Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

PENGUINS RETURN ON EMPTY


Picture by Katherine Ponganis

Any of us who wish to take to the water have no choice but to don scuba diving gear before plumbing the depths. Yet many ocean going mammals and birds regularly dive on a single breath. One of the avian world's champion divers is the emperor penguin, which routinely dives for several minutes while searching for food. Intrigued by the bird's dive tolerance, Paul Ponganis from the Scripps Institution of Oceanography at the University of California San Diego decided to investigate how the birds handle perilously low oxygen levels. From previous studies, Ponganis and his colleagues knew that towards the end of a dive, oxygen levels in the penguin's air sac, which provides additional ventilation for the lungs, fell even lower than those experienced by birds at extreme high altitudes. Wondering how the birds manage their oxygen stores during a dive, the team decided to investigate penguins' blood oxygen levels as they foraged beneath the sea ice (p. 4279).

In previous field seasons, Ponganis and his team had developed a system where they fitted a tiny oxygen probe inside the penguin's air sac and collected oxygen level data in a microprocessor carried on the bird's back. This time, Ponganis wanted to measure the penguins' vascular oxygen levels. Adapting techniques that he practices routinely as a cardiac anaesthesiologist, Ponganis inserted the probe into a vein or artery before releasing the penguins from an isolated ice hole, allowing the birds to come and go freely for several days before retrieving the probe and backpack crammed with oxygen data.

Scrutinising the data from 130 dives, the team was amazed to see that a few birds' venous blood oxygen levels had fallen almost to zero by the end of a dive and almost a third of the returning divers had blood oxygen levels where humans black out. And when the team compared lung, arterial and venous blood oxygen levels at the end of long dives, they were virtually indistinguishable. 'Emperor penguins clearly push the limits of hypoxemia' says Ponganis 'and are capable of "returning on empty"' he adds.

But how do penguins pull off this remarkable feat? Ponganis believes that the answer lies partly in the penguin's haemoglobin. He suspects it must have very different oxygen carrying characteristics from most ducks and flying birds. According to Ponganis, his idea builds on Bill Milsom's work in the 1970s and David Jones's from the 1980s. He explains that Milsom suggested that haemoglobins with a high oxygen affinity (that are able to bind oxygen at very low concentrations) may allow divers to use their respiratory oxygen stores more completely. And in 1986, David Jones found that the Pekin duck was unable to use all of its oxygen stores; the duck was at the point of 'imminent cardiovascular collapse' despite having used only 75% of its respiratory oxygen. Based on these ideas, Ponganis suspects that the emperor penguin's haemoglobin may have a high oxygen affinity to allow the bird to fully exploit its oxygen reserves during a dive and is keen to investigate this idea during his current stay in Antarctica.

10.1242/jeb.014787

Ponganis, P. J., Stockard, T. K., Meir, J. U., Williams, C. L., Ponganis, K. V., van Dam, R. P. and Howard, R. (2007). Returning on empty: extreme blood O_2 depletion underlies dive capacity of emperor penguins. *J. Exp. Biol.* **210**, 4279-4285.

MUSSELS TAKE THE STRAIN

Watching a coastal winter storm, no one can fail to marvel at the persistence of mussels battered by the waves. Tethered to the rocks by robust fibres known as byssal threads, scientists have long been intrigued by the fibre's mechanical properties. The thread's major component is a form of collagen, similar to that found in muscle tendon, but at strains where muscle tendons fail, mussel byssal threads just keep on stretching, easily doubling their length. And more remarkably, a few hours after yielding the stretchy threads self-heal, regaining most of their original stiffness. Intrigued, J. Herbert Waite and student Matthew Harrington began investigating the byssal thread's collagen like proteins, focusing in particular on preCol D to find out how it contributes to the thread's unique performance (p. 4307).

Harrington explains that preCol D is the main component of the thread nearest the


Picture by Matthew Harrington

end anchored to the rocks. Each PreCol D protein comprises several subdomains, and suspecting that the individual domains contributed different properties to the thread's remarkable sturdyness, Waite and Harrington decided to compare preCol D proteins from *Mytilus edulis*, *M. galloprovincialis* and *M. californianus* to see if they could learn more about each domains' functions.

Having sequenced the three *M. californianus* preCols, Harrington compared them with the preCols from the other two species. The collagen domains were essentially identical across all three species, but the sequences from some of the flanking domains differed dramatically.

Harrington explains that one of the PreCol D domains is very similar to another superprotein: spider silk. Spider silk is incredibly stiff, and when Harrington aligned the amino acid sequences of the silk-like domain from the three species, he found that the *californianus* protein had gained an extra stretch of amino acids, similar to the insertions found in the stiffest spider silks, which could explain why *californianus* byssal threads are so much stiffer than the other two. 'Mussels have taken a page from the spiders' book' says Harrington 'using the same types of insertion to stiffen the thread'.

Shifting focus to another small PreCol D domain, peppered with histidine amino acids, the pair realised that the positions of the histidines in the domain were essentially the same across all three species: 'we thought the histidine residues must be important' says Harrington. As histidine amino acids coordinate metal ions, the team wondered whether the histidines from neighbouring preCols could cross-link with each other via metal ions, adding to the stiffness of the thread. Harrington began testing, altering the histidine sidechains' ability to form cross-links and measuring the impact on the thread's stiffness. Sure enough the byssal thread's stiffness varied as the team altered the histidine residues, confirming that the histidine-rich domain probably contributes to the thread's stiffness by cross-linking.

The pair also suspect that the histidine domain contributes to the thread's self-healing. Harrington explains that when collagen in tendon ruptures, strong covalent links in protein chains have been ripped apart; they cannot reform, so the tendon is permanently damaged. But when byssal threads are stretched to breaking point, the team suspect that the weaker histidine crosslinks fail, reforming once the storm has passed 'giving the thread the ability to yield and give' says Harrington.

10.1242/jeb.014753

Harrington, M. J. and Waite, J. H. (2007). Holdfast heroics: comparing the molecular and mechanical properties of *Mytilus californianus* byssal threads. *J. Exp. Biol.* **210,** 4307-4318.

FLYING ON THE EDGE


Picture by Chris Blokhuis

As a fruit fly twists and dives through the air, two major environmental forces act on its gyrating body: inertia and friction. While the insect's inertia is entirely down to its body mass, it was less clear how significant air friction is on the tiny aeronaut. Fritz-Olaf Lehmann explains that the insect's light wings contribute little to the insect's mass, and so virtually nothing to their inertia. However, they comprise a significant surface area. Could they contribute significantly to the friction on the tiny aviator? Curious to know how friction contributes to a fly's manoeuvrability, Lehmann and his postdoc Thomas Hesselberg calculated the damping coefficient due to frictional forces acting on a fly's flapping wing during a saccadic turn and found that it was 100 times greater than the friction acting on the body alone (p. 4319). Far from being insignificant, friction appears to be a major force on the fly's activities and could help rapidly turning flies to stop. Hesselberg and Lehmann realised that instead of actively breaking, the sticky air could halt a rapidly turning fly. Following the simulations the pair decided to test frictional effects on the insect's flight in a controlled environment.

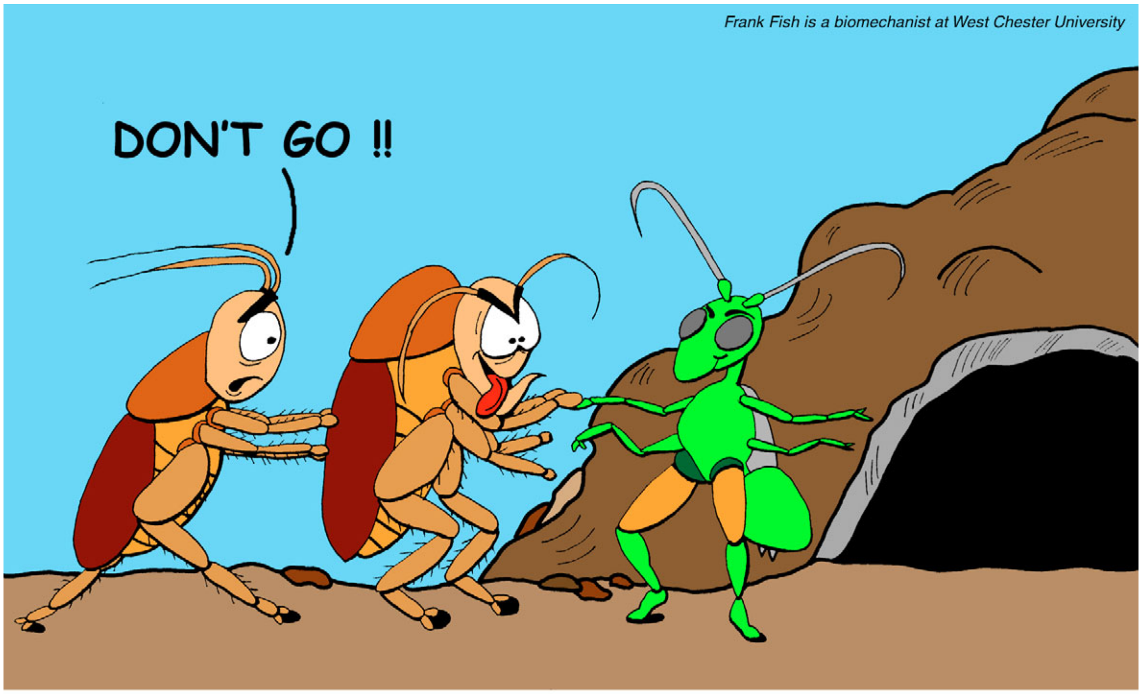
Tethering fruit flies inside a high-tech flight arena, Hesselberg and Lehmann tested the insects' responses to a simulation of the insect's view as it turned towards a moving target. Hesselberg designed a complicated feedback control system that forced the insect to steer as it flew is if approaching a moving object, feeding back the insect's behaviour into the simulation. By altering the simulated view, the pair could fool the insect's flight control system and trick it into behaving as if it were flying through thick and normal air, so that the team could measure the insect's steering accuracy to see how well it manoeuvred at high and low friction. The team found that the tethered fly coped reasonably well flying through most of the simulations, except when the damping was especially high or at low (normal) conditions; failing to turn when the damping was high, as if the air was too thick; and turning uncontrollably when the damping was low, as if normal air is too thin to stop the rotation.

One reason that has been suggested for control failure when the insect is flying in low damping (normal) air is that the fruit fly's visual system cannot respond fast enough to rapid turns, forcing a freely flying insect to rely on other sensory systems, such as the gyroscopic halteres. However, when the flies are tethered, the halteres are no longer able to supply information for precise flight control. With only their eyes to guide them, tethered insects have to rely on exceptionally precise wing beat control, between 1-2°, to control turning; which is beyond even these aviators' talents. However, Lehmann and Hesselberg found that the flies could successfully control flight when they increased the damping above normal levels to a point where the flies only needed a wing beat accuracy of 3-4°, suggesting that flies could manoeuvre accurately using their eyes alone if the sensory information was delivered as fast as information from the halteres. All of which suggests that fruit flies are flying on the edge, depending on every control and sensory system available to them.

10.1242/jeb.014779

Hesselberg, T. and Lehmann, F.-O. (2007). Turning behaviour depends on frictional damping in the fruit fly *Drosophila*. *J. Exp. Biol.* **210,** 4319-4334.

ZOMBIE 'ROACHES



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IT WASN'T A GOOD THING WHEN SHE SAID THAT SHE WANTED HIM TO COME HOME FOR DINNER.

When a cockroach is stung by a jewel wasp, the hapless victim is in for a deeply unpleasant experience. After the venom has robbed the cockroach of its free-will to walk, the wasp leads the victim back to a burrow to be colonised by its larva and consumed from the inside out. How the wasp's venom turns cockroaches into zombies has long intrigued Frederic Libersat. Knowing that the venom seems to alter the activity of neurones that release the neurotransmitter octopamine, Libersat and his colleagues at Ben-Gurion University of the Negev decided to see whether the venom incapacitates its victims by interfering with octopamine release (p. 4411).

First the team tested whether the venom targeted the octopamine system by seeing if they could restore cockroach free-will by administering octopamine agonists (mimics) to stung cockroaches. Injecting octopamine agonists into the insect's circulatory system, the team restored the insect's ability to roam free. Next, knowing that octopamine releasing neurones were directly connected to regions of the brain targeted by the wasp attack, the team decided to try to reverse the toxin's effects by injecting an octopamine agonist directly into the stung brain regions. Amazingly, the zombies recovered significantly when injected in the protocereberum, part of the central brain, but not when injected in the subesophageal ganglion.

By directly replacing octopamine with the octopamine agonist in the protocereberum, the team had mostly restored the cockroaches ability to walk independently. Libersat and his team suspect that 'venom injection into the head ganglia selectively depresses the motivation to move by modifying the release of octopamine as a neuromodulator in restricted regions of the cockroach brain'.

10.1242/jeb.014761

Rosenberg, L. A., Glusman, J. G. and Libersat, F. (2007). Octopamine partially restores walking in hypokinetic cockroaches stung by the parasitoid wasp *Ampulex compressa*. *J. Exp. Biol.* **210,** 4411-4417.

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