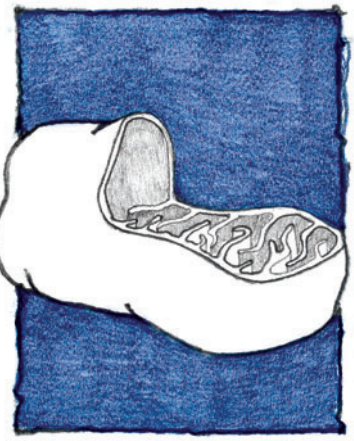
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

AEROBIC CAPACITY



RATS THAT CAN'T RUN CAN'T HIDE FROM RISK FACTORS

At a time when transgenic organisms are all the rage in biological research, it's nice to know that some hypotheses can still be tested with experimental animals created the old-fashioned way: through selective breeding. Since 1996, Steven Britton and Lauren Koch of the University of Michigan have used rats bred for high and low aerobic exercise capacity to address numerous questions about exercise performance, cardiac function and cardiovascular regulation. Their latest study of these rats – a collaboration with colleagues at the Norwegian University of Science and Technology, the Medical College of Ohio, and Williams College (Massachusetts) – probes the apparent link between aerobic capacity and cardiovascular and metabolic diseases.

Aerobic capacity, abbreviated $\dot{V}_{O2max}$, is an animal's maximum rate of oxygen consumption per unit time. It is often measured by having an animal run on a treadmill that gets progressively steeper until the animal becomes exhausted. At the cellular level, $\dot{V}_{O2max}$ reflects the ability of the mitochondria to use oxygen for ATP production. Individuals with a low $\dot{V}_{O2max}$ typically have a lower-than-normal density of mitochondria in their muscle cells, the chief oxygen consumers during intense exercise.

Beginning with a mixed population of rats, the researchers mated the best treadmill runners with each other and did likewise for the worst runners, testing the offspring's running ability and selecting the extreme individuals for the next round of mating. After 11 generations of this selective breeding, the population of high-capacity runners could run about 350% farther than the low-capacity runners during a progressive treadmill test. The team found that the low-capacity runners exhibited a variety of symptoms associated with an increased risk of cardiovascular and metabolic diseases. These included high blood pressure, impaired relaxation of blood vessels, insulin resistance, impaired cardiac pumping ability, and elevated triglyceride and free fatty acid levels in the blood. To add insult to injury, the low-capacity runners were also fatter than the high-capacity runners.

These data add new weight to previous suggestions that a low $\dot{V}_{O2max}$ *per se* can lead to the development of symptoms like those listed above. If there is indeed a cause-and-effect relationship, it may well involve the mitochondria; several transcription factors and enzymes essential for mitochondrial function were 60–90% lower in the low-capacity runners' muscle than in the high-capacity runners' muscle. However, the possible connections between these proteins and cardiovascular variables such as blood pressure remain open to speculation at present.

To what extent can exercise training protect individuals from the risks associated with low $\dot{V}_{O2max}$? When the team trained low-capacity runners on treadmills for six weeks, they noticed that several measures of cardiac function improved along with $\dot{V}_{O2max}$. However, they did not quantify the effects of training on the other above-mentioned risk factors; presumably, the team will examine these in a subsequent report. Aerobically impaired rats and humans will undoubtedly await these results with interest.

10.1242/jeb.01648

Wisløff, U., Najjar, S. M., Ellingsen, Ø., Haram, P. M., Swoap, S., Al-Share, Q., Fernström, M., Rezaei, K., Lee, S. J., Koch, L. G. and Britton, S. L. (2005). Cardiovascular risk factors emerge after artificial selection for low aerobic capacity. *Science* **307**, 418-420.

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PYTHONS PAY THE PRICE FOR PROTEIN

Devouring an unsuspecting victim poses a serious challenge for pythons' guts. During the long gaps between meals, pythons cut their energy costs by keeping their metabolic rate low. But when they guzzle a large meal, their metabolic rate soars as they fire up their digestion. This post-feeding increase in metabolic rate is called specific dynamic action (SDA). All animals pay this 'tax' on food processing, but the degree of metabolic upregulation depends on the meal. In mammals, digesting fats and carbohydrates requires minimum upregulation, while the highest SDA levels accompany diets rich in protein. Thus, pythons eager to gorge on protein are perfect models to identify the causes of metabolic responses to feeding.

Marshall McCue, Albert Bennett and James Hicks from the Department of Ecology and Evolutionary Biology in Irvine, California were intrigued by the metabolic tax caused by different diets, and set out to quantify the effect of meal composition on SDA in pythons. They fed 18 Burmese pythons (*Python molurus*) pureed meals with identical energy content but varying amounts of carbohydrates, lipids and proteins. To compare the metabolic costs of digesting prepared meal mixtures with those of processing an entire carcass, they also offered the snakes whole mice. To monitor how pythons' metabolic rate adjusted to cope with different meals, the team served these dishes to food-deprived snakes while continuously measuring the reptiles' oxygen consumption. A large increase in metabolic rate (a large SDA response) would reveal which part of the pythons' meal carries the highest metabolic cost.

McCue and his colleagues found that python SDA is highly dependent on the meal's chemical composition. Because pythons are carnivorous, the Californian group was not surprised to find that fat and carbohydrate meals passed through the snakes' intestines without considerable SDA response; they weren't being digested, except for glucose and sucrose, which induced relatively high SDA. Instead, the group discovered that *de novo* protein synthesis, the new formation of proteins from prey amino acids, causes the greatest increase in pythons' metabolic rate. To demonstrate the influence of de novo protein synthesis on SDA, the group chemically prevented the process by administering a protein synthesis inhibitor to the amino acid mixture that they fed the snakes. They found that SDA dropped over 70%. The importance of protein synthesis in metabolic upregulation was also supported by the group's discovery that digesting complete protein mixtures, which consisted of all the amino acids found in whole mice, induced higher metabolic rates than snake menus with an 'incomplete' mix, deficient in certain amino acids. In addition, they found that digesting simple proteins such as gelatin and collagen caused lower levels of metabolic upregulation than the complex proteins in whole-mice dishes. The team concluded that, due to protein synthesis after eating prey amino acids, pythons pay the biggest metabolic price for digesting protein, not for digesting fat or carbohydrates.

But pythons don't normally eat pureed meals, so the team wanted to find out if a python's habit of cramming a whole animal into its gut carries a cost. McCue and his co-workers were astonished to find that pureed python meals induced 36% greater SDA than wolfing down an intact mouse. They concluded that mechanical digestion of prey doesn't bear metabolic costs. Since snakes fuel SDA with the energy stored within their prey, pythons apparently mobilize nutrients more effectively from a prey soup than from a complete carcass.

10.1242/jeb.01653

McCue, M. D., Bennett, A. F. and Hicks, J. W. (2005). The effect of meal composition on specific dynamic action in Burmese pythons (*Python molurus*). *Physiol. Biochem. Zool.* **78**, 182-192.

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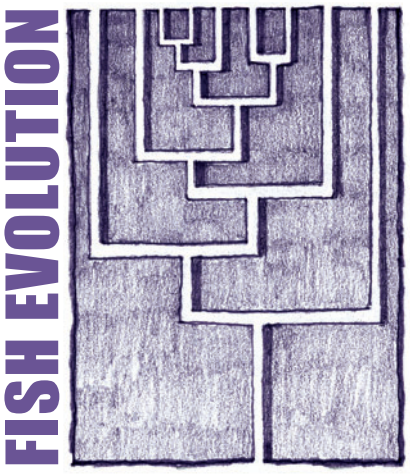

LOVELY *RETIA*

Unless you're in the Dead Sea, where the salty water makes floating easy, you'll have to put some effort into staying afloat. Likewise, staying at a constant depth below the sea's surface takes a lot of effort. For fish, putting in effort to maintain their depth in the sea would make finding prey or avoiding predators even more difficult than it already is. To get around this problem many fish have resorted to using a wide variety of strategies to achieve neutral buoyancy. This has clear advantages; since neutrally buoyant fish don't have to work to maintain a particular depth, their muscles are free to perform other behaviours.

Of the many innovations that fish use to achieve neutral buoyancy, the gas-inflated swimbladder is perhaps the most striking. However, the swimbladder doesn't work alone; it requires several other physiological and anatomical innovations, including a network of capillaries called the *rete mirabile* that's responsible for increasing the amount of dissolved oxygen around the swimbladder, which diffuses into and inflates the swimbladder.

How did these innovations evolve? That's the question Michael Berenbrink and colleagues at the University of Liverpool set out to answer in a recent *Science* article. They started by focussing on the evolution of another *rete* that's responsible for getting oxygen to the fish's eye. Mapping the presence and absence of the eye *rete* onto a fish phylogeny, the team showed that it evolved once, about 250 million years ago. When they mapped the presence and absence of the swimbladder *rete* onto the same phylogeny, they found it evolved several times, but only after the fish already had the eye *rete*.

But how do the *retia* deliver their oxygen loads to the eye and swimbladder against an oxygen gradient? It turns out that fish have a special form of haemoglobin that shows a Root effect; that is, in acidic conditions its oxygen affinity changes and it offloads oxygen, even at high oxygen tensions. Since this special form of haemoglobin would appear to be necessary for the *retia* to function properly, the team reasoned that it must have evolved before the first *rete* – the eye *rete* – appeared. To find out, Berenbrink and his colleagues measured the Root effect in 49 fish species and mapped this onto their phylogeny. Sure enough, they found that this Root-effect haemoglobin evolved only once, before the evolution of the eye *rete*. They point out that those fish that have lost the eye and swimbladder *retia* also show reductions in their Root effect, suggesting that the need for oxygen delivery maintains the Root effect in fish.

Having Root-effect haemoglobins in their bloodstream presents an interesting problem for fish; exercise and hypoxia lead to increased blood acidity levels, which may cause haemoglobins with strong Root effects to offload their oxygen in inappropriate places. Berenbrink and his colleagues discovered that another innovation apparently evolved to protect fish from this eventuality: a sodium–proton exchanger that regulates acidity in red blood cells. They show that this exchanger's activity increased after the eye *rete* evolved and its activity is reduced when the eye *rete* is secondarily lost. This suggests that the exchanger probably evolved to solve fishes' acidity-regulation problem.

This analysis by Berenbrink and his team provides insights into the evolution of a unique suite of anatomical and physiological innovations that may have contributed significantly to the buoyant success of the ~22,000 bony fish species alive today.

10.1242/jeb.01651

Berenbrink, M., Koldkjær, P., Kepp, O. and Cossins, A. R. (2005). Evolution of oxygen secretion in fishes and the emergence of a complex physiological system. *Science* **307**, 1752-1757.

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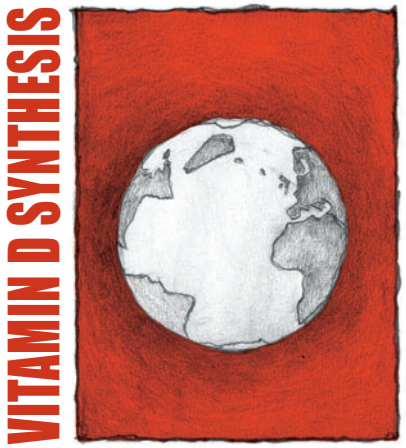

JAMAICAN LIZARDS' BALANCING ACT

In vertebrates, vitamin D plays an important role in maintaining the body's calcium levels through the calcium–phosphorous hormonal regulation system. Animals with low vitamin D levels suffer from calcium deficiency, which leads to numerous maladies. Thus, animals need to maintain their vitamin D stores to stay healthy, and do so either by absorbing vitamin D from their food or by producing it themselves. However, producing your own supply of vitamin D requires ultraviolet-B (UV-B) radiation, and since animals clearly differ in the time they spend sunbathing, the relative importance of these two vitamin D sources varies considerably among animals. Generally, creatures that eat food with low vitamin D levels appear to rely mostly on UV-B-generated vitamin D, while nocturnal and other animals that rarely see sunlight appear to be unable to generate their own and presumably depend on rich dietary vitamin D sources instead.

Ferguson and colleagues at Texas Christian University wondered if this general pattern of attaining vitamin D applies to closely related species that live in the same place, but experience different UV-B and dietary vitamin D availabilities. To find out, they compared the natural UV-B exposure, the skin's ability to photobiosynthesize (generate its own) vitamin D, and dietary vitamin D levels of two Jamaican lizards: *Anolis lineotopus merope*, a shade-dweller, and *Anolis sagrei*, which prefers to bask in the sun.

To quantify natural UV-B exposure, Ferguson and colleagues watched adult lizards of the two species for nine continuous hours, recording each lizard's location and sun-exposure (whether they were sitting in full-sun, filtered-sun or shade) every five minutes. To assess the amount of UV-B irradiation the lizards experienced in these locations, on a subsequent day they re-enacted each lizard's movements on the observation day using artificial 'lizard models'. The models were ampules that contained provitamin D, which is converted to vitamin D in proportion to UV-B exposure. To measure how effective the lizards were at generating their own vitamin D, the team assessed skin sensitivity for vitamin D biosynthesis by exposing skin samples from each species to 0, 20, 40 or 60 minutes of UV-B irradiation and measuring vitamin D production. Finally, they assessed the lizards' natural levels of dietary vitamin D by determining vitamin D levels in the stomach contents of wild-caught lizards.

The team found that the shade-dweller *A. lineotopus merope* experienced less UV-B irradiation than the sun-loving *A. sagrei* but, unexpectedly, they didn't compensate for this by eating vitamin D-rich food; in fact, the sun-lovers had more vitamin D in their food. So how do the shade-dwellers cope? Ferguson et al. provide an explanation: the shade-dweller's skin is much more sensitive to UV-B-induced vitamin D biosynthesis than that of *A. sagrei*; they are simply better at producing vitamin D, despite the lack of sun. Ferguson et al. argue that the reduced skin sensitivity of *A. sagrei* to vitamin D biosynthesis may reflect a lesser need for self-generated vitamin D, since there is enough of it in their diet, and a greater need for UV-B sunscreen to protect their skin from the damaging influence of UV-B. Thus, it appears that these lizards can adjust their skin's UV-B sensitivity, so they can strike a balance between avoiding UV-B damage and generating their own vitamin D.

10.1242/jeb.01652

Ferguson, G. W., Gehrmann, W. H., Karsten, K. B., Landwer, A. J., Carman, E. N., Chen, T. C. and Holick, M. F. (2005). Ultraviolet exposure and vitamin D synthesis in a sun-dwelling and a shade-dwelling species of *Anolis*: are there adaptations of lower ultraviolet B and dietary vitamin D_3 in availability in the shade? *Physiol. Biochem. Zool.* **78**, 193-200.

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STRETCHY TO THE CORE

One of the main differences between eukaryotes and bacteria is that eukaryotes sequester their genetic material into organelles, with most of the DNA enclosed by a structure known as the nuclear envelope. The nuclear envelope is a double membrane studded with pore complexes that regulate the movement of molecules into and out of the nucleus. In higher eukaryotes, its inner membrane is lined with a two-dimensional fibrous network called the nuclear lamina. Little is known about the structure or function of the lamina, but it is known that the proteins that make it up belong to the diverse family of cytoskeletal proteins known as intermediate filaments. Intermediate filament proteins assemble into 10 nm-diameter filaments that are believed to impart cells with mechanical integrity. It is likely that the nuclear lamins do the same for the nucleus. Recently, Rowat and colleagues tested the hypothesis that the nuclear lamina behaves as an elastic solid capable of mechanically reinforcing the nucleus.

Rowat's team tested specifically whether the nuclear envelope deforms like an elastic solid or flows like a fluid when the nucleus is sucked into a micropipette. Labelling the lamin network with green fluorescent lamin proteins allowed them to analyse whether the distance between lamin molecules changes as the nucleus is drawn into the pipette. The researchers reasoned that, if the lamina is an elastic solid, the fluorescence intensity should spread out where the lamina is stretched and increase where it is compressed. Alternatively, if molecules in the lamina can diffuse freely, as in a fluid, deformation should have no effect on the average fluorescence intensity.

The team found that when the nuclear envelope in both living mammalian cells and isolated nuclei is pulled into a micropipette, the fluorescence intensity is greater than average at the mouth of the pipette and decreases exponentially as you move from the mouth inward. This suggests that the lamina is compressed at the mouth and stretched inside the pipette, which supports the idea that the nuclear envelope behaves like an elastic solid when it is supported by a lamina. Furthermore, they found evidence of buckling folds that radiated out from the mouth of the pipette in the direction of tension, which would not occur if the lamina is a two-dimensional fluid. But what happens when the deforming force is removed – does the nucleus spring back to its original shape? For quick deformations, Rowat's team found that the answer is yes, but for longer deformations (more than ten seconds), relaxation is slower. In other words, like most biological materials, the nuclear lamina is best described as a viscoelastic solid.

This work could have profound implications for understanding a mysterious group of genetic diseases known as laminopathies, which are caused by mutations in the lamin-encoding gene. These diseases are typically characterized by defects in muscle, adipose and nervous tissues, but one unique variant causes a rare syndrome in which patients appear to age prematurely and die before their teens. The cell nuclei of patients with laminopathies often exhibit irregular shapes and there is evidence that they are mechanically fragile. Rowat's team is next going to measure how nuclei isolated from these patients stand up to mechanical stress, which is an important first step to understanding the mechanisms underlying laminopathies. Furthermore, by exploring what goes wrong when nuclear lamina function is disrupted, these studies will illuminate the selective pressures that were responsible for the appearance of the nuclear lamina in higher eukaryotes.

10.1242/jeb.01650

Rowat, A. C., Foster, L. J., Nielsen, M. M., Weiss, M. and Ipsen, J. H. (2005). Characterization of the elastic properties of the nuclear envelope. *J. Roy. Soc. Interface* **2**, 63-69.

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THAT ALL-IMPORTANT TAN…

The arthropod exoskeleton is both a blessing and a curse. It provides mechanical protection, a permeability barrier and a lightweight skeleton; but it must be moulted regularly as it becomes constricting when the animal grows. The multi-stage moulting process involves the growth of a new flexible cuticle underneath the old one, the detachment from and shedding of the old one (ecdysis), then the expansion of the new one before it is finally tanned – that is, made rigid by chemical crosslinking. Classic neck-ligation experiments (tying the neck with ligature silk to prevent soluble brain factors from reaching the rest of the body) showed that brain-derived factors are responsible for moulting. Since then, it has become clear that moulting is under multiple, sequential neurohormonal control. In March this year, two research groups simultaneously revealed an important missing link in this pathway – the last step in the process, the tanning hormone bursicon.

Bursicon is released from the insect's head after ecdysis; if an insect's neck is ligated soon enough after moulting, its cuticle does not tan. Painstaking work has shown that bursicon acts through the second messenger cyclic AMP. Second messengers signal the arrival of hormones (like bursicon) at the cell surface to target molecules in the cell. Bursicon's effects can be mimicked in neck-ligated insects by injecting their abdomen with either cyclic AMP analogues or a central nervous system-derived peptide fraction of around 30 kDa in size, too large to be a neuropeptide. That is, bursicon is a protein hormone, explaining why it has proved hard to identify.

To determine bursicon's peptide sequence, one of the groups laboriously purified

bursicon from the cockroach *Periplaneta*. They located partial sequences in bursicon that matched a known class of mammalian hormone, the cystine knot hormones. These hormones are thrown into characteristic 3-D shapes by internal disulphide bridges between cysteine residues, and typically form dimers (complexes made up of two proteins). The sequence of the cockroach hormone also matched closely a single gene in the *Drosophila* genome, *CG13419*, marking it as a likely candidate gene that codes for bursicon. A strong candidate receptor for the hormone was also known; another *Drosophila* gene, *rickets*, showed a very similar mutant phenotype to the effects of neck ligation, and *rickets* mutants could be rescued by injection of cyclic AMP. However, the big problem was that the peptide encoded by *CG13419* itself had no effect on the receptor encoded by *rickets*. Something was missing!

The insight that came to both groups was that bursicon might be a heterodimer (a complex of two different proteins) rather than a homodimer (a complex of two of the same protein). That is, the *CG13419* gene product needed to associate with a different cystine knot protein before it became active. The two groups came to the same answer by different routes; one noticed that the honeybee homologue of *CG13419* contained a second cystine knot sequence, which matched a further *Drosophila* gene; the other looked for *Drosophila* genes similar to *CG13419*. Both identified *CG15284* as a candidate to form the heterodimer with the protein encoded by *CG13419*.

Now, everything fits together. Both groups showed that the two proteins, when co-expressed *in vitro*, form a 35 kDa protein, close to the original mass predicted from the cockroach bursicon. This heterodimer also potently activated the *rickets* receptor *in vitro* to generate cyclic AMP; they had found the missing link. In addition, synthetic bursicon displayed strong tanning activity when the groups injected it into neck-ligated insects. Finally, *CG13419*, *CG15284* and *rickets* were all expressed at times consistent with their playing a role in ecdysis. The close similarity between the proteins encoded by these three *Drosophila* genes and those in other insects suggests that this pathway for cuticle hardening is tightly conserved, at least across insects.

10.1242/jeb.01649

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