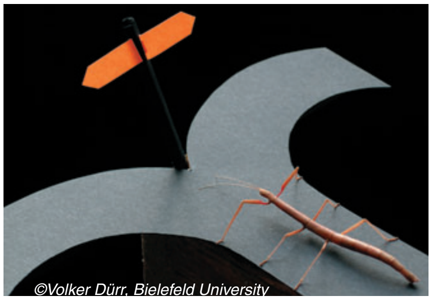
Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

SWERVING STICK INSECTS


©Volker Dürr, Bielefeld University

Watch a stumbling toddler and you'll see that we struggle to learn how to control just two legs. But spare a thought for the humble stick insect; it has to coordinate 18 leg joints as it ambles around. Volker Dürr and Wiebke Ebeling at Bielefeld University analysed how stick insects negotiate bends to understand the behavioural coordination that underlies walking (p. 2237 and p. 2253).

To see how stick insects initiate changes in direction during walking, Dürr and Ebeling examined the sequence of stick insects' leg movements during straight and curved walking. They tethered stick insects onto an air-cushioned Styrofoam ball and videotaped them as they sauntered across the top of the ball. At the same time, they recorded in which direction the insects walked by tracking the rotation and backward and sideward translations of the ball using motion sensors. They filmed the insects walking in a straight line and then rotated a black and white pattern around the insects – a visual motion stimulus that they knew would provoke the insects to start turning.

To examine how quickly the insects adjusted their legs as they turned, Dürr and Ebeling measured the rate of change of 13 kinematic parameters per leg, including stride direction, length and duration, and lift-off and touch-down positions. Then they related the time course of these parameters to the insects' walking behaviour. The time at which Dürr and Ebeling recorded a change in an animal's walking path served as a reference point to identify which parameters change faster than the insect's change of direction. They reasoned that the parameters that change faster than the change in walking direction are the ones that actively initiate an insect's turn, and those that change at a slower rate than the insect's turn do not play an active role in turning.

Dürr and Ebeling found that the movement parameters changed at different rates for each of the insects' six legs, so turning is a carefully orchestrated process in which each leg has a different function. They noticed that the two front legs change their step direction faster than the insect's turning response, and concluded that stick insects' front legs actively initiate turning. But they were surprised to find that the strongest changes were not necessarily the fastest ones. 'A parameter's magnitude doesn't tell you everything,' Dürr concludes, 'you also need to consider the time course of events.'

So how do stick insects orchestrate their walking? 'We can model it using simple coordination rules,' Dürr says. An insect's six legs coordinate their lift-off times by 'telling' each other which state they are in. Examining his behavioural data, Dürr found that the strength of these signals is not constant between legs, but adapts depending on whether the insect is walking in a straight line or a curve. Dürr and his collaborators in Bielefeld will incorporate all of these behavioural findings into computer simulations of insect walking. By testing these updated algorithms on a 6-legged robot in the lab, they might eventually manage to adapt leg coordination to the environment's current demands, enabling such a robot to march across the rugged terrain that thwarts wheeled robots.

10.1242/jeb.01694

Dürr, V. and Ebeling, W. (2005). The behavioural transition from straight to curve walking: kinetics of leg movement parameters and the initiation of turning. *J. Exp. Biol.* **208**, 2237-2252.
Dürr, V. (2005). Context-dependent changes in strength and efficacy of leg coordination mechanisms. *J. Exp. Biol.* **208**, 2253-2267.

UV SETS THE BEE CLOCK


Picture by Ed Chang

If you see a bee flitting between flowers, it's a good bet that it's relying on UV light for its deft navigation. Given that we've known for almost a century that bees can see UV, we know surprisingly little about the nature and location of UV visual pigments in bees' brains. To explore the possible functions of such pigments, Adriana Briscoe and Johannes Spaethe set out to locate UV visual pigments in bumblebees' brains (p. 2347).

Briscoe explains that insects' visual pigments consist of opsin proteins. If you want to find out where an opsin protein is expressed, the obvious place to look is in an insect's retina. But Briscoe knew that some butterfly visual pigments are only expressed in the butterfly's brain, not in its retina. Could the same be true for bumblebees?

Before they could tackle this question, Briscoe and Spaethe needed to identify the gene for bumblebees' UV-sensitive opsin protein. They extracted RNA from bumblebee eyes, and from it sequenced a putative UV opsin gene. To find out if their sequence really encodes a UV opsin, they searched other insect genomes for similar sequences. They found that their putative bumblebee UV opsin only produced hits to other known insect UV opsins. But was their bumblebee opsin a functional UV-sensitive visual pigment? To show that the bumblebee sequence contains a critical amino acid residue that confers UV sensitivity, Briscoe and Spaethe aligned their bumblebee sequence with the known UV opsin sequence of *Drosophila*. Briscoe was relieved to find that 'the bumblebee sequence contained the same mutation found in *Drosophila* that we know is functionally important to make a UV-sensitive pigment.'

Now, Briscoe and Spaethe could probe where this UV opsin protein is expressed in the bumblebee brain. They had an anti-opsin antibody for a butterfly UV visual pigment, which they discovered also revealed UV opsin expression in bumblebees' retinas and brains. In the bumblebee retina, they found that the ommatidia – the repeating structures that make up the compound eye – come in three types; they can contain two, one or no UV opsin-expressing cells. Briscoe didn't expect to find such heterogeneity because honeybees, bumblebees' closest relatives, are thought to have just one ommatidial type. Briscoe and Spaethe also found that the UV opsin is expressed in bees' ocelli, the three simple eyes that detect polarised light and allow bees to forage until dusk. 'This is the first opsin to be localised in bee ocelli,' Briscoe says.

But when Briscoe and Spaethe examined bumblebees' brains, they were in for a few more surprises. They found UV opsin expression in various brain parts, but two in particular caught their eye – the optic and antennal lobes, the regions that process vision and olfaction. Briscoe and Spaethe knew that some insects express period, a circadian clock protein, in the outer layer of the optic lobe. Curious to find out if this is also true for bumblebees, they searched for period expression in bumblebee brains. Sure enough, bumblebees express period in the optic and antennal lobes. Since period and the UV opsin are both expressed in the optic and antennal lobes, Briscoe and Spaethe conclude that UV light might play a role in bumblebee circadian rhythm regulation, mediated through these two brain regions.

10.1242/jeb.01691

Spaethe, J. and Briscoe, A. D. (2005). Molecular characterization and expression of the UV opsin in bumblebees: three ommatidial subtypes in the retina and a new photoreceptor organ in the lamina. *J. Exp. Biol.* **208**, 2347-2361.

CRICKETS FLUSH OUT TOXINS

Picture by Doug Neufeld

Insects have an astounding ability to cope with environmental challenges. Doug Neufeld and his colleagues have discovered one of the reasons why insects can devour almost anything and emerge unscathed. They reveal that the insect 'kidney', the Malpighian tubules, can flush harmful substances like pesticides out of insects' bodies (p. 2227).

Reflecting on the fact that insects happily eat plants that contain toxins or have been sprayed with herbicides and insecticides, Neufeld reasoned that insects must be able to deal with this toxic melange. He suspected that organic anion transporters in insects' Malpighian tubules might play a role in the disposal of environmental toxins. He knew that the fluorescent dye fluorescein is a good tracer for organic anion transport processes, because it is pumped into the Malpighian tubules by these transporters. To show that insects can excrete toxins through organic anion transporters, Neufeld needed to show that a toxin stops the transport of fluorescein into the tubules. If it does, the toxin is either blocking fluorescein transport or is being transported itself in favour of fluorescein, Neufeld explains.

Teaming up with Ross Kauffman and Zachary Kurtz, Neufeld set out to test whether toxins are transported into insects' Malpighian tubules. Examining Malpighian tubules from crickets under a microscope, the team pumped solutions containing fluorescein and each of various test toxins across the tubules. To see if fluorescein was still being transported in the presence of each toxin, they measured how quickly the tubules started glowing, using a photometer to detect how much light was given off. They found that several toxins stopped fluorescein uptake by the tubules, including a plant alkaloid, herbicides and two insecticide metabolites (produced when an insect breaks down an insecticide).

But are these toxins really being transported by the tubules? To find out, Neufeld measured the precise amounts of the two insecticide metabolites in cricket Malpighian tubules, using high performance liquid chromatography to analyse which compounds were present in tubule tissue. Sure enough, both of the insecticide metabolites were present in the tubules. But this didn't convince Neufeld. To be sure that the cells were transporting the metabolites, he killed the tubule cells. He found that only one of the metabolites was still being accumulated in the tubule. 'This metabolite probably just sticks to the tubule, but is not actually transported across the tubule cell,' Neufeld concludes. But the other metabolite was only accumulated when the tubules were alive, so crickets' tubule cells do excrete this metabolite.

Neufeld reveals an intriguing application of these findings. If insects can purge insecticides, they can evade eradication. But if we can entice insects to gobble up an inhibitor that blocks insecticide metabolite transport so the insects can't expel the toxins anymore, 'we could make insecticides much more effective,' Neufeld speculates.

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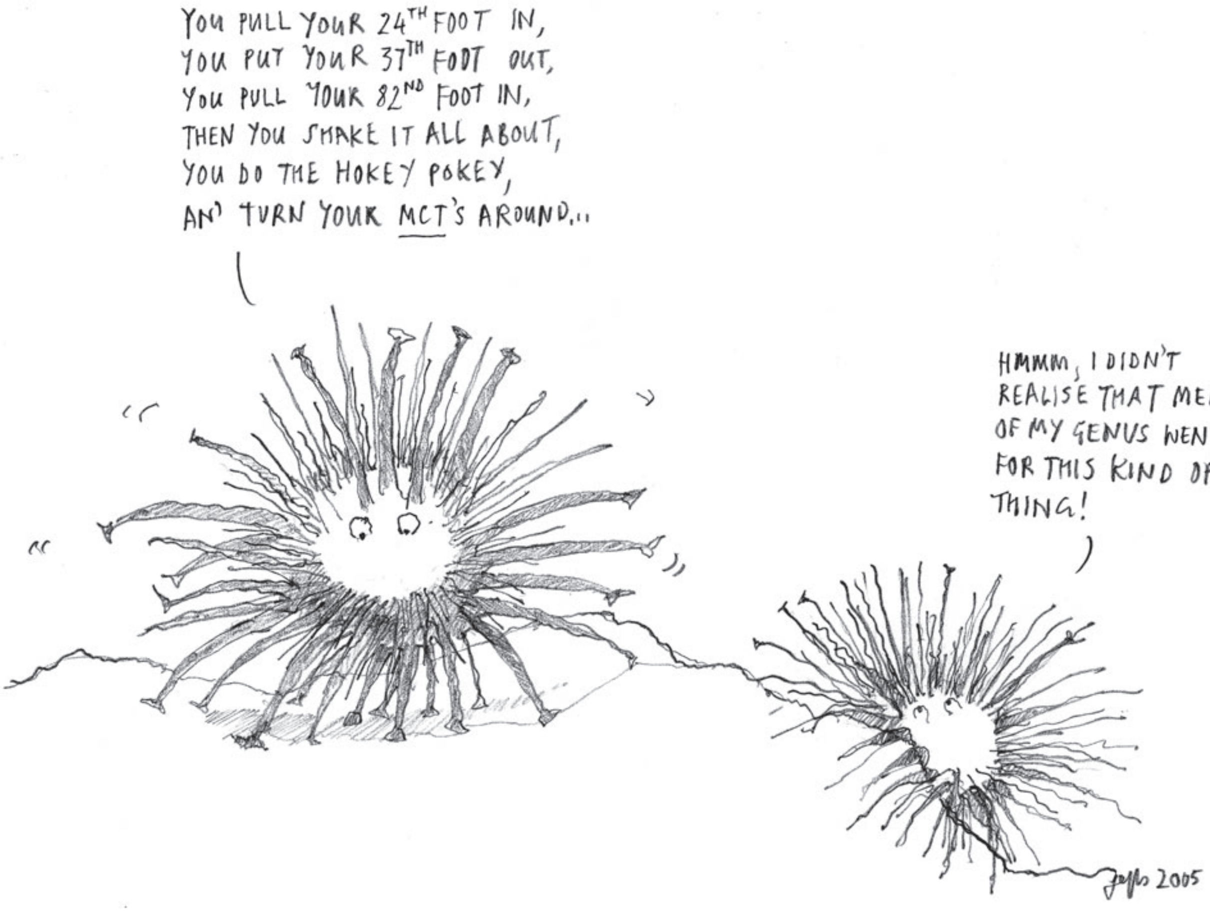
Neufeld, D. S. G., Kauffman, R. and Kurtz, Z. (2005). Specificity of the fluorescein transport process in Malpighian tubules of the cricket *Acheta domesticus*. *J. Exp. Biol.* **208**, 2227-2236.

MUTABLE FEET GET A GRIP



Pete Jeffs is an artist living in Paris

Life can be tough if you're a small creature on a seaside rock; one minute you're on the move, and the next you need to cling on as waves threaten to sweep you away. Patrick Flammang and his Belgian colleagues reveal the trade secret of rock-clambering sea stars and sea urchins: their numerous tiny hydraulic tube feet contain mutable tissues that allow their owners to get a toehold when the sea gets too rough (p. 2277).

Flammang explains that many echinoderms, including sea stars and sea urchins, possess mutable collagenous tissues (MCTs) that can switch from being flexible to rigid in seconds, thanks to special secretory cells. To find out if sea stars and sea urchins have mutable tissues in their feet, Flammang and his team immersed the tube feet of the little sea-dwellers in solutions that they knew would disrupt secretory cells. Then they tested the mechanical properties of the tube feet by stretching each tube foot to breaking point. They knew that other echinoderm mutable tissues become stiffer after bathing in these cell-disrupting solutions, and noticed exactly the same effect in the tube feet of sea stars and sea urchins. They also found that both species' tube feet became more flexible in calcium-free seawater, just as other mutable tissues do. And when they examined the tube feet under a microscope, they saw cells that are remarkably similar to the secretory cells found in other mutable tissues. The team concludes that sea stars and sea urchins possess mutable tissues in their feet, which act as shock-absorbers to help the creatures resist pounding waves. But they also noticed that sea urchin tube feet are stronger, stiffer and tougher than the larger tube feet of sea stars, which they suspect reflects how the creatures walk; sea urchins use their tube feet to drag themselves forwards, while sea stars' tube feet act as flexible levers.

10.1242/jeb.01693

Santos, R., Haesaerts, D., Jangoux, M. and Flammang, P. (2005). The tube feet of sea urchins and sea stars contain functionally different mutable collagenous tissues. *J. Exp. Biol.* **208**, 2277-2288.

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