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Inside JEB

BED BUGS LESS SELECTIVE THAN THOUGHT

Vincent Harraca

It wasn't long ago that bed bugs were just an uncomfortable memory from a bygone era. However, the blood-sucking critters have recently staged a remarkable comeback, and with it new industries have emerged with the aim of tracking and eradicating the irritating pests. Vincent Harraca from Lund University, Sweden, explains that traps could be used to monitor bed bug infestations, 'but so far the traps are not efficient because we do not know how to attract bedbugs into them', he says. Human odour is complex, containing as many as 400 different components, and as no one had identified the key components that attract the insects to home in on juicy victims, Harraca, Camilla Ryne, Göran Birgersson and Rickard Ignell decided to find out which compounds in human odour are irresistible to bed bugs (p. 623).

But Harraca's first challenge was to collect samples of human odours, 'And to do that you need bags that do not release any smell', explains Harraca. 'The best ones are oven bags for roasting chicken or turkey, but they do not make human-sized oven bags', he chuckles. Resourcefully heat-sealing the transparent bags together, Harraca was then faced with another dilemma: convincing his volunteers to climb inside them naked for 2.5 h while he collected their odours on filters. Having successfully collected samples from eight volunteers, Harraca teamed up with Birgersson to isolate the individual components constituting each odour. Then he tested which odours the insect could sense by recording the electrical activity in the insect's odour receptors with a tungsten electrode.

Having isolated 100 different compounds from the human odour samples, Harraca admits that he was surprised to see that the insects only responded to five. And when he identified the compounds – four aldehydes and a ketone (sulcatone) – he was even more surprised, because these compounds are emitted by the skins of all vertebrates. He admits that the team had thought that the insects would react to scents such as geranyl acetone and lactic acid that are produced by humans in large quantities. However, instead of specifically sniffing out people, bed bugs are happy to sniff out any warm blooded creature to snack on.

The team then decided to find out how natural human odour affects the blood sucking insects' behaviour. Constructing a double-decker arena from Petri dishes, Harraca placed a semi-circle of filter paper soaked in body odour in one side of the lower chamber of the arena and then allowed a bed bug to explore the odour in the upper chamber for 7 min while he filmed its movements.

Realising that the bed bugs were repulsed by the most concentrated odour samples, Harraca repeated the process with more dilute body odours and found that the insects were not strongly attracted to human scents alone. Explaining that they are also attracted to warmth and carbon dioxide, he suggests that bed bugs are guided by a combination of senses to locate slumbering victims. Harraca also suspects that the relative proportions of ketones and aldehydes in an individual's scent might alter their attractiveness for bed bugs as the pests were more motivated to explore one person's odour, which differed subtly from the others.

10.1242/jeb.070060

Harraca, V., Ryne, C., Birgersson, G. and Ignell, R. (2012). Smelling your way to food: can bed bugs use our odour? *J. Exp. Biol.* **215**, 623-629.

Kathryn Knight

PARVALBUMIN AFFECTS FISH ATHLETICISM

How quickly you escape a predator or whether you catch the next meal is one of the key factors that decides whether you contribute to the greatest experiment on earth: evolution. Athletic performance varies enormously from individual to individual, and muscle performance is probably the main determinant of whether you're a Usain Boult or one of the stragglers. Frank Seebacher and Isabel Walter from the University of Sydney, Australia, reasoned that the biochemical machinery that regulates how hard and fast muscle contracts determines whether zebrafish are elite athletes or laggards. The duo decided to investigate the muscle biochemistry of zebrafish to identify key components that determine a fish's athleticism (p. 663).

Setting the fish a swimming challenge, the duo compared the animals' endurance and sprint performance, expecting to find that the best sprinters had the poorest endurance. However, they found no correlation, so the fish had not traded off one performance against another.

Next, they looked to see if increasing the fish's levels of a calcium handling protein – parvalbumin, which is involved in the regulation of muscular contraction – affected the fish's endurance and sprint performance; and it did, improving their performance measurably. 'This is the first demonstration that parvalbumin plays a role in determining whole-animal performance', say Seebacher and Walter.

Finally, the duo investigated the fish's metabolic machinery and found that the high performance fish – the fast sprinters and the fish with the greatest endurance – had higher expression of the muscle biochemistry regulators PPARδ and PGC-1α. They also found that the fastest sprinters were able to release energy faster because they had higher levels of the ATP-release enzyme creatine kinase, as well as having higher expression levels of dihydropyridine receptor and sarcoplasmic reticulum calcium ATPase (SERCA) 2, both of which regulate the release of calcium. And when they looked at the muscles of the high endurance swimmers, they found that those fish had higher levels of ryanodine receptor and SERCA 1, which are also involved in calcium regulation in the muscle.

Seebacher and Walter say, 'We have identified a number of molecular traits that can explain differences in sprint and sustained locomotor performance in individuals'. They add, 'Understanding how these traits function and what regulates their expression will provide new insights into locomotion, its evolution and into associated diseases'.

10.1242/jeb.070094

Seebacher, F. and Walter, I. (2012). Differences in locomotor performance between individuals: importance of parvalbumin, calcium handling and metabolism. *J. Exp. Biol.* **215**, 663-670.

Kathryn Knight

Rheb REGULATES CRAB MUSCLE ATROPHY

Moulting their shells and misplacing limbs, crabs continually depend on their versatile musculature to reshape their bodies. According to Donald Mykles and colleagues from Colorado State University and the University of Wisconsin – Stevens Point, USA, the massive claw muscle of blackback land crabs withers by as much as 78% before they can slip their shells off. However, Mykles and his colleagues made an interesting discovery in 2010. Instead of shutting down protein synthesis to allow the muscle to wither, the crabs ramp it up 11-fold by shutting down myostatin expression – which usually keeps protein synthesis in check. Knowing that myostatin regulates protein expression in mammals *via* the mTOR (metazoan target of rapamycin) signalling cascade, which controls mRNA translation, Mykles decided to investigate the mTOR pathway in blackback land crabs to find out whether it also regulates muscle rebuilding in crabs during moulting (p. 590).

Stimulating crabs to moult, the team collected muscles from the animals and searched for the mRNA of four key mTOR signalling proteins – Rheb, mTOR, Akt and s6k. The crabs expressed all four genes, confirming that the mTOR pathway is active in moulting crab's atrophied muscle.

Next, the team measured the expression levels of each gene in crabs that had been induced to moult using methods that raise the animals' moulting hormone (ecdysteroid, which regulates myostatin levels) over different time scales. They found that *Rheb* levels increased in the moulting crabs' claws muscles and in the atrophied thoracic muscles of crabs that had lost a limb. Mykles says 'Rheb is a key activator of mTOR. Its up-regulation by moulting indicates that Rheb plays a role in the stimulation of mTOR-mediated protein synthesis by ecdysteroids'.

However, when the team investigated the mechanism by which *Rheb* is upregulated in the two muscles, there were differences. They found a strong correlation between the levels of *Rheb* in the crabs' claws and the ecdysteroid levels, suggesting that ecdysteroid does regulate protein synthesis *via* mTOR in the claw muscle. Yet, when they compared the ecdysteroid levels and *Rheb* levels in the thoracic muscles – that wither in response to limb loss – the team found no correlation. Ecdysteroid hormone does not control muscle atrophy in response to limb loss.

Analysing the expression patterns of *Rheb* and myostatin in atrophied claw and thoracic muscles, Mykles and colleagues also saw different expression relationships between the proteins in the two tissues. 'If blackback land crab myostatin plays a role in both types of atrophy, any unifying mechanism must reconcile the differences between myostatin and *Rheb* expression in the two muscles', the team says.

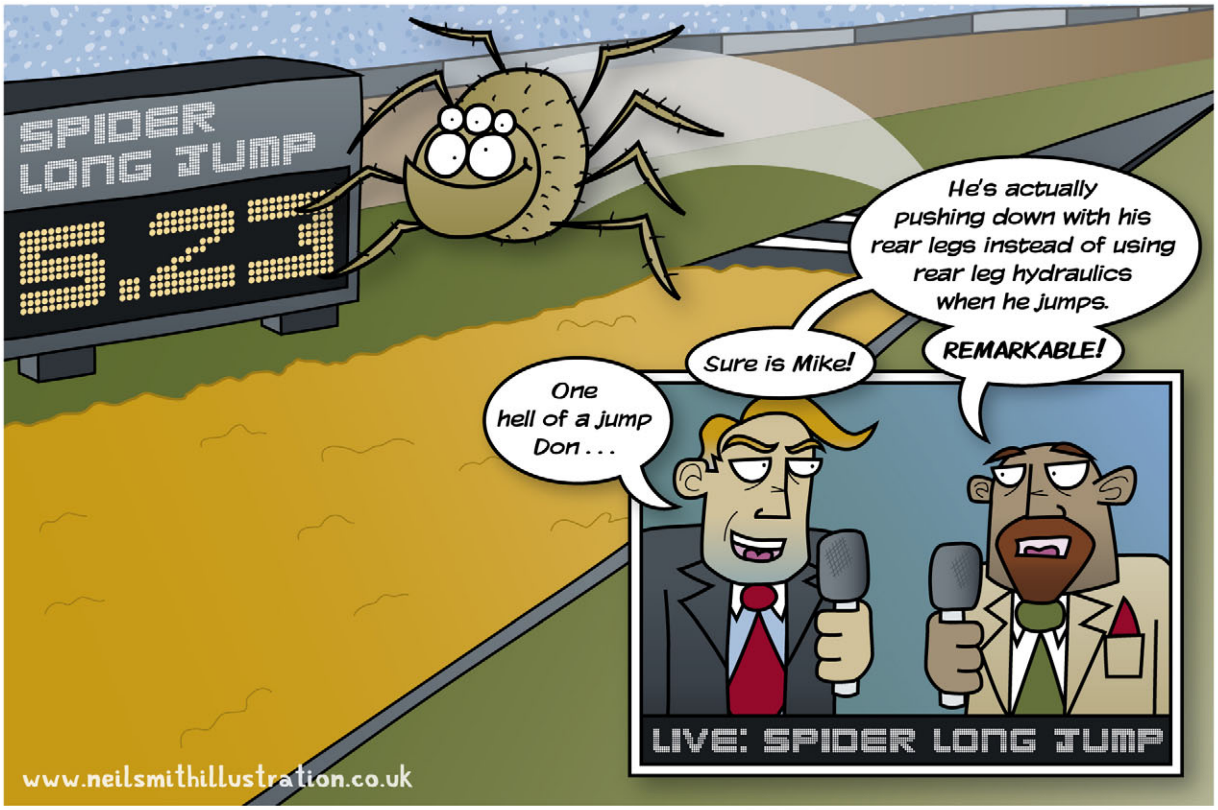
Having shown that *Rheb* is a key component of the signalling pathway that regulates muscle atrophy in crabs, the team says that it is keen to build, 'a mechanistic understanding of the interactions between ecdysteroids and the signalling pathways that control protein metabolism in crustacean skeletal muscle'.

10.1242/jeb.070078

MacLea, K. S., Abuhagr, A. M., Pitts, N. L., Covi, J. A., Bader, B. D., Chang, E. S. and Mykles, D. L. (2012). Rheb, an activator of target of rapamycin, in the blackback land crab, *Gecarcinus lateralis*: cloning and effects of molting and unweighting on expression in skeletal muscle. *J. Exp. Biol.* **215**, 590-604.

Kathryn Knight

SPIDERS FLEX LEG WITH MUSCLE TO TAKE OFF



Watching spiders move is enough to send shivers up some people's spines, but for others they are an inspiration. Tom Weihmann from Friedrich-Schiller University, Germany, explains that robotics engineers would love to design robots based on the arachnid's unique hydraulic legs that manoeuvre nimbly over uneven and treacherous terrain. According to Weihmann, spiders have no leg extension muscles. Instead they flood their joints with haemolymph to extend their limbs. However, recent work in Reinhard Blickhan's lab had cast doubt on their use of the hydraulic leg extension mechanism during jumps. Weihmann explains that *Cupiennius salei* spiders extend the rear pair of legs when they leap. However, instead of using hydraulic extension to push off, he thought that they may flex muscles at the joint between the body and the leg to press the whole leg down and push off. But Weihmann needed proof, and to get this he measured the ground reaction forces generated by leaping *Ancylometes concolor* spiders' hind legs during a push off (p. 578).

Carefully manoeuvring the large spiders so that one of their rear feet rested on a tiny force plate, Weihmann gently nudged the animals and filmed them as they hurled themselves into the air. Analysing the forces acting on the spider's rear foot as it launched itself forward, Weihmann, Michael Günther and Blickhan realised that the ground reaction force was always angled upward and positioned behind the leg. Knowing that the spider's back end essentially pivots around its centre of mass as it pushes off with all of its feet, Weihmann and his colleagues explain that the spider can only generate the upward-directed force that sends it flying if it presses the entire hindmost leg down by flexing the muscles at the joint between the leg and body.

So, the hydraulic leg extension mechanism cannot account for *A. concolor*'s impressive leaps and Weihmann is keen to learn more about the scuttling animals' peculiar legs.

10.1242/jeb.070086

Weihmann, T., Günther, M. and Blickhan, R. (2012). Hydraulic leg extension is not necessarily the main drive in large spiders. *J. Exp. Biol.* **215**, 578-583.

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