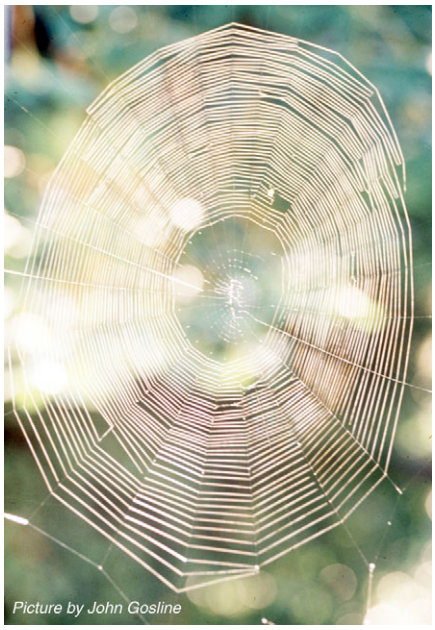
Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

SPIDER SILKS CAN BE SPRINGS OR RUBBER


Picture by John Gosline

It's stronger than steel and nylon, and more extensible than Kevlar. So what is this super-tough material? Spider silk; and learning how to spin it is one of the materials industries' Holy Grails. John Gosline has been fascinated by spider silks and their remarkable toughness for most of his scientific career. He explains that if we're to learn how to manufacture spider silk, we have to understand the relationship between the components and the spun fibre's mechanical properties; which is why he is focusing on major ampullate silk, one of the many silks that spiders spin. According to Gosline, spiders use major ampullate silk for draglines and to build the frame and radial structures in webs, all of which have to deform and absorb enormous amounts of energy without fracturing. Comparing the amino acid sequences of major ampullate silk proteins from *Araneus diadematus* and *Nephila clavipes*, Gosline realised that the sequences differed on one count; *Araneus* silk is relatively rich in the amino acid proline, while proline levels in *Nephila* silk are very low. Curious to know how the presence of proline affects the silks, Gosline and his student, Ken Savage, set about comparing the silks' mechanical properties to find out how the amino acid affects spider silk toughness.

However, obtaining consistent spider silk samples is a problem. Gosline explains that spiders adjust the way they manufacture their silks depending on their circumstances, so he and Savage left the spiders roaming free so that the strands of dragline silk that they dropped were as uniform as possible. Having established a reliable silk supply, Savage set about testing the silks' mechanical properties (p. 1937). Gently stretching the dry silk while measuring the force on it, the team quickly realised that the silks behaved almost identically; the presence of proline had little or no effect on dry silk. However, when Savage began investigating the hydrated silk it was a completely different story. For a start, the wet *Araneus* silk shrank and swelled much more than the proline deficient *Nephila* silk. Savage also tested the silk's stiffness, and found that the *Nephila* silk was almost ten times stiffer than the *Araneus* silk. Finally, knowing that regions of the silk proteins stack to form microscopic crystals in a fibre, Savage measured the fibre's birefringence to see how the two silks compared and if the organisation of the proteins in the silk fibre changed when they were damp. The proteins in the *Nephila* silk were always more organised than the proteins in the *Araneus* silk, regardless of whether they were wet or dry. And as Savage stretched the silks, the degree of organisation in the hydrated *Nephila* silk increased much more than the *Araneus* silk.

Gosline realised that the different mechanical properties could be accounted for by the silk proteins' amino acid composition. According to Gosline, proline amino acids are famed for breaking up the organised three-dimensional structures that protein chains fold into, so protein structures with high proline content would be poorly organised in comparison to proteins with little or no proline. *Araneus* silk contains 16% proline, found mostly in linker regions between the protein's crystalline structures, which would make the linkers flexible and randomly arranged. Gosline realised that if this was the case, the hydrated silk might behave like an elastic band. *Nephila* silk, on the other hand, has a very low proline content in the linker regions, allowing the linkers to form a relatively well organised crystalline structure and behave more like a stiff spring. Gosline and Savage decided to investigate both silks' stretchiness to see if they were more rubber-like or spring-like.

Stretching samples of the hydrated silks, Savage gently raised and lowered the temperature from 30 to 10°C while carefully measuring the minute forces required to maintain the extension (p. 1948). For *Nephila* silk the force remained essentially constant as the temperature changed, a clear indication of spring-like elasticity. However, for the proline-rich *Araneus* silk the force varied in direct proportion to the temperature, behaving like a rubber-band. So proline-rich spider silks extend like floppy rubber bands, while spider silks with low proline levels behave more like rigid springs.

Having found that proline amino acids have a dramatic effect on the mechanical behaviour of hydrated spider silks, Gosline and Savage are keen to find out why the behaviour of the dry silks is almost indistinguishable and what the functional significance is of the different proline contents.

10.1242/jeb.020354

Savage, K. N. and Gosline, J. M. (2008). The effect of proline on the network structure of major ampullate silks as inferred from their mechanical and optical properties. *J. Exp. Biol.* **211** 1937-1947.
Savage, K. N. and Gosline, J. M. (2008). The role of proline in the elastic mechanism of hydrated spider silks. *J. Exp. Biol.* **211** 1948-1957.

ANTS RELY ON DUAL NAVIGATION

Scurrying across the scorching desert floor, *Cataglyphis* ants spend most of the day searching for food. However, having roamed significant distances in search of a tempting treat, the intrepid insects are faced with the tricky task of finding their way home. According to Rudiger Wehner, from the University of Zurich, the ants rely on a host of navigational systems, including local landmarks and their own internal map of the path taken (path integration). But with a selection of navigational strategies to choose from, how do they use this information to find their way home? Do they combine and process information from each system, coming up with a single homing strategy before they embark, or do they use several navigational systems simultaneously, relying to varying degrees on each as they proceed along? Wehner knew the only way to find out was to put two of the ants' navigational systems into conflict and see how the insects fared (p. 1868).

Travelling to the Tunisian desert, where he has been studying ant navigation for over 30 years, Wehner, Patrick Bregy and Stefan Sommer set about testing the insects' homing strategy. First they trained individual ants to shuttle back and forth between their nesting site (marked with a large black cylinder landmark) and a feeder stocked with tempting biscuit crumbs. Once an insect had got the hang of foraging at the feeder, the team transported it in the dark to an identical test site several hundred metres away; only this time the cylinder had been removed too. Releasing the insect, which thought it was still at the feeder, Wehner was pleased to see that it headed straight towards the site where the nest should have been. Deprived of landmarks, the insect had resorted to following its 'path integration' internal map. But what would happen if the team messed about with the ant's landmarks by moving the cylinder?

Repositioning the cylinder to one side of the ant release site, the team released another insect and charted its route. The ant headed off in the direction where it expected the nest to be, but veered off towards the cylinder before arriving at the location where the nest should have been. Repeating the test with 30 more insects, the team found that the ants' courses always deviated towards the cylinder before they successfully returned homewards. And when the team tested the insects with the cylinder located 5, 10 and 15 m along the ants' homeward route, the insects consistently drifted toward the landmark before finding their way home.

The team realised that instead of deciding on a single strategy before embarking on their journey, the ants were simultaneously using their path integration and landmark navigation systems as they returned home, relying more heavily on path integration than the landmark's location. 'The ants didn't neglect the landmark, even when it was in the wrong place' says Wehner.

10.1242/jeb.020362

Bregy, P., Sommer, S. and Wehner, R. (2008). Nest-mark orientation *versus* vector navigation in desert ants. *J. Exp. Biol.* **211**, 1868-1873.

COLD CANE TOADS CUT PROTON LEAK


Picture by Kerry Withers

Mitochondria are the tiny cellular energy generators that produce the energy rich chemical ATP, which powers our every move. Mitochondria consume oxygen to pump protons out of the mitochondrial matrix, which generates an electrical gradient that ultimately drives ATP synthase to produce ATP. But not all protons contribute to ATP synthesis. Some leak back across the mitochondrial inner membrane, producing heat and providing protection from toxic reactive oxygen species. But proton leak is energetically costly, and when times are hard, most creatures reduce it to cut back their metabolic expenditure. Curious to know how ectothermic creatures cope in challenging conditions, Martin Jastroch and his colleagues Magdalene Trzcionka and Martin Klingenspor in Marburg, Germany and Kerry Withers in Toowoomba, Australia, decided to investigate how cane toad mitochondrial metabolism responds to cold and hunger (p. 1911).

Travelling from Northern European to Withers' tropical Queensland laboratory, Trzcionka acclimated one group of toads at a comfortable 30°C while the other half was cooled to a chilly 10°C. Feeding half of the warm toads while starving the remaining animals, and dividing the cold toads in the same way (half fed, half starved), Trzcionka measured their metabolic rates after several days and found that the temperature had a dramatic effect. The warm acclimated toads' metabolic rates were four times the metabolic rates of the cold acclimated toads, while their diet (or lack of) had virtually no effect on the animals' metabolic rates. The cold toads had reduced their metabolic costs.

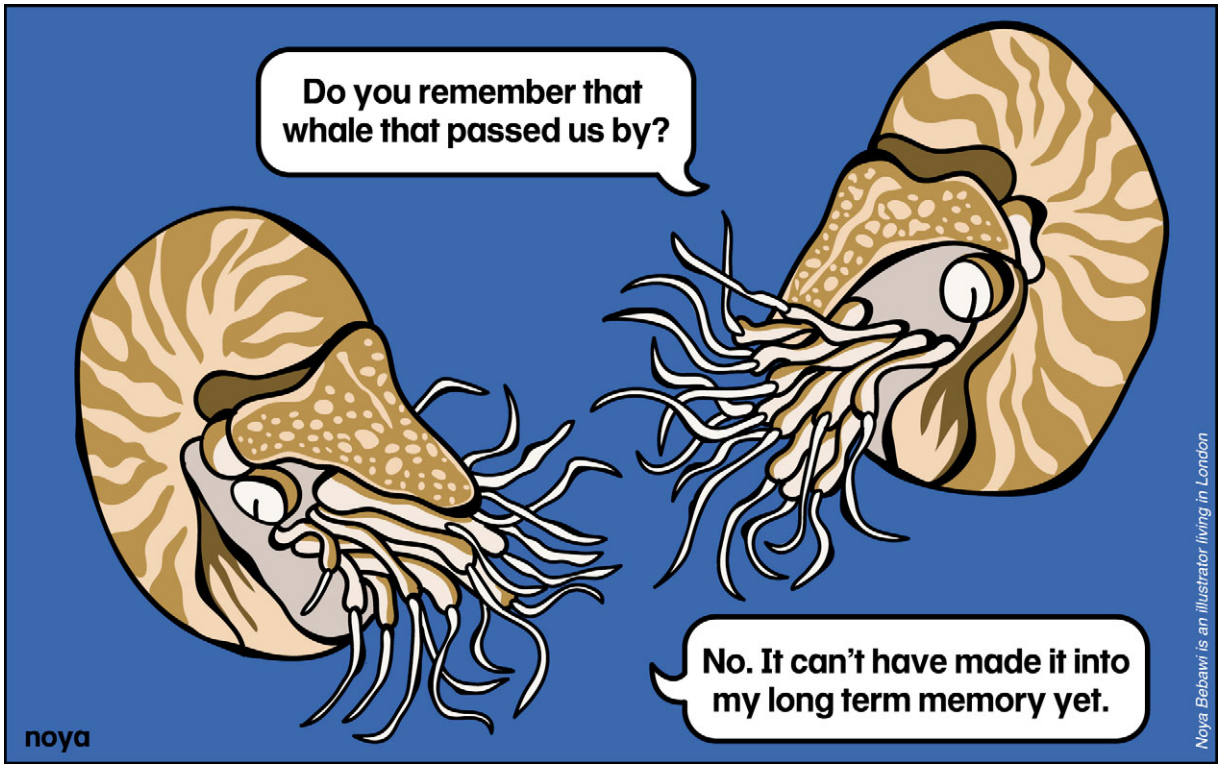
Next Trzcionka tested how the toads' mitochondria had responded to cold and starvation by measuring their mitochondrial proton leak rates. Jastroch admits that this was a particularly ambitious task. 'These experiments are usually only undertaken by experienced scientists' explains Jastroch; let alone a student early in her career in a laboratory 16 000 km from her supervisor. But after months of preparation and with a reliable Skype connection for technical support from Jastroch, Trzcionka successful measured the mitochondrial proton leak rates from the toads' muscle and liver tissues. Adaptation to the cold and starvation had hardly affected proton leak in skeletal muscle at all. But it was a different matter in the liver. The cold acclimated amphibians had significantly reduced their proton leak rate in the liver, and when they added in the effects of starvation the team was surprised to see that the proton leak rate increased even though the amphibians had reduced their energy consumption by reducing the rate at which they pumped protons out of the mitochondrial matrix.

Curious to know how the toads regulated proton leakage, Trzcionka measured the levels of mitochondrial proteins known to participate in proton leak. However, none of the protein levels correlated with the protein leak levels, leaving Jastroch keen to find out which mechanisms regulate cane toad's mitochondrial proton leak when conditions are metabolically challenging.

10.1242/jeb.020388

Trzcionka, M., Withers, K. W., Klingenspor, M. and Jastroch, M. (2008). The effects of fasting and cold exposure on metabolic rate and mitochondrial proton leak in liver and skeletal muscle of an amphibian, the cane toad *Bufo marinus*. *J. Exp. Biol.* **211**, 1911-1918.

LIVING FOSSIL MEMORIES



Nautiloids are the sole surviving family of externally-shelled cephalopods that thrived in the tropical oceans 450–150 million years ago. However, in the intervening years their modern soft bodied relatives dumped the shell and developed complex central nervous systems; which makes Nautilus ideally suited to discover the 'evolutionary pathways that led to the development of the complex coleoid [soft bodied cephalopod] brains' say Robyn Crook and Jennifer Basil. Knowing that the simple Nautilus brain lacks the structures required for memory in more sophisticated cephalopods, Crook and Basil decided to test the living fossil's memory (p. 1992).

Training Nautilus pompilus to associate the smell of food with a blue light, the cephalopods eventually learned to respond to a flash of blue light by extending their tentacles. Then the scientists tested the cephalopods memories with a flash of light 3 min, 30 min, 1 h, 6 h, 12 h and 24 h after training. Amazingly, Nautilus remembered their training for up to an hour before the memory was lost, but then the memory returned 6 h later, lasting up to 24 h. Nautilus has both short and long term memory, just like modern cephalopods, despite lacking the same brain structures.

Crook and Basil are optimistic that the unsophisticated Nautilus brain could teach us how modern cephalopod brains evolved.

10.1242/jeb.020370

Crook, R. and Basil, J. (2008). A biphasic memory curve in the chambered nautilus, *Nautilus pompilius* L. (Cephalopoda: Nautiloidea). *J. Exp. Biol.* **211**, 1992-1998.

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