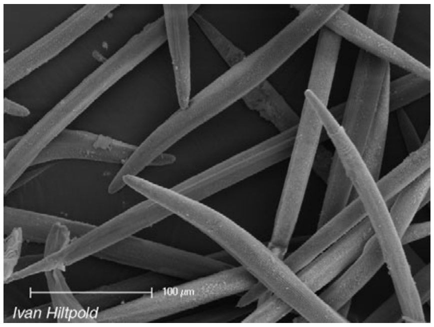
Inside JEB highlights the key developments in *The Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

NEMATODES VANQUISH WESTERN CORN ROOTWORM



Ivan Hiltpold

The larva of *Diabrotica virgifera virgifera* beetles wreak havoc on maize. Feasting on the plants' roots, they are estimated to cause $1 billion of damage every year in the US. Ted Turlings from the University of Neuchâtel, Switzerland, explains that the pest, known as western corn rootworm, only arrived in Serbia in the 1990s, but since then it has marched through at least 11 European countries. 'Pesticides work to control the pest, but they are not environmentally friendly,' explains Turlings and adds, 'When it arrived in Germany in 2007 they wanted to eradicate it but the pesticide that they used killed millions of bees.' Looking for an alternative, more ecological, form of pest control, Turlings wondered whether predatory nematodes (microscopic worms) that munch on insects could defeat the pest. Knowing that *Heterorhabditis bacteriophora*, which kills western corn rootworm larvae, is relatively unresponsive to an alarm signal, (*E*)-β-caryophyllene, released by the infested roots, Turlings wondered whether he could improve *H. bacteriophora*'s response to caryophyllene in a bid to produce an effective biopesticide (p. 2417).

Using an 'olfactometer' (six tubes radiating out from a central point) packed with damp sand for the nematodes to crawl through, Ivan Hiltpold inserted capillaries into the sand, which released different odours at the end of three of the olfactometer's arms. Then he released *H. bacteriophora* nematodes at the centre of the olfactometer and allowed the nematodes to choose which odour they tracked. Timing how long it took 500 nematodes to reach the end of the trail in the caryophyllene arm of the olfactometer, Hiltpold collected the worms and allowed them to breed. Gathering the offspring 10 days later, he tested their responses to the three odours and again selected the 500 nematodes that reached the end of the caryophyllene trail first for breeding. Repeating the selection process 6 times, Hiltpold improved the nematode's performance significantly, decreasing the time it took 500 worms to reach the end of the caryophyllene trail from 10 h to 2 h.

Next Hiltpold tested how improving the nematode's response to caryophyllene had impacted on their potency. Sprinkling the selected nematodes directly on the pest larvae and waiting to see how many larvae died, he was relieved to find that the selected nematodes were only slightly less infectious than their forebears. This loss of potency could be overcome easily by the worm's increased response to caryophyllene, but how would the selected nematodes perform in a field?

'We couldn't test the nematodes in Switzerland because the western corn rootworm is not present yet, so we had to travel to Hungary,' says Turlings. Teaming up with Stefan Toepfer and Ulrich Kuhlmann from CABI Europe-Switzerland, who had access to western corn rootworm infected fields sown with two varieties of maize (one that produced caryophyllene and another that did not), Turlings' colleague, Mariane Baroni, sprayed solutions of the selected nematodes between the rows of maize in some plots and sprayed solutions of the unselected nematodes on other plots in the same fields. Then the team waited to see whether the selected nematodes offered any protection against the pest.

They did. The variety of maize that released caryophyllene was healthier than the variety that did not release caryophyllene after treatment with the selected nematodes; and the selected nematodes killed more pest larvae near the caryophyllene releasing maize than the unselected nematodes did.

Turlings says that this result is encouraging, but admits that there is more to be done before the nematodes can be used commercially. For instance, US varieties of maize have lost the caryophyllene alarm signal and application of the biopesticide is costly and problematic, but Turlings is optimistic that his team can crack both of these problems to add the nematodes to the maize farmer's arsenal.

10.1242/jeb.047852

Hiltpold, I., Baroni, M., Toepfer, S., Kuhlmann, U. and Turlings, T. C. J. (2010). Selection of entomopathogenic nematodes for enhanced responsiveness to a volatile root signal helps to control a major root pest. *J. Exp. Biol.* **213**, 2417-2423.

PREDATOR ODOURS DON'T BOTHER BATS

Despite their nocturnal and aerial lifestyle, bats are still at risk from predators. Weasels and stoats can scale the walls of bat roosts and young and old bats are in danger from foxes if they fall. Tess Driessens from Vrije Universiteit Brussels, Belgium, and Björn Siemers from the Max Planck Institute for


Stefan Greif

Ornithology, Germany, wanted to know how bats recognise predators. 'It might be important for bats to assess whether or not predators are there when they inspect new roosts,' explains Driessens. While sound and visual cues could be helpful warnings when predators are in residence, they are of little help if a predator is absent when a bat investigates a new roost. However, odours can linger long after a predator has departed. Curious to find out whether bats react to odours left by potential predators, Driessens and Siemers decided to find out whether bats fear odours left by foxes, weasels and stoats (p. 2453).

'Synthetic predator odours such as TMT [found in fox faeces] and 2-PT [found in weasel and stoat odours] induce innate fear responses in rodents so we decided to use these synthetic olfactory cues and the odour of a natural least weasel to compare bat responses,' explains Driessens. Travelling to the Max Planck Institute's Tabachka Bat Research Station in Bulgaria, Driessens and Siemers collected greater mouse-eared bats as they returned to their cave after a night of foraging. The duo then took the animals to the lab to test their sense of smell before releasing the animals back at their roost.

Putting individual bats in a Y-shaped maze, Driessens placed a cotton pad that carried the scent of either a least weasel, $1.8\times10^{-2}\,\mathrm{mol\,l^{-1}}$ TMT or $1.8\times10^{-4}\,\mathrm{mol\,l^{-1}}$ 2-PT in one arm of the maze and a cotton pad soaked with the odourless solvent (DEP – used to dissolve TMT and 2-PT) in the other arm. Then she filmed the bat's behaviour for 8 min, recording and scoring the animal's activity levels and whether it avoided the predator's odour. Cleaning the maze with ethanol so that no trace of the smell was left, Driessens then tested the bat's response to an equally unpleasant odour, either basil extract or goat smell, which does not terrify rodents, to see if the bats were just avoiding the smell because they didn't like it, or they avoided it because it terrified them. If the bat was frightened by the fox and weasel scents, Driessens expected it to become inactive and avoid the TMT or 2-PT arm of the maze, while remaining active in the maze when the acrid odour was around.

But after testing the bats, Driessens found that they did not respond differently to the two types of odour. They remained equally active in both experiments and were happy to visit both arms of the predator maze. The bats weren't bothered by the predators' smells.

So why didn't the greater mouse-eared bats avoid fox and weasel odours when encounters with either animal could prove fatal? Initially the duo was concerned that the bats couldn't smell the predator odours in the maze. However, when they considered the bat's olfactory threshold, which is similar to that of humans, and tested the smells on colleagues – who regularly work in smelly bat caves and had no problem picking up the stench – they were convinced that the bats must have been able to smell the odours.

Driessens suspects that the bats may be ignoring the odours because they have other more pressing concerns than predation when choosing a roost. Alternatively, bats could be so familiar with the odours of cohabiting weasels and foxes that they no longer perceived the odours as a threat.

10.1242/jeb.047860

Driessens, T. and Siemers, B. M. (2010). Cave-dwelling bats do not avoid TMT and 2-PT – components of predator odour that induce fear in other small mammals. *J. Exp. Biol.* **213**, 2453-2460.

FRUIT FLIES DETECT SLOPES WITH TWO SENSES

When a fruit fly selects a target, the insect locks it in its sight (fixates) and homes in. 'But less was known about what happens when they actually reach those objects,' explains Alice Robie from the California Institute of Technology. Explaining fixation, Robie's supervisor, Michael Dickinson, says, 'It is often described as what happens when you hang a carrot in front of a donkey and it keeps following the carrot forever. We were curious about what happens when the "donkey" actually gets to the carrot. Andrew Straw in my lab has been working on developing software that allows us to track flies with high accuracy so we had the technology to allow us to see how the flies explore a simple but interesting landscape under conditions where we could know their position and velocity at all times' (p. 2494).

But first Robie and Dickinson had to design their landscape. Filming the insects' movements from a single position, the duo settled on a landscape of cones arranged in an arena so that they could always see the fly's position and calculate the insect's vertical position as it scaled the heights. Robie built 4 cones ranging from 36 mm to 10 mm high each with the same surface area but with sides ranging from a steep 75 deg slope to a shallow 30 deg slope. Then she released individual flies, which were hungry and so highly motivated to explore their surroundings, into the arena and filmed them for 10 min in infra-red light. Robie was instantly struck that the flies explored all four cones equally, but once they'd found the highest cone they scaled it and spent more time there than on the shorter shallower cones. 'We were surprised that they showed such a strong preference for the tallest, steepest object,' says Dickinson.

Curious to know how the insects identified the tallest cone, Robie switched off the lights and filmed them with infra-red light to see how they coped in the dark. Without their sight, the flies could no longer fixate on the cones, so their paths became more wiggly as they explored the arena, but once they had stumbled upon the highest cone they reacted as if the lights were on, scaled it and stopped at the top. The insects were using some sense other than vision to identify the tallest cone.

Knowing that the insects sense gravity with sensors in their antennae (Johnston's organs) Robie wondered if the insects could use these gravity sensors to identify the steepest (and highest) cone. Putting a dab of glue on the joint between the second and third antennal segments to inactivate the Johnston's organs, Robie waited to see if the insect could identify the tallest cone by vision alone. Again the fly succeeded.

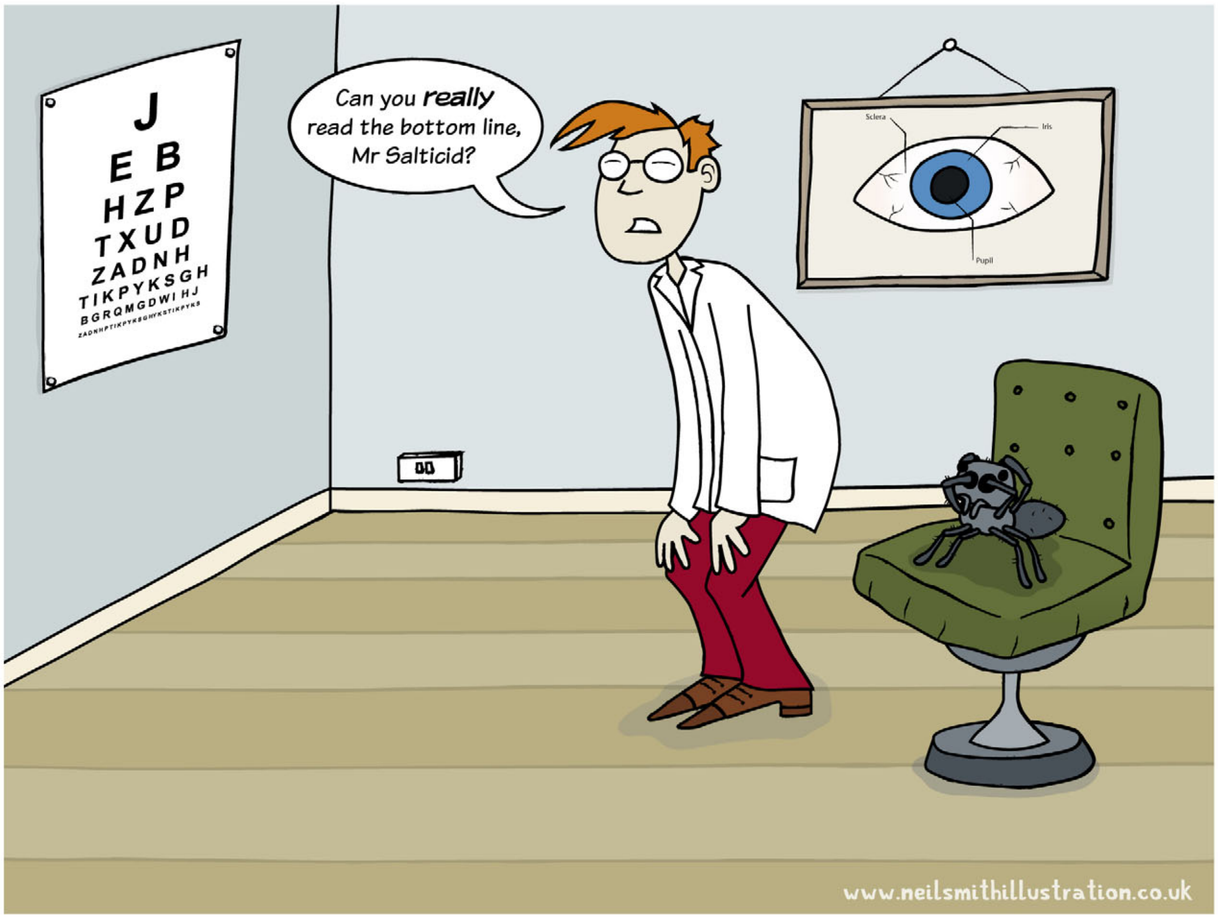
Finally, Robie decided to see whether a fly deprived of sight and its gravity sensors could identify the tallest cone, but this time it could not. 'The movie was extraordinary,' says Dickinson, 'they would go up the cone over the top and down the other side. It was like they just didn't know they were on an object.'

Dickinson admits that he was surprised that the flies were unable to identify the tallest cone when deprived of both senses. He says, 'The animal is covered with mechano receptors, especially on the legs, so we were almost certain that they could use information from their legs to tell them they are on a steep object.' However, having convinced himself that fruit flies only require two senses to identify steep slopes, Dickinson is keen to find out more about the neural circuits that control how flies explore their environment.

10.1242/jeb.047878

Robie, A. A., Straw, A. D. and Dickinson, M. H. (2010). Object preference by walking fruit flies, *Drosophila melanogaster,* is mediated by vision and graviperception, *J. Exp. Biol.* **213**, 2494-2506.

SECOND PAIR OF EYES GIVE *S. VESTITA* A GOOD VIEW



Jumping spiders are famed for having up to four pairs of eyes. Together, the eyes comprise a modular visual system that gives the spider a good view of the world. But how does each pair of eyes contribute to the arthropod's vision? Explaining that the second pair of eyes (anterior lateral eyes) flank the central pair, Daniel Zurek and his colleagues from Macquarie University, Australia, wondered whether *Servaea vestita* spiders use the second pair of eyes to identify movement in the environment and to decide whether or not to orient towards it (p. 2372).

Covering four of the spiders' eyes with removable silicone blinds, the team showed them tethered live flies and movies of moving dots and tested the partially sighted arthropods' responses to the movements. The team also compared the responses of fully fed and hungry females with those of fully fed and hungry males, to see whether hunger motivated the spiders to orient in the direction of passing potential meals.

Analysing the partially sighted arthropods' responses, the team found that they could stalk and attack flies using their anterior lateral eyes alone. The spiders also oriented in the direction of fly sized dots moving at a walking pace, but ignored large fast dots that could have been hungry predators and small slow dots that resembled insects that were too small for the arthropods to eat. And when the team compared the males' and females' responses, the females were far more motivated to orient than the males, probably because their energy demands are higher.

Zurek says, 'Even when the spiders were confined to visual input from this secondary pair of eyes, they could respond to targets that are very hard for other animals to see, and were able to detect, stalk and attack flies, which was unexpected.'

10.1242/jeb.047845

Zurek, D. B., Taylor, A. J., Evans, C. S. and Nelson, X. J. (2010). The role of the anterior lateral eyes in the vision-based behaviour of jumping spiders. *J. Exp. Biol.* **213**, 2372-2378.

Kathryn Knight
kathryn@biologists.com