More Fun Than Fun: How Do Animals Recognise their Kin

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The Indian paper wasp, with a paper nest – with its hexagonal cells, eggs, larvae, pupae and adult wasps. Photo: Thresiamma Varghese.

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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.

Honey bee workers almost never reproduce. Instead, they spend their entire lives working for the welfare of their colony and their queen's offspring. They may even die in defence of their colony as their every act of stinging is an act of suicide. Clearly, honey bee workers challenge Darwin's theory of natural selection. Darwin certainly thought so, calling them a "special difficulty, which at first appeared to me insuperable, and actually fatal to my whole theory".

In the mid-20th Century, the British-Indian biologist J.B.S. Haldane was the first to make light of this difficulty when he realised that saving two of his drowning brothers would compensate the risk of his death while attempting to do so. Another British biologist W.D. Hamilton independently discovered this extension to natural selection, that has come to be known as kin selection. With rigorous mathematics, Hamilton showed that a gene causing altruistic behaviour could indeed be favoured by natural selection, provided the altruism is directed towards close genetic relatives.

Kin selection theory was a watershed moment in evolutionary biology, especially for those attempting to discover the evolutionary logic of ecology and behaviour. Kin selection spawned several sub-disciplines – evolutionary ecology, behavioural ecology, sociobiology, evolutionary psychology and their various cousins. Practitioners of these sub-disciplines grew exponentially as did their research papers, books and journals and even university departments.

Showing that animals and plants and even microbes used genetic relatedness as the guiding principle to dispense cooperation, altruism, and even selfishness and spite, became the holy grail of this brave new brand of biologists. And they succeeded admirably, despite some mathematical controversy about how best to measure the evolutionary benefits of helping kin.

The social bee

An interested bystander might have noticed that for some 15 years into the resulting frenzy, no one seemed to have had the time or sangfroid to ask whether animals have the capacity to distinguish their relatives from non-relatives! This is not a trivial question – even for humans, the possibility of kin detection is quite controversial. Finally, in 1979, a young man named Les Greenberg, working under the mentorship of Charles D. Michener, the world's authority on every kind of bee – and there are some 17,000 of them – had the alacrity, or naïvety, to look for the elephant in the room.

While Michener studied all kinds of bees, many of his students studied a social bee (*Lasioglossum zephyrum*) that builds tunnels in the soil in which the queen bee lays eggs, and the worker bees rear her brood. The Michener lab had perfected the art of rearing these bees in simple artificial nests with a pinch of soil between two glass plates.

The beauty of this arrangement is that the researchers can be privy to the goings-on inside the transparent nest. The worker bees routinely leave the nest to locate and bring back pollen and nectar. The bees always have to be wary of predators or bees of other colonies trying to sneak into their nests with nefarious designs.

Thus, they station one bee to guard the entrance of the nest. Much like our security staff, the guard bee scans (in this case smells) all incoming bees and decides who can be safely let in; guard bees can effectively block their entrance to the nest if they wish to keep an unwelcome guest out. The guard bees generally let their own nest mates in and keep all others out. Although all the bees in a nest may be quite closely related, this discrimination by the guards is based on familiarity and does not prove that they can recognise their relatives without prior familiarity.

In 1979, Greenberg used the guard bees to examine whether the bees can tell their relatives apart from non-relatives. In a clever move, he placed bees of his choice, one at a time, at the entrance to the nests. All the bees he placed were unfamiliar to the guards and the other bees in the nest. But because Greenberg was breeding bees from known parents, he knew the genetic relatedness of the test bees to the guard bees. Thus, Greenberg presented the guard bees with their sisters, aunts and nieces, cousins and unrelated individuals.

A Lasioglossum bee on mountain mint, somewhere in Pennsylvania. Photo: Beatriz Moisset, CC BY-SA 3.0

His question: could the guard bees infer these relationships by smelling the introduced bees? Greenberg found that the probability of acceptance of the introduced bees by the guard bees was significantly positively correlated with the genetic relatedness between the guard bee and the introduced bee.

Thus, the closer the relatedness between the guard bee and the presented bee, the higher the rate of acceptance. This was the first clear experimental demonstration of kin recognition without prior familiarity. Not surprisingly, this finding created quite a buzz, prompting the exploration of kin recognition abilities in every imaginable group of animals, and even plants and microbes.

The Vespiary

I too caught the kin recognition bug. By the mid-1980s I had already decided to devote my career to understand every aspect of the biology of the Indian paper wasp (*Ropalidia marginata*). This is a remarkable species frequently nesting in and around human habitation all over peninsular India. We had perfected techniques to observe the wasps both in nature and in the laboratory. We routinely transplanted their nests with all the brood and adults to laboratory cages of varying dimensions in our unique laboratory, which we had christened the Vespiary. The next aspect of their biology that deserved our attention was the possibility of kin recognition.

The Vespiary – a unique laboratory to house wasps in cages of different sizes, including kitchen jars used in nestmate discrimination experiments (foreground). Photo: Thresiamma Varghese

We could not breed the wasps as Greenberg had done with his bees. So, we had to get at kin recognition indirectly by studying nestmate discrimination. As the name implies, nestmate discrimination is the ability of the wasps to discriminate between wasps that belong to their nests and those that do not. We placed three wasps in a ventilated plastic box to test for discrimination and observed how they behaved toward each other. Two of these wasps belonged to one nest (nestmates of each other) while the third belonged to a different nest (non-nestmate of the other two wasps).

We gave tolerant behaviours such as approaching and smelling each other without aggression high scores. Conversely, we gave low scores to aggressive behaviours such as fighting or lunging at each other. Adding up all the scores, we obtained a ‘Tolerance Index’ for every pair of wasps. We could thus measure the level of tolerance among nestmates and between non-nestmates. If the two nestmates were more tolerant of each other than they were of the non-nestmate, we concluded that there was nestmate discrimination.

I was fortunate that three excellent students Arun Venkataraman, Swarnalatha Chandran and Padmini Nair, joined me in conducting these experiments. We collected nests from many places in and around Bangalore to employ in these experiments. We made sure that wasps we called non-nestmates were derived from nests collected at least 10 km apart; this was necessary to avoid the possibility that the two nests were not recently derived from the same parent nest. We conducted four experiments.

In experiment 1, we tested adult wasps present on their respective nests at the time of collection. However, we isolated each test wasp in a separate box for several days before the experiment. We found that these wasps readily distinguished between their nestmates and non-nestmates. The two wasps drawn from the same nest were more tolerant of each other than each of them was to the non-nestmate. Such discrimination could be based on memory of familiarity because the two nestmates had stayed together on the same nest before the experiment. That's not true kin recognition.

In experiment 2, we did not use the adult wasps. We cut the nest with the pupae in two halves and kept them separately. We tested the adult wasps that emerged from the pupae after we cut up the nest. Thus, even the two nestmates in this experiment had never seen or smelled each other. Nevertheless, we found that, even the unfamiliar nestmates were more tolerant of each other than they were of the non-nestmate. Although on separate halves, the two nestmates would be related to each other having emerged from the same nest. But how did they know this?

nestmate recognition experiment team (clockwise from top-left): Arun Venkataraman, Swarnalatha Chandran, this author and Padmini Nair – three decades after the experiment. Photos: Swarnalatha Chandran

We can assume that each wasp has a smell on its body, which we can call its 'label'. Every wasp must also have in its brain a 'template' against which it can match the labels of other wasps and decide how closely they are related. If each wasp's label and templates are the product if its genetic makeup, then true kin recognition is possible without prior familiarity. Our close relatives' labels and templates would be similar to our own on account of close genetic relatedness.

On the other hand, if the wasps acquire the labels and templates by being together with and smelling their nest or nestmates, true kin recognition is not possible. The wasps will consider as their relatives, any wasps they are exposed to, even if they are unrelated, simply because they have jostled with each other. Experiment 2 does not tell us if the wasps' labels and templates are self-based or acquired from the fragment of the nest or wasps emerging on their fragment. To know the source of the labels and templates, we performed a third experiment.

The C-section

In experiment 3, we removed each emerging wasp from its nest within 1 or 2 minutes of emergence, in the hope of denying it an opportunity to learn the smell of its nest or nestmates. This was a challenge but also a lot of fun. The wasps emerged from their pupal cases at all times of the day or night over several days. To remove every newly emerging wasp in less than two minutes after emergence, one of us had to keep our eyes focussed on the nests 24 X 7. During the daytime, the four of us took turns, keeping watch for one or two hours at a time. In the evenings, one of us took the nest home in a taxi, keeping up the vigil and isolating emerging wasps during the journey, much to the amusement of the taxi driver.

Upon reaching home, we had the enthusiastic participation of our entire families vying with each other to stay awake and watch. Thus, we got enough wasps which we hoped were sufficiently naïve. Alas, these naïve wasps gave us inconclusive results. They came close to making nestmate discrimination but were not very good at it. We worried that they might have begun to acquire and learn their nest-specific smell in those one or two minutes before we removed them. So, we needed yet another experiment.

In experiment 4, we removed the wasps before they were born, i.e., before they emerged from the pupae. We cut open the pupal caps about 24 hrs before the expected time of emergence and raised the wasps in an incubator for the remainder of their development. We jokingly called this procedure "C-section"!

If these C-section wasps could also discriminate between their nestmates and non-nestmates, their labels and templates must be self-based. If they cannot discriminate, then that is because they need to learn and acquire the nest-specific smell after emergence. We found clear evidence that the wasps in the 4th experiment did not discriminate between nestmates and non-nestmates – they treated everybody the same. Thus, we showed that nestmate discrimination in *R. marginata* depended on labels and templates acquired by learning and not genetics. This finding has serious implications for how effectively kin selection might work.

As you can imagine, we had great fun doing these experiments, watching hundreds of wasps interact with nestmates and non-nestmates, and especially, keeping all-night vigils for emerging wasps. Apart from a few plastic boxes, we needed very little to conduct these fascinating experiments and make these important discoveries. To be sure, our friends and family learned all about the wasps and understood the reasons for our heightened motivation to study what might otherwise appear rather obscure.

Life on Earth is highly variable so that we can't expect that what is true for our wasps is necessarily true for other species. There may be different species where each individual generates its labels and templates based on its genetic makeup be capable of true kin recognition. I recently came across an interesting study in fish that proves this point.

Mitchel J. Daniel and F. Helen Rodd from the University of Toronto, Canada and the Florida State University, Tallahassee, USA have studied kin recognition in the Trinidadian guppy (*Poecilia reticulata*), also intending to discover whether the templates used by these fish are self-referenced or family-based. They used an ingenious experimental design. They mated a virgin female with a single male in one fish tank. After she gave birth to offspring, they moved the mother to a different tank and mated her with a different male.

The offspring born in the two tanks would be maternal half-siblings, having the same mother but a different father. However, despite having the same mother, the offspring in the two tanks had no contact with each other. If they nevertheless recognise each other as being more related to each other than non-kin, then their templates must be self-referenced and not family-referenced. And that is exactly what they found. So Trinidadian guppies are quite different from paper wasps; the fish are capable of true kin recognition without prior experience while paper wasps are not. The phenomenon and mechanism of kin recognition has great relevance even for humans, not least in the context of tolerance between mother and child during pregnancy.

Male and female wild form guppies (Poecilia reticulata). Photo: Per Harald Olsen, GNU Free Documentation

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The diversity of life on Earth is vast and largely unexplored. Fewer than 10% of all species on the planet even have a name, let alone be investigated for the amazing and diverse ways in which natural selection has adapted them to survive in their respective environments.

There are endless opportunities awaiting fun and adventure-loving naturalists from around the world and especially from the underdeveloped countries – as Michael Robinson once remarked, there are many biologists where there is little biodiversity and few biologists where there is much biodiversity. All we need is to debunk the myth that science can only be practised by the privileged few with big labs and much money.

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