More Fun Than Fun: How Do Insect Societies Deal With Infectious Diseases?

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A bivouac nest of the army ant Eciton hamatum*. Picture taken at La Selva Biological Station, Costa Rica. Colony size estimates for this species range from ~50,000 to 500,000. Photo: Daniel Kronauer*

RAGHAVENDRA GADAGKAR

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.

The evolutionary success of parasites does not depend on causing disease, much less on killing their hosts. Instead, it relies on how best parasites can use their hosts' bodies to grow and multiply and infect new hosts.

Disease and death are merely unavoidable collateral damages. Colonies of social insects such as those of ants, bees, wasps and termites present parasites with a paradise of sorts: they contain densely packed individuals that are also rather genetically similar and hence of similar susceptibility. Not surprisingly, parasites are the scourge of insect societies. But insect societies have not only survived this scourge but are among the most evolutionarily successful and ecologically dominant members of Earth's terrestrial fauna.

The solution to this apparent paradox lies in the fact that there is, and has been for millions of years, a Machiavellian tug of war between insect societies and their parasites. This tug presents evolutionary biologists with an equally enchanting paradise to discover the power of natural selection, or the lack of it, especially when both warring parties use the same process (natural selection) to succeed. Evolutionary biologists have rejoiced in this paradise with great pleasure and success.

Almost everything I have been reading about this ongoing contest between *Homo sapiens* and SARS-CoV-2 during the last 12 months has made me ponder about insect societies and how they deal with

their parasites and the diseases they suffer on account of them. There have been hundreds of memorable studies on social insects and their parasites, giving me a wide choice from which to pick a few of my favourites to reminisce.

Hygienic honey bees

One of my all-time favourite stories is the discovery of 'hygienic behaviour' in honey bees by a combination of serendipity and clever experimentation. The serendipity was on account of Edward Brown, a beekeeper in Iowa. American foulbrood (AFB) is a disease caused by the bacterium *Paenibacillus* larvae, which kills the larvae and pupae of the European honey bee (*Apis mellifera*). Beekeepers usually burn and destroy diseased colonies quickly so that the bees don't get much of a chance to develop resistance through natural selection.

There was, however, one famous exception. Edward Brown purchased a large number of empty combs from beekeepers whose colonies had been killed by the disease. Brown's interest was to extract the wax from these combs. He rather cleverly, or so he thought, allowed his bees to steal any leftover honey from these combs before melting them. As it happened, his bees not only stole honey but also the disease-causing bacteria. Many of Brown's bees died, but some survived, and those that survived developed natural resistance. Brown had thus unknowingly selected for honey bees that were resistant to AFB.

Walter Rothenbuhler, a professor in the departments of zoology and entomology at the Ohio State University, was intrigued and decided to investigate. He found that the resistance developed by Brown's bees to AFB was of a most interesting kind. The bees evolved a specific form of hygienic behaviour – their workers uncapped the cells containing dead pupae and removed their corpses – leading to them being called 'hygienic bees'. Rothenbuhler's observations and experiments showed that resistance to the disease, including the behaviours of uncapping and removing corpses, was genetically determined.

In fact, he was able to cross hygienic bees with the normal non-hygienic (wild-type) bees and show that two separate genes were involved in conferring resistance: one gene for uncapping and another for removing. He experimentally produced bees with one of these genes in the mutated form and the other in wild-type form. Thus, some of his bees uncapped cells containing sick pupae but failed to remove the corpses, while others failed to uncap cells but removed diseased pupae if Rothenbuhler kindly uncapped them.

Only if they had both mutated genes were they capable of uncapping as well as removing the diseased pupae. This was one of the early demonstrations of the genetic basis of complex behaviour.

A little protozoan rises in challenge

Another favourite story of mine is the battle between bumble bees and one of their microscopic parasites. Bumble bees are largely restricted to the northern hemisphere, although some occur in higher altitudes in the southern hemisphere.

Left: A nest of the bumble bee *Bombus terrestris* (Photo: Paul Schmid-Hempel). Right: A scanning micrograph of its protozoan parasite *Crythidia bombi* (6-8 µm X 2-3 µm; flagellum 3 µm; photo: Boris Baer).

The European bumble bee (*Bombus terrestris*) has an annual colony cycle. The queen's daughters are workers and help the queen with rearing her offspring. Towards the end of the season, however, the queen begins to lose control over her workers. The latter develop their ovaries, revolt against the queen and lay a few eggs of their own (they can only produce sons, not daughters) before the colony completely disintegrates.

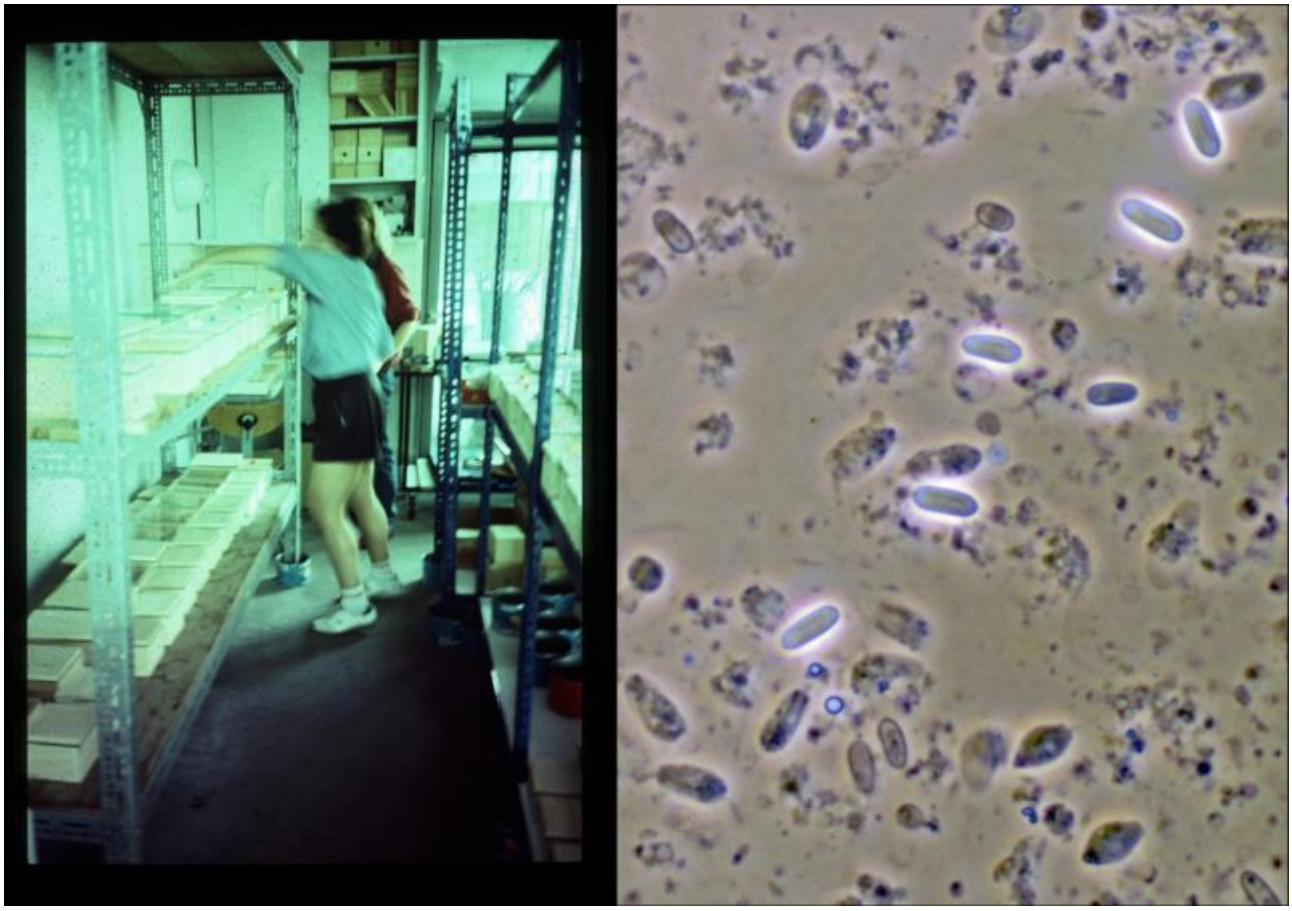
Successful queens must therefore produce many sons and daughter queens before their workers revolt. The sons mate and die while the daughter queens mate and hibernate to start new colonies the following spring.

Crithidia bombi is a Trypanosome parasite that lives off bumble bees. Trypanosomes are microscopic parasitic protozoans – single-celled animals – with a rounded or longish body that moves around by the corkscrew-like motion of its flagellum. The best-known examples are *Trypanosoma brucei*, which causes sleeping sickness, and *Trypanosoma cruzi*, which causes Chagas disease in humans.

Crithidia bombi, however, lives in the intestines of the bumble bees and is transmitted through contact with infected individuals or their faeces. The parasite depends on an unbroken chain of transmission from queens of one generation to queens of the next generation.

Jacqui Shykoff and Paul Schmid-Hempel at ETH Zurich experimentally infected some bumble bee colonies in their laboratory with *Crithidia bombi* so that they could compare them with uninfected control colonies. They found that infected queens develop their ovaries slowly and poorly compared to uninfected queens. This is because the parasite uses the host machinery, on a part-time basis, to make more copies of itself and spread to the queen's daughters and through them to other colonies.

But because queens have poorly developed ovaries, they would produce fewer daughter queens and sons before their workers revolt, making it less likely that the parasite will get efficiently transmitted to the next generation. If we think that this is the price that the parasite has to inevitably pay, we would be wrong. The parasite has other tricks up its sleeve.



Left: Jacqui Shykoff when working towards her PhD at the University of Basel. Right: Photo in light microscopy of the faeces probe of a worker of *Bombus terrestris* shows a mixture of Nosema bomb in spores (bright oval forms, naturally infected) and cells of *C. bombi* (pear-shaped with flagellum, experimentally infected). Photos: Regula Schmid-Hempel, ETH Zurich

When the parasite jumps from the mother queen to her daughter workers, the workers also develop their ovaries slowly and poorly so that it takes longer for them to lay their own eggs and start revolting against the queen. Thus, the queens have more time to enlist the cooperation of their workers and produce new daughter queens and sons for the next generation, enhancing the chances of transmission of the parasite.

As a result, the parasite and the queens more or less break-even but at the cost of the workers, who don't get to produce their own sons. Machiavellian indeed! I suspect that natural selection can't easily come to the rescue of the workers who lose out in this game because the 'losing' workers can nevertheless gain indirect fitness by raising the queen's offspring, by the process of kin selection. Host-parasite tugs-of-war can take on new dimensions of complexity when we deal with social insects.

Altruistic bees with a civic sense

My third favourite example from the last century is the insightful work of a dear friend, Michał Woyciechowski, a professor of environmental sciences at the Jagiellonian University, Krakow.

Honey bee workers perform the various tasks necessary for their colony's well-being in an organised manner, changing their tasks as they age. Typically, bees work at home during the first half of their lives, cleaning, building and nursing. They spend the second half working outside their nests, undertaking the more risky and hazardous tasks of foraging for food and bringing it back to the nest. Their rates of mortality increase dramatically when they begin to work outdoors.

Why do young bees work under the protection of home and old bees go out and take greater risks, rather than the other way around?

From an evolutionary perspective, the honey bees' schedule seems to make sense: by taking fewer risks early in life and greater risks later, the workers live longer on average. And this should be good both for the individual worker bee as well as for the colony.

Woyciechowski and his colleagues put this idea to a rigorous test. First, they built a mathematical model to predict the extent of risk-taking, not as a function of age but of life expectancy. (Notice that life expectancy can be different even at the same age if the individual is sick or if the environmental conditions change.)

The great advantage of such a model is that they could test its predictions by experimentally altering the life expectancy of the bees. It isn't possible to experimentally change the age of a bee but one can change its life expectancy. They were thus able to test the predictions of their model with simple field and laboratory experiments.

Michał Woyciechowski during field work in Krakow, May 2019.
Photo: Raghavendra Gadagkar

In the laboratory, they infected some honey bee workers with a protozoan parasite, Nosema apis, and treated other bees with carbon dioxide, and confirmed that both treatments lowered the bees' life expectancy.

In the field, they demonstrated that workers with lower life expectancy did indeed undertake more risky tasks: they started to work outside the nests earlier in life and did so in worse weather conditions than untreated bees. Their results were so robust that, even among infected bees, those infected earlier in life started foraging even earlier than those infected a little later.

For me, the most poignant result is that sick bees leave their nests earlier in life to start foraging and thus incur a greater risk to themselves rather than take sick leave and wait to recover. But by leaving early, they reduce the chances of infecting other bees in the nest and thus perform an act of altruism.

Woyciechowski has argued that division of labour in honey bees and other social insects is so organised that individuals with lower life expectancy undertake more risky tasks even in the absence of disease. Such an arrangement comes in handy to deal with disease when necessary. An effective response to infection is built into their everyday lifestyle and is not only based on doing something radically different when sickness strikes – something for us to think about.

Social networks that limit transmission

Unsurprisingly, disease research has become truly modern in the big data and high-throughput era of the 21st century. In 2013, Danielle P. Mersch, Alessandro Crespi and Laurent Keller of the University of Lausanne developed a fancy new technique to simultaneously and automatically track the spatial positions and interactions of ants. Believe it or not, they glued strips with two-dimensional bar codes to the backs of the ants and had a camera attached to a computer to monitor their movements.

Their technique is as visually impressive as are their statistics: "We used a tracking system to continuously monitor individually tagged workers in six colonies of the ant *Camponotus fellah* over 41 days… more than 9 million interactions… a total of 2,433,250,580 ant positions and 9,363,100 social interactions"!

Nathalie Stroeymeyt (photo: Laurent Keller) marking *Lasius niger* ants with 1.6 mm barcoded tags for the queen and 0.7 mm tags with for the workers, as seen on the right (photo: Timothée Brütsch)

These researchers more recently teamed up with Nathalie Stroeymeyt, Anna V. Grasse and Sylvia Cremer to use their technique to study how social networks of ants might be adapted to deal with disease.

First, they tested the hypothesis that the networks of social interaction in an ant colony should be so designed that, in addition to permitting efficient communication and division of labour, they should also minimise the spread of disease. They called this preparedness for disease 'constitutive organisational immunity'.

Tracking all physical contacts between all pairs of ants in 22 colonies of the ant *Lasius niger*, they computed several global properties of their interaction networks. Network scientists have now developed a number of statistical measures that succinctly capture different features of the networks. Some of the measures pertain to the positions of individual members in relation to the whole network, such as whether an individual is relatively central or peripheral to the network. Others pertain to the global characteristics of the networks, such as whether interactions are uniformly distributed across the network or whether they are concentrated in some corners.

Using several individual and global properties of the ant networks, Nathalie Stroeymeyt and her colleagues showed that the network of interactions is designed to inhibit rather than enhance the transmission of infections across the colony. They showed this rather cleverly.

They simulated infection in a computer model and showed that infection would spread more slowly in the kind of networks observed in ant colonies, versus a random network. Next, they experimentally infected their ant colonies with the fungus *Metarhizium brunneum*. They found that now the ants modified their interaction patterns to further halt the spread of the disease, perhaps at the cost of some efficiency in communication and division of labour. They labelled this 'induced organisational immunity'.

We see once again that there is evidence of preparedness to deal with disease even before disease strikes, followed by new emergency measures after disease.

Sanitary ants are prudent caregivers

We know that social insects care for sick individuals: they have individuals in their colonies that specialise in caring for the sick. An important component of such 'sanitary care' involves grooming the infected individuals to remove external parasites and applying antimicrobial poisons onto the bodies of the infected individuals.

But aren't the caregivers at a greater risk of catching the infection and spreading it to the rest of the healthy colony? We also know that caregivers develop immunity to the pathogens they encounter in the line of duty and are partly protected against future exposure to the same pathogen. This is called 'social immunisation'.

But what about exposure to a different pathogen?

Sylvia Cremer and her colleagues at the Institute of Science and Technology in Klosterneuburg, Austria, recently studied the garden ant (*Lasius neglectus*) by experimentally infecting them with the fungal pathogens *Metarhizium robertsii* and *Beauveria bassiana*.

Their studies show that caregivers are protected to some extent against future exposure to the same parasite. However, they are even more susceptible to other pathogens than before. The caregivers are prudent enough to switch tactics: they reduce grooming and step-up poison application when confronted with an ant infected with a different pathogen.

Thus, the sanitary ants give what the authors call "risk-averse healthcare" service. Indeed, it is remarkable that the ants can change their behaviour based on their own immunisation status and the identity of the pathogen being encountered.

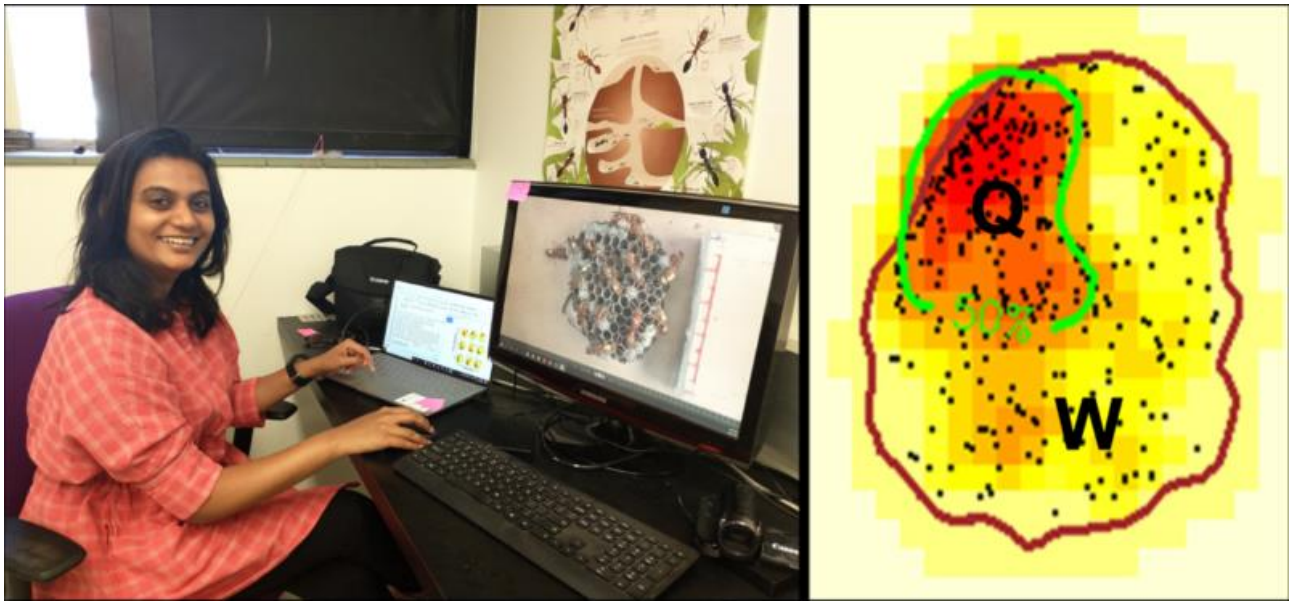
Wasp queens stay safe

Compared to bees and ants, social wasps have been rather neglected when researching disease, perhaps because their colony sizes are quite small. But I am happy to say that my student Nitika Sharma has made a beginning. Nitika studied the Indian paper wasp *Ropalidia marginata*, as most of my students have done. But she has blazed an altogether new trail in my group.

Nitika asked whether each wasp has a favourite place to sit on the nest. I was very sceptical, but she found that they do indeed. Nitika video-recorded several wasp colonies and manually extracted the spatial locations of each wasp every six minutes over three days. She then constructed what are called 50% kernel density estimation maps.

These maps show the locations on the nest where the wasps spend 50% or more of their time. Nitika showed that the 'unexpected' (at least for me) non-random space use by the wasps is designed for efficient food distribution while simultaneously minimising the spread of disease, i.e. organisational immunity.

Her result that impressed me the most was that the queens minimised their contacts with the workers, especially the foragers who are, of course, more likely to be carriers of disease.



Nitika Sharma (photo: Ishani Sinha) extracting spatial data from a video recording of a colony of the primitively eusocial wasp *Ropalidia marginata* (see screen) to construct estimates of the density of spatial locations of the wasps, as shown on the right. The area where the queen is found for 50% or more of its time in marked 'Q' and 'W' is where workers spend 50% or more of their time.

Social immunity

In a landmark paper published in 2007, Sylvia Cremer, Sophie Armitage and Paul Schmid-Hempel from Germany, Denmark and Switzerland respectively, introduced the concept of 'social immunity'. Social immunity has now become one of the hottest topics in research. Although these authors complain that today everyone likes to use the term social immunity so that it has lost its original focussed meaning, I think this is a sign of success.

Recent research on social immunity has shown that there are many parallels between individual physiological immunity and social immunity. The most striking parallel is captured by the evocative phrase "care-kill" dichotomy.

At first, social immunity (like individual immunity) attempts to prevent the onset and spread of infections. Presumably, this is adequate in most instances. But when the infection agents break through this early defence, social immunity (like individual immunity) switches track to isolate and eliminate infected individuals – to kill if care fails.

I think you will agree that the dynamics of host-parasite interactions in insect societies are fascinatingly complex and surprisingly relevant to the human condition. It is my fond hope that humans will be curious enough to understand how other creatures deal with disease and have the sapience to adapt the knowledge so gained to suit our needs.

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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