasitise and destroy each other in the game of one-upmanship? Beekeepers rely on harmony and cooperation in the hive to make their living.



How social life in honey bees can be turned into a parasitic lifestyle. Image: Robin Moritz and Robin Crewe

Dysfunction in the hive?

Two well-known honey bee researchers, Robin Moritz from the University of Halle in Germany and Robin Crewe from the University of Pretoria in South Africa, have now taken a holistic view of the various features of honey bees that make them seem less than perfect harmonious societies. In a recent book ruthlessly entitled *The Dark Side of the Hive* (2018), Mortiz and Crew tear apart the long-held perfectionist view of honey bee societies and conclude:

> "The honey bee colony is thus far from being a harmonious, cooperative whole. It is full of individual mistakes, obvious maladaptations, and evolutionary dead ends. Conflict, cheating, worker inefficiency, and curious reproduction strategies all occur."

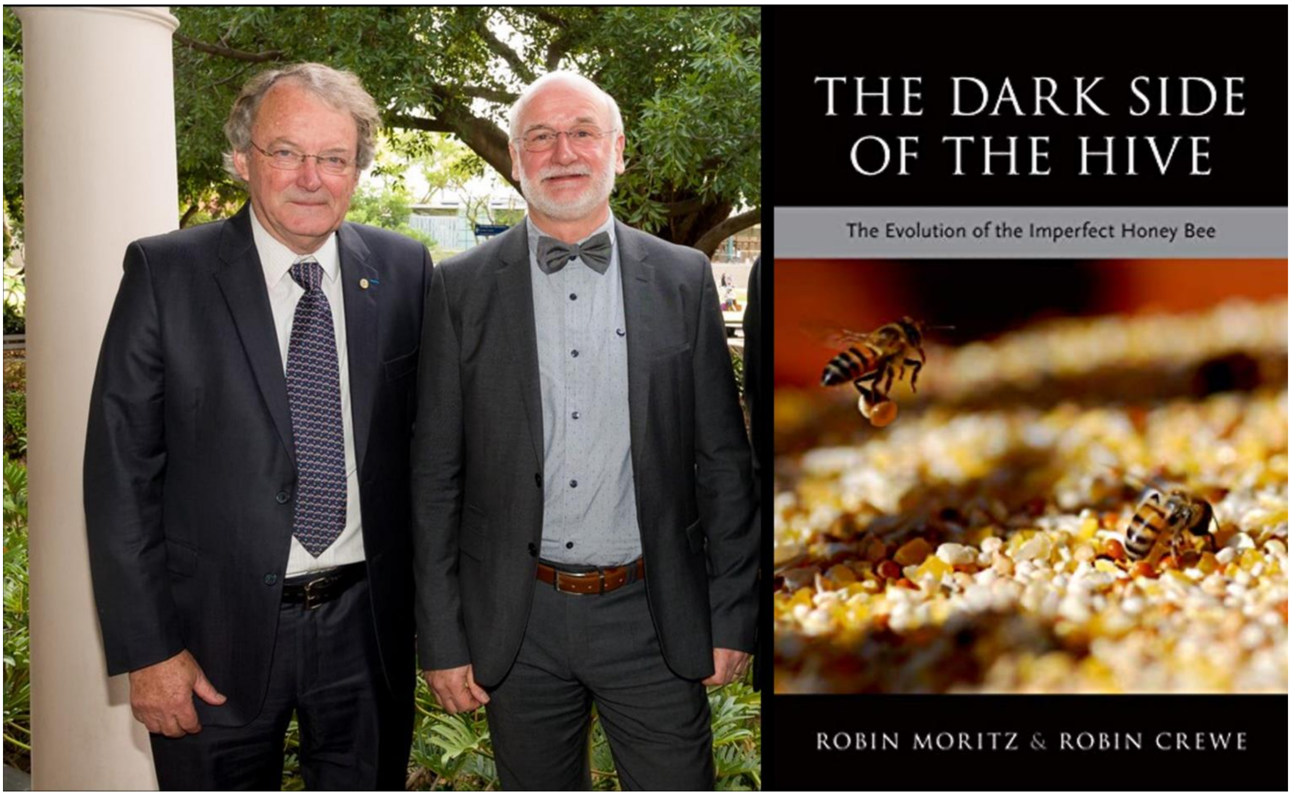
The Dark Side of the Hive is one of the most shocking science books I have read. Brimming with out-of-the-box thinking and a large dose of heresy, Moritz and Crewe tear into the prevailing complacent and admiring view of honey bees and look at the dark side of every aspect of honey-bee biology.

To take just one example, they list all the problems associated with the 'difficult diet' of the honey bees. Their vegan diet, with its exclusive dependence on nectar and pollen, presents myriad problems. The bees have to transport large quantities of liquid. Since a bee can transport only about 25 mg of nectar at a time, they need some 400,000 foraging trips to gather the nectar to make one kilogram of honey.

To give us a feel for what that means, we are told that this is equivalent to a return flight to the Moon and back! So, how do the bees solve this problem?

The queen produces hundreds of thousands of workers to share the burden. But this means a massive investment in the non-reproductive worker force to produce just a few queens and drones, not to mention the difficulties of housing and managing the large population of workers.

Nectar is a dilute sugar solution and will quickly go bad and ferment, so they expend a considerable effort to evaporate the water and concentrate it into a thick syrup. But that poses its own problems as viscous honey can stick to the bees' bodies, block their trachea and kill them by asphyxiation. So they spend much time and energy constantly grooming themselves.



Left: Robin Crewe and Robin Mortiz, authors of The Dark Side of the Hive. Right: The cover of their book shows honey bee workers maladaptively collecting seeds from bird feeders, mistaking them for pollen.
Photo: Robin Moritz and Robin Crewe

The story with pollen is not much different. Pollen is fine dust that the bees must harvest and bring home in large quantities while constantly cleaning themselves to prevent their trachea being blocked. Digesting pollen presents its own problems. The thick indigestible coating of the pollen must be excreted in large amounts and going out of the hive to do so presents another challenge, especially in the winter.

The pollen diet presents an even more significant challenge to the larvae, who don't defecate until they become pupae. Besides, to preserve pollen and maintain its nutritional quality, the bees process it and make 'bee bread'.

The ancestors of honey bees mass provision their larvae, i.e. they add all the food required for the development of the larvae into the cells before laying an egg in it. Honey bees, however, have evolved progressive provisioning: they frequently feed the larvae, altering the diet and adding secretions as appropriate. This is a huge undertaking.

In this vein, Moritz and Crewe find fault with every aspect of honey bee biology. I found their approach to bee biology absolutely fascinating, although it appeared perverse at first, I must confess. I knew most of the facts they present but had not thought about them in this light.

Robin Moritz told me in an email that he and Crewe were inspired to write *The Dark Side of the Hive* because of their conviction that "Nobody is perfect (and bees definitely not) … but then there is no need to be perfect in order to become evolutionarily successful."

Moritz and Crewe don't just indict the honey bees. With powerful arguments, they indict the research strategies and methodologies of scientists involved in honey bee research.

"The picture of harmony and success is compelling, sometimes perhaps so compelling that it might easily preclude asking critical questions about such obvious efficiency," they write. And "The perfection that is perceived to exist in their social organisation is a function of a particular experimental focus on the colony as a whole rather than exploring the idiosyncrasies of its individual members," they argue.

Finally, they tell us that they instead, "explore the situations in which individual interests are pursued often at the expense of the colony, and … show that the solutions that have evolved are often less than optimal."

One of their main criticisms concerns a central debate in evolutionary biology. Does natural selection act to make the best colonies fit enough to compete with other colonies, or does it make the best individuals fit enough to compete with other individuals, even within the same colony? The answer must be 'both'. But the interesting unknown is how the trade-off between the two levels of selection plays out in different situations. This should be the topic of much future research.

The Dark Side of the Hive has had a profound effect on me. It has shown me how I was blind to the possible alternate interpretations of well-known facts. It has made me worried about my interpretation of other fields of knowledge. I recommend that not only students of honey bees and other social insects but also all biologists should read *The Dark Side of the Hive* by Moritz and Crewe alongside other wonderful books such as *Biology of the Honey Bee* by Mark L. Winston, *The Wisdom of the Hive* and *Honeybee Democracy* by Thomas D. Seeley and *The Spirit of the Hive* and *Art of the Bee* by Robert E. Page, which focus primarily on the bright side of the hive.

The true essence of what we have learnt about honey bees in this two-part article is that honey bees have an uncanny ability to manage conflict, display a semblance of normalcy and become evolutionarily successful despite great scope for conflict and inherent dysfunctional tendencies – making them even more impressive and more worthy of the epithet "a prime favorite of the gods," as William Morton Wheeler lyrically described them almost a century ago. And surely there is something for us humans to learn from the bees here.

Raghavendra Gadagkar is a Department of Science and Technology (DST) Year of Science Chair Professor at the Centre for Ecological Sciences at the Indian Institute of Science, Bengaluru.

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