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

SQUID SHORTEN THICK FILAMENTS FOR FAST CONTRACTION

Growing up is never easy, especially when the world seems intent on holding you back. As every fluid dynamicist knows, the smaller you are, the more you have to struggle to overcome viscous forces. And hatchling squid are no exception. Starting out at a diminutive 0.05 g the tiny aquanauts have to overcome enormous viscous forces, but this resistance dwindles to almost nothing once the youngsters are nearly fully grown. Joe Thompson explains that 'the physics of locomotion changes dramatically over a narrow range of sizes for squid'. He and Bill Kier were puzzled how the growing squid cope with the transition from a highly viscous world to the thinner world encountered by their elders. The team knew that some creatures change their body shapes dramatically as they grow in stature, but magnified hatchlings and mature adult squid appear virtually indistinguishable. Thompson and Kier decided to investigate the developing squid's jetting technique to see if anything changes as the youngsters age (p. 433).

Working with squid ranging in size from 5 mm infants up to 40 mm veterans, Thompson filmed the cephalopods with high-speed video cameras to analyse their mantle jetting styles. Filming the larger animals was relatively straightforward, but capturing the tiny youngsters' movements proved much trickier; they shot out of the camera's field of view before Thompson could record their escape jet routine. Carefully tethering the tiny squid in place Thompson was able to film the animals' pumping mantles as they attempted to propel themselves forwards. Analysing the mantle pumping rates, the team realised that the smallest squid pulsed their mantles at a much faster rate than the largest animals, with some of the smallest managing 13 muscle lengths per second while the larger squid could only sustain 3 to 5 muscle lengths per second.

Thompson explains that Danny Weiss and J. Siekman had suggested several decades ago that short-pulsed jets produce more thrust than long sustained jets, and this could explain how the rapidly pumping hatchlings overcome viscosity. But how were the youngsters able to contract their mantles so much faster than their elders? Was the squid's mantle muscle changing as they matured? Bill Kier already knew that some squid modify arm muscle tissue to contract rapidly by decreasing the length of the thick myosin filaments. Could this squid have adopted the same strategy in its mantle muscle?

The team decided to take a closer look at the developing squid's mantle muscle. Turning to transmission electron microscopy, Sonia Guarda helped Thompson painstakingly prepare and section the delicate muscle samples for the high-resolution microscope. Looking at the larger squid's muscle first, Thompson and Kier saw thick filaments ranging in length from 1.2 to 3.4 μm, but when they looked at the smallest squid's thick filaments 'we had to zoom way in to see them' says Thompson. The filaments were significantly shorter, ranging in length from 0.7 to 1.4 μm.

Thompson admits that modifying the thick filament length to achieve a high shortening velocity is a novel concept for muscle physiologists familiar with vertebrate muscle, which vary muscle biochemistry to raise the shortening velocity. Although it may not be a common solution to the problem, Thompson suspects that other invertebrates may also modulate their muscle function by altering the filament structure.

10.1242/jeb.02079

Thompson, J. T. and Kier, W. M. (2006). Ontogeny of mantle musculature and implications for jet locomotion in oval squid, *Sepioteuthis lessoniana*. *J. Exp. Biol.* **209**, 433-443.

FREEZE-TOLERANCE EXTENDED BY RAPID COLD HARDENING

In the midge world, *Belgica antarctica* larvae are something akin to superheroes. Resistant to anoxia for weeks, tolerant of pH ranging from 3-12 and able to survive losing 65% of their body water, these creatures are bywords for tough. But possibly their most remarkable characteristic is their ability to survive being frozen solid. An Antarctic veteran, Richard Lee has been intrigued by the residents of this remote continent ever since his first visit as a postdoc in 1980. Puzzled by *B. antarctica*'s stress tolerance, Lee, David Denlinger and their colleagues wondered whether the insect's larvae would acquire increased cold tolerance as a result of a previous exposure to a cold snap. Lee explains that this phenomenon, known as 'rapid cold hardening', is well studied in many freezing intolerant insects, but has never been reported in creatures that miraculously survive being frozen alive. Could *B. antarctica*'s remarkable freeze-tolerance be extended by rapid cold hardening (p. 399)?

Lee and Denlinger headed south with postdocs Joseph Rinehart and Scott Hayward. The team was also accompanied by a local high school science teacher, Luke Sandro, who related his experiences doing science in Antarctica to colleagues and students over the internet in daily web-logs. Working long hours in the austral summer, the team gathered thousands of midge larvae from the mud and penguin detritus around the US's Palmer Station.

Back in the base's lab, the team tested the larvae's freeze-tolerance by cooling them to –10°C; 75% of the larvae died. But when they precooled the larvae for an hour at –5°C before plunging them to –10°C, the insects' tolerance increased dramatically, with the death rate falling to less than 15%. The larvae had undergone rapid cold hardening.

Shipping some of the untreated larvae in icepacks back to the northern hemisphere, Lee was surprised to find that the insects had serendipitously become acclimated to winter conditions by the time they arrived in Ohio. Michael Elnitsky, Lee's graduate student, began testing the winter acclimated insects' cold tolerance, finding that they survived much lower temperatures than the summer-adapted larvae in Antarctica. But how would these winter-adapted insects respond to a dose of rapid cold hardening?

Elnitsky precooled the larvae to –5°C for 1 hour before freezing them at –15°C for 24 hours. This time, more than 85% of the larvae survived a temperature that was fatal for the summer larvae. In fact, most of the larvae survived –20°C after rapid cold hardening. Lee, was astonished; rapid cold hardening had extended the limit of freeze tolerance significantly.

But how are the insects able to cold harden so quickly? First Lee and Elnitsky tested to see whether an episode of rapid cold hardening caused the insect to produce protective antifreeze molecules, but could find no evidence. Next, they began investigating how tissues responded to the cold, with and without rapid cold hardening. The team found relatively high levels of cell death in tissues that had not been rapidly cold hardened, while the death rates were much lower in rapidly cold hardened cells. Lee believes that the physiological basis of rapid cold hardening is cellular, but finding the mechanism is going to be a tricky task.

10.1242/jeb.02040

Lee, R. E., Jr, Elnitsky, M. A., Rinehart, J. P., Hayward, S. A. L., Sandro, L. H. and Denlinger, D. L. (2006). Rapid cold-hardening increases the freezing tolerance of the Antarctic midge *Belgica antarctica*. *J. Exp. Biol.* **209**, 399-406.

PROCTOLIN MEDIATES EFFECT THROUGH cGMP

Every time we twitch, even the tiniest movement, no matter how strong or weak, is coordinated by complex networks of neurones. But invertebrates orchestrate muscular contractions with a much simpler neural network. They achieve fine control by modulating muscle nerve signals. Sabine Kreissl from the University of Konstanz in Germany explains that one signal-modulating molecule, the neuropeptide proctolin, modulates many neuromuscular processes in the isopod *Idotea emarginata*. Released from neurones, proctolin binds receptors on muscle cells, and enhances depolarisation induced muscle contraction. But 'coupling the receptor to its target in the cell is a black box' explains Kreissl. Curious to discover the muscle cell signalling cascade that is triggered by the neuropeptide proctolin, Kreissl and her colleagues Berit Philipp and Nicole Rogalla began investigating the contents of proctolin's black box (p. 531).

Knowing that proctolin's effects could be simulated by stimulating components of the cAMP signalling pathway, Philipp began investigating cAMP levels in muscle cells to see if a cAMP dependent pathway mediated proctolin's effect. But when she measured the levels of cAMP in muscle cells, they failed to respond to proctolin. Next, Rogalla tested whether cAMP might regulate muscle contraction. She inhibited a key component of the cAMP signalling pathway and measuring the degree to which the contraction was enhanced. If a cAMP pathway regulated the contraction, she expected the contraction to become weaker; but the contraction strength was unaffected. cAMP did not play a role in transmitting the proctolin signal. Some other signalling cascade must mediate the neuropetide's effects.

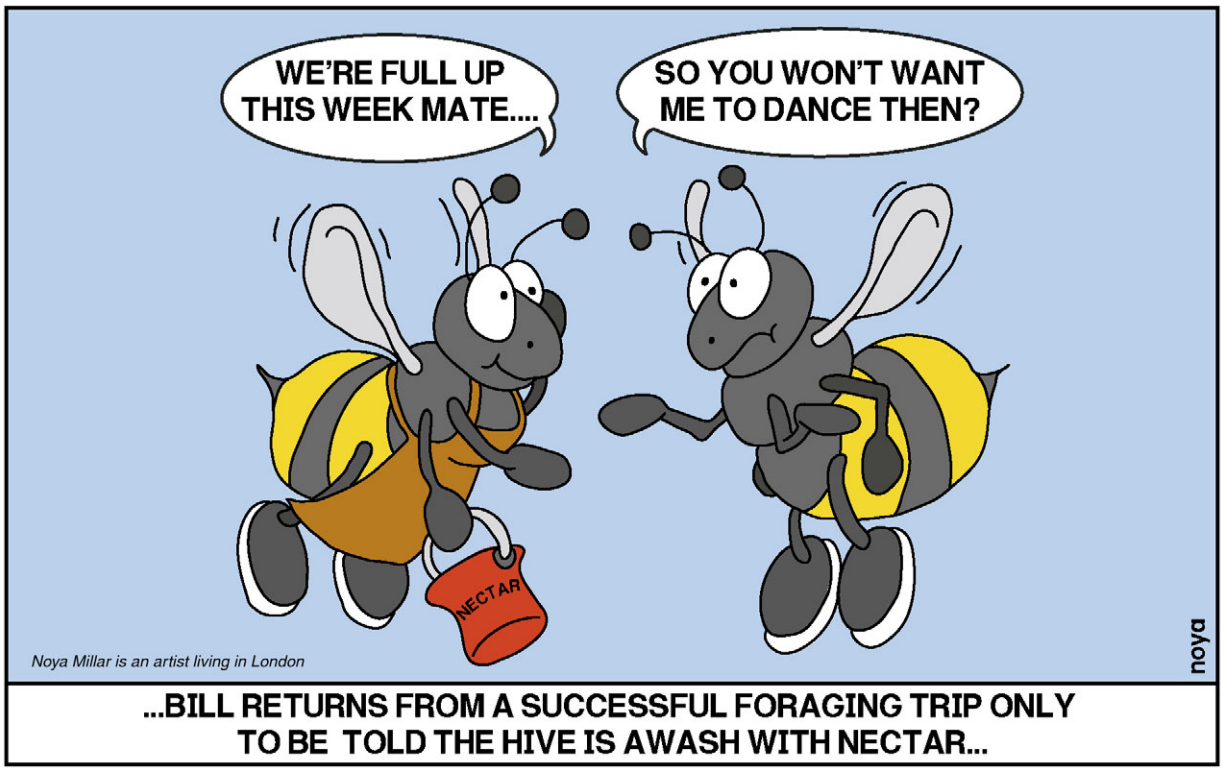
Turning to the literature, Kreissl realised that another signalling molecule could be involved in modulating muscle contractions; cGMP. Knowing that cGMP stimulates muscle relaxation in smooth muscle, Kreissl suspected that falling levels of the signalling molecule could in turn cause muscle contraction. Following Kreissl's hunch, Philipp measured the levels of cGMP in the isopod's muscle cells after exposure to proctolin, and found that the signalling molecule's level fell. And when Rogalla investigated cGMP's effect on the strength of the muscle cell's contraction by augmenting its cGMP levels, she found the strength of contraction diminished. cGMP must play a role in proctolin signalling. 'cGMP had never been implicated before in proctolin signalling' Kreissl says but it seemed as if it may hold the key to proctolin's black box. However she still didn't know which signalling pathway linked proctolin to cGMP.

Back in the library, the team discovered that protein kinase C (PKC) was a component of the cGMP signalling pathway in vertebrates. Knowing that a PKC pathway had already been implicated by other scientists in proctolin signalling, Kreissl decided to inhibit the protein to see if this was the pathway that mediated proctolin's muscle cell effects. Measuring the muscle cell contraction while applying the protein kinase C inhibitor, Rogalla saw the contraction decrease, and when she supplemented the inhibited cell's cGMP supply with an artificial analogue, she saw the contraction gain force again. Proctolin was mediating its effect in the muscle through a protein kinase C dependent cGMP pathway.

10.1242/jeb.02078

Philipp, B., Rogalla, N. and Kreissl, S. (2006). The neuropeptide proctolin potentiates contractions and reduces cGMP concentration *via* a PKC-dependent pathway. *J. Exp. Biol.* **209**, 531-540.

FORAGERS GET THE MESSAGE



Returning from a successful foraging trip, a well-loaded forager bee has two things on its mind; offloading its cargo and telling the rest of the hive the good news. But communication is often a two-way thing. Rodrigo de Marco decided to investigate Martin Lindauer's original suggestion that assistant bee's receiving nectar from the returning forager might influence the forager's enthusiasm to communicate its find (p. 421).

Designing an enclosed environment where he could control the nectar inflow to the hive while supplying a single trained bee with a regulated nectar intake, De Marco filmed the trained bee's dance sequences when the hive was awash with nectar and when it had fallen on harder times. Analysing the insect's responses, De Marco realised that the probability of the forager bee dancing declined when the hive was well supplied with nectar, while unloaded bees danced more enthusiastically when the hive was short of nectar. The forager was able to respond to tiny fluctuations in the hive's nectar supply and pitch its dances accordingly.

De Marco suspects that the forager recognises how eagerly the assistant unloads the nectar cargo, playing its message down in times of plenty and dancing frenetically when nectar is scarce.

10.1242/jeb.02077

De Marco, R. J. (2006). How bees tune their dancing according to their colony's nectar influx: re-examining the role of the food-receivers' 'eagerness'. *J. Exp. Biol.* **209**, 421-432.

Kathryn Phillips
kathryn@biologists.com