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

TOTAL RECALL

Picture by Jeff Wilson (RSBS photography, ANU)

Smells can be very powerful triggers of memories in humans, but what about in other animals? Judith Reinhard and colleagues at The Australian National University wondered whether scents could trigger navigational memories in honeybees. They designed a set of compelling behavioural experiments to see if honeybees could use scents to help them make their way back to a known food source (p. 4373).

Reinhard knew that honeybees have a keen sense of smell and are notoriously good at navigating to sweet-smelling flowers. But could these animals associate an attractive scent with a visual memory of the route to a specific location? The team needed to show that sniffing a particular smell only triggered a navigational memory in specific bees: those that linked the odour to a memory of the location of a tasty food reward.

Rigging up an artificial feeder, the team first tempted a group of honeybees to home in on an attractive rose scent, luring them with a tasty sugar snack. The insects quickly learned to navigate their way towards the location of the rose-scented feeder. The team expected that rose scent released in the beehive would trigger the bees' memory of the location of the rose feeder and send them off in the right direction. But would the smell trigger a foraging frenzy in all the other bees in the hive too? The team decided to train a second set of bees in the hive to forage at a second feeder, this time lured by a tangy lemon smell. They hoped that when bees in the hive caught a whiff of rose, lemon-trained bees would stay put while the rose-trained bees would set off in a hurry, using the navigational memory triggered by the scent.

Sure enough, when the team fanned rose aroma through the trained bees' hive, nearly all the rose-trained bees went flocking to the site of the feeder despite the fact that it no longer smelled of rose, while the lemon-trained bees stayed safely tucked up at home. The rose scent had triggered a navigational memory in the rose-trained bees only. Reinhard was pleased with this clear evidence that a scent triggers a very specific navigational memory in honeybees.

The next step was to see how many scents the brainy bees could remember. The team found that a single bee could associate two different scents with two different locations. They were even more impressed to find that bees could learn to associate two different scents with two different colours, even when the coloured feeders were moved around to different locations. The bees were doing so well that Reinhard was confident that they would be able to remember three different locations associated with three different scents. She was astonished when she discovered that this was a step too far. Intrigued by the limits to the insects' amazing memory, Reinhard is now setting out to investigate how complicated things can get before the bee's brain is baffled.

10.1242/jeb.01364

Reinhard, J., Srinivasan, M. V., Guez, D. and Zhang, S. (2004). Floral scents induce recall of navigational and visual memories in honeybees. *J. Exp. Biol.* **207**, 4373-4381.

DAPHNIA BREATHES EASY

Understanding how millimetre-sized animals function poses unique problems for biologists, because processes that work in large animals often cannot be scaled down simply. But as far as Ralph Pirow is concerned, the tiny crustacean *Daphnia* is the perfect model system to study oxygen transport processes, precisely because of its minute size. Oxygen is transported in animals by diffusion or convection, depending on the animal's size. While diffusion takes care of oxygen delivery in something as small and simple as a fish egg, circulatory convection takes over when things are scaled up in larger creatures. But intermediate sized animals like *Daphnia* might use a mixture of the two. Pirow and Rüdiger Paul at the University of Münster wondered how oxygen is transported in the little crustaceans: do they rely on diffusion or convection? They were surprised to find that it all depends on the oxygen levels in the environment (p. 4393).

Pirow explains that *Daphnia* masterfully adapt to changing oxygen levels. When the little creatures find themselves starved of oxygen they produce haemoglobin, an oxygen transport molecule, to help them cope. So, do *Daphnia* with different levels of haemoglobin transport oxygen differently? To find out, Pirow, Paul and Christopher Bäumer decided to compare oxygen profiles inside the bodies of normal-oxygen-adapted and low-oxygen-adapted *Daphnia*.

They reared one population of the transparent minicrustaceans at normal oxygen levels and a second population in low oxygen conditions. The low-oxygen-population went from transparent to a bright red colour as the animals pumped up production of haemoglobin. While the low-oxygen-adapted population became haemoglobin-rich, the population reared in normal oxygen conditions remained haemoglobin-poor. The team was now ready to visualise oxygen levels inside animals from the two populations. They injected an oxygen-sensitive phosphorescence probe (which emits more light as oxygen levels decrease) into the crustaceans' circulatory system. The light intensity images provided by the probe allowed the team to see two-dimensional oxygen profiles in *Daphnia*'s haemolymph circulation.

Examining a cross-section through the middle of the animal's body under a microscope, Pirow saw steep oxygen gradients in haemoglobin-poor *Daphnia* but fairly flat oxygen gradients in haemoglobin-rich animals. He explains that the steep slopes of the oxygen gradients in the bodies of the haemoglobin-poor animals indicate diffusion-based oxygen transport, as 'you can imagine the oxygen rolling down the slopes from regions of high oxygen to regions with less oxygen'. The presence of haemoglobin, acting as a buffer that stabilizes the release of oxygen from the haemolymph to body tissues, restricts the haemolymph oxygen concentration to a narrow range, smoothing out the oxygen gradients in the body. The gentle oxygen gradients in the haemoglobin-rich *Daphnia* indicate that, as haemoglobin levels increase, the animals switch from a diffusion-dominated to a convection-dominated oxygen transport system. 'Oxygen can be transported at much flatter internal oxygen gradients by convection than by diffusion' says Pirow, 'so animals with high haemoglobin levels have the advantage that they can cope much better with oxygen-deficient habitats'.

If having lots of haemoglobin is so useful, why aren't all *Daphnia* bright red? Many aquatic animals, like *Daphnia*, are transparent in a bid to escape unwanted attention from predators. Having high levels of haemoglobin may help the creatures survive in low oxygen habitats, but turning bright red also makes them much easier to spot by a hungry fish. To avoid becoming lunch, *Daphnia* may have to pay the price of struggling for air in oxygen-starved water.

10.1242/jeb.01365

Pirow, R., Bäumer, C. and Paul, R. J. (2004). Crater landscape: two-dimensional oxygen gradients in the circulatory system of the microcrustacean *Daphnia magna. J. Exp. Biol.* **207**, 4393-4405.

OSTRACODS' OXYGEN PUZZLE

According to palaeontological records, two billion years ago atmospheric oxygen levels were a tenth of the levels they are today. Prehistoric water-breathing crustaceans presumably adapted to these low levels, but when oxygen levels rocketed, some creatures took advantage of the metabolic advantages offered and allowed their blood oxygen levels to rise. However, crustaceans seem to have stuck to their old-fashioned low-oxygen habits; they have remarkably low blood oxygenation levels even to this day. Oddly enough, despite the large evolutionary gap between crustaceans and warm-blooded animals, modern mammalian brains share the low oxygenation levels of crab and crayfish circulation. Jean-Charles Massabuau and Laure Corbari at Bordeaux University wondered if this striking similarity was evidence that the crustaceans' prehistoric oxygen regulation strategy was preserved over evolutionary time (p. 4415).

Massabuau reasoned that if he could show how primitive water-breathers were able to regulate their tissue oxygenation levels in prehistoric times, this would reveal an early adaptation strategy that could have maintained low tissue oxygenation levels in animals throughout the course of evolution. Massabuau needed a primitive animal, so when Pierre Carbonel told him about tiny creatures called ostracods that hadn't changed much in the past 500 million years, Massabuau realised he had found an 'open window into the past' to study ancient oxygen regulation strategies.

The team decided to focus on the ostracods' breathing apparatus. Ostracods are minute crustaceans, complete with little appendages that beat rhythmically to waft water into the animal's breathing cavity, where gas exchange occurs by diffusion. Wondering whether the diminutive creatures were able to adjust their breathing rate at different oxygen levels, the team designed miniature aquaria, allowing them to change the oxygenation levels in the mini-habitats. The team videotaped the crustaceans to see whether the animals would beat their breathing appendages faster as oxygen levels dropped. The team were surprised when they saw that the ostracods didn't change the beating frequency of their breathing apparatus at different oxygen levels. Clearly, the ostracods couldn't regulate their ventilation in response to changes in water oxygenation. So how had the animals coped for millions of years?

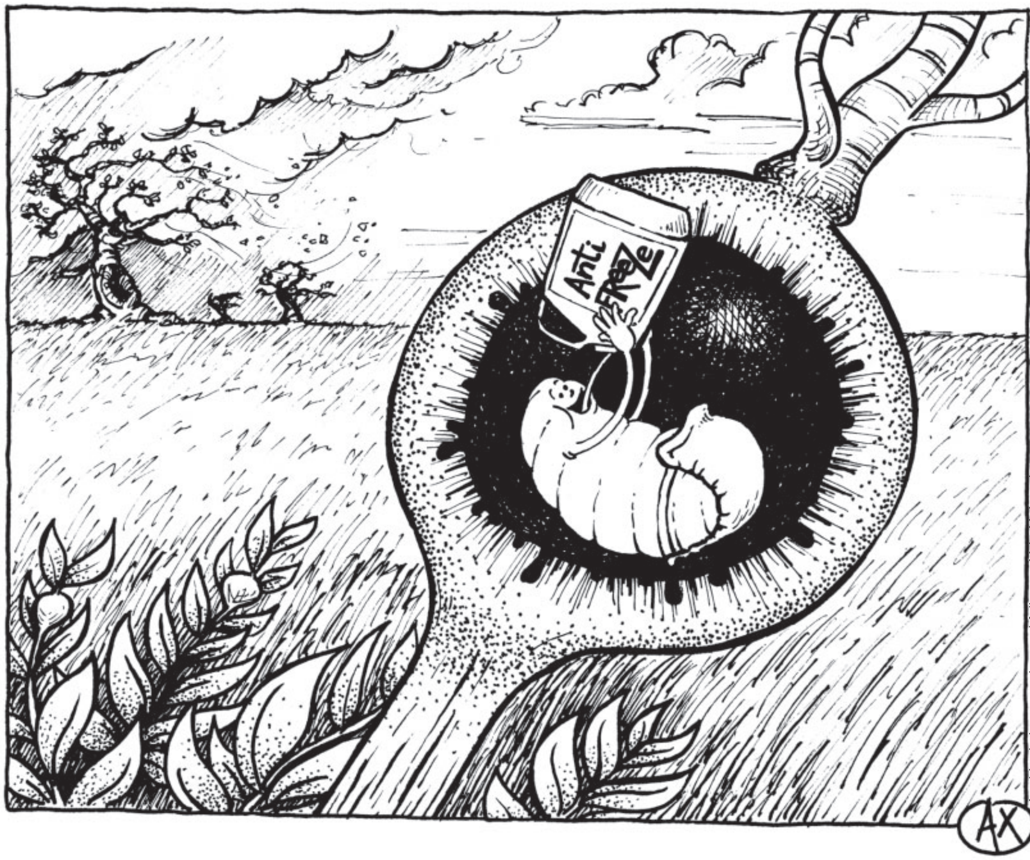
The team suspected that ostracods might regulate their oxygen by choosing to live at particular oxygen levels. To test this, they needed to study the distribution of the little sediment-dwellers in their natural habitat. They didn't have to travel far to collect sediment core samples: the Bay of Arcachon in France, where ostracods live, is right outside their lab. The researchers then determined the ostracods' preferred oxygen levels by measuring the oxygen profile of each sediment sample, freezing and slicing up the samples and counting the number of animals in each slice. The team found that the crustaceans were escaping both oxygen-rich and oxygen-depleted regions and migrating to sediments where the oxygen levels were slightly higher than those required by their tissues. The ostracods were regulating their body oxygenation simply by crawling to their comfort zone.

So 500 million years ago, these tiny crustaceans were already managing their tissue oxygenation levels using a behavioural rather than a physiological strategy, which could have been conserved over evolutionary time. But what is the evolutionary advantage to maintaining such low body tissue oxygenation? 'One explanation is that metabolism produces oxygen free radicals, which damage cells', says Massabuau, 'so maintaining low oxygen levels may protect an animal's tissues from free radical attack'. It seems ostracods may have a very good reason for being stuck in the past.

10.1242/jeb.01363

Corbari, L., Carbonel, P. and Massabuau, J.-C. (2004). How a low tissue O_2 strategy could be conserved in early crustaceans: the example of the podocopid ostracods. *J. Exp. Biol.* **207**, 4415-4425.

How Goldenrod Gall Flies Cope with Larval Dehydration
(do not try this at home)



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ANTI-FREEZE CUTS BUG'S WATER LOSS

It's tough being a bug in winter, yet some creatures, like goldenrod gall fly larvae, miraculously manage to survive icy winters and emerge unscathed in spring. But surviving frosty conditions is only part of the problem; since water is locked up in ice, bugs are also in danger of drying out. So how do the larvae manage to overwinter? Jason Williams and colleagues at Miami University wondered whether the larvae gradually develop both cold tolerance and resistance to water loss as temperatures plummet between autumn and winter (p. 4407).

Williams explains that in early autumn, few larvae can survive subzero temperatures. However, by winter the bugs develop freeze-tolerance, churning out sugar-like substances called cryoprotectants that act as anti-freeze, so that the youngsters can endure a frosty –20°C. Even more impressive, goldenrod gall fly larvae become desiccation resistant and can compete with desert beetles when it comes to cutting their water losses. But are these two remarkable abilities linked? From early autumn to mid-winter, the team measured larval survival rates after freezing, as well as the bugs' cryoprotectant levels and resistance to water loss. They found that a seasonal decline in water loss rates was directly linked to boosted cryoprotectant levels in the insects' circulatory systems. Williams concludes that the gall flies' anti-freeze doesn't just stop the insect from turning into an icicle; it may also conveniently protect larvae from the perils of dehydration.

10.1242/jeb.01366

Williams, J. B., Ruehl, N. C. and Lee, R. E. Jr (2004). Partial link between the seasonal acquisition of cold-tolerance and desiccation resistance in the goldenrod gall fly *Eurosta solidaginis* (Diptera: Tephritidae). *J. Exp. Biol.* **207**, 4407-4414.

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