

**INSIDE JEB**

## Migrating birds use fully reduced FADH<sup>-</sup> for navigation



European robin (*Erithacus rubecula*). © Francis C. Franklin/CC-BY-SA-3.0, via Wikimedia Commons.

To us it seems miraculous: a migrating bird can embark from its wintering grounds and successfully return to the breeding site that it may have left months before. Guided only by their internal compass, birds and many other migrating species sometimes cover thousands of kilometres before arriving home. Yet, how these intrepid voyagers detect the earth's magnetic field for navigation is a topic of hot debate. Roswitha Wiltschko, Wolfgang Wiltschko and Christine Nießner from the Goethe-Universität Frankfurt, Germany, explain that some birds, such as chickens and migratory robins, are thought to 'see' the magnetic field superimposed on their vision when the Earth's magnetic field interacts with a specially activated form of a protein called cryptochrome (Cry1a), which absorbs UV to green wavelengths in cone cells in the retina. However, it was not clear which of the two possible activated forms of the Cry1a protein is essential for the navigators to detect magnetic fields.

Wiltschko explains that plants absorb blue-green light using a form of cryptochrome where the chromophore – the part of the protein (flavin adenine dinucleotide, FAD) that is responsible for the protein's light sensitivity – is partially reduced to a semiquinone by UV and blue light. However, in a second step, the semiquinone can be further reduced by UV, blue and green light to produce

FADH<sup>-</sup> and it is this form that can then be reoxidised to produce a pair of electrons (a radical pair) that is essential for the detection of magnetism.

Knowing that exposure to different light colour combinations can produce the semiquinone and FADH<sup>-</sup> forms of Cry1a and that the Cry1a protein changes shape depending on whether it is carrying the semiquinone or FADH<sup>-</sup> chromophore, the Wiltschkos and Nießner produced an antibody that could distinguish between the two incarnations of the protein to discover more about which form of FAD is used by magnetism-sensitive birds for steering (p. 4221). However, instead of testing their theories on a migratory species, the team first investigated which forms of the Cry1a protein chickens produce under white and coloured lights.

Explaining that chickens orient naturally, Nießner and Susanne Denzau took birds that had been kept in normal daylight and tested whether they were able to produce the fully reduced form of FADH<sup>-</sup>. Exposing the birds to UV (373 nm), blue (424 nm), turquoise (502 nm) or green (565 nm) light, the duo then used the antibody to test whether the birds had been able to produce FADH<sup>-</sup> and found that all of the animals did. Then – reasoning that chickens that have been exposed to green light alone could only produce FADH<sup>-</sup> if they had access to a supply of semiquinone produced during earlier exposures to blue and UV wavelengths – the team isolated the birds in the dark for 30 min before exposing them to the four test wavelengths of light. If the team's ideas were correct, the birds that had been bathed in green light could not produce FADH<sup>-</sup>, as the supply of the essential semiquinone intermediate would have already run out. Using the antibody to test chickens' eyes, Nießner and Denzau could see that the birds that had been exposed to the blue and UV wavelengths had produced FADH<sup>-</sup>; however, as predicted, there was no FADH<sup>-</sup> in the retinas of the birds that had been kept in green light.

So, the chickens were capable of producing FADH<sup>-</sup> Cry1a as well as the

semiquinone form of Cry1a; but which form of Cry1a do migrating birds use to set their bearing? This time, the Wiltschkos turned to a well-established migratory species, the European robin (p. 4225) to test how well birds that had been exposed to combinations of light colour that produced either the FADH<sup>-</sup> or semiquinone forms of Cry1a were able to set their bearings.

Plunging the birds into darkness for an hour, the team recorded which directions they wanted to fly under blue, turquoise or green light. Sure enough, the birds that were attempting to take off under the blue and turquoise lights set the correct northerly bearing as they were able to produce FADH<sup>-</sup>. However, the birds that were attempting to take off under green light were completely disorientated, trying to head in easterly and westerly directions. Without a supply of semiquinone, the birds were unable to produce the FADH<sup>-</sup> that is essential for magnetism detection. However, when the team monitored the birds' attempts to migrate under the three colours of light after spending the day in white light, even birds that had been exposed to green light successfully set northerly bearings, although by the second hour, the green light birds had run out of semiquinone – and FADH<sup>-</sup> in turn – and become disorientated again.

The robins clearly required FADH<sup>-</sup> to produce the radical pair that is essential to detect the Earth's magnetic field, as they lost the ability to navigate under green light when their supply of semiquinone Cry1a ran out. The team is now keen to find out how radio frequency fields, which disrupt birds' magnetic compasses, affect how Cry1a detects magnetic fields.

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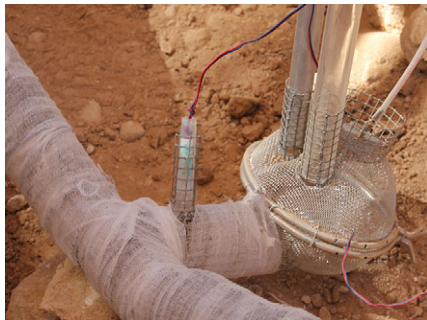
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Inside JEB highlights the key developments in The Journal of Experimental Biology. Written by science journalists, the short reports give the inside view of the science in JEB.

## Burrows ventilated by eddy penetration



Test burrow nest chamber, made of two kitchen sieves. Photo credit: Inbal Brickner-Braun.

Just because we aren't burrow dwellers doesn't mean that we can't appreciate the challenges of a subterranean lifestyle. Anyone who has travelled on an underground system cannot have failed to notice the stale atmosphere in the tunnels and it has long been assumed that burrowing species must contend with high concentrations of carbon dioxide in their subterranean lairs. However, a team from Ben-Gurion University of the Negev, Israel, and ESF-SUNY, USA, suspected that burrow dwellers' homes might be better ventilated than we assume. Inbal Brickner-Braun, Daniel Zucker-Milwenger, Avi Braun, Berry Pinshow, Scott Turner and Pedro Berliner explain that although CO<sub>2</sub> levels in the burrows of some rodent species were found to be high, carbon dioxide measurements in the burrows of other rodents were essentially the same as those on the surface. So, the team decided to find out how the burrows of one small

species of rodent, Sundevall's jird, are ventilated (p. 4141).

The team say that various processes may contribute to the ventilation of burrows. They explain that air might be forced through the tunnels by the inhabitants moving like pistons, moved by convection or diffusion, or driven by air movements at the surface. Intrigued, the team built artificial jird burrow systems to find out whether eddies from the surface could replenish air deep inside a burrow.

Improvising with kitchen sieves to build the nest chamber and light-wire mesh for the burrow walls, Brickner-Braun and Zucker-Milwenger constructed two, 2 m long U-shaped tunnels, each of which descended 60 cm down from the surface and was connected to the nest chamber at the deepest point by a short tunnel. Wrapping one burrow in plastic (to seal it and allow air movement along the tunnel by convection alone) and the other in medical gauze (to permit diffusion of gases across the unsealed walls), the team buried the simulated burrows in light soil, and aligned them with the direction of the prevailing wind. Then they waited for windy days to measure the air temperature in the burrow – in the hope of seeing puffs of warm air driven by surface air currents penetrate the tunnel – to find out whether atmospheric turbulence can drive air circulation through subterranean burrows.

Analysing the temperature profiles of the burrows, the team saw that eddies from

the surface were able to travel deep into the burrows, but they never reached the nest in the most remote regions.

However, when the team simulated the presence of a mother with four pups inside the burrows by pumping CO<sub>2</sub> into the nest chambers, they could see that air currents at the surface dramatically affected the chamber's CO<sub>2</sub> levels. The CO<sub>2</sub> concentration in the sealed burrow fell spectacularly from 25,660 ppm (65 times atmospheric CO<sub>2</sub> levels) at the lowest wind speeds to ~4000 ppm at wind speeds of 4 m s<sup>-1</sup>. Meanwhile, in the unsealed burrow with both entrances plugged – so that gases could only leave or enter by diffusion across the burrow walls – the CO<sub>2</sub> levels were unaffected by wind speed, remaining between 8600 and 10,400 ppm. However, when both mechanisms of gas exchange were possible, the nest CO<sub>2</sub> measurements fell as low as 2800 ppm at the top wind speed of 3.0 m s<sup>-1</sup>.

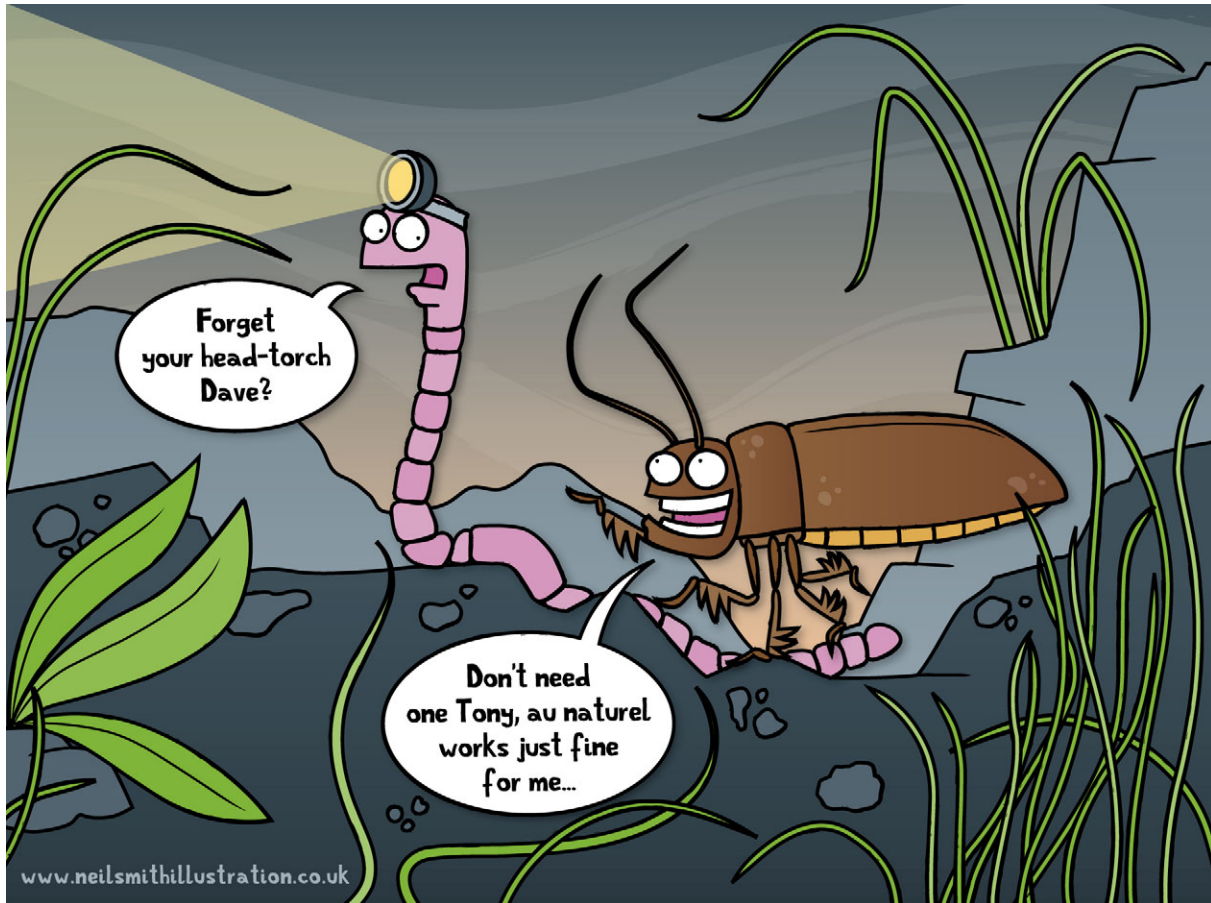
The team say, 'The nest chamber seems not to be directly ventilated by eddy penetration'; however, they suspect that fresh air carried into the burrow probably increases the CO<sub>2</sub> gradient between the nest and main channel to boost its diffusion away from the chamber and keep the air fresh.

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Brickner-Braun, I., Zucker-Milwenger, D., Braun, A., Turner, J. S., Pinshow, B. and Berliner, P. (2014). Ventilation of multi-entranced rodent burrows by boundary layer eddies. *J. Exp. Biol.* **217**, 4141-4148.

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## Cockroaches pool light signals to see in dark



Scuttling around in the dead of night, American cockroaches tend to rely most on touch and their sense of smell for guidance. However, the insects always keep an eye open for a dark bolt hole to scamper into. Given that the animals are fine-tuned to head for dark corners, Matti Weckström and his colleagues from the University of Oulu, Finland, were curious to find out how sensitive the cockroaches' vision is in extremely dim light. The team decided to use the insects' tendency to turn in the same direction as their surroundings – when the image on their eye of the surroundings moves – to find how sensitive their eyes are and to learn more about how they process visual information at very low light intensities (p. 4262).

The team placed individual cockroaches on a roller ball – which the insects could only touch with their feet to indicate in which direction they were moving. Then they displayed images of moving gratings illuminated by light at intensities ranging from a brightly lit room (500 lx) to a dark moonless night (0.005 lx) and measured the insects' reactions. They were impressed to see that the cockroaches' vision was fantastically sensitive, allowing the animals to see gratings moving in light as low as 0.005 lx when each photoreceptor was only picking up one photon every 10 s. And when the team analysed the cockroaches' responsiveness, they realised that the insects were pooling and processing the signals from thousands of light-sensitive

cells to detect motion at these low light levels.

Weckström and his colleagues say, 'The cockroach visual system for motion detection has to rely on unknown neural processing in the deeper ganglia in order to cope with the inescapably deteriorating spatial resolution', adding that they hope to apply the lessons that they learn from the sensitive insects to design better automatic nocturnal vision systems.

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Honkanen, A., Takalo, J., Heimonen, K., Vähäsöyrinki, M. and Weckström, M. (2014). Cockroach optomotor responses below single photon level. *J. Exp. Biol.* **217**, 4262–4268.

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