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.

COCKROACHES HOLD BREATH TO CONSERVE WATER


Natalie Schimpf

When some insects stop rushing around and settle down, they switch to a discontinuous respiration mode where they cycle between closing, 'fluttering' (rapidly opening and closing) and opening the spiracles that they breathe through. Why inactive insects switch to this form of interrupted breathing is hotly debated. Natalie Schimpf from The University of Queensland explains that there are several possible reasons: the insects could hold their breath to conserve water; discontinuous breathing could have evolved to improve respiration underground where oxygen is scarce and carbon dioxide levels high; or the animals could reduce oxygen levels in their tracheoles (breathing tubes) by holding their breath to prevent damage from the reactive atmospheric oxygen. And with evidence stacking up for and against each hypothesis, there was no sign of the debate dying down. However, when Craig White introduced Schimpf to the respiration phenomenon during her undergraduate lectures, the student noticed that no one had ever tested whether adult insects could adjust their discontinuous breathing patterns in response to long exposures to altered atmospheres. Only pupae had been tested. Together with Robbie Wilson, she and White quickly realised that testing whether adult insects can adjust their breathing patterns could help them unravel the reason behind insects using a discontinuous breathing pattern (p. 2773).

Deciding to work with cooperative cockroaches, the team obtained male *Nauphoeta cinerea* from a local pet food supplier; cockroaches are a lizard delicacy. Having overcome her distaste for the scuttling insects, Schimpf supplied them with dry and humid atmospheres; atmospheres with oxygen levels ranging from 5 to 40%; and atmospheres with carbon dioxide levels ranging from 0.3 to 6% for 5 weeks. Once the insects had acclimated to the new atmospheres, Schimpf settled individual adults in a darkened respirometry chamber and measured the gases exhaled by the inactive animals. Recording the cockroaches' responses to atmospheres with different oxygen, carbon dioxide and humidity levels, it was clear that the animals could alter their discontinuous gas exchange breathing pattern in response to different atmospheres. But would their new breathing patterns shed light on why the insects hold their breath?

After weeks of recording the cockroaches' gas exchange patterns, the team joined up with Philip Matthews to see whether any of the theories stood up to the tests. Reasoning that the insects would keep their spiracles closed for longer in a high carbon dioxide atmosphere if discontinuous breathing had evolved to help them cope underground, the team saw that the insects kept their spiracles closed for less time. So *N. cinerea* did not evolve discontinuous gas exchange to cope with a subterranean lifestyle. And when they analysed the breathing patterns of the insects from the low and high oxygen environments, instead of closing their spiracles for longer in high oxygen atmospheres to protect themselves from oxidative damage, the insects opened their spiracles for longer. So that ruled that theory out too.

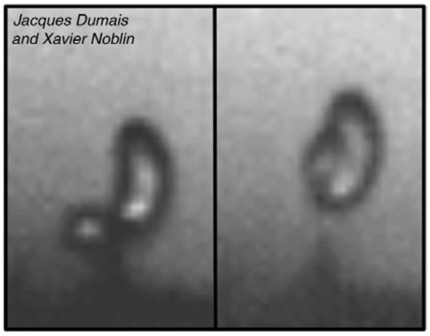
That only left the water conservation theory. Would the cockroaches open their spiracles for longer in humid conditions and keep them closed in the dry to prevent respiratory water loss? They did; inactive *N. cinerea* use discontinuous gas exchange to conserve their body fluids. So discontinuous gas exchange seems to be essential for water conservation in *N. cinerea*, but the jury is still out on whether other insects hold their breath to conserve water.

10.1242/jeb.036129

Schimpf, N. G., Matthews, P. G. D., Wilson, R. S. and White, C. R. (2009). Cockroaches breathe discontinuously to reduce respiratory water loss. *J. Exp. Biol.* **212**, 2773-2780.

FUNGI OPT FOR WATER POWERED BALLISTICS

How basidiomycete fungi hurl millions of spores into the atmosphere has intrigued scientists for almost a century. Sitting atop a microscopic structure, the sterigma, each spore is immobile until a tiny drop of water, known as Buller's drop, condenses on it. Within seconds the spore is catapulted into the air, but how the droplet sends its spore flying wasn't clear. Physicist Xavier Noblin from the Université de Nice-Sophia-Antipolis explains that various theories had been suggested: maybe the droplet was actually a bubble and the spore blasted off



when the bubble exploded; or maybe the droplet suddenly fused to the spore by surface tension, launching them both into the air. But no one had ever calculated the droplet and spore's energetics to find out how the droplet launches the spore on its way. Having joined Jacques Dumais' plant biomechanics lab in Harvard in 2004, Noblin was in the perfect place to apply his knowledge of the physics of droplets to the puzzling question of how fungi launch spores (p. 2835).

Knowing that the microscopic spores were fired off at a speed of about 1 m s^{-1}, Dumais, Noblin and Sylvia Yang realised that they would have to use a super-fast high-speed camera to have a chance of seeing what happened to the droplet as the spore tore free of its launch site. Fortunately, Dumais had access to two cameras that could record movies at a remarkable 250,000 frames s^{-1}, but it still wasn't clear if this would be fast enough to capture the droplet's movements. 'We knew we would get the spore motion,' says Noblin, 'but we weren't sure we'd get the droplet motion'. And the team had no control over when, and in which directions, the spores would take off, so having chosen a spore that looked as if it might be about to fly, the team would set the camera rolling and hope that they captured the moment of take off.

Amazingly, they did. After weeks of filming, the team's patience was rewarded when they captured the instant when the expanding droplet suddenly touched the banana-shaped *Auricularia auricula* fungus spore and fused to the depression in the spore's surface as it took off. The launch was over in less than 8 μs (two frames in the movie) and the trio could clearly see the spore tumbling as it flew through the air. Next the team filmed *Sporobolomyces* yeast spores, and saw exactly the same process as the droplet initially grew before contacting the spore and fusing to its surface to send the spore flying.

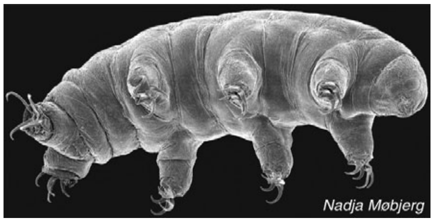
But how is a single water droplet able to send a spore rocketing from its launch site? Noblin and Dumais suspect that as surface tension fuses the droplet to the surface of the spore, surface tension energy is released, which powers the droplet's movement, smashing it into the spore, sending both of them flying. Noblin adds that the spore essentially 'jumps off' the sterigma, powered by surface tension energy as the droplet collides with the spore.

So rather than develop complex catapult systems to hurl their spores into the atmosphere, some fungi have evolved a simple ballistic system that only requires water to produce one of the planet's most amazing microscopic ballistic performances.

10.1242/jeb.036145

Noblin, X., Yang, S. and Dumais, J. (2009). Surface tension propulsion of fungal spores. *J. Exp. Biol.* **212**, 2835-2843.

HOW NORTHERN TARDIGRADES WEATHER WINTER



Tinier than a pinhead, tardigrades are some of the toughest creatures on the planet. Some are so tough that they have even survived being sent into space. While *Halobiotus crispae* isn't at the top of the list of tardigrade tough guys, it's still pretty robust. Enclosing itself in a cyst-like second skin while hibernating during winter in the icy waters around Greenland, the marine tardigrade eventually sheds its protective outer layer in the spring before becoming active during the short northern summer. Curious to find out more about the tardigrade's strategy for survival, Nadja Møbjerg, Reinhardt Møbjerg Kristensen and their colleagues from the Universities of Copenhagen and Roskilde decided to test the resilience of a local population of *H. crispae* from the Danish coast. However, the Danish tardigrades prefer to hibernate during the relatively warm Danish summer, becoming active in late winter and early spring. Would the two populations use the same strategies to survive the effects of ice and salt water, or had they come up with alternative approaches to weather their local conditions (p. 2803)?

Collecting tardigrades from the sediments at the bottom of the Danish Isefjord, Møbjerg and her colleagues, Kenneth Agerlin Halberg and Dennis Persson, tested the tiny animals' resistance to freezing. Teaming up with Hans Ramløv and Peter Westh, they gently cooled the active and hibernating animals and measured the exact moment when the tiny creatures froze. Amazingly, the tardigrades remained unfrozen until the temperature dropped to –20°C, when both active and hibernating animals froze. Somehow the animals are able to prevent their body fluids from freezing, remaining supercooled until the temperature plummeted to –20°C. And when the team thawed the animals, the hibernating animals were absolutely fine, having survived the icy experience.

Next the team tested the Danish tardigrades' responses to saline conditions by immersing the animals in seawater ranging from a salinity of 2 ppt to 40 ppt and found that the active animals seemed able to cope better with the drastic changes. And when they measured the volume of the animals' bodies, it was clear that the animals reacted very differently to high and low salt concentrations. The animals' bodies initially swelled by 60% when transferred to dilute seawater, before shrinking back down to their normal size 2 days later, while the animals that were transferred to more concentrated seawater initially shrunk by 40% before slowly reinflating over the next day to a new smaller, but stable, body size.

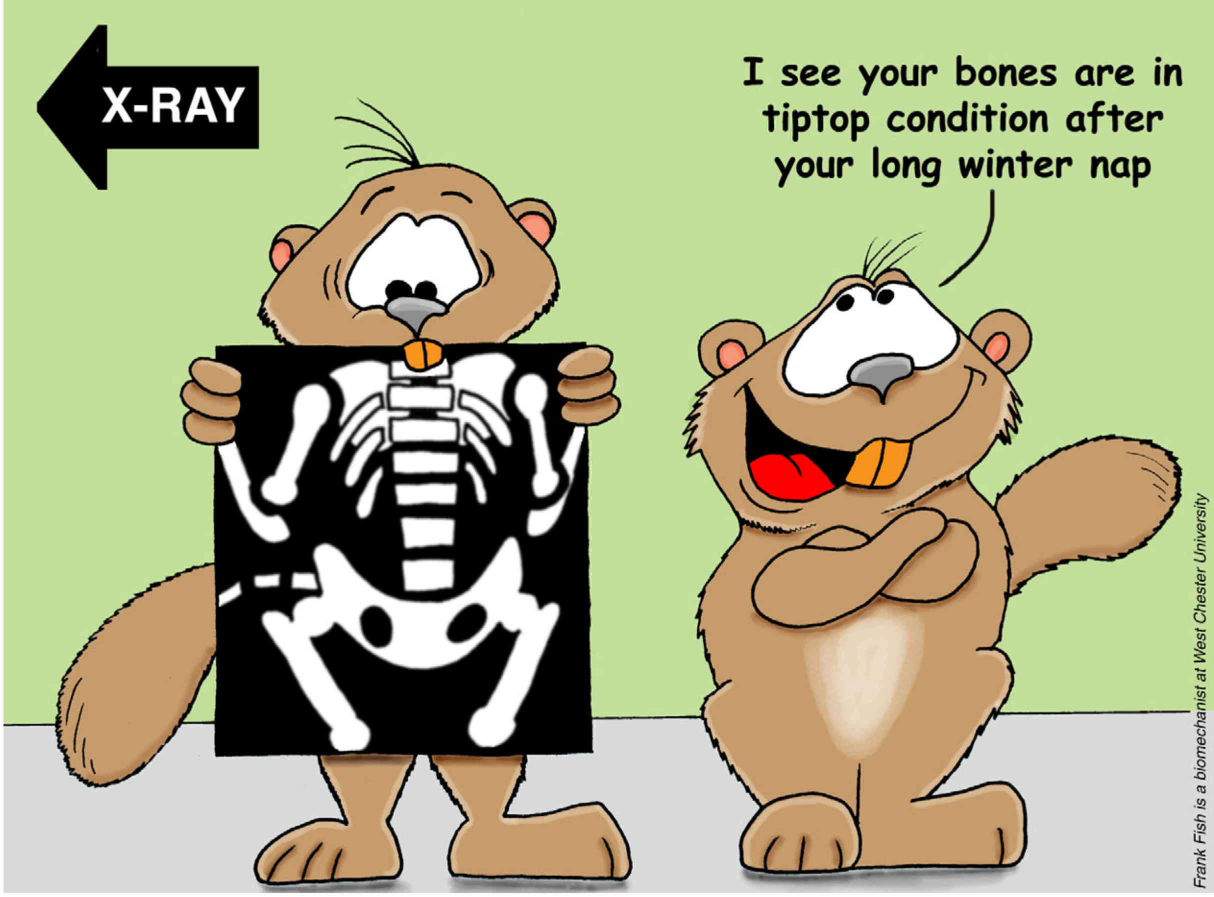
Wondering what effect the tardigrades' dramatic size change had on their body fluids, Halberg carefully extracted nanolitre samples of body fluid from the microscopic beasts and measured the number of particles dissolved in the fluid. No matter what salt concentration the tardigrades were exposed to, their body fluids were always slightly more concentrated than the environment surrounding them. And when the team tested the effects of salt and ice on the Greenland *H. crispae* population, they found that the animals use the same strategies for survival as the Danish tardigrades.

Having found how different *H. crispae* populations protect themselves from the damaging effects of salt and ice, Møbjerg suspects that all *H. crispae* use the same approaches, and is keen to find out more about the ways the tiny creatures regulate their body fluid composition to survive in some of the planet's most inhospitable environments.

10.1242/jeb.036152

Halberg, K. A., Persson, D., Ramløv, H., Westh, P., Kristensen, R. M. and Møbjerg, N. (2009). Cyclomorphosis in Tardigrada: adaptation to environmental constraints. *J. Exp. Biol.* **212**, 2803-2811.

HIBERNATING SQUIRRELS' BONES WEATHER WINTER WELL



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It is clear to anyone that has watched astronauts being carried from their rockets that microgravity and disuse play havoc with our bones. But hibernating animals routinely experience months of immobility during every winter, and yet resume normal activity within hours of waking up in spring. Curious to find out how hibernating animals' bones fare during months of winter disuse, Frank van Breukelen and his colleagues from the University of Nevada tested hibernating golden-mantled ground squirrels' bones to see how well they stood up to months of inactivity (p. 2746).

Comparing the strength and stiffness of the bones of hibernating inactive and active ground squirrels, the team found that the dormant ground squirrels' bones were in fine condition. They were as strong and stiff as those of the active animals'. So months of winter inactivity did not affect the ground squirrels' bones.

However, when van Breukelen and his colleagues compared the strength of active summer squirrels and squirrels that had been inactive during the summer, they were surprised to see that the inactive squirrels had suffered bone loss, much like inactive humans and other mammals.

van Breukelen is now keen to understand how hibernating ground squirrels maintain their bones during months of inactivity and is curious to find out whether the cool winter conditions play a role in protecting the animals' bones from atrophy.

10.1242/jeb.036137

Utz, J. C., Nelson, S., O'Toole, B. J. and van Breukelen, F. (2009). Bone strength is maintained after 8 months of inactivity in hibernating golden-mantled ground squirrels, *Spermophilus lateralis*. *J. Exp. Biol.* **212**, 2746-2752.

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