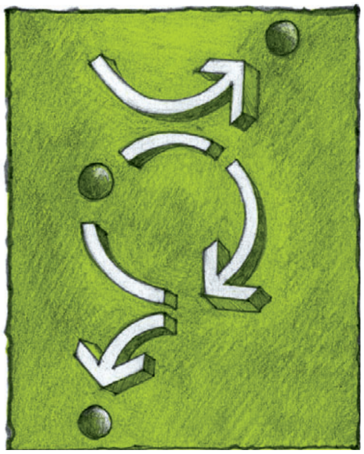
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

ROYALACTIN



ACTIVE COMPONENT IN HONEYBEES' ROYAL JELLY DISCOVERED

Considering their role in pollination, honeybees are among the most beneficial insects on earth. This is because they are social insects that share tasks and live in hives consisting of thousands of workers and one queen. Although the workers and queen are genetically identical, the queen has a larger body, develops in a shorter time and lives much longer than a worker bee. Moreover, the queen is the only bee in the hive that lays eggs. It has been known for more than 100 years that larvae continually fed on royal jelly – a special diet secreted by workers – transform into queens. However, the identity of the jelly's active component was unknown. In a recent study published in *Nature*, Masaki Kamakura from the Toyama Prefectural University, Japan, performed exciting experiments identifying one component of royal jelly that transforms workers into queens.

Royal jelly is a quite peculiar juice. It is a complex mixture of different nutritional ingredients and hence not easy to analyse, in particular because some of the components are unstable. But it was this characteristic that helped the Japanese scientist to find the queenmaker. He harvested royal jelly from queen-less workers, stored it for different lengths of time, and tested whether it was still able to induce queen differentiation in larvae. Long-term storage decreased the jelly's biological activity significantly. Then he went on to analyse the jelly's protein composition. He recognised that only a few proteins were degraded over time, and he concluded that one of them must carry queenmaker activity. Among the candidates was a 57 kDa protein, which he named royalactin. Indeed, when he purified this protein from royal jelly and added it to a diet that was not able to induce queen differentiation, the larvae developed into queens. Kamakura obtained a similar result when he used artificial royalactin that had been synthesised by bacteria.

Because of the lack of genetic tools in bees, the underlying signalling pathway is difficult to examine. Therefore, Kamakura switched to *Drosophila* and performed an ambitious experiment where he fed fly larvae with a medium containing either active royal jelly or inactive royal jelly supplemented with royalactin. The result from this simple but brilliant experiment was stunning, as the flies acquired many of the attributes of queen bees. Both diets led to a shortened developmental time, an extended life span and an increase in the flies' body size. Moreover, he observed similar effects when he overexpressed royalactin in the flies' fat body. Next, Kamakura worked out the signalling pathway for royalactin-mediated changes by testing its action in mutant flies defective in central components of several signalling pathways. In doing so, he showed that royalactin acts through a mitogen activated protein kinase pathway initiated by the epidermal growth factor receptor (Egfr) in the flies' fat body. This signalling cascade appears to also function in bees, as knockdown of *Egfr* expression by RNA interference suppressed queen differentiation in bee larvae reared on royal jelly.

Kamakura has done an incredible job and he must have worked day and night to find the active ingredient in royal jelly and decipher its mode of action. However, many questions remain unanswered and insect scientists around the world will now go on to further examine how royalactin works precisely and address the question of whether similar proteins have comparable functions in other social insects such as ants.

10.1242/jeb.049874

Kamakura, M. (2011). Royalactin induces queen differentiation in honeybees. *Nature* **473**, 478-483.

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REWRITING THE NEURAL CODE FOR RUNNING

When an animal is standing still or moving slowly, it's relatively easy to figure out what a specific muscle might do. Once the animal starts moving more quickly, the question gets a lot hairier – the body and the legs are swinging around; sometimes the legs hit the ground and sometimes they're in the air; and bones and joints have their own springiness. If we could answer the question, though, we'd be one step closer to understanding how animals run, jump, fly or swim as well as they do – and perhaps a step closer to building robots that could do the same thing.

Some groups have turned to computer models to examine how muscles and body interact during fast movements. But Simon Sponberg, Andrew Spence, Thomas Libby, Chris Mullens and Robert Full at the University of California, Berkeley, developed an alternative method to examine this interaction in running cockroaches.

They implanted recording and stimulating electrodes in a cockroach's middle leg, in the ventral femoral extensor muscle – a muscle that produces force to extend the leg and raise the body up or, conversely, to slow the body from falling down. Indeed, previous work, in which the muscle was excised from the body, had suggested that its primary function is as a brake, absorbing energy by slowing the body down.

Sponberg and his colleagues wanted to look at how the muscle functions in the animal during running. So, rather than pulling the muscle out, they developed a system that recorded the normal muscle action potentials from the nervous system, then used the stimulating electrode to rewrite that neural command – all in real time as the cockroach ran in an open arena. They couldn't erase the command entirely, but they could extend it for longer than normal or start it earlier. To see the effects of changing the neural command, they had the cockroaches carry acceleration-sensing backpacks and used high-speed video to accurately and rapidly track leg and body motions.

What they found was almost completely different from what they'd predicted based on older work. If the muscle normally operates like a brake, they expected that extending the normal command would make it brake even harder. Instead, it did the opposite, and started producing energy, lifting the body higher. Interestingly, the changes persisted into the following stride – for which they hadn't changed the neural command – and tilted the cockroaches up.

When the researchers started the neural command earlier, rather than extending it later, they found yet another effect – the animal started to turn. The earlier the command started, the stronger the turn.

In a companion paper, Sponberg and colleagues considered how the muscle changed its function during running. They found that the effect was due to a purely mechanical positive feedback between the muscle force and the leg position during the stance phase – no sensory inputs were needed. With more spikes, the muscle produced more force, extending further, which allowed it to produce even more force and extend even further.

Studying these neuromechanical feedback loops is challenging, but they hold the key to understanding the amazing flexibility of animal locomotion.

10.1242/jeb.049858

Sponberg, S., Spence, A. J., Mullens, C. H. and Full, R. J. (2011). A single muscle's multifunctional control potential of body dynamics for postural control and running. *Phil. Trans. R. Soc. Lond. B* **366**, 1592-1605.
Sponberg, S., Libby, T., Mullens, C. H. and Full, R. J. (2011). Shifts in a single muscle's control potential of body dynamics are determined by mechanical feedback. *Phil. Trans. R. Soc. Lond. B* **366**, 1606-1620.

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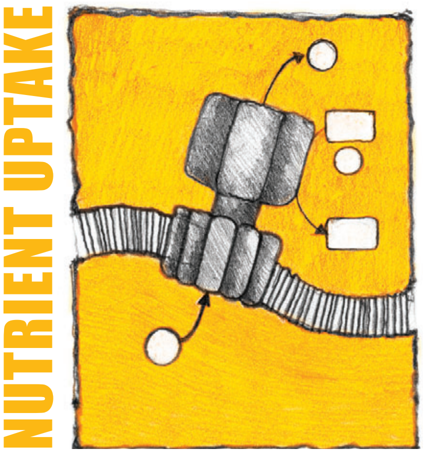

HAGFISH GO WITH MORE THAN THEIR GUT

The eel-like hagfish is notorious in the animal kingdom because of its repulsively fascinating production of defensive slime and its fondness for burrowing into and munching its way out of decaying carcasses. Unlike other vertebrates, the ancient hagfish is an osmoconformer, meaning it keeps most of its internal ions at about the same concentration as in the surrounding seawater. This characteristic reduces the need to develop impermeable skin to regulate ion and water movement. It also presents the possibility of direct nutrient acquisition (feeding!) across the skin, a trait previously thought to exist only in invertebrates like mussels and worms. This notion prompted Chris Glover of the University of Canterbury, New Zealand, and two Canadian colleagues, Carol Bucking and Chris Wood, to investigate the feeding potential of hagfish at a new level.

The research trio baited Pacific hagfish off Vancouver Island and transported their catch to the nearby Bamfield Marine Sciences Station, where they set about collecting target tissues. First, the team prepared hagfish gills, controlling the water composition that entered the gill, and collecting what passed through it. They also injected radioactively labelled amino acids into the water flowing to the gill to follow the movement of these nutrients. Much to their excitement, the gills readily absorbed the nutrients.

Glover and colleagues stretched the skin of the hagfish across the mouth of a vial containing a solution equivalent to the internal fluids of a hagfish. With the skin intact, the team immersed the vial in a solution that contained either food colouring dye or radioactive amino acids so that they could monitor whether nutrients could cross the barrier. The dye did not penetrate the skin for at least 12 h, demonstrating the integrity of the skin barrier. However, nutrient uptake did occur across the skin, just as it had across the gills. Nutrient uptake profiles for both the gills and skin were sigmoidal in shape and dependent on sodium, with a large linear increase in uptake occurring only at high amino acid concentrations at the gills. These characteristics indicate that, with the exception of very high concentrations of nutrients, absorption occurs *via* specific transport pathways, not by diffusion alone. Such types of transport system are already known to exist in vertebrate tissues, relying upon the inherent sodium gradient between the extracellular and intracellular space to give nutrients like these a free-ride across the cell membrane. As hagfish are thought to be the oldest living link to the ancestral vertebrate, these results suggest that early aquatic vertebrates may have utilized similar feeding strategies, with a more specialized digestive system evolving later, along with the development of osmoregulatory strategies.

As the hagfish's food supply can be unreliable and they are capable of surviving for months between meals, when fine dining opportunities present themselves, this ancient vertebrate must take advantage, justifying its tendency for gluttony and perhaps contributing to the evolution of multiple surfaces capable of nutrient acquisition. Such a strategy proves particularly effective for an animal like a hagfish, which immerses itself completely within its carcass of choice. This research reveals a novel means of feeding in vertebrates and is the first account of nutrient absorption across a tissue other than the gut. As a writhing hagfish might advise, go with more than your gut!

10.1242/jeb.049866

Glover, C. N., Bucking, C. and Wood, C. M. (2011). Adaptations to *in situ* feeding: novel nutrient acquisition pathways in an ancient vertebrate. *Proc. R. Soc. Lond. B*, published online before print, doi: 10.1098/rspb.2010.2784.

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FLIGHT IN RAIN COSTS BATS DEAR

Flying in the cluttered rainforest is challenging at the best of times, and it is particularly challenging for little bats when it rains. In 1971, Donald R. Griffin showed how high humidity and rain interferes with echolocation, which could explain why many bats won't fly during a downpour. In addition, having wet fur could increase the energetic costs of thermoregulation and decrease the aerodynamic properties of the bat by causing the fur to clump together. However, no one knew how much of an impact getting wet might have on bats in flight. In a recent study, Christian C. Voig and his colleagues from the Leibniz Institute for Zoo and Wildlife Research, and from the Freie Universität Berlin, Germany, measured the cost incurred by bats when flying in the rain.

The researchers studied 10 Sowell's short-tailed bats (*Carollia sowelli*) at La Selva Biological Station in Costa Rica. Each bat was exposed to three treatments in random order. In the first treatment, the researchers allowed the bats to fly in dry conditions; the second treatment consisted of wetting the bats with tap water and then allowing them to fly; and during the third treatment, the bats were exposed to moderate rain while flying. Voig and his colleagues measured the metabolic rate of the bats before, during and after flight to determine the cost of flight during these three different conditions.

The metabolic rate of dry bats while flying conformed to the team's expectations for mammals of that size. However, the metabolic rate of the wet bats was more than twice as high as that of the dry bats, and it didn't matter how the animals had been drenched. Although the additional weight of water itself could partially explain the higher cost of flying while wet, the researchers point out that a twofold increase in metabolic rate would have required the average 18 g bat to be carrying 25 g of water, which is unlikely as dry and wet bats weighed about the same. The increase in flight costs was also not due to changes in flight pattern caused by the raindrops, because the wet bats that had been doused with tap water experienced the same increase in metabolic rate as the bats that had been rained on.

However, Voig and his team of researchers noticed that water caused the bats' fur to clump, which could decrease the aerodynamic properties of the fur, increasing the costs of lift and thrust during flight. They also suggest that the cooling effect of water evaporating from the bat's fur and wing membranes increases the cost of thermoregulation and could therefore increase the cost of flight.

Off all living and extinct vertebrates, only three groups have evolved flight: pterosaurs, birds and bats; but of these three groups, only bats are covered with fur. Although fur aids in bats' thermoregulation by providing insulation, Voig's study shows that having fur can be a drag if you are a hungry little bat trying to forage in the rain.

10.1242/jeb.049882

Voig, C. C., Schneeberger, K., Voig-Heucke, S. L. and Lewanzik, D. (2011). Rain increases the energy cost of bat flight. *Biol Lett.*, published online before print, doi: 10.1098/rsbl.2011.03013.

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