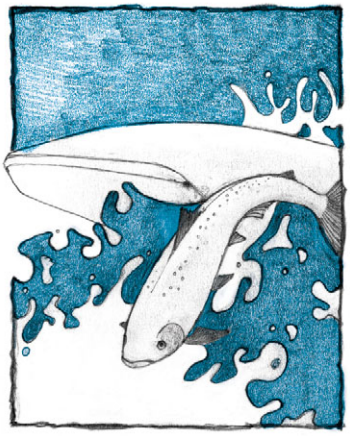
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

NEMATOCYST FUNCTION



NANO-FAST NEMATOCYSTS

The group Cnidaria, which includes jellyfish, anemones, corals and the familiar freshwater *Hydra*, is not generally known for its speed. In fact, it was only in the mid 18th century that they were first accepted as animals by biologists, having been categorized alongside plants for centuries. However, in a twist of historical irony, a recent paper by Nüchter and colleagues has demonstrated that these animals carry out one of the fastest mechanical processes in the animal kingdom.

One of the keys to the cnidarians' diversity and longevity (11 000 extant species, 600 million years on Earth) is undoubtedly their invention of the nematocyst. Nematocysts are explosive cellular structures that fire a tiny barbed harpoon into predators and prey and inject a painful and paralyzing cocktail of chemicals. While contact with nematocysts by humans is more often uncomfortable than fatal, small crustaceans, worms and even fish often do not survive the encounter.

Discharge of the harpoon tip or 'stylet' was known to be fast, but no one knew precisely how fast. Using even the fastest high-speed cameras, the transition of the nematocyst from the undischarged to the discharged state appeared instantaneous, with no intermediates in between. However, high-speed camera technology has improved enormously in the past few years, and in this study, Nüchter and colleagues used a state-of-the art camera to finally resolve the kinetics of nematocyst discharge. With exposure times as short as 200 ns, they were just able to capture nematocysts in motion for the first time.

What they found was truly astounding. Discharge of the barbed stylet was complete in about 700 ns (0.0000007 s), which makes it one of the fastest biological events ever measured. The average velocity of the stylet was about 14 m s^{-1}, with a final velocity of about 25 m s^{-1}. This means that nematocysts are faster than the fastest human sprinter and somewhere between lions and pronghorn antelopes in terms of maximum speed. Of course, nematocysts don't keep this speed up for very long. In fact, the researchers found that the average distance travelled by the stylet was about 13 μm, which is perfectly adequate for a weapon that is discharged by direct contact with its target. While the speed of discharge is impressive, the acceleration is even more so. The team found accelerations that were 1–5 million times the acceleration due to gravity (***g***). This kind of acceleration is difficult for humans to imagine, especially if you consider that we tend to black out at sustained accelerations greater than only 6 ***g***. In 2004, Patek and coworkers demonstrated in a *Nature* paper that mantis shrimps are capable of accelerating their striking limbs at more than 10 000 ***g***, which at the time was the highest acceleration recorded from any organism. Nematocysts appear to achieve accelerations that are 500 times greater.

The upshot of all this is that the discharged stylet strikes its target with enough energy to pierce even the tough armour of exoskeleton-sporting prey (and sometimes human skin). Once the armour is breached, a long, coiled tubule everts from the nematocyst capsule through the stylet and into the victim. Further contraction of the capsule forces neurotoxins through the tubule, paralyzing and often killing the victim. The researchers found that tubule eversion was considerably slower than stylet discharge, with kinetics in the leisurely millisecond range.

With the kinetics of discharge now much clearer, researchers will undoubtedly turn their attention to other unresolved questions such as how energy is stored in the capsule and how that energy is released and converted into the kinetic energy of the stylet and tubule. Stay tuned for more studies of these fascinating microscopic weapons.

10.1242/jeb.02466

Nüchter, T., Benoit, M., Engel, U., Özbek, S. and Holstein, T. W. (2006). Nanosecond-scale kinetics of nematocyst discharge. *Curr. Biol.* **16**, R316-R318.

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BENEFICIAL BLUEBERRIES

Exercise is commonly viewed to be beneficial for one's health. However, you may be surprised to learn that exercise can be detrimental too. Previous study in mammals, including humans, has shown strenuous exercise to produce free radicals, which increase the presence of reactive oxygen species that induce oxidative damage in cells and tissues. Specifically, reactive oxygen species have been shown to play an important role in the etiology of numerous serious ailments such as cancer, Alzheimer's disease and heart disease.

Nevertheless, there may be a simple way to continue exercising without suffering the consequences of the associated oxidative damage. Numerous fruits contain antioxidant compounds such as polyphenols and flavanoids that have been shown to protect against oxidative stress by functioning as reducing agents, singlet oxygen quenchers, and helpers in the repair of damaged DNA bases or protein amino acids. Thus, maintaining a diet supplemented with fruits high in antioxidant compounds could potentially serve to sustain the body's antioxidant levels and prevent exercise-induced oxidative damage.

Kriya Dunlap and associates at the University of Alaska Fairbanks were interested in determining whether a diet supplemented with a fruit high in antioxidant compounds would indeed elevate an animal's total antioxidant power and protect against oxidative muscle damage associated with exercise. As such, Dunlap's team devised an experiment to investigate whether supplementing the diet of Alaskan huskie (*Canis lupus familiaris*) sled dogs with blueberries would prevent oxidative muscle damage in these animals following strenuous exercise. The team maintained two groups of 12 dogs for two months with minimal exercise on either (1) a normal dog food diet or (2) a supplemented diet in which 2% of the daily food intake was blueberries. At the end of the two-month acclimation period, both groups of dogs were exercised on two consecutive days for 30 min each day at 70% of their $\dot{V}_{O_2}$ max. Blood samples were taken prior to, immediately following and 24 and 48 h post-exercise for measurements of plasma creatine kinase and isoprostane – indicators of muscle damage – and total antioxidant power. Measurements were compared to a control group of dogs that were fed a normal dog food diet over the two-month adaptation period but were not exercised at its conclusion.

The team found that supplementing the diet of young healthy sled dogs with blueberries failed to attenuate the muscle damage associated with the exercise regime. Both groups of exercised dogs exhibited a slight, but not unusual, amount of muscle damage following exercise. However, the blueberry-fed dogs did have a greater total amount of antioxidants present in blood plasma immediately post-exercise, but not at 24 or 48 h following exercise. From this finding, Dunlop's team surmise that the blueberry-fed dogs had the potential to be better protected against the deleterious effects of oxidative stress. Thus, it appears the old saying holds true, 'you are what you eat'!

10.1242/jeb.02468

Dunlap, K. L., Reynolds, A. J. and Duffy, L. (2006). Total antioxidant power in sled dogs supplemented with blueberries and the comparison of blood parameters associated with exercise. *Comp. Biochem. Physiol.* **143A**, 429-434.

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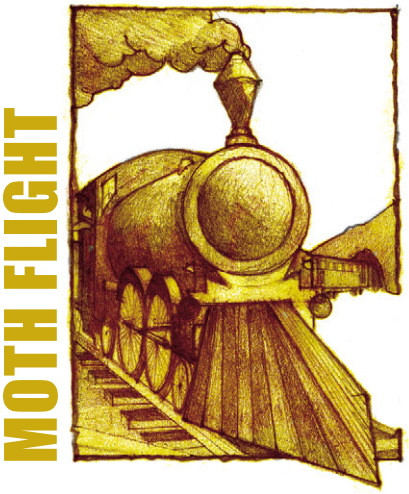

MEASUREMENTS AT THE LEADING EDGE IN INSECT FLIGHT

In recent years, a variety of techniques have been developed that allow the air movements around the wings of flying animals to be measured. Such techniques have been primarily applied to birds, or scaled-up models of insects. A key challenge for animal flight aerodynamicists has been to measure the flows, or wake, around the wings of life-sized insects. Whilst smoke can be used to visualise the form of the wake generated by a flying insect, it doesn't really give much quantitative information that can be used to determine, for example, the mechanical costs of flight. Richard Bomphrey and co-workers, at Oxford and Cranfield Universities, sought to determine, quantitatively, the patterns of airflow around a flying hawkmoth.

The animal they studied had a wing length of a mere 52 mm, so the technical challenges in measuring wakes from such a small animal are obviously quite profound. They conducted experiments on the moth while tethered in a wind tunnel. The tether was instrumented, to allow six force components to be measured during flapping. The movement of air around the wings was determined by using a digital particle image velocimetry (DPIV) system. The system used a laser to generate a 0.4 mm-thick sheet of light, produced in 5 ns pulses. Small smoke particles (less than 10 μm in size) were introduced into the flight tunnel, and the DPIV system automatically tracked the movement of the smoke particles within the wake. Analysis of the wake was triggered when the force produced by the moth was highest during the downstroke of each wingbeat. The tether of the moth could be moved horizontally in the wind tunnel, so that

DPIV measurements could be made at different points behind the insect. Experiments were conducted at two wind speeds (1.2 and 3.5 m s^{-1}).

The experiments showed that hawkmoths generate a curved, elliptical, vortex loop, arising from the leading edge of the wing and having a width equal to the wingspan of the insect. Bomphrey and colleagues show that the velocity profiles they observed matched very closely those predicted by existing theories for the aerodynamics of flapping flight of insects. The team found that the forces produced by air movement within the vortex loops could account for 85% of the forces measured on the tether to which the moth was attached. During the experiments, the team found that when the wings 'clapped' together, two and a half times as much aerodynamic force was produced than during downstrokes. Whilst not every wingbeat cycle contains a 'clap', they occur sufficiently often to supplement the force generated in the downstroke.

Despite collecting impressive data on the airflows around the moth, the team are planning to continue their work and use a stereoscopic DIPV system to improve the accuracy of measurements by determining airflows in all three planes.

10.1242/jeb.02464

Bomphrey, R. J., Lawson, N. J., Taylor, G. K. and Thomas, A. L. R. (2006). Application of digital particle velocimetry to insect aerodynamics: measuring the leading-edge vortex and near wake of a hawkmoth. *Exp. Fluids* **40**, 546-554.

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INSECT MIGRATION

DUE SOUTH?

Every year, hundreds of animals migrate across the globe. Some, like the Arctic tern (*Sterna paradisaea*), make spectacular journeys traversing the globe from north to south whilst others, like the merlin (*Falco columbarius*), make more modest journeys between Iceland and the UK. Migration is not just confined to birds, amphibians and mammals – some insect species are also known to migrate. Indeed, insects like the monarch butterfly (*Danaus plexipus*) make spectacular migrations across North America, leaving sites in the southern USA and Mexico in March and reaching as far north as Canada by May. In birds, mammals and amphibians a great deal of information is available not only about their migratory routes but also about the cues that trigger the migratory movements of individuals. By contrast, relatively little is known about the cues that trigger migration and the movements of individual insects during their migrations.

Like monarch butterflies, some dragonfly species are known to migrate north between March and June and south between August and October across the North American continent, travelling long distances. In preparation for these journeys, the dragonflies may accumulate fat stores just like song birds preparing to migrate. The rules that dragonflies use to guide their migration may also be similar to those of song birds. Martin Wikelski from Princeton University and his colleagues set out to determine what rules dragonflies use to guide their migration and whether they are similar to those used by song birds. During September and October 2005 they monitored the movements of 14 adult common green darner dragonflies (*Anax junius*) captured in New Jersey by attaching small radio transmitters to their ventral thorax. These radio transmitters were light enough (~300 mg) that the green darners were still able to make their migratory flights. These flights were tracked both on the ground using conventional radio telemetry and in the air from Cessna aeroplanes.

All of the dragonflies made migratory flights during the period that they were being monitored. The migratory flights occurred during the day and tended to occur on days when the wind speed was low and there had been a drop in temperature during the previous night. Surprisingly, the direction of the wind did not affect whether the dragonflies migrated and, since the dragonflies' flight direction didn't differ much from the wind direction, this often resulted in zigzagging flight paths. The average direction of the migratory flights was almost due south, approximately 186° from north, taking the dragonflies to warmer climes. The dragonflies did not migrate continuously but instead had distinct stopover days. During their migratory flights some of the dragonflies encountered the ocean, causing them to change direction and avoid prolonged flight over open water.

These rules governing the migration of green darner dragonflies are remarkably similar to the rules that govern the migratory behaviour of song birds. Song birds are known to take stopover days, to migrate in response to decreasing nocturnal temperatures, to avoid flying in high winds and to avoid extended flights over open water. The similarities between the rules governing dragonfly and songbird migration suggest that the rules are reliable and that variables, such as night-time temperature, are good indicators of changing weather patterns, which could trigger departure.

10.1242/jeb.02467

Wikelski, M., Moskowitz, D., Adelman, J. S., Cochran, J., Wilcove, D. S. and May, M. L. (2006). Simple rules guide dragonfly migration. *Biol. Lett.* doi:10.1098/rsbl.2006.0487.

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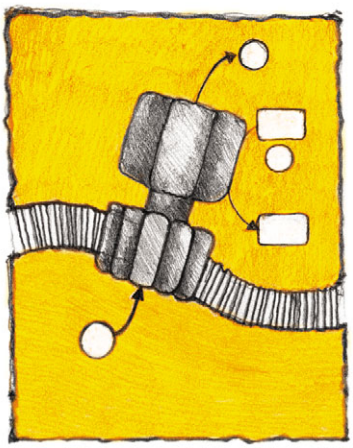
CALCIUM SIGNALLING



BAD CRAC LOWERS YOUR RESISTANCE

Calcium is a ubiquitous second messenger, with vital roles in nerve, muscle and indeed in nearly every cell. It is thus surprising that, despite tens of thousands of researcher years, one of the great puzzles in calcium signalling, whether the calcium signal comes from outside the cell or from stores inside, appears only now to have been solved. For most cells, the answer is both; when a ligand binds to its receptor on the cell surface, it triggers formation of inositol trisphosphate, which activates the internal component of the signal *via* a calcium channel on the endoplasmic reticulum (the main intracellular calcium store). Further calcium rushes in from the outside through calcium release-activated calcium (CRAC) channels in the cell's outer membrane. The external signal appears to be triggered indirectly by the first calcium wave, when depletion of the endoplasmic reticulum calcium store seems to signal back to the plasma membrane, but virtually nothing was known about the structure or regulation of this elusive channel. Over the years, physiologists have posited many candidates for this signal and its target, but none have stood the test of time. However, a rare human disease and a hardcore functional genomics technology have finally identified the CRAC channel.

Human severe combined immunodeficiency (SCID) is a tragic disease in which children have to live in isolation bubbles. One type of the disease is caused by T cells failing to respond to pathogens and has previously been shown to be caused by the loss of CRAC channel function. Classical human disease mapping identified a short region on chromosome 12 associated with SCID that contains several calcium-related genes that could possibly activate CRAC, but it was not possible to identify which of these genes was responsible for the children's loss of immunity. So the authors turned to a clever *Drosophila* screen to find out which calcium-related gene could be responsible for the loss of CRAC function.

It is now possible to knock down expression levels of nearly every *Drosophila* gene, by exposing cultured *Drosophila* cells to interfering RNA (RNAi) targeted at each of the insect's genes. Using this interference RNA technique, the team found two calcium-related genes that directly affected CRAC function: one was a gene called *dStim*, already implicated in CRAC signalling, and the other encoded a channel-like molecule. A human homologue of this *Drosophila* channel gene occurs right in the middle of the chromosomal region implicated in human SCID. Could this channel prove to be the CRAC channel itself? The team put the human homologue of *Drosophila* CRAC, Orai1, back into T cells from SCID patients and found that the biophysical properties previously described for the CRAC channel were restored and normal immune function was resumed by the T cells. Orai1 is the CRAC channel.

CRAC signalling is ubiquitous and so many other functions should be impaired. Why then do SCID patients only show an immune deficit? In humans, there are three *Orai* genes, and Orai1 is immune specific. So CRAC function in other tissues is probably provided by the other genes.

Although decades of physiology delimited the properties of CRAC signalling very precisely, it is fair to concede that physiology alone might never have provided this intriguing answer to the CRAC problem, showing yet again how powerful a multidisciplinary approach can be.

10.1242/jeb.02465

Feske, S., Gwack, Y., Prakriya, M., Srikanth, S., Puppel, S. H., Tanasa, B., Hogan, P. G., Lewis, R. S., Daly, M. and Rao, A. (2006). A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function. *Nature* **441**, 179-185.

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