OUTSIDE JEB

Spiders move like ants to fool predators



Ants are fierce predators. They move in their hundreds, ruthlessly searching for prey and devouring anything in their path. When attacked, they can swarm the offending predator, often overpowering it with their strength and numbers. Ants are therefore risky prey – but is this something that other invertebrates can exploit to their own advantage?

Spiders have their own suite of adaptations to avoid predators. They can run fast, jump high and even selfamputate their own legs to escape, and some species do something even cleverer: they disguise themselves as ants. They wave their forelegs around like antennae and even their thin, tapered bodies are shaped like those of ants. It has the desired effect; most predators mistake them for ants and leave them alone. Some of these myrmecomorphs – ant-mimics – even move like ants. Spiders run in a stop-start fashion, turning on the spot, whereas ants typically move continuously, changing direction while walking. Yet the degree of similarity between the movements of these spiders and the ants that they mimic remained untested until Ximena Nelson and Ashley Card, from the University of Canterbury in Christchurch, New Zealand, decided to investigate.

The team compared the locomotion of four species of Myrmarachne 'ant spiders' with that of ants and some non-ant-like spiders from the same local habitat. By filming them walking and analysing the movements, Nelson and Card confirmed that the movements of the ant-mimic spiders were remarkably similar to those of ants, with longer bouts of continuous

movement in contrast to the stop-start motion of their non-ant-mimic cousins.

As this ant-like movement appeared to be so accurate, the authors surmised that locomotion must be an important part of the mimicry. To test this, they created two animations of the ant-mimic spiders – one moving like a spider and the other like an ant – and showed them to some predatory spiders to measure their responses. Ideally, the team would have liked to investigate the benefits of the movement mimicry on a predator that dislikes ants, such as Portia fimbriata, a predatory jumping spider, but they are so wary of anything that even resembles an ant that they won't even approach an ant-like animation, making it impossible to test their reactions. Instead, the team presented the animations to a local predator that specialises in eating ants another jumping spider, Sandalodes bipenicillatus – to see whether it would be fooled. The reactions of these spiders were pretty conclusive; they spent more time looking at the animation with ant-like movement, and were more likely to visually track and attempt stalking than when shown the animation with spiderlike movement. So, it seems as though the mimicked motion of the Myrmarachne is enough to fool the specialist ant predator, suggesting that it would also be a convincing enough disguise to protect them from attack by species such as P. fimbriata.

In some ways, these results raise more questions than they answer. Previous work has shown that Myrmarachne's physical resemblance to ants is enough to deter ant-hating predators. So if these spiders mimic ants so accurately that they risk being eaten by specialist ant predators, why do it? Nelson and Card suggest that it could be due to the way predators size up their prey, simultaneously processing multiple characteristics such as number of legs, colouration and movement. Depending on experience, the predator may require more of these characteristics to tick the 'antlike' box for them to be convinced by the disguise, possibly leaving these antmimics in the clear. This work also

emphasises the need to understand more about how invertebrate predators identify their prey, which in turn would help researchers learn how these adaptive traits came to be. However, in the meantime, we must doff our caps to these spidery masters of disguise.

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Understanding faecal attraction in roaches



Much of our common knowledge of cockroach behavior is gained in that brief interval between flicking on the kitchen light and racing to stomp on the little buggers. But the chaotic movements we observe while roaches scurry for their lives belies a far more interesting truth: behind our cupboards, roaches live highly organized social lives. Indeed, social contact among roaches is so crucial for their development and well-being that roaches kept on their own exhibit 'isolation syndromes': they develop more slowly, are reluctant foragers and show diverse deficiencies in mating and reproduction. If social contact is so important, how do young roaches ensure they stay together? New research published in *Proceeding of the National* Academy of Sciences by Ayako Wada-Katsumata and her colleagues from North Carolina State University in the USA provides compelling evidence that the key to aggregation lies in the alluring bouquet of roach poop.

Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

It has long been known that faeces are potent attractants for developing roaches. But the source of the chemicals that coordinate aggregation had remained unknown. While most researchers argued that aggregation pheromones are produced by the cockroaches themselves, the North Carolina team considered a novel alternative: that the pheromones were instead derived from gut bacteria excreted into the roach faeces.

To test this idea, the team reared germ-free roaches and then measured the attractiveness of their germ-free faeces to other roaches. In short, they didn't much like it. Irrespective of developmental stage, roaches significantly preferred extracts of 'normal' faeces to those isolated from sterile insects. But when uncontaminated roaches were recolonized with their endogenous microbial community, their faeces regained their appeal.

So what makes normal roach faeces so attractive? When the team compared the chemical profiles of normal with germfree poop, they identified a series of volatile compounds that were only found in the former. Furthermore, they could pin the production of a majority of these compounds to a set of six bacterial species that were resident in the cockroach guts. If these microbes were absent, or present with reduced diversity, test roaches lost their interest.

Cockroaches are some of our most pernicious pests. They carry disease, violate our food and (often) induce a general sense of queasy revulsion; we just don't like them, and we certainly don't want them in our homes. By understanding the factors that bring them together, Wada-Katsumata and colleagues hope to design a synthetic pheromone cocktail to bait roaches to their doom.

But this may be harder than it seems. Other researchers have also identified attractor compounds in roach faeces, but these only partially overlap with the volatiles discovered in this study. Moreover, the roaches in the present study didn't much care for the other chemical cocktail. At the moment it remains unclear whether this is due to the different methods used by different researchers, or to differences in the gut flora carried by different roach populations. If the latter,

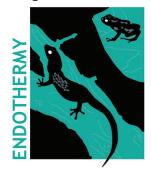
are these differences manifest at the level of individual colonies, or of individual families? Can a good whiff of poop identify a parent, a sibling or a suitable mating partner? The approaches and insights developed in this work provide a clear path towards answering these questions.

10.1242/jeb.130047

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Hot-blooded lizard illuminates endothermy origins



Understanding the evolution of endothermy in birds and mammals is a central question in evolutionary physiology, because thermal biology intertwines with numerous ecologically important life-history traits, such as body size and shape, foraging ranges, growth rates and the production of offspring. Theories about the selective drivers for the evolution of endothermy have been debated for decades, and include: the prevention of overheating during locomotion; expanded ability to sustain exercise; the generation of heat during digestion; and parental control of the reproductive environment. The discovery that a lizard (Salvator merianae) develops endothermy when arousing from hibernation and preparing to reproduce by growing gonads, mating and depositing yolk in their eggs, recently reported by Glenn Tattersall and colleagues in Science Advances, informs this debate in a number of ways.

The authors measured the environmental temperature and the body temperatures of adult male and female lizards weighing approximately 2 kg throughout the year. During the fall and winter, the lizards hibernate in underground burrows for 5–6 months. However, the researchers discovered that between days 160 and 180 of the hibernation fast, the night-time body temperature of both the males and females was raised markedly above the ambient temperature. At the end of the hibernation, the lizards resumed activity, basking during the day to supplement their own heat production and returning to the burrows at night. The team noticed that the lizards' body temperatures remained elevated throughout the night, by up to 10°C above the burrow temperature, during the period when they were preparing to reproduce, whereas at other times of the year the nocturnal body temperatures equilibrated with the burrow. And when the authors placed fasting adults that were prepared to reproduce in a thermostatically controlled chamber for 8 days, they learned that the lizards maintained a body temperature that was higher than that of their surroundings without the benefit of heat generated by digestion or the insulation of their burrows. However, when the lizards were disturbed, their body temperature fell, perhaps because of heat loss caused by increased blood flow to the limbs, skin, tail, head or other peripheral tissues. So heat production and the ability to regulate body temperature were not associated with either feeding or activity in this lizard.

The discovery that these lizards are able to maintain a body temperature that is greater than their surroundings is important in several ways. It refutes conventional wisdom, which holds that small animals that lack body insulation cannot raise body temperatures significantly, and complements previous work showing that, during reproduction, some species of python raise their body temperature up to 13°C above the surroundings when brooding eggs in insulated nests. It also shows that the ability to maintain a raised body temperature is not an oddity of one family of snake, but fits within a pattern of greater thermal stability during the reproductive period observed even in fully endothermic species, such as birds and mammals. Also, embryos are generally less tolerant of thermal

fluctuations than adults, so it is not surprising to find strong selection for characteristics that confer thermal stability during development. Convergent evolution is one of the strongest lines of evidence for the significance of a characteristic; therefore, the discovery by Tattersall and colleagues of the convergent evolution of reproductive endothermy in this lizard supports the hypothesis that the same selective pressure drove the evolution of endothermy in birds and mammals.

10.1242/jeb.138156

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Primed to fight: mom's meals make baby stronger



Expectant mothers have a long list of dietary dos and don'ts to worry about to give their babies the best start in life. Sushi, alcohol and raw eggs are all off the menu, and folic acid supplements suddenly become a necessity. The same appears to be true for the red flour beetle (Tribolium castaneum). Beetles that eat a diet contaminated with different species of bacteria produce eggs that are better primed to fight off bacterial pathogens, and recent research from Andreas Vilcinskas' laboratory at the Fraunhofer Institute for Molecular Biology and Applied Ecology in Giessen, Germany, shows us why.

Despite the earlier belief that invertebrates cannot mount an immune response, recent research has shown that invertebrates that encounter a pathogen such as a bacterium or fungus can subsequently defend themselves against that type of pathogen. Even more surprisingly, this priming can be passed on to subsequent generations in a process called transgenerational immune priming (TGIP). Using the red flour beetle, which is a commonly used model organism, Vilcinskas and his team set out to test two possible mechanisms for transgenerational immunes priming: one in which different genes are activated or inactivated by changes in DNA methylation in the eggs, and a second in which the eggs are directly exposed to the bacteria in the mother's body.

The team first looked at changes in the expression of stress- and immune-related genes in eggs laid by parents that were fed three different types of bacteria, each with different cell walls: *Escherichia coli*, *Micrococcus luteus* and *Pseudomonas entomophila*. They found upregulation of these genes, which turned out to be specific to the bacteria that the parents had eaten, with the eggs responding most strongly to the diet that included *M. luteus*.

Next, the researchers decided to look at whether there were wholesale changes in how many genes were turned 'on' or 'off' in the eggs by measuring the amount of DNA methylation in the eggs of parents fed the different bacterial species. However, they found no differences among the eggs laid by moms with different diets, so they moved on to the second hypothesis: that the eggs themselves became contaminated with the bacteria that their parents consumed.

To test this theory, Vilcinskas and colleagues fed beetles a very special meal - E. coli attached to a fluorescing particle that the team could use to identify where bacteria ended up in the bodies of the mothers that had consumed the meal. Using fluorescent microscopy to track the location of the bacteria, they found that not only did E. coli translocate from the gut of the mother beetles to the fat body, but also that E. coli could be detected between the wall of the developing egg and the mother, as well as on the eggs in the oviduct. The researchers concluded that the eggs were primed for an immune response because they were exposed to bacterial pathogens from their mother's diet well before they were laid and exposed to the external world.

So it turns out that expectant red flour beetle mothers can arm their offspring to deal with pathogens long before they are laid by eating a diet that primes an immune response when bacteria that have been consumed reach unlaid eggs in the mother's body. And fathers can also influence their offspring's immune function, but how this occurs remains a mystery that the researchers hope to tackle sometime soon.

10.1242/jeb.130054

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MMPs re-gift sight to zebrafish



When Prince Charming is blinded by his fall from Rapunzel's tower, her tears of joy restore his vision. In reality, losing one's sight is a life-long sentence because the human optic nerve – like most of the mammalian nervous system - has a limited capacity for self-repair. Yet there are many examples in the animal kingdom of growth and reinnervation of the central nervous system following injury. For example, zebrafish experience full functional recovery from optic nerve damage in as little as 3 weeks! What's their secret? Certainly there are many, but a group headed by Lieve Moons at the University of Leuven, Belgium, recently unlocked one. Their study, published in the Journal of Comparative Neurology, describes how a group of restructuring enzymes called matrix metalloproteinases (MMPs) work together to help the retina re-communicate with the brain after optic nerve damage.

To regain sight after optic nerve damage, the sensory neurons of the retina must regrow their axons through the optic nerve and establish new connections with the optic tectum of the brain, where visual signals are integrated. Moons' research team wanted to establish the timeline of MMP activity during this repair process. They began by crushing the optic nerves of anesthetized zebrafish with forceps and then allowed the fish to recover for up to 3 weeks. At defined times during this recovery period, the team looked at the expression changes of specific MMPs in the retinas of some of the injured fish using immunohistochemistry and western blots. They noted dynamic changes in the expressions of two particular MMPs, MMP-13a and MMP-2, in the retina

during the first week of recovery when axon regrowth peaks and approximately 60% of the optic tectum is reinnervated.

To fully lift the veil on the role of MMPs in 'un-blinding' zebrafish, Moons' team reasoned that if they blocked MMP activity during the first week of recovery, then retinal axons would fail to reinnervate the optic tectum. So, they repeated the optic nerve crushing experiment, but this time they injected the injured eyes with either saline or an MMP inhibitor to block MMP activity. One week later, Moons' team applied biocytin – a chemical label that is taken up by the axons and travels to their terminals – to the optic nerve, thus allowing the team to figure out whether the axons have found their way to the optic tectum. When they

checked the zebrafish brains, the team found far less biocytin in the optic tectums of fish given the MMP inhibitor compared with fish given saline. This means that MMPs are crucial for helping axons regrow during the first week after injury to the visual system. Perhaps Rapunzel's magic was merely a healthy dose of MMPs.

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