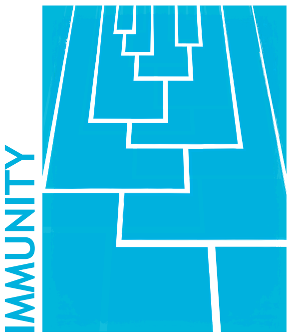
OUTSIDE JEB

Flour beetles evolve to arrest their killers



The ability to thwart infection is one of the most remarkable inventions in animal evolution. But despite its importance, immunity does not work the same way in all animals. Whereas vertebrate adaptive immunity has memory and specificity, meaning that it can use a first encounter with a pathogen to arm itself against subsequent encounters with the same pathogen, invertebrates, like insects, lack this capacity – or so it was thought. Recent evidence supports the idea that invertebrate immunity is far more sophisticated than had been believed, even showing evidence for memory and specificity. But how does immunity evolve, and can it improve?

An international team of scientists led by Joachim Kurtz from the University of Münster, Germany, recently addressed this question. Kevin Ferro, now at the University of Arizona, USA, and Robert Peuß, now at the Stowers Institute in Kansas City, USA, conducted an experiment where they exposed flour beetles (*Tribolium castaneum*) to different bacterial pathogens and studied how their ability to overcome infection evolved over many generations. Their design was based on a procedure called 'immune priming' where beetle larvae are first injected with heat-killed bacteria and then subsequently challenged with a potentially lethal infection of the same or a different bacterial species later in life.

The logic of the experiment was simple. If immune priming induced immune memory, larvae would be protected during their second exposure to the same pathogen. This, in turn, would allow them to survive to adulthood and to breed the next generation of larvae, which, over several generations, would select for beetles with an improved ability to beat infection. In other words, they would experimentally evolve beetles with enhanced adaptive immunity.

After only 14 generations, and nearly 50,000 animals, it worked! The beetles evolved increased survival after priming and challenge. More impressively, the magnitude of this improvement was higher if each generation of larvae were primed and challenged with the same pathogen, a so-called specific challenge, than with a mismatched pair of pathogens, an unspecific challenge. In short, memory and specificity evolved readily. But how did it occur?

To identify mechanisms underlying enhanced immunity, the research team compared the gene expression profiles of primed beetles that evolved with a specific challenge each generation with those evolved with an unspecific challenge. Unsurprisingly, there were hundreds of differences between the groups. And while there were no diagnostic genes that simply explained improved immunity on their own, there were several tantalizing targets in well-known immune effectors that were significantly up- or down-regulated in the specific group, including genes for antimicrobial peptides and iron capture. Notably, several of these pathways serve crucial functions in vertebrate immunity, suggesting striking conservation of the mechanisms of pathogen defence across animals.

Beetles are hugely diverse, so much so that the 19th century biologist J. B. S. Haldane once quipped that God must have had an 'inordinate fondness for beetles'. One can only imagine how Haldane would have reacted had he known about the massive numbers and incredible diversity of bacteria. Beetles, indeed all creatures, face unpredictable challenges from the innumerable quantities of microbes that they encounter at every stage of their lives. Most microbes are harmless, but if left unchecked by an active and flexible immune system that can respond to novel challenges, even these species would surely kill us. Although I'm excited by the cleverness and scope of this experiment, I'm not remotely surprised at the outcome. The massive success of beetles virtually guaranteed the result, at least in broad strokes. Figuring out how it all works promises many exciting discoveries to come.

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Ferro, K., Peuß, R., Yang, W., Rosenstiel, P., Schulenburg, H. and Kurtz, J. (2019). Experimental evolution of immunological specificity. *Proc. Natl. Acad. Sci. USA* **116**, 20598-20604. doi:10.1073/pnas.1904828116

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Why a sea snake needs a hole in its head

Sea snakes are renowned predators that display a suite of adaptations, including paddle-like tails for swimming and glands to expel excess salt, which equip them for the marine realm. Yet, as the snakes still require air to breathe, they remain reliant on excursions to the surface, which expose them to predators and limit their ability to hunt prey underwater. Aquatic snakes somewhat alleviate this need by having thin skin, which permits some degree of oxygen uptake across the body wall into their blood. But gas exchange via this route is restricted, as skin on the

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whole must retain its integrity to protect the body, so it remains a barrier to the diffusion of gases. However, a new discovery by Alessandro Palci from Flinders University, Australia, and colleagues sheds light on how some species have developed an additional specialised respiratory organ fit for the purpose.

It has long been known that some species of sea snake have a peculiar foramen, or hole, in the top of their skull. This attracted the attention of Palci's team, who pondered why the snakes need this hole in their head. To resolve its function, they began studying the anatomy of blood vessels in the head of the annulated sea snake (*Hydrophis cyanocinctus*) using CT scans of preserved specimens, as well as with microscopic analysis of dissected tissues. To provide a comparison, they also investigated the same anatomy in a closely related land snake, the taipan *Oxyuranus scutellatus*, as well as two other sea snakes, the olive sea snake (*Aipysurus laevis*) and Stoke's sea snake (*Hydrophis stokesii*), which do not have the unusual enlarged openings in their skulls.

In the annulated sea snake, the team saw a dense bed of blood vessels, termed a plexus, on the top of the skull that was absent in their terrestrial cousin, the taipan, and the other sea snakes. This plexus primarily consists of thin-walled veins, which eventually converge in a single vessel that penetrates the skull, via the conspicuous foramen, and passes into the brain. To ascribe a role to the vessels, the authors considered a number of hypotheses – including the possibility that the bed of blood vessels supplies the salt secretion glands – but none except for a role in respiration made anatomical sense. The team believe that the dense meshwork of blood vessels, lying just beneath a thin layer of skin, provides a route to boost oxygen in the blood in order to keep the brain alive. To strengthen their theory, the team made calculations, which suggest the additional oxygen that diffuses into the blood via the plexus could satiate the brain three times over.

It remains to be established whether this unusual labyrinth of blood vessels successfully prolongs dive duration and why only some species of sea snakes require it, but for now this study describes a curious, novel adaptation for cerebral oxygenation that may assist these diving specialists to prosper underwater.

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Palci, A., Seymour, R. S., Van Nguyen, C., Hutchinson, M. N., Lee, M. S. Y. and Sanders, K. L. (2019). Novel vascular plexus in the head of a sea snake (Elapidae, Hydrophiinae) revealed by high-resolution computed tomography and histology. *R. Soc. Open Sci.* **6**, 191099. doi:10.1098/rsos.191099

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Starved snails move slowly through resource-rich environments

In nature, animals have to cope with constantly changing landscapes due to shifts in land use and the developing climate crisis. Changing landscapes can affect the resources available to animals and may alter how they move through their environment to find and eat food. While there is a wealth of information surrounding how a landscape affects an animal's movement, significantly less is known about how an animal's movement pattern varies over time within a changing landscape. To test how patterns of movement change through time in landscapes that vary in the amount of available food, researchers from Washington University of St Louis, USA, built their own tiny landscapes for the freshwater snail, *Physa acuta*, to cruise through.

First, Carl Cloyed and colleagues travelled to Illinois where they collected snails from the waters of the Mississippi River to bring back to the laboratory. In the wild, the snails graze on algae, so the researchers constructed tiny artificial 'landscapes' on panes of glass with varying amounts of algae (0%, 25%, 75% or 100% coverage). To ensure that the algae were distributed randomly across the glass, the researchers made a grid of 100 squares and removed algae from random patches. After depriving the snails of food for 1 day to ensure that they were hungry, the researchers placed the snails onto their temporary homes and set up video cameras with tracking software to measure their crawling speed and movement patterns.

The researchers initially examined whether the presence of food affected the snails' speed. Because the snails should have been hungry and keen to satisfy their hunger at the beginning of the experiment, the researchers predicted that the molluscs would move slower in patches with food. Indeed, the movies confirmed their hunch, revealing that the snails moved faster when searching for food in barren patches and slower in algae-rich patches where they could gorge themselves. This supports the idea that internal states, like hunger, might drive animals to alter their speed depending on their environment.

Similarly, the researchers predicted that the snails' movement patterns would differ depending on whether the molluscs were passing through regions of plenty or algae-free patches. Sure enough, the snails in bare patches moved in long straight lines to search better for food and the snails surrounded by abundant algae moved back and forth in short stretches to consume as much as possible. This suggests that the snails alter their movements depending on their local environment.

Cloyed and colleagues then wanted to test how the snails' movement patterns changed as they roamed extensively over a 24 h period, comparing the molluscs' movements during the first 12 h with their movements during the second half of the day. Initially, the snails moved in straight lines, but during the second half of the experiment, they took shorter, windier paths. This suggests that the presence of resources affects not only the snails' movements at a single patch but also their movement patterns across their environment.

This is one of the first projects to experimentally demonstrate how an animal's landscape can affect its movement patterns over time. Movement characteristics – such as moving quickly in a straight line when hungry – could be included in models of animal movements to help us understand better the consequences of landscape changes on animal behaviour.

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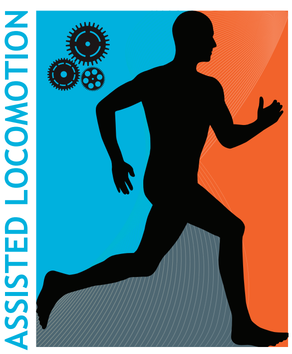
Smart suit reduces energy of both walking and running



Humans walk when going slowly and run to go fast, and switch between the gaits to use the one that is most efficient for the speed at which they are moving. In a bid to improve our efficiency even further, engineers have designed a range of different assistive devices worn around the legs – so-called exosuits, which take advantage of springs, clutches or electric motors – to reduce the energy required to walk or run. But usually, these assistive suits only benefit a single gait type, because the muscles in our legs activate in different patterns depending on whether we are walking or running. To overcome this limitation, a research team from Conor Walsh's lab at Harvard University, USA, set out to produce an assistive exosuit that could reduce energy expenditure during both walking and running by sensing the gait type that wearers used.

The team built a 5 kg suit to fit around a person's hips with cables running down the thighs from an electrical motor on the lower back. The motor shortens the cables to assist the hip muscles, such as the hamstrings and glutes, but responds differently depending on whether sensors embedded in the fabric detect the body is moving up–down – indicating walking – or down–up, when running. In a series of experiments, the team tested whether athletes save energy when wearing the suit, whether the suit can detect when athletes walk or run and whether the suit is versatile enough to provide energy savings across a wide range of walking and running conditions.

To test the effectiveness of the suit, the team recruited nine athletes to walk on a treadmill at 1.5 $\mathrm{m\,s^{-1}}$ and run at 2.5 $\mathrm{m\,s^{-1}}$ while measuring their energy use from their oxygen consumption. The suit reduced the energy cost of walking by 9.4% during assisted walking and by 4.0% while running – savings that should translate into improved athletic performance.

To show that the suit accurately detects when the person wearing it is walking or running, the team performed a series of tests involving treadmill walking and running, from speeds of 0.5 to 4.0 $\mathrm{m\,s^{-1}}$ while climbing up and down slopes, ranging from –10% downhill to 20% uphill. The suit did extremely well in almost all cases, correctly identifying the type of gait type used by the athlete on 99.98% of occasions. In addition, it was able to produce energy savings of up to 22.8% when the athletes were running at speeds ranging from 2.5 to 3.0 $\mathrm{m\,s^{-1}}$ on the flat and when walking uphill. The team also tested how well the suited volunteers coped when walking and running across different terrains, from normal ground to muddy tracks and snowy landscapes; the results indicate that the suit could benefit people in a wide range of challenging situations, such as firefighters and search-and-rescue teams, who often need to move fast while carrying heavy loads. The team also suggests that the suit may benefit patients, such as stroke victims, who require some support when struggling to walk during recovery.

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