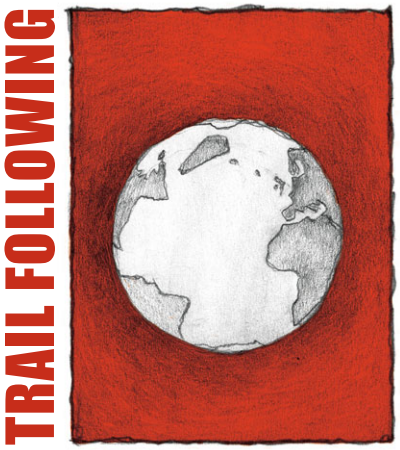
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB



SNAIL TRAILS

It doesn't take a particularly experienced naturalist to recognize that snails and slugs – gastropods – leave a trail of mucus wherever they go and that the gooey material is both secreted by the animals and intimately tied to their movement. What may be considerably less appreciated is the metabolic cost associated with producing such trails. Estimates of the energy needed for mucus production across a number of species suggest that approximately one-quarter of consumed energy is used in making these tracks. Given its high cost, investigators are interested in the potential role(s) of mucus secretion in marine and terrestrial gastropods beyond its role in locomotion. For example, might trails be used in food capture or navigation? Various gastropod species track trails laid down by other individuals, so Mark Davies and Janine Blackwell, from the University of Sunderland, recently performed a series of experiments to test the possibility that tracking snails may save energy by using another snail's mucus.

To test their hypothesis about energy savings in gastropod trail following, Davies and Blackwell used the widespread, intertidal common periwinkle, *Littorina littorea*, collected from Whitburn, UK. The investigators allowed individual 'marker' snails to lay down single mucus trails on an array of connected microscope slides. In some cases they used 'tracker' snails to create double trails consisting of a second layer of mucus by placing a second animal directly onto a marker snail's path, where it would secrete its own mucus trail onto the original marker's trail that it followed.

To quantify whether tracker snails secreted less mucus than marker snails, the biologists then flooded the glass slides with a fluorescent dye and used fluorescence microscopy to measure the thickness of single and double trails. All trails were convex in cross-section, and double trails were significantly thicker at their midpoint (on average 46.8 μm) than single trails (on average 35.4 μm). By estimating the area under curves that describe mucus thickness as a function of trail width, the investigators showed that double trails possess only 27% more mucus than single trails, suggesting that tracker snails secrete much less energetically costly material when they follow a previously laid trail.

Davies and Blackwell also studied the rate of mucus decay caused by environmental exposure, for example to waves and the weather, to find out if this would affect the trails laid down by tracker snails. To measure mucus decay, they took microscope slides with single and double trails to a midshore site and fixed them horizontally onto a frame attached to a flat rock, leaving them for one of four time periods: one tidal cycle, two tidal cycles, one week and 2.5 weeks. Again examining trails using fluorescence microscopy, they found that trail thickness decayed with increasing exposure time, such that by 2.5 weeks no signs of marker trails remained. Moreover, tracker snails placed onto marker trails secreted more mucus the longer the marker trail had been exposed, showing that the amount of marker mucus laid down affects how much mucus a tracker secretes.

Assuming mucus composition remains comparable between single and double trails, it is clear that tracker snails produce much less mucus than is found in the original paths laid by marker snails. Thus, in some ways like cross-country skiers, gastropods appear to gain energetic benefits when following in the track of another individual, and more recent trails confer greater benefits. I now have new-found respect for those snails I've observed in aquaria forging paths that others can follow.

10.1242/jeb.001073

Davies, M. and Blackwell, J. (2007). Energy saving through trail following in a marine snail. *Proc. R. Soc. Lond. B* **274**, 1233-1236.

Gary B. Gillis
Mount Holyoke College
ggillis@mtholyoke.edu

AGING SNAKES

Things wear out with use, and the more intensely we use them, the faster they wear out. This is certainly true for cars, shoes and other objects in our modern world, and might also be true for mammals. About 100 years ago, the German physiologist Max Rubner applied this principle to the aging process in mammals and postulated a negative relationship between an animal's energy turnover and its rate of aging. This 'rate-of-living' theory agreed with the 'free-radical' theory of aging, which suggested that oxidative stress was caused by the by-products of respiration, reactive oxygen species (ROS). Oxidative stress is higher in animals with higher energy turnover rates and thus could explain why mice live shorter lives than elephants.

Kylie Robert and her colleagues from Iowa State University and Santa Clara University, California, recently tested how aging and lifespan are related to physiological and behavioural differences in six different species of colubrid snakes: either long-lived, with a lifespan greater than 15 years, or short-lived, with a lifespan less than 10 years. Reptiles are very suitable models to test whether metabolism affects longevity, because they have a low rate of metabolism and age slowly but still exhibit a wide array of life spans ranging from <2 years up to records of 150 years.

First, the team tested the rate-of-living theory in the snakes, measuring their oxygen consumption to assess the animals' metabolic rates. They found no relationship between metabolic rate and lifespan, thus contradicting the theory, because the older animals did not have a lower metabolic rate. Since the relationship between metabolic rate and the aging rate is absent in many comparisons in endotherms, Robert and her collaborators were not too concerned that they missed finding one in an ectotherm.

The team suspected that lifespan could also be related to slithering performance, with faster slitherers being better able to escape predators and therefore live longer. To test this idea, the researchers measured the locomotory performance of long-lived and short-lived snakes by recording their locomotory speed, with the help of photocells, over 1 m on a linear racetrack. Consistent with their expectations, Robert and her team observed that longer-lived snakes had increased locomotory performance compared with shorter-lived species. They concluded that in snakes, behavioral traits like locomotory performance influence the avoidance of predation and may even affect the evolution of lifespan. Besides, they also observed that faster snakes also behaved more aggressively, therefore defending themselves better, which helps them reach a longer lifespan.

Finally they turned their attention to cellular physiology and quantified oxidative stress in the two groups of snakes to find out if the free-radical theory of aging applied in the snakes. They measured production of the ROS hydrogen peroxide in mitochondria, finding that short-lived snakes produced significantly more hydrogen peroxide than the longer-lived species. Together, the group's findings clearly confirm the free-radical theory of aging, with longer-lived species producing fewer ROS and suffering less oxidative stress. The authors explain that their study is the first to address ROS production using reptilian species as the model system and shows that colubrid snakes are promising subjects for aging studies.

10.1242/jeb.001081

Robert, K. A., Brunet-Rossinni, A. and Bronikowski, A. M. (2007). Testing the 'free radical theory of aging' hypothesis: physiological differences in long-lived and short-lived colubrid snakes. *Aging Cell* **6**, 395-404.

Teresa Valencak
Veterinary University Vienna
Teresa.Valencak@vu-wien.ac.at

NEURONS VIE FOR RECRUITMENT

When a memory is formed in the brain, connections are strengthened between neurons requiring transcription of genes that are dependent upon CREB, a transcription factor that binds to DNA and regulates gene expression. However, this gene expression need not occur in every neuron involved in the memory but only in a subset of the neurons. How is a neuron selected to participate in a memory trace? Jin-Hee Han and colleagues from the University of Toronto, Canada demonstrate in a recent *Science* article that neuronal selection during memory formation is not a random process but depends upon the neuron's relative CREB activity at the time of learning, such that a neuron with relatively high CREB activity would be selected over a neighboring neuron with low CREB activity. These data indicate that neuronal competition plays an important role not only in development but in the adult brain as well.

Mice deficient in CREB have difficulty learning to fear a tone that was previously associated with a foot shock. To find out how many neurons need CREB in order to form a memory, the team took CREB-deficient mice and micro-injected a viral vector expressing CREB into the lateral amygdala, a brain area essential for learning this task. This 'viral' CREB is capable of initiating gene transcription in infected neurons.

In order to visually track which neurons were infected with the viral vector, the team fused CREB to a green fluorescent

protein (CREB–GFP). They found that the mice could form a memory associating the tone with a foot shock even though only ~18% of neurons glowed, showing that they were infected by CREB–GFP. This clearly shows that CREB is only required in ~18% of neurons of the lateral amygdala to form this memory.

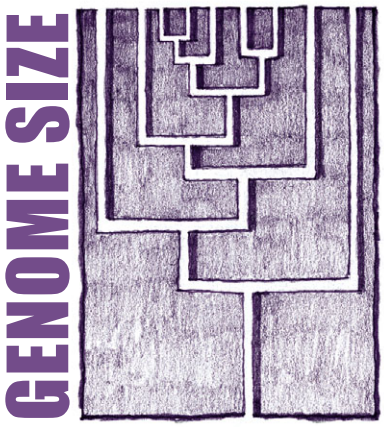
In order to show that those neurons infected with CREB–GFP were the same neurons that were recruited to the fear memory trace, the authors looked for neurons that were positive for RNA of the activity-dependent gene, *Arc*, which is transcribed when neurons fire. *Arc* RNA found in the nucleus acts as a molecular marker, showing that a neuron was active in the preceding 5–15 min. The authors demonstrated that neurons containing *Arc* RNA were also positive for CREB–GFP. Thus, the neurons that had increased CREB function were the ones that were activated and recruited to form the fear memory trace.

What happens if you decrease CREB function in ~18% of neurons in a mouse that has normal CREB levels to begin with? Nothing; there were no memory impairments in this scenario because the remaining neurons with intact CREB function were able to out-compete the CREB-deficient neurons for inclusion in the memory trace. The authors propose that, during learning, neurons are selected to participate in a memory trace as a function of their relative CREB activity at that time. These findings could offer an explanation of why emotionally arousing stimuli result in such strong memories for associated cues. Stress activates the noradrenergic system, and the stress hormone norepinephrine activates CREB, meaning that emotionally arousing stimuli may cause CREB activity to increase in more neurons and prime them for memory selection.

10.1242/jeb.001065

Han, J.-H., Kushner, S. A., Yiu, A. P., Cole, C. J., Matynia, A., Brown, R. A., Neve, R. L., Guzowski, J. F., Silva, A. J. and Josselyn, S. A. (2007). Neuronal competition and selection during memory formation. *Science* **316**, 457-460.

Susan Sangha
Westfaelische Wilhelms Universitaet Muenster
sangha@uni-muenster.de



SOARING WITH SMALLER GENOMES

One of the most fascinating things about birds is their ability to fly. When birds first arose from theropod dinosaurs, a group that includes the well-known and terrifying carnivore *Tyrannosaurus rex*, they were the first vertebrates showing powered flight. The origin of flight was a huge evolutionary transition and probably depended on several avian traits. One such trait is believed be the exceptionally small genome size of birds. Genome size has a significant influence on cell size because a smaller genome can be contained within a smaller nucleus, and a smaller nucleus leads to a smaller cell, which is less costly to maintain. Some researchers believe that the small genomes of birds created energy-saving conditions beneficial for flight. However, unlike more familiar avian characteristics such as feathers, wings, efficient lungs and high body temperatures, the evolutionary history of genome size in birds was unknown. To investigate this area further, Chris Organ from Harvard University and his colleagues decided to explore the genome sizes of the dinosaur ancestors of birds.

But how can genome size be determined in extinct dinosaurs, whose only earthly remains are fossils? Fossils usually preserve only the mineralized parts of an organism, and small cellular details are typically lost. However, cells in bone called osteocytes are an exception. In living animals, individual osteocytes reside in small mineralized pockets called lacunae, and lacunae size can be measured from fossils. Because Organ and colleagues knew that cell size is related to genome size, they worked out the correlation between osteocyte size and genome size across living vertebrates. By using this correlation they could calculate the genome size of both theropod and non-theropod dinosaurs by simply measuring the size of their lacunae.

Interestingly, the researchers found that theropod dinosaurs had genome sizes as small as birds. On the other hand, ornithischian dinosaurs such as *Triceratops*, which didn't give rise to birds, had much larger genomes, similar to those of living reptiles. From these observations, the authors concluded that an abrupt reduction in genome size occurred in early theropods, well before the origin of birds.

Organ and colleagues were also interested in which parts of the genome had been reduced. In addition to the small portion of our genome made up of protein-coding genes, there is a large portion made up of so-called interspersed repetitive elements, which do not code for proteins or regulate gene expression. The authors knew that genome size is related to the number of interspersed repetitive elements in the genome, since larger genomes have a higher proportion of these elements. By correlating genome size to the percentage of interspersed repetitive elements in the genomes of living vertebrates, Organ and colleagues inferred the percentage of these elements in theropod dinosaurs, finding that the genomes of theropods had fewer interspersed repetitive elements than those of ornithischians.

The decrease in genome size in the close ancestors of birds was probably caused by reductions in the non-protein coding regions of the genome. This change likely occurred before the origins of flight, along with the appearance of other pre-flight features such as an efficient respiratory system and high body temperature. So, despite millions of years of evolution, it appears that *T. rex* and turkeys aren't that different!

10.1242/jeb.001099

Organ, C. L., Shedlock, A. M., Meade, A., Pagel, M. and Edwards, S. V. (2007). Origin of avian genome size and structure in non-avian dinosaurs. *Nature* **446**, 180-184.

Graham R. Scott
University of British Columbia
scott@zoology.ubc.ca

LACTATE: GOOD, BAD OR BOTH?

During high-intensity exercise, hard-working muscles quickly fatigue as they anaerobically break down glycogen, forming lactate, which causes the familiar 'burning' feeling. This creates acidic conditions, which probably compromises calcium transport in the muscles, in turn reducing force development. Until recently, this scenario would have been the standard textbook answer, however recent compelling evidence shows that lactate might actually be beneficial, rather than detrimental, to force development in working heart and skeletal muscle. During strenuous exercise, working muscles lose potassium (K^+), raising the extracellular K^+ concentration, which makes muscle cells less excitable and decreases force production. As muscles lose K^+, lactate and circulating catecholamines – stress hormones such as adrenaline – also accumulate in the bloodstream. These compounds can counteract the negative effects of increased extracellular K^+ concentration on muscles. In a recent paper, Frank de Paoli and co-workers from the University of Aarhus, Denmark, set out to investigate the combined effect of catecholamines and lactate on isolated rat skeletal muscle exposed to high concentrations of extracellular K^+.

The team incubated rat soleus muscle in a temperature-controlled bath at 30°C, where they also controlled K^+, lactate, adrenaline and carbon dioxide levels. To determine the effect of extracellular K^+ concentration on muscle contraction, they stimulated the muscle and measured its force generation as they raised K^+ concentration in the bath from 4 to 15 mmol l^{-1}. They found that this reduced force generation by 85%.

By adding lactate to the bath, the team found that force production recovered slightly in a dose-dependent manner, with 20 mmol l^{-1} lactate having maximal positive effect. Adding physiological levels of adrenalin (10^{-5} mol l^{-1}) improved force production further under high K^+ conditions, and when added together with 20 mmol l^{-1} lactate, the additive effect of the two compounds led to an almost full recovery of force production.

De Paoli and co-workers found that the adrenaline-induced force recovery was caused through improved excitability as a consequence of an increased Na^+–K^+ pump activity in the muscle cell membrane. This pump actively moves K^+ back into the cell, and Na^+ out, increasing the chemical K^+ gradient, which helps to re-polarise the muscle membrane and makes contraction more likely. By contrast, lactate had no effect on Na^+–K^+ pump activity, instead helping force recovery by decreasing intracellular pH. In short, a decreased intracellular pH enhances force production through a decrease in chloride channel activity, which in turn affects the balance of all the ions on either side of the membrane, again leading to a re-polarised membrane potential. Adrenaline did not change intracellular pH; hence, the protective effects of lactate and adrenaline on muscle excitability and force generation occur through two distinct mechanisms that have an additive effect.

These results suggest that circulating catecholamines and development of acidic conditions during exhaustive exercise may improve muscles' tolerance to elevated K^+ levels. This implies that during high-intensity activity with high extracellular K^+ and adrenaline, lactate actually serves as a performance-enhancing chemical, rather than being the cause of muscle fatigue. These exciting results were, however, all obtained using isolated muscles in a dish at a relatively low temperature. Only future experiments will determine whether the mechanisms outlined in this paper contribute significantly in live animals with intact contracting muscles.

10.1242/jeb.001107

de Paoli, F. V., Overgaard, K., Pedersen, T. H. and Nielsen, O. B. (2007). Additive protective effects of the addition of lactic acid and adrenaline on excitability and force in isolated rat skeletal muscle depressed by elevated extracellular K^+. *J. Physiol.* **581**, 829-839.

Johnnie B. Andersen
University of Aarhus
Johnnie.andersen@ki.au.dk