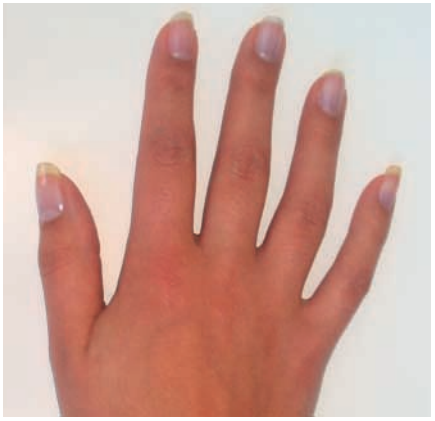
Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

SCRATCHING THE FINGERNAIL'S SURFACE



Dedicated nail biters, pay attention. You know what happens when you're nibbling a particularly annoying piece of nail? They never seem to rip downwards, only ever across. Well, Roland Ennos and a team of undergraduate researchers can now explain why your fingernails only splinter in one direction. They have discovered that the central layer of the nail is effectively reinforced by long thin cell structures, directing fractures across the nail no matter which way you try to rip (p. 735).

Ennos admits that this piece of research was inspired by his own nail nibbling habits, but with well-trimmed nails of his own, he needed a willing nail donor before he could begin investigating the tissue's biomechanical properties. Fortunately, his student Stephanie Shayler was happy donating her talons to science. After harvesting 3 mm-long fragments, Shayler slit the nail at the tip and tried tearing downwards. But the nail always resisted the downward rip; the nail seemed to be reinforced along its length. Puzzled by the nails' anisotropic characteristics, Shalyer used precision cutting equipment to cut the nail lengthways, and across, to test her nails' toughness. The nails were twice as tough along their length than across their width. More surprisingly, Shayler's nails were almost as tough as horses' hooves!

But how was the nail structured to resist rips in one direction, and not the other? Using a scanning electron microscope, Shayler and Ennos could clearly see three keratin-laden layers. The top and bottom layers were both made up from 'flat overlapping slate-like sheets' explains Ennos, and could rip in almost any direction. But when Ennos saw the core layer, sandwiched between the outer two, it was clear why nails only rip in one direction. The middle layer was built up from long thin keratin-packed cells, arranged with their long axes across the nail's width, resisting cracks that might penetrate to the nail's bed. But how tough was the central layer relative to the outer two?

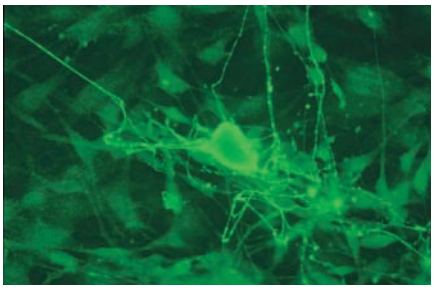
This time Laura Farren set about trying to isolate each of her fingernails' layers to test their toughness. But peeling the layers apart proved tricky, until Farren realised that they were relatively easily separated when her fingernails were soft after a swim. Using the same precision cutting equipment, Farren tested each layer's toughness and discovered that the central layer was four times tougher along its length than its width, and six times tougher than either the upper or lower layers.

So why does the nail need the brittle outer layers when the fibrous core seems to provide most of the nail's inner strength? Ennos explains that, although the core layer strengthens the nail along its length, nails are still relatively fragile across their width. He suspects that it's the tile-like outer layers that give long talons their bending strength, but he adds 'this study has only started to scratch the surface of fingernail design'.

10.1242/jeb.00837

Farren, L., Shayler, S. and Ennos, A. R. (2004). The fracture properties and mechanical design of human fingernails. *J. Exp. Biol.* **207**, 735-741.

GRAPES' SWEET PAIN



Lets face it, grapes are irresistible; at least humans seem to think so. But some birds are less sure. Bruce Bryant

explains that methyl anthranilate in grape skins is so unpleasant to birds that a dose is enough to trigger vigorous head shaking 'in an effort to rid themselves of the offensive substance'. But how methyl anthranilate inflicts pain on its feathered victims wasn't clear, until Bruce Bryant and his colleagues at the Monell Chemical Senses Center began teasing apart the cellular mechanisms of pain transduction in chicken neurons (p. 715)

Bryant explains that all creatures have a well-developed suite of defensive senses that detect danger, called nociceptors. Nociceptors from the head signal painful encounters with irritants *via* the trigeminal ganglion, and methyl anthranilate is no exception. Bryant and his colleagues Michael Kurnellas and Michael Kirifides were keen to get to the molecular mechanisms of the painful message, so they began tracing how the neurons registered an encounter with methyl anthranilate.

First Kirifides began mapping the neurone's concentration response, by increasing the cell's methyl anthranilate exposure from 10 to 300 μmol l^{-1}, and watched their calcium levels rise. The cells reached a peak at 300 μmol l^{-1} methyl anthranilate. 'In the pain field these levels [of methyl anthranilate] are very high' explains Bryant, 'but not unrealistic'. And when the team tested the proportion of cells that responded to methyl anthranilate, they found that almost 50% of trigeminal neurons responded to the irritant.

But what was the molecular mechanism of the painful encounter? The team decided to investigate calcium signalling in the neurones by starving the cells of calcium and tracking their response to the irritant. Bryant explains that the neurone's calcium signal originates from one of two sources, either calcium stores within the cell or an extracellular source, so the team removed the extracellular source of calcium and tested the cells' response to methyl anthranilate. If the signal's calcium originated from reserves in the cell, the cell could still respond to methyl anthranilate, but if external calcium was necessary for the neurone's signal, removing it would abolish the signal. There was no response. Methyl anthranilate was stimulating the neurone's calcium uptake, but how was it getting in?

Bryant knew that some calcium uptake channels are activated by voltage changes across the neurone, sometimes caused by sodium uptake, so the team decided to test whether a voltage gated channel was involved in the bird's aversion response by removing sodium from the fluid bathing the cells and treating them with methyl anthranilate. Again the cells failed to respond to the irritant. Bryant realised that sodium entry into the cell changes the membrane's potential and activates the calcium channel, to mediate the bird's painful experience.

But why are Bryant, Larry Clark and the US Department of Agriculture intrigued by avian aversion to certain flavours? 'Up to 5% of cattle feed is eaten by birds' Bryant explains, which can amount to significant losses for a farmer afflicted by a hungry flock. Bryant and Clark hope that if they can understand how foul tastes, that mammals barely notice, affect birds it might be possible to design non-lethal repellents that drive the feathered pests away, leaving cattle to dine, unaccosted.

10.1242/jeb.00836

Kirifides, M. L., Kurnellas, M. P., Clark, L. and Bryant, B. P. (2004). Calcium responses of chicken trigeminal ganglion neurons to methyl anthranilate and capsaicin. *J. Exp. Biol.* **207**, 715-722.

BLUEFIN'S COLD HEART HITS ROCK BOTTOM

Cruising the planet's oceans, bluefin tuna are true giants of the deep; Barbara Block describes them as 'majestic fish'. Yet very little was known about the physiology or habits of these gentle giants, as working with the fish in captivity seemed impossible. But being situated in Monterey allowed Block and her lab to establish a collaboration with their next-door neighbour, the Monterey Bay Aquarium, to build a unique facility housing bluefins weighing up to 50 kg. For the first time, Block and her students could begin studying these enigmatic creatures in ways they had only dreamed of before.

One of the main questions that puzzled Block about the bluefin was the fish's ability to forage and breed in waters ranging from the tropics to the poles (p. 881). Block explains that unlike other teleosts, whose body temperature is in equilibrium with their surrounding water, tunas are significantly warmer as they retain heat in many tissues thanks to complex counter-current heat-exchanging blood vessels. Yet the fish breathe through gills that constantly chill their blood and hearts to ambient temperature. How could the fish's heart function over such a wide thermal range?

Keen to know how bluefin's hearts weather the enormous temperature range, Block and her team set out to sea in a fishing boat to collect juvenile fish. Sailing 500 miles into the Pacific Ocean, the scientists successfully caught more then 20 juvenile bluefins, transferring them to an onboard storage tank, ready for the long journey back to Monterey and their new home in the Tuna Research and Conservation Center's massive tanks.

Back on land, Jason Blank, Jeffery Morrissette, Thomas Williams and Block prepared to begin testing the fish's cardiac function. Block explains that 'keeping the heart going is a challenge' and adds that the experiments are limited by diffusion, so they must be done on small fish. It took the team almost a year to master the experimental techniques they would need to successfully test the fish's cardiac function, but by then the fish had grown too large for their hearts to be sustained by the perfusion technique. The team had to wait another year before the juvenile bluefins returned to the Mexican coast, and then they were ready to continue making cardiac recordings.

Gently dropping the temperature from 30°C to 2°C, Blank and Morrissette saw the fish's heart rate decline from over 150

beats min^{-1} at 30°C to 13 beats min^{-1} at 2°C. While the strength of the fish's maximal heart beat was relatively unaffected by the massive temperature change, the dramatic fall in heart rate caused the bluefin's maximal cardiac output to plummet from 106 ml kg^{-1} min^{-1} at 25°C to 18.1 ml kg^{-1} min^{-1} at 2°C. Most surprisingly, the bluefin's heart continued functioning at temperatures where other tuna hearts failed. And when Block and her team began investigating the cellular basis of the heart's thermal tolerance, Ana Landeira found that the heart tissue expressed high levels of SERCA 2, one of the pump proteins responsible for regulating the heart's contraction, 'fitting nicely with the organismal study' Block adds.

Block suspects that it is the bluefin heart's exceptional thermal tolerance that permits the creature's nomadic lifestyle, allowing it to exploit environments that are simply too cold for other tuna species. And it could also explain some of the fish's intriguing diving behaviour. The fish seem able to descend to enormous depths where the temperature is barely above freezing. Block suspects that even the bluefin's heart can't tolerate such low temperatures for long, so the fish 'bounce' back to warm in waters at the surface.

10.1242/jeb.00835

Blank, J. M., Morrissette, J. M., Landeira-Fernandez, A. M., Blackwell, S. B., Williams, T. D. and Block, B. A. (2004). *In situ* cardiac performance of Pacific bluefin tuna hearts in response to acute temperature change. *J. Exp. Biol.* **207**, 881-890.

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