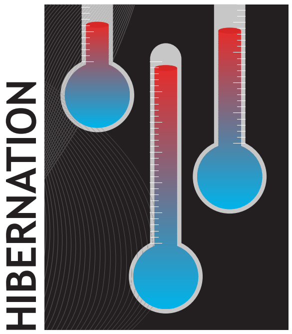
OUTSIDE JEB

Hibernating ground squirrels keep their mitochondria supercomplex



One of my favorite things about science fiction is when the genre takes inspiration from the natural world. For example, tales involving deep-space travel often begin with the protagonist awakening from a hibernation-like state – allowing them to get around the problem of aging as they traverse the stars. Here on Earth, some mammals use hibernation to decrease their metabolism to near-undetectable levels, which helps them survive low temperatures and lack of food during winter seasons. Achieving this kind of metabolic stasis in addition to prolonging survival after trauma are just two of the many important applications that studying hibernation could provide.

One key to understanding how hibernators – such as the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) – lower their metabolism is to study how they adjust their mitochondria, as these organelles provide most of the energy (in the form of ATP) required for life and cellular activity. Until recently, it was believed that mitochondrial enzymes produce ATP by passing electrons around as they randomly collide into each other. But Amalie Hutchinson and colleagues from the University of Western Ontario in Canada wanted to understand whether these enzymes were consistently bound together – known as supercomplexing – and whether these associations change between seasons and as these animals lower their metabolism when they hibernate.

The team captured thirteen-lined ground squirrels in Carman, MB, Canada, and brought them back to their lab in London, ON, Canada, where they were housed under conditions simulating summer temperatures (∼21°C) and natural day length in Carman. As winter approached, the scientists lowered the environmental temperature to 4°C and shortened the day length to 1 h light and 23 h dark to simulate winter hibernation conditions. They also collected mitochondria from the heart, liver and brown adipose tissue – which uses mitochondria to generate warmth – at three time points: summer, when the animals were active; when the animals had briefly arisen and were active during the winter hibernation; and during winter hibernation. The researchers then measured the amounts of supercomplexes and their protein make-up using techniques that separate mitochondrial enzymes but leave supercomplexes intact.

The scientists found that rather than changing the total amount of supercomplexes between seasons or winter conditions these squirrels seem to shuttle one specific mitochondrial enzyme (complex III, which is a massive protein made up of 11 subunits) between larger and smaller supercomplexes. As these squirrels transitioned from being active in the winter to resuming hibernation, the amount of complex III in larger supercomplexes decreased in brown adipose tissue – which may be important for rapidly lowering their metabolism. In contrast, the amount of complex III in larger supercomplexes was greatest in the liver in winter, while complex III was more associated with smaller supercomplexes in the heart in the summer. These changes in heart and liver mitochondria could represent an important part of the seasonal changes in the metabolism of these animals.

Hutchinson and colleagues have shown that something as remarkable as completely shutting down your metabolism could involve very subtle changes in mitochondrial supercomplexes. That said, it's probably going to be a while before we can use the lessons learned from these squirrels to safely send people into deep space. In the meantime, much remains to be learned about how these hibernators signal these shifts in enzyme interactions and their ultimate effects on mitochondrial function.

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Hutchinson, A. J., Duffy, B. M. and Staples, J. F. (2022). Hibernation is super complex: distribution, dynamics, and stability of electron transport system supercomplexes in *Ictidomys tridecemlineatus*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **323**, R28-R42. doi:10.1152/ajpregu.00008.2022

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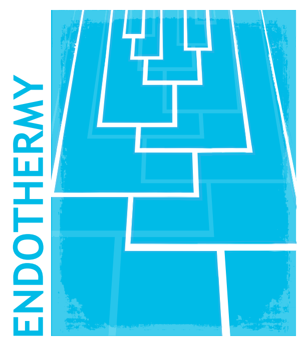
Dinosaurs weren't so cool



Absurdly, birds are dinosaurs. Palaeontologists insist they're close relatives, but to nearly everyone else these animals are polar opposites. Birds are graceful, musical, fluffy and so athletic that many travel between continents without resting. In contrast, dinosaurs hardly seem graceful. They're at once fierce, scaly monsters and fodder for goofy cartoons because of features like *Brachiosaurus*'s oversized neck or *Tyrannosaurus*'s clumsy arms. But despite their apparent differences, somehow, birds are theropods – the dinosaur group that includes *T. rex*. This bird–dinosaur connection is increasingly clear in the fossil record, which includes fossils of feathered and winged dinosaurs, suggesting many weren't as reptilian as movies depict. And now, in the biggest departure yet from Jurassic Park, new evidence shows plenty of dinosaurs weren't even cold blooded (ectothermic). Like mammals, birds are warm blooded (endothermic). Birds have the highest metabolisms of all living animals and much of this energy is spent on keeping their bodies warm. But when did their warm-blooded metabolisms evolve? Was it recent, or did ancestral dinosaurs heat themselves, too?

To investigate, a team of scientists from across the USA and Spain, led by Jasmina Wiemann and Derek Briggs (Yale University), found a way to check an animal's metabolism by scanning their bones, even after they are fossilized, as some biological molecules remain. But it's unrealistic to extract those molecules, because you can't grind up precious artefacts to test what's inside. Instead, the researchers used harmless light to scan the fossils without destroying or even touching them. When illuminated with infrared light, each biological chemical responds with its own unique light scattering signature. Using this signature, the researchers could tell which biomolecules were preserved in the fossilized eggshells, teeth or bones, and some turned out to be metabolic waste products. With these fossil scans, the researchers revealed details about how much energy the dinosaurs burned, even though they had been extinct for millions of years.

The scientists applied this technique to dinosaur fossils to detect any remaining biological molecules – as well as the bones of modern-day reptiles, mammals and birds, whose metabolic rates are already known – to estimate the dinosaurs' metabolic rates. Surprisingly, the metabolisms of the dinosaurs were more varied than expected. Some had slow metabolisms, including plant-eaters such as the spikey *Stegosaurus* or the three-horned *Triceratops*, more in line with the metabolisms of modern cold-blooded reptiles. However, others had faster, more bird-like metabolisms, such as the flying pterosaurs or the turkey-sized predator *Velociraptor*. In fact, many dinosaurs, including *T. rex*, were warm blooded.

The researchers then made a family tree to track when and how warm bloodedness evolved, and it seems that the dinosaurs and closely related reptiles that lived at the time evolved warm-blooded metabolisms more than once. In addition, investing in heating paid off: every time they evolved warm bloodedness, they quickly evolved a new way of moving. For example, plesiosaurs – marine behemoths that grew almost as long as a ten-pin bowling lane – probably began to swim as they became more warm blooded. And metabolism appears to have increased in pterosaurs right before they started to fly. In addition, dinosaurs such as *Velociraptor* and *T. rex* increased their metabolic rates before they learned to walk on two legs. Remarkably, birds evolved from these bipedal dinosaurs when their arms were free to evolve into wings, probably benefiting from their ancestors' high metabolisms to fuel flight.

Despite this work, I still can't imagine dinosaurs with bird-like grace, but this is why this study is so fascinating: it bridges the bird–dinosaur divide. Our simplistic notions of dinosaurs are dissolving, causing us to rethink how we typecast dinosaurs as bland reptilians. In reality, dinosaurs were more diverse and interesting than anything in Jurassic Park.

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Birds get a leg-up from their beaks

Since our early ancestor took those first wet steps out of the water and onto land, one rule has united all their descendants: we are forbidden to have an odd number of limbs. We can lose limbs along the way, but our embryological development constrains us to have paired legs. Rules are of course there to be broken and some birds have indeed found a way to bend them.

If you watch a climbing bird, say a parrot, you'll notice that they will occasionally grasp the inclined surface with their beak as they make their way up. Melody Young, a graduate student at the New York Institute of Technology (NYIT), USA, was also curious about this. Given that birds have swapped their front legs for wings, getting a leg-up from their beaks could be a big help.

Young, together with their NYIT colleagues Edwin Dickinson, Nicholas Flaim and Michael Granatosky, set out to investigate whether parrots truly power their stride using their beaks as a third leg. They tested the climbing prowess of six rosy-faced lovebirds (*Agapornis rosiecollis*), by observing their head, tail and body movements and by measuring the forces they exerted whilst ascending a climbing frame. The incline of the surface was gradually raised, to see whether the lovebirds' technique changed with the steepness of the climb.

Initially, the lovebirds were happy to walk along on two legs, without any beak assistance. But, as soon as the climbing frame was raised up to a 45 deg incline, the birds started using their beaks and their tails to assist their climb. The frequency of beak and tail use increased each time that Young made the surface steeper. Once the climbing frame was vertical, the birds used their beak and tail in rhythm with every step. This confirmed the received wisdom that birds climb with help from their beaks and tails, but are they really propelling themselves upwards by using one of these structures as a third limb?

Young and colleagues had the answer. They looked at the force data, to see whether, aside from the feet, any other body part was actively pulling the parrots upwards. During vertical climbing, they found that the beak produced just as much propulsive force as the birds' legs, showing us that the lovebirds were powering their ascent with their beaks as well as their feet in equal measure. They also used their beaks to keep them attached to the vertical surface, gripping on whilst their feet pushed-off with each step.

However, the lovebirds used their tails differently, producing low forces, much lower than those produced by the feet or the tail. This showed Young and colleagues that lovebirds use their tail more like a balancing aid. Imagine using a crutch or a walking stick as you climb a hill, not necessarily using it to push or pull your body forward, but more as a way of stopping yourself toppling over.

Parrots have bent the rules of our ancestors and use their beak as a third limb, and this is no mean feat; they have super-sized their neck muscles to haul themselves upwards. In fact, these muscles match or even beat comparable forces generated by the arms of human rock-climbers. Considering that parrots also defend themselves and eat with their beaks, this piece of anatomy is truly multi-functional, an evolutionary Swiss army knife.

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Crowded three-spined stickleback embryos are at a disadvantage

The idea that early-life conditions can have lasting effects on an individual's fitness is widely accepted. One factor that influences the condition of early-life stages in animals is the number of youngsters competing for the same resources, such as food. Ulrika Candolin and colleagues from the University of Helsinki, Finland, had previously found that an increased number of embryos in three-spined stickleback (*Gasterosteus aculeatus*) nests decreases the hatching success of embryos. However, fathers can improve the embryos' chances of hatching successfully by fanning their nests more, to give youngsters more oxygen and get rid of waste. Yet, the effects of an overcrowded nest after hatching, with an increased number of youngsters in the limited space, were unknown. To figure this out, the same team determined whether the number of embryos in three-spined stickleback nests would impact their ability to survive and grow after hatching.

Candolin and colleagues caught three-spined stickleback adults in the Baltic Sea off the coast of Finland and then, when the males were ready to mate, the team coaxed the fish into building tunnel-shaped nests with algae and sand by showing them a fertile female, before allowing them to mate with one, two or four females to vary the number of eggs in each nest. Then, the team allowed the embryos to grow in the nests while their fathers cared for them until they hatched. Three days after hatching, the researchers recorded how many of the embryos hatched successfully and measured their length. The researchers also separated a group of 20 hatchlings from each nest into individual tanks to develop with the same amount of space. Four weeks later, the team measured their length and survival, to look at the impact of the number of embryos packed into a nest later in life.

They found that the stickleback embryos from larger clutches, with more eggs crammed inside a nest, hatched 6 days earlier and were smaller than those from the smaller clutches that had more space while developing. The team also found that the proportion of eggs that hatched successfully was greater in the nests that were crowded with more eggs. However, 4 weeks after hatching, the youngsters from the larger clutches of eggs had caught up and there was no difference in their body length from that of youngsters that developed in smaller numbers. Unfortunately, the hatchlings from the larger clutches of eggs also had lower survival rates than those that had developed in smaller clutches, even though all the hatchlings were given the same amount of space to develop in. The lower survival rate occurred regardless of how much the fathers fanned their eggs, which meant they did not entirely neutralize the damaging effects of being raised in a higher density nest. The team suggested that this may explain why fathers eat some of their own embryos; it could be a strategy for maintaining an optimal number of youngsters in their nests to give the ones that survive the best start in life.

It seems that stickleback fathers must maintain a delicate balance between the number of offspring in their nests and the youngsters' ability to survive. However, Candolin and colleagues point out the need for more research on the mechanisms that cause embryos to be smaller when they hatch and to hatch early, in addition to the impact that this may have later in life. Most importantly, they stressed that researchers should consider the conditions in which they raise embryos when investigating the impact of early-life experiences as they grow older.

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Friends warn friends about feasts and famines

Many years ago, Eleanor Roosevelt famously quipped, 'Learn from the mistakes of others. You can't live long enough to make them all yourself'. Little did Eleanor know that her words would not only apply to human behaviour but also to the physiology of a highly social songbird. The red crossbill (*Loxia curvirostra*) is a nomadic species whose flocks travel far and wide in search of foraging grounds abundant with conifer seeds. As seed production can be unpredictable from year to year, red crossbills are often challenged by periods of food deprivation that can have devastating impacts on their body condition. In her recent study, Jamie Cornelius from Ohio State University, USA, shows that red crossbills can learn from the mistakes of their food-deprived friends, inducing coping mechanisms that prepare them for challenging food conditions ahead.

Animals can gather information about the environment through their own experiences, but may also glean information by observing others. Thus, Cornelius questioned whether red crossbills that observe the deterioration of their underfed friends would make behavioural and physiological adjustments to prevent themselves from meeting that same fate. She tested this idea by housing pairs of birds in side-by-side cages. One bird in each pair was selected to be a well-fed onlooker, keeping an eye on its adjacent neighbour for 3 days, while the neighbour was placed on a strict diet and only permitted to eat during two short feeding windows per day. Following the 3 day experience, Cornelius then restricted the diet of the well-fed onlooker to determine whether observing their famished neighbour's predicament had changed how they responded to food scarcity.

Cornelius discovered that when the onlooker birds were placed on meagre rations, they lost less weight. They ate more during the brief windows when food was available, as though their neighbour had warned them to 'eat what you can, when you can'. In addition to this behavioural change, Cornelius also found physiological changes. Warned of a dwindling food supply, the onlooker birds kept their flight muscles in tip-top shape, which is important when food conditions are so grave that birds must fly long distances in search of better dining opportunities. The onlooker birds also had larger intestines, which Cornelius thinks improves how efficiently food is digested and ultimately helps to reduce their weight loss during periods of famine.

As with any good study, Cornelius's findings left her with many more questions. She learned that knowledge gained by observing others in their surroundings can alter bird behaviour and physiology, but how these changes are mediated remains a mystery. Luckily, she has a hunch. Her previous work demonstrated that red crossbills that are forewarned of poor environmental food conditions by their friends mount a large stress response when food is scarce. The stress response involves elevating levels of the stress hormone corticosterone, which is thought to enhance survival in birds during periods of environmental stress by increasing foraging behaviour. Thus, corticosterone may act as a messenger between the brain and the rest of the body, helping to mediate between behaviour and physiology.

For red crossbills, it seems that Eleanor Roosevelt's edict to 'learn from the mistakes of others' could be a matter of life and death. If birds don't learn from the misfortunes of others, they may not survive long enough to make the same mistakes themselves. Fortunately, the hardships endured by some in the flock could caution others to avoid meeting the same fate.

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