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

TONGUE BALLISTICS

CHAMELEONS' COOL TONGUES DON'T MISS A SHOT

Cold is normally avoided by ectothermic animals, which rely on environmental temperature to keep their body temperature high. When exposed to cold, metabolic rate slows down, muscles stiffen, and escaping from predators or catching a meal becomes increasingly difficult. This is why the distribution of chameleon species across a wide range of thermal habitats, from deserts to alpine zones, is so intriguing. In 1982, Stephen Reilly reported that some species of chameleons can even feed at body temperatures as low as 3.5°C, allowing them to take advantage of thermal niches other lizards can't exploit. Although these observations were made almost 30 years ago, the answer to how chameleons are able to catch prey at very low temperatures had remained elusive until now.

Christopher Anderson and Stephen Deban, from the University of South Florida, USA, believe the secret is in their tongues. Chameleons are sit-and-wait predators; they ambush their prey by rapidly projecting their sticky tongue once their prey is within reach. This means they don't need fast leg muscles to catch their prey, they need a fast tongue instead. Interestingly, chameleons possess a unique mechanism of tongue projection that relies on rapid elastic recoil of collagen tissue, which works somewhat like a 'bow and arrow'. Anderson and Deban set out to determine whether this elastic-recoil mechanism confers thermal independence to chameleon tongue projection.

Using a high speed camera, the team recorded tongue projection events of veiled chameleons (*Chamaeleo caliptratus*) catching crickets that had been placed at different pre-set distances. They then measured peak acceleration, peak velocity and peak mass-specific power of the tongue projections, as well as the tongue retractions, which do not use elastic recoil but instead are driven by contraction of the hyoglossus muscle.

The chameleons were able to project their tongues and capture the crickets over the same range of distances whether they were at 15, 25 or 35°C. Although the performance of tongue projection decreased by 10–19% from 25 to 15°C, even at 15°C performance was maintained at an extremely high level; the average peak velocity was 3.4 $m\,s^{-1}$, average peak acceleration was 357 $m\,s^{-2}$ and average peak mass-specific power was 1892 $W\,kg^{-1}$. The drop in performance of tongue retraction, on the other hand, was much more pronounced, exhibiting decreases in peak velocity, acceleration and mass-specific power of between 42 and 63% over a 10°C range. These results confirm a high degree of temperature independence of tongue projection and a large temperature dependence in the performance of the tongue retraction.

The large differences in thermal dependence between tongue projection and retraction support the hypothesis that the elastic-recoil mechanism is responsible for the high degree of temperature independence observed during chameleon ballistic tongue projection. During this process, temperature-dependent muscle contraction 'loads' the tongue before launch. The relatively temperature-independent elastic-recoil mechanism then powers the tongue's projection, which launches it towards the prey with impressive speed and acceleration. The temperature independence of this process allows chameleons to feed early in the morning when it is too cold for other lizards to hunt and to inhabit thermal habitats other lizards can't exploit, which gives them a leading edge when competing for food. Now, you can't tell me that's not a cool tongue.

10.1242/jeb.036640

Anderson, C. V. and Deban, S. M. (2010). Ballistic tongue projection in chameleons maintains high performance at low temperature. *Proc. Natl. Acad. Sci. USA* **107**, 5495-5499.

Viviana Cadena
Brock University
viviana.cadena@brocku.ca

COLD ADAPTATION

HOW WOOLLY MAMMOTH BLOOD CHEATED THE COLD

The woolly mammoth vanished just after the last Ice Age but may be the best-understood prehistoric species because their massive size and demise in a geographic freezer made for near-perfect fossilization. Indeed, the fossil record has illuminated much of what we know of this animal regarding anatomical adaptations to the cold, e.g. minimizing heat loss with thick fur, thick oily skin, blubber, and small ears and tail. Interestingly, scientists have also determined that the woolly mammoth descended directly from Asian elephants that originated in tropical Africa 5–7 million years ago. What kind of evolutionary adaptations allowed a massive tropical elephant that is excellent at eliminating excess heat to move into and survive the frigid Arctic? Until recently, none of the fossilized evidence could be connected to how this animal once functioned because physiological and biochemical characteristics do not fossilize.

Based on their knowledge of blood physiology adaptations in Arctic species alive today, Kevin Campbell from the University of Manitoba in Canada and 14 colleagues from across the globe wondered if this Ice Age creature possessed similar adaptations that allowed it to move to frigid climates. In a typical mammal, haemoglobin, the O_2 binding protein in the blood, releases O_2 with very slight increases in temperature, thus allowing beneficial site-specific oxygen delivery to warm, working muscles. However, the haemoglobin of contemporary Arctic species is insensitive to temperature so that O_2 delivery to cold extremities and appendages is maintained, despite having a warmer core, saving energy and minimizing heat loss. The team hypothesized this would be the woolly mammoth's strategy, but how do scientists analyse blood from an animal that went extinct 10,000 years ago?

Campbell's group extracted DNA from three permafrost-preserved Siberian mammoths that lived 43,000 years ago. From this DNA, they sequenced haemoglobin genes, and converted the sequences into mRNA. They then inserted the mRNA into *E. coli* bacteria, which manufactured the mammoth's haemoglobin. Next, the team used atomic modelling and found structural differences in mammoth haemoglobin resulting from three amino acid substitutions not found in Asian and African elephants. Finally, they performed physiological and biochemical experiments on the reassembled haemoglobin to determine how the structural differences affect function.

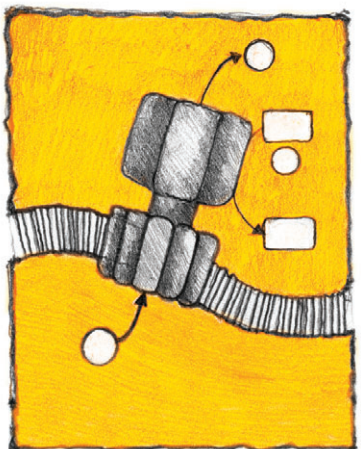
The mammoth haemoglobin functioned over an extremely wide temperature range compared with their tropical elephant cousins. This could be due to more chloride binding sites on the molecule, which changes how much heat is released during binding. Arctic reindeer possess similar binding sites, and elephant haemoglobin has the binding cluster but it is not used. Campbell's team believes these three substitutions in the haemoglobin sequence set mammoths apart from their elephant cousins, allowing them to oxygenate tissues even at very low temperatures, preventing costly heat loss. The team's unique multi-disciplinary approach has resulted in the first discoveries about key molecular and physiological adaptations in an extinct species. They think that the physiology behind cold-adaptation may be what facilitated the woolly mammoth's rapid expansion across a frozen environment that is no longer available for scientists to survey.

10.1242/jeb.036624

Campbell, K. L., Roberts, J. E. E., Watson, L. N., Stetefeld, J., Sloan, A., Signore, A. V., Howatt, J. W., Tame, J. R. H., Rohland, N., Shen, T.-J., Austin, J. J., Hofreiter, M., Ho, C., Weber, R. E. and Cooper, A. (2010). Substitutions in woolly mammoth hemoglobin confer biochemical properties adaptive for cold tolerance. *Nature Genetics* **42**, 536-540.

Jodie L. Rummer
University of British Columbia
rummer@zoology.ubc.ca

WATER RECEPTOR



A TASTE FOR WATER

Whether we like sweet wine, salty tomato juice or bitter lemon depends on our individual preferences: each to his own. Regardless of which flavour of beverage we prefer, from a chemical point of view it is more or less the same as they are all composed primarily of water. But do we also have a taste for water? Although water is essential for us, astonishingly little is known about how we sense it and regulate its uptake. However, we can learn how we may taste water from studying insects, as there is a general agreement among scientists that these little creatures have a taste for water. A research team from the University of California-Berkeley led by Kirstin Scott have now published an exciting study in *Nature* providing new insights into the molecular basis of water taste in *Drosophila*.

Drosophila and other insects have a unique set of gustatory sensory neurons in their mouthparts that participate in the detection of various tastes. Among them are also neurons known to respond to water, but by an unknown mechanism. In order to identify the neurons' water receptor, Peter Cameron, a graduate student in Scott's lab, compared gene expression in the mouthparts between control flies and mutant flies lacking all taste neurons. One of the genes, whose expression was significantly decreased in the mutant flies, was *pickpocket 28* (*ppk28*), a gene encoding an ion channel of the degenerin/epithelial sodium channel (ENaC) family. PPK28 was a promising candidate for the wanted water receptor, as ion channels are known to be involved in the detection of different tastes. Indeed, when Peter Cameron tested which gustatory neurons actually make PPK28, he could not detect it in neurons known to sense sweet or bitter tastes, but he found it in neurons known to participate in water sensing.

Next, he monitored neuronal activity in response to different taste solutions by using a genetically encoded fluorescent Ca^{2+} sensor expressed in the water-sensing neurons of living flies. He found higher neuronal activity when he stimulated the flies' mouthparts with pure water and lower activity when he applied solutions containing salts, sugars, acids or bitter substances. The higher the concentration of the added substance, the lower the resulting neuronal activity, suggesting that the water receptor responds to changes in the relative water content and hence is an osmosensitive receptor. Then Peter Cameron's colleague Makoto Hiroi recorded electrical signals from gustatory neurons of the taste-sensing organs (sensilla), comparing control and mutant flies lacking a functional PPK28 channel. The sensilla of the mutant flies failed to respond to water, in contrast to the control flies, which did. Moreover, the mutant flies also changed their behaviour as they drank significantly less water. Finally, to determine whether PPK28 is directly involved in water sensing, the team genetically manipulated bitter-sensing neurons as well as human embryonic kidney cells, which do not have any taste receptors, to make them produce PPK28, and again recorded Ca^{2+} signals to monitor whether they could detect water. They successfully converted the bitter-sensing neurons into water-sensing ones, and even conferred this type of responsiveness on human embryonic kidney cells, which lack the taste response.

Scott and her colleagues have provided solid evidence that a sodium channel of the degenerin/ENaC family functions as a water receptor by sensing differences in the solution's osmolarity. The PKK28 receptor may therefore serve as a framework for studying water sensing and osmosensing in other animals and even humans. Whether the identified receptor type could also be involved in central osmosensation, which is essential to control the osmolarity of our extracellular body fluids, remains unclear, as this would require extreme sensitivity to minute osmolarity changes.

10.1242/jeb.036632

Cameron, P., Hiroi, M., Ngai, J. and Scott, K. (2010). The molecular basis for water taste in *Drosophila*. *Nature* **465**, 91-95.

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

AURAL SENSITIVITY

I HEAR WITH A LITTLE HELP FROM MY FRIENDS

The vertebrate ear is extraordinarily sensitive, able to discern faint whispers but still find meaning in extremely loud noises, and capable of tuning in to an important conversation over the cacophony of a city street. But individual hair cells, the sensory cells that detect sound vibrations, do not seem to be sensitive enough to account for how well the ear works.

Instead, groups of hair cells in the inner ear gain some of their extraordinary sensitivity by their mechanical interactions with their neighbors, according to new research published in the *Proceedings of the National Academy of Sciences*.

Jérémie Barral and Kai Dierkes and their colleagues at the Institut Curie in Paris, France, and the Max Planck Institute for the Physics of Complex Systems in Dresden, Germany, knew that individual hair cells actively produce force, wiggling back and forth, and that the wiggling could help to selectively and non-linearly amplify faint sounds at a frequency close to the wiggling frequency. But the wiggling also produces noise. For a single hair cell, the noise should swamp out any benefit.

The scientists hypothesized that connections between the hair cells might serve to damp out noise without impeding the non-linear amplification process. Hair cells in a living ear are coupled together by a springy, gelatinous matrix. Perhaps that mechanical linkage is the key to the ear's sensitivity.

To test their hypothesis, Barral and Dierkes built an innovative device that linked an isolated hair cell to a computational simulation of other hair cells. They isolated a group of hair cells from a bullfrog's inner ear, dissolved away the gelatinous matrix, and attached a probe to a single hair bundle. The probe could both apply force and measure the movement of the hair bundle. Then they measured the frequency and amplitude of spontaneous oscillations in the cell, and used these and other measurements to produce a 'cyber clone', a computational simulation of a hair cell with the same properties as the real one. The simulations ran quickly enough that they could use their probe to apply forces to the real hair cell as if it were connected by springs to one cyber clone on each side.

Once the cyber clones were running, the group examined the interaction between cells. With no linkage and no sound, the real cell and its cyber clones wiggled back and forth as they normally do, out of sync and fairly noisy. With springs connecting them, the cells rapidly synchronized and became much less noisy. At the same time, the amplification of external vibrations – sound – increased dramatically. For faint sounds, the coupled cells were nearly twice as sensitive as any one alone.

Twice as sensitive is still not very good, at least compared with the performance of the whole ear, which can be nearly 100 times more sensitive than a single hair cell. But the hair cell only benefited from two neighbors. Increasing the size of the group to 9×9 cyber clones pushed the amplification up to a realistic gain of 52 dB – about 60 times that of an individual cell – close to the levels achieved in the mammalian cochlea. Thus, it seems that relatively small groups of hair cells, connected by a springy matrix, can reach the extraordinary performance seen in the whole ear.

10.1242/jeb.036616

Barral, J., Dierkes, K., Lindner, B., Jülicher, F. and Martin, P. (2010). Coupling a sensory hair-cell bundle to cyber clones enhances nonlinear amplification. *Proc. Natl. Acad. Sci. USA* **107**, 8079-8084.

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