Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

FOLLOW THE NOSE

Every one of us has experienced a sleepless night courtesy of an annoying buzzing mosquito trying to bite us. What presumably prevents us most from sleeping is the helpless admission that the mosquito will achieve its aim a soon as we doze off. They do so even in complete darkness, attracted by our body odors. However, we are not completely at the mercy of our tormentors. Some of us will fight before surrender by taking up an insect repellent. Most of them contain *N*,*N*-diethyl-*m*-toluamide (DEET), a compound that efficiently wards off many biting insects. The common view is that the deterring effect of DEET is because it smells bad to the insects. Recently, however, the research team of Leslie Vosshall from the Rockerfeller University in New York reported in *Science* that this view is wrong.

The attraction that we exert on female mosquitoes is largely odor mediated. With specialized sensory organs called sensilla they sniff human body emanations such as lactic acid in our sweat or CO_2 and octenol in our breath. The insect's sensilla harbor olfactory sensory neurons that fire electrical impulses in response to a particular attractant. By measuring these odor-evoked electrical impulses at a sensillum of the *Anopheles* mosquito, Vosshall's team at first examined the effect of DEET on the sensory neuron responses to octenol and CO_2. While DEET had no effect on CO_2-evoked responses, it inhibited firing of neurons responding to octenol.

As neurobiology is much better understood in fruit flies than in mosquitos, the scientists switched to carrying out behavioral experiments in *Drosophila*. They established an assay where the flies were given the choice of two different trap vials to enter. After setting up different experimental conditions they recorded the number of flies in each vial. If both vials were loaded with food, the flies took the bait and distributed themselves equally between them. However, if one of the two baited vials was treated with 10% DEET, a common concentration in repellent sprays, the flies avoided the DEET-treated vial. Surprisingly, the flies were happy to enter the DEET-scented vial when no food was present. It appeared as if the flies couldn't smell the food in the presence of DEET rather than just being warded off.

Then the scientists went further, testing whether the DEET effect involves odor receptors known to respond to particular smells including octenol. For this purpose they engineered frog egg cells producing various types of *Drosophila* and *Anopheles* odor receptors on their cell surface. When these eggs were exposed to different human body odors, the scientists could record inward cation currents indicating that the receptor had 'smelled' the scent. In the presence of DEET a subset of the tested receptors, including the *Anopheles* octenol receptor, were far less responsive to the odor, as the measured currents were significantly reduced. DEET was blocking the ability of the *Anopheles* octenol receptor to detect the tell-tale human scent.

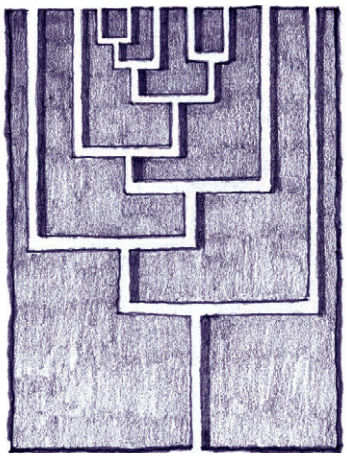
Vosshall and her colleagues have shown that DEET is not a repellent in the real sense but prevents the mosquito from smelling us. Their nifty trick of using frog egg cells as a sort of 'midget nose' may help to identify new compounds that protect us even more effectively from mosquitoes, including those transmitting serious diseases.

10.1242/jeb.011437

Ditzen, M., Pellegrino, M. and Vosshall, L. B. (2008). Insect odorant receptors are molecular targets of the insect repellent DEET. *Science* **319**, 1838-1842.

Hans Merzendorfer
University of Osnabrueck
merzendorfer@biologie.uni-osnabrueck.de

BEHAVIOUR



ONISCOPHAGOUS ARACHNIDS: OR EATING ROLLERS

Woodlice – slow-moving terrestrial isopods also known as 'pill bugs' – would appear to be a juicy treat for any predator. They are the main component of many ground-dwelling fauna and can be found in large numbers. But it appears that, in general, these isopods have few invertebrate predators: only a few animals prey upon them occasionally, including harvestmen, centipedes and ants. Yet one genus of tropical ant, *Leptogenys*, includes species that are exclusively oniscophagous: they eat nothing but woodlice and are equipped with specially adapted mouthparts to kill and carry their prey. Some spiders also dine occasionally on woodlice. For example, non-web-building *Dysdera* spiders, found around the Mediterranean and in Europe, are also thought to feed on woodlice with their unusually long, curved mouthparts, known as chelicerae. Milan Řezáč, Stano Pekár and Yael Lubin from Israel and the Czech Republic wondered whether the size of a spider's chelicerae is a good indicator of their dining preference and style.

Selecting five species of *Dysdera*, each with different shaped chelicerae, the team starved the spiders for 2 weeks before presenting them with a single live lunch, ranging from woodlice and centipedes to springtails, bugs and flies. As soon as the spiders caught their prey, the victim was removed, to ensure that the arachnids remained hungry enough to strike again. The team found that species with unmodified, slightly curved chelicerae, never went for woodlice, while the species with long, hook-like appendages all captured woodlice, and in the case of *D. abdominalis*, which has very elongated chelicerae, this was the only food they would take.

To investigate how these different cheliceral designs might procure each species an advantage when faced with the behaviour and morphology of woodlice, Řezáč and coworkers offered each spider a choice of dining – a rolling woodlouse, a 'clinging' non-rolling woodlouse and a fly. They found that *Dysdera* spiders used three grasping tactics – each specific to their cheliceral shape.

Species with elongated chelicerae used a 'pincer' tactic, capturing the woodlouse with their extended fangs, one on the isopod's back, the other on its belly. If this was done quickly enough, the hapless woodlouse did not have time to roll up. One species with concave chelicerae used the 'fork' tactic, spearing the woodlouse through its belly before it rolled up. Finally, one species with long, flattened chelicerae used the 'key' tactic, in which the fangs were slid like a stiletto between the plates of the isopod's armour, succeeding even if the woodlouse had rolled up.

Particularly interesting was the finding that *D. abdominalis* spiders, with their elongated chelicerae, were unable to transport prey with their mouthparts, and had to use their legs and pedipalps to hold the victim, making it tricky to walk. This may explain why not all *Dysdera* species have adopted an onicophagous strategy – in an environment where there are many predators, stumbling along with your prey may be disadvantageous.

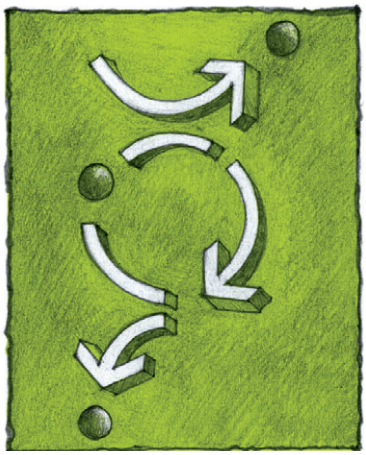
But why haven't other spiders developed large chelicerae to exploit abundant woodlice supplies? The team point out that in many arachnids male chelicerae are the focus of intense sexual selection pressure from females. This does not appear to be the case in *Dysdera,* but in other species chelicerae are used in mating not eating; this could keep woodlice off the menu for most species, leaving the isopods to carry on pottering, unperturbed by arachnid predation.

10.1242/jeb.011445

Řezáč, M., Pekár, S. and Lubin, Y. (2008). How oniscophagous spiders overcome woodlouse armour. *Journal of Zoology* **275**, 64–71.

Matthew Cobb
University of Manchester
cobb@manchester.ac.uk

DIVING PHYSIOLOGY



NO DEPTH TO NEUROGLOBIN

Discovered less than a decade ago, and reported in all vertebrate phyla, the function of the oxygen-binding neuronal heme protein neuroglobin has yet to be determined. Suggested roles include oxygen storage or transport, regenerating NAD^+ under anaerobic conditions, or acting as a scavenger of toxic reactive oxygen species. Very low oxygen levels (hypoxia) increase neuroglobin levels in the mammalian brain, and neuroglobin increases reduce the damage following experimentally induced stroke in animal models. However, its oxygen binding characteristics and low tissue concentrations suggest that neuroglobin is not primarily an oxygen storage compound. The apparent pro-survival role of neuroglobin under low oxygen conditions led Terrie Williams and her colleagues at the University of California Santa Cruz to wonder whether diving mammals, which naturally experience levels of hypoxia that would cause brain damage in most vertebrates, express higher levels of neuroglobin than non-divers.

The team compared the brain protein neuroglobin and the more widespread cellular cytoglobin in both diving and terrestrial animals: eight kinds of stranded or humanely killed dolphins and whales, two pinnipeds and the sea otter, plus coyotes, foxes, bobcats and mountain lions from government trapping programs (or roadkill). They measured total globin protein content from gray matter samples and then calculated the neuroglobin/cytoglobin levels by subtracting the hemoglobin absorbancy from the total globin content. The researchers also determined neuroglobin and cytoglobin mRNA levels for the mountain lion, bobcat, and five marine mammal species.

Diving marine mammals are known to have higher blood oxygen carrying capacities,

and this was reflected in the group's results. Brain hemoglobin levels were higher in marine mammals compared with terrestrial ones, with a near 10-fold difference in hemoglobin concentration between mountain lions and the pelagic diving pilot whale. Similarly, there was a 3-fold range in neuroglobin/cytoglobin levels among the 16 species tested, with a distinct clustering of terrestrial, swimming and diving specialists. Surprisingly, though, neuroglobin levels were not highest in the divers but in the swimmers, which generally dive for short periods only but spend time in fast, aerobic swimming. There was in fact an inverse relationship between neuroglobin levels and maximum dive time in marine mammals. Williams' group thus hypothesized that deep divers rely preferentially on circulating globins while 'sprinters' enhance intracellular globin stores.

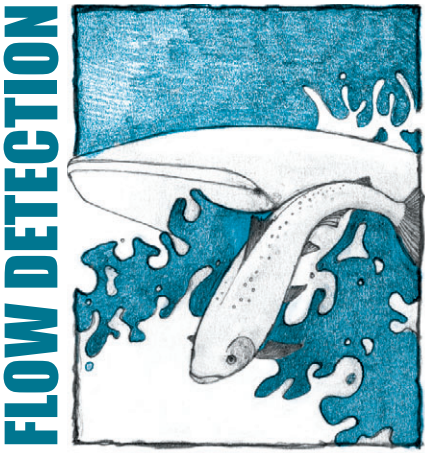
Interestingly, a link between activity level and intracellular heme proteins is supported by the terrestrial exception to the rule: the bobcat had neuroglobin levels comparable to swimming specialists and significantly higher than other terrestrial animals. The bobcat is an ambush predator rather than a good swimmer, so a high globin level in both groups implies that the neuroglobin is not associated with hypoxia tolerance but instead benefits highly active species.

The authors suggest that neuroglobin facilitates oxygen movement from blood to neural tissues and thus provides a secondary level of neuronal protection from hypoxia in animals that cannot significantly increase circulating hemoglobin levels. Diving mammals can increase the number of circulating red blood cells because the decreased heart rate and vasoconstriction typical of divers ameliorate the negative impact of highly viscous blood, and thus divers can maintain oxygen gradients even in hypoxia. Active sprinters, with elevated heart rates and blood flow, instead appear to increase intracellular globin levels to ensure adequate oxygen delivery, because you can't catch the prey if you pass out on the way!

10.1242/jeb.011452

Williams, T. M., Zavanelli, M., Miller, M. A., Goldbeck, R. A., Morledge, M. C. D., Pabst, D. A., McLellan, W., Cantin, L. P. and Kliger, D. S. (2008). Running, swimming, and diving modifies neuroprotecting globins in the mammalian brain. *Proc. R. Soc. B* **275**, 751-758.

Sarah Milton
Florida Atlantic University
smilton@fau.edu



HOW FISH SENSE STEADY FLOW

Many fish orient to steady water flow. This behavior, called rheotaxis, is key for river fish like salmon that migrate up- or downstream, or simply have to accommodate the ever-present river flow. How do fish sense the water motion?

It seems likely that the lateral line sense must be involved. The lateral line is an array of flow-sensing hair cells that run in lines along a fish's body. Indeed, if you pharmacologically disable the lateral line, many fish are not able to orient to flow very well.

But that's what's weird. The lateral line doesn't respond well to steady fluid motion. It has two types of sensor: canal neuromasts, which are embedded in pores below the fish's skin, and peripheral neuromasts, which stick out into the flow. Canal neuromasts only respond to differences in the flow speed between each end of the pore, so they can't sense steady flow. Peripheral neuromasts, on the other hand, respond to flow speed, but since they rapidly adapt to different steady flow speeds, it seems likely they wouldn't help much with rheotaxis either.

Boris Chagnaud, working in Horst Bleckmann's laboratory at the University of Bonn, realized that the physiological and behavioral data didn't match up. On the one hand, behavioral studies showed that knocking out the lateral line degrades rheotactic behavior, but on the other hand, the physiology indicates that the lateral line shouldn't be able to sense steady flow in the first place.

The resolution turns out to be simple. Steady flow doesn't exist. Even in highly controlled laboratory conditions, there are always turbulent fluctuations in the water motion, and these fluctuations move downstream at the mean flow speed. The researchers put an anesthetised fish in a laboratory flow tank designed to produce steadily moving water with very low turbulence. To measure the flow speeds, they tracked small particles in the water using a technique called particle image velocimetry. Even though the flow had little turbulence, they found fluctuations in the flow velocity near the fish's body. Points further along the fish's body had the same fluctuations, but slightly later in time, showing that the turbulence was moving downstream at the steady flow speed. The researchers were able to quantify this effect using a mathematical technique called cross-correlation, an estimate of the similarity in the fluctuations in two measurements when one measurement is delayed relative to the other. In this case, the cross-correlation time delay represents the time it takes a turbulent fluctuation to move along the fish's body.

In other words, if fish know the spacing between their lateral line sensors, they ought to be able to estimate the flow speed by looking at the cross-correlations between different neuromasts. To see whether this might be possible, Chagnaud measured the signals from pairs of neuromasts in the lateral line of goldfish. He found many pairs with high cross-correlations, and that the correlation time delay decreased as he increased flow speed.

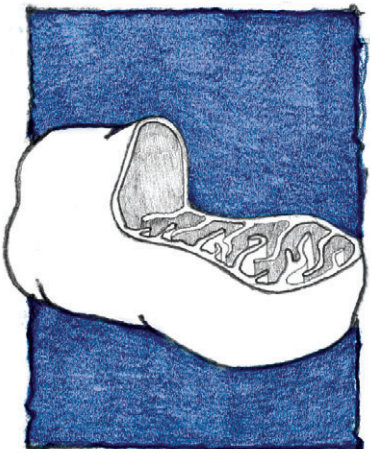
Fish can't really perform fancy cross-correlation analyses like the researchers did. Instead, they might use what's called a 'coincidence detector', known from studies of vision and hearing. Neurons in the brain will only respond when the signals from two different neuromasts reach the brain simultaneously, but the signals take different amounts of time to reach the brain. Such 'delay lines' allow sets of neurons to respond to different time delays. With a set of different delay lines, the neurons are, in effect, performing a cross-correlation. These neurons still need to be located, but Chagnaud's work shows they could, in principle, allow fish to extract information on steady flow speed, even with lateral line sensors that don't respond to steady flow.

10.1242/jeb.011429

Chagnaud, B. P., Brucker, C., Hofmann, M. H. and Bleckmann, H. (2008). Measuring flow velocity and flow direction by spatial and temporal analysis of flow fluctuations. *J. Neurosci.* **28**, 4479-4487.

Eric Tytell
University of Maryland, College Park
tytell@umd.edu

UNCOUPLING PROTEINS



COOL CROCS

Although north Australian winters are extremely mild by any standards (around 20°C), Australian crocodiles still have to adjust to these chilly conditions relative to their scorching summers. The ability of ectothermic animals to remain functional and active when exposed to cold environmental temperatures requires a suite of typical physiological adjustments, known as the cold acclimation response. Among these adjustments, mitochondrial levels increase in order to maintain energy production when reduced temperatures slow biochemical reactions. This led a University of Sydney team, Tonia Schwartz, Shauna Murray and Frank Seebacher, to investigate whether the improved metabolic capacity resulting from cold acclimation is associated with protective mechanisms against reactive oxygen species (ROS), a possibly damaging by-product of increased metabolic capacity. The group first hypothesised that mitochondrial uncoupling proteins (UCPs) found in other vertebrates are also present in reptiles, given their suggested role in ROS protection. Second, the authors proposed that the expression of the genes coding for these proteins would increase when crocodiles are exposed to winter temperatures.

Initially, the researchers had to find out whether UCPs are expressed in crocodiles, as they had not been reported in reptiles previously. Using UCP gene sequence information from various vertebrates, the team designed primers that would target and amplify all UCP genes and found that crocodiles apparently express three UCP genes. Next the team began to study the evolution of this gene family by performing a phylogenetic analysis of the crocodile UCP genes relative to UCP gene sequences from organisms ranging from plants to fish, amphibians, birds and mammals. They found that one of the three crocodile UCP genes is homologous to the vertebrate *UCP2* gene, and two cloned genes are homologous to vertebrate *UCP3*. Like birds, crocodiles do not appear to have *UCP1* homologues; however, the two *UCP3* genes group with the birds' single *UCP* gene, agreeing with their shared common ancestor.

While it is unclear what the functional roles of UCP2 and UCP3 are, the cold acclimation experiment provides a good starting point to explore their potential protective role against ROS. After acclimating crocodiles to temperatures mimicking winter and summer conditions for a month, the team collected liver and skeletal muscle samples to measure the level of the reptile's UCP genes using quantitative real-time PCR. When exposed to colder winter conditions, crocodiles up-regulate the expression of *UCP2* and *UCP3* in their liver, but not their skeletal muscles. The authors' previous work also showed that the activity of some Australian crocodile mitochondrial enzymes increases in the liver during cold acclimation, in agreement with the increase in mitochondrial levels.

In accordance with their hypotheses, the team have shown that UCP gene expression increases when ectothermic reptiles are acclimated to the cold. The correlated changes in mitochondrial oxidative capacity, which would lead to increased ROS production in liver, also suggest the potential role of UCPs in protection against ROS. The functional basis of these observations remains to be clarified. Nevertheless, the increase in UCP gene expression in response to cold might be another component of the cold acclimation response in ectotherms.

10.1242/jeb.011460

Schwartz, T. S., Murray S. and Seebacher, F. (2008). Novel reptilian uncoupling proteins: molecular evolution and gene expression during cold acclimation. *Proc. R. Soc. B* **275**, 979-985.

Charles Darveau
University of Ottawa
cdarveau@uottawa.ca