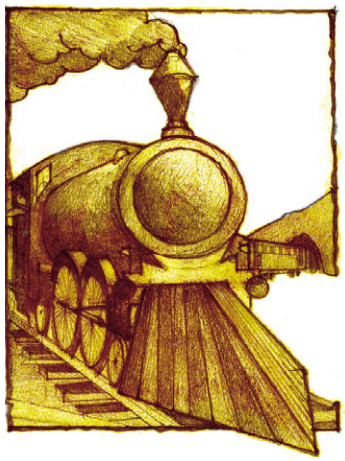
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

FLIGHT



INSECT WINGS FLIP FOR FREE

Flap, flip, back, flip… that's (roughly) how insects beat their wings – the 'flap' and 'back' are the down- and upstroke, and the 'flip' is when they flip their wings over at the end of each stroke. The flips set the wing at the correct angle to produce lift force during the strokes, but the rotational motion during the flip also generates substantial lift itself.

Insects' proficiency at wing flipping sets them apart from birds and helps to make them such agile flyers. Now, recent computational simulations from researchers at Cornell University suggest that insects may get the flip for free – fluid dynamic forces naturally flip the wing over at the end of a wingbeat, without the need for any muscular forces.

It's not a new idea that fluid dynamic forces may help the wing flip (the correct tongue-twisting terms are 'pronation' for the flip at the beginning of downstroke and 'supination' for the flip at the beginning of upstroke). But without today's powerful computers, no one had been able to test the idea.

So Attila Bergou, Sheng Xu and Jane Wang developed a computer simulation of a flapping insect wing. They measured the kinematics of both the fore- and hindwings of a tethered dragonfly. Then they used code they had developed previously to calculate the fluid motion around a two-dimensional slice through each wing, about halfway along their length. The team estimated the mechanical power required to flip the wing over, including fluid and inertial forces. Negative power values indicate that fluid forces tend to flip the wing over.

Sure enough, the researchers found negative power at the ends of the up- and downstrokes. As the wing starts to slow down, fluid forces tend to flip the wing over. But this observation didn't explain the mechanism. Fluid forces take many forms – forces due to acceleration, or to pressure, or to friction – but which effect was responsible for the flip?

To answer this question, they used a different mathematical model, a much simpler one, called a 'quasi-steady' model. After tuning several parameters in the simple model, they found that it matched up fairly well with the much fancier simulation. Not only that, but it conveniently allowed them to separate different types of fluid dynamic forces.

The dominant effect turned out to be acceleration. As the wing begins to slow down at the end of a wing beat, the air around it must also slow down. Since air has mass, it resists that deceleration, applying a force that pushes primarily on the center of the wing. Since the wings are attached to the insect's body near the front edge, not at the center, the fluid's 'acceleration reaction' causes a torque that tends to flips the wing over.

Does this fluid-assisted wing flip occur in reality? To find out, the researchers realized they could exploit the asymmetry of fluid and muscular forces. Fluid dynamic forces are strongest at the tip of the wing, while muscular forces are applied at the base. If fluid forces cause the flip, then the wing should start twisting at the tip. Inspecting the high-speed movies in detail, Bergou saw a distinct twisting wave, starting at the tip and propagating towards the base.

It seems that insects really can flip their wings for free.

10.1242/jeb.011320

Bergou, A. J., Xu, S. and Wang, Z. J. (2007). Passive wing pitch reversal in insect flight. *J. Fluid Mech.* **591**, 321-337.

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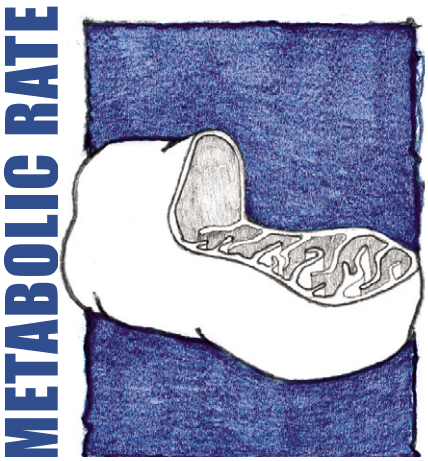

THE PACE OF LIFE

To explain why some of us are svelte and others accumulate more reserves, we often refer to our metabolism and its intensity. This internal pace of life when we are at rest is also called basal metabolic rate. However, why and how the intensity of our basal metabolism varies between individuals or even species is not easy to explain. A recent theory suggests that the chemical composition and physical properties of cellular membranes function as a 'metabolic pacemaker'. The activity of proteins floating in a phospholipid membrane will depend on the membrane's composition and will thus have a generalized effect on cellular activity and metabolism. So far, the theory seems to stand up as observations of many species and groups of animals have shown a relationship between cellular membrane phospholipid composition and basal metabolic rate. Paweł Brzęk, Katarzyna Bielawska, Aneta Książek and Marek Konarzewski from the University of Bialystok in Poland have investigated the mechanism that could explain variations in basal metabolism within a species and the role of membranes as metabolic pacemakers.

By selectively breading mice with higher or lower than average metabolic rate for more than 20 generations, the team has ended up with mice that have the same body mass but a 20% difference in metabolic rate at rest. Next, the team used their selected mouse lines to test the hypothesis that higher metabolic rate would be associated with an increase in the size of metabolically active organs (such as the heart, liver and kidneys) in combination with changes in the cell membrane phospholipid composition of these organs. Following the metabolic rate measurements, Brzęk's group weighed internal organs and analyzed the composition of membrane phospholipids of the liver and kidneys. It turned out that mice selected for higher metabolic rate had larger livers, kidneys and hearts, as predicted. The differences suggest a genetic correlation between the size of these organs and basal metabolic rate. In other words, evolutionary changes in basal metabolic rate are correlated with changes in size of these metabolically active organs. Still, the magnitude of the difference in metabolic rate could not be accounted for solely by variation in organ mass.

Next, the team investigated cellular membrane composition. The membrane pacemaker theory predicts that increased cellular metabolism is associated with a change in the membrane's composition (an increase in the occurrence of double bonds – unsaturation – in lipid chains). Of the two organs studied, both the liver and kidneys showed some differences in membrane composition between the high and low metabolic rate mice, but not according to the theory. Liver membranes from mice selected for a high metabolic rate had fewer double bonds in their lipid chains. The authors' observations clearly do not support the membrane pacemaker theory to explain small changes in basal metabolic rate within a species, at least not with the selection regime imposed. Still, the observed differences for many unsaturated lipids is puzzling and the team suspects that other aspects of cellular metabolism may also contribute to basal metabolic rate variation, such as altered mitochondrial enzyme activity.

Evolution of metabolic rate is hard to track but studies such as that of Brzęk and colleagues are helping to bridge the gap between the large variations found across species, and more subtle differences between individuals.

10.1242/jeb.011361

Brzęk, P., Bielawska, K., Książek, A. and Konarzewski, M. (2007). Anatomic and molecular correlates of divergent selection for basal metabolic rate in laboratory mice. *Physiol. Biochem. Zool.* **80**, 491-499.

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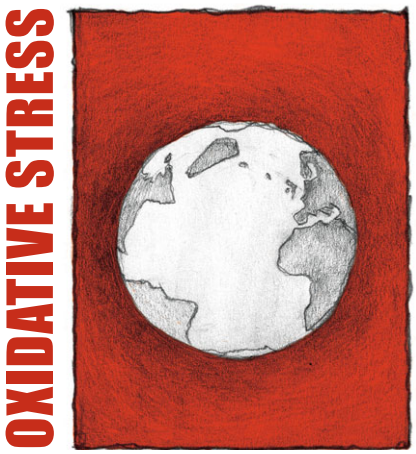

THAWING NO PROBLEM FOR SUPERCOOL TURTLES

In the world of stroke and heart disease research, it is understood that a great deal of the damage from ischemia (reduction in blood flow) is due not only to the lack of oxygen when blood flow is cut off but also results from the restoration of blood flow and oxygen to the tissue. When oxygen floods back into the system, the mitochondrial electron transport chain functions inefficiently, resulting in a burst of reactive oxygen species (ROS). These highly reactive compounds may continue damaging proteins, lipids and DNA for hours to days after the initial ischemic insult. But animals that hibernate, or go for extended periods without oxygen, experience and then recover from greatly reduced respiration, heart rates and blood flow, and thus provide a natural model of ischemia and recovery without apparent tissue damage. Hatchling painted turtles (*Chrysemys picta elegans*) that hibernate in shallow natal nest chambers during their first winter after hatching not only hibernate but may actually survive by supercooling or actual freezing. This led Patrick Baker and his colleagues at Miami University to wonder how well frozen or supercooled turtles handled what could be the extremely stressful period of thawing and restoration of blood flow, with its potential for ROS production.

Turtle eggs hatched in the laboratory were gradually cold acclimated through the autumn down to 4°C. Then the team exposed the hatchlings to 48-h bouts of supercooling (down to –6.0°C in an environment free of nucleating agents), freezing (down to –2.5°C, with ice to induce nucleation) or hypoxia, before allowing the youngsters to recover for 24 h and taking tissue samples to see how the turtles responded to the icy conditions.

Knowing that animals that experience hypoxia switch to anaerobic respiration and

generate lactate, the team measured the hatchlings plasma lactate levels and found that all of the hatchlings experienced hypoxia or anoxia (little or no oxygen).

The team also measured total antioxidant capacity in the hatchlings' plasma and brain and liver tissues; rather than measure levels of individual antioxidants, Baker and his colleagues opted to measure the overall ability of the tissues to prevent free radical accumulation when hydrogen peroxide was added to the reaction medium. They also looked for markers of oxidatively damaged proteins and lipids. The scientists found no significant increase in damaged lipids or proteins in any group compared with control animals kept at 4°C, nor were there differences in total antioxidant capacity of the tissues. This implies that the youngsters' ability to fight oxygen free radicals is not upregulated during hypoxia or cooling, but that intrinsic antioxidant levels are sufficient to fight ROS damage.

Baker and co-workers found that the antioxidant capacity of the hatchling painted turtles is comparable to that of several other animals, including a freeze-tolerant and an anoxia-tolerant frog, and the laboratory mouse (which as a warm-blooded mammal could be expected to have a high antioxidant capacity). Interestingly, the hatchlings' antioxidant capacity was higher than in the adult anoxia-tolerant turtle *Trachemys scripta*, which also can withstand anoxia and reoxygenation without damage, although this may be due in part to the suppression of ROS formation in addition to antioxidant levels. Additional experiments are planned to determine if the differences between hatchlings and adults are ontogenetic or taxonomic.

But at least we know this much: when it comes to resisting oxidative stress, these turtle babies are supercool!

10.1242/jeb.011353

Baker, P. J., Costanza, J. P. and Lee, R. E., Jr (2007). Oxidative stress and antioxidant capacity of a terrestrially hibernating hatchling turtle. *J. Comp. Physiol. B* **177**, 875-883.

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COMMUNICATION

THE SMELL OF DANCING BEES

In 1947, pioneer ethologist Karl von Frisch reported that foraging bees, on returning to the hive, perform a 'waggle dance' on the surface of the honeycomb. The dance, a figure-of-eight movement in which the worker waggles its abdomen in the middle, is used to transmit information about the location of a food source to other workers. In subsequent decades, the waggle dance has revealed some of its secrets, but a fundamental mystery remains: how do bees know that a dancing forager is on the comb? A beehive is a dark, crowded and noisy place, full of rustling bees, and the space between honeycombs is often extremely narrow – the insects cannot use visual cues to see if one of their sisters is dancing.

A group of researchers led by David Gilley from Tucson, Arizona, decided to see whether chemical communication could help explain the ability of the bee dance to recruit workers – this is what happens in bumblebees, which do not dance. Using a technique called solid phase micro-extraction, the authors put a highly absorbent fibre into the narrow space above the 'dance floor' and then placed the fibre into a gas chromatograph to identify the substances that had stuck to the fibre. They found that the air around dancing forager bees contained substantial quantities of four long-chain hydrocarbons (two alkanes and two alkenes) whereas the air around non-dancing foragers showed significantly lower levels.

To test whether these chemicals had a behavioural function, they dissolved synthetic versions of three of these compounds in hexane, allowed the mixture to evaporate and injected the gas into an experimental bee hive where workers were dancing naturally. They discovered that the number of bees leaving the hive increased when the synthetic compounds were injected, compared to a hexane control.

These data suggest that dancing bees emit a set of pheromones that encourage foragers to leave the hive and forage, following the directional and distance information encoded in the angle and duration of the dance. The authors suggest that these pheromones may act by attracting foragers to the 'dance floor', where they would then come into contact with the dancing bee.

However, we are still far from understanding the full story. How specific is the bees' response? The authors used only hexane as their control – it is possible that bees require a particular set of compounds or that they show similar responses to a wide range of stimuli. How does the message get transmitted? The substances involved are relatively heavy hydrocarbons (23°C and 25°C), which ooze out from underneath the cuticle. These waxy substances are widely used by insects for chemical communication, but they are not really 'odours' and they are generally considered not to be volatile.

Furthermore, cuticular hydrocarbons are very sticky and would end up smeared all over the honeycomb, taking a substantial amount of time to evaporate. How do bees recognise the difference between a new signal emitted by a dancing bee and one left a few minutes, hours or even days earlier? Perhaps the bustling activity of the hive means that every worker around the stimulus rapidly gets a tiny amount of these substances on them, and the intense signal emitted by the dancer is soon 'dissolved' among hundreds or thousands of insects.

By adding chemical communication to the rich mystery of the bee waggle dance, Gilley and his colleagues have shown that the buzz of the beehive includes its smell – or rather, its taste.

10.1242/jeb.011346

Thom, C., Gilley, D. C., Hooper, J. and Esch, H. E. (2007). The scent of the waggle dance. *PLoS Biol.* **5**(9), e228.

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SIZE MATTERS

One fundamental problem in biology is the question 'what mechanisms restrict the size of animal tissues and organs?' This question is not only relevant to development but also applies after maturation, when many tissues and organs, such as the liver, continuously grow and diminish in order to regenerate. Although much progress has been made in understanding the genetic programs that control cell growth, cell division and cell survival, little is known about the mechanisms that sense and limit tissue and organ size. However, new insight into the underlying program is provided by a recent *Cell* article; a team of American scientists led by Duojia Pan reveals that the Hippo signalling pathway universally controls size in metazoan organisms.

The Hippo signalling pathway has been worked out recently in *Drosophila* and is a protein phosphorylation cascade, initiated by the activation of the Hippo (Hpo) kinase. The pathway ultimately phosphorylates and inactivates a transcriptional co-activator called Yorkie (Yki). As Yki promotes cell growth, survival and proliferation, its inactivation results in suppression of growth. But precisely how the Hippo pathway regulates Yki activity wasn't clear.

To answer this question, Pan's team expressed components of the Hippo signalling cascade together with a tagged version of Yki in *Drosophila* cells so that they could track the protein's location after phosphorylation. They found that Yki is excluded from the nucleus when Hippo signalling induces Yki phosphorylation, preventing it from accessing its target genes in the nucleus and suppressing cell growth.

Knowing that 14-3-3 proteins, involved in protein re-localization, bind to motifs containing phosphorylated serine or threonine residues, the team scanned Yki's amino acid sequence for 14-3-3 binding motifs. They found a potential binding site including serine 168 (Ser168) as a candidate for phosphorylation. In a series of immunological experiments they demonstrated that this serine is the only residue that is phosphorylated by the Hippo cascade and that 14-3-3 only binds Yki after phosphorylation.

The role of Yki phosphorylation in growth suppression was finally confirmed by *in vivo* studies using transgenic flies that produce a mutant Yki where Ser168 is replaced by an alanine. The mutant flies showed excessive tissue growth, showing that the Ser168 phosphorylation of Yki is needed to suppress tissue growth.

Having established a growth-suppressive signalling pathway in *Drosophila*, Pan's team wanted to know whether the Hippo cascade also plays a role in higher animals. Finding a conserved serine in the mammalian Yki homologue YAP suggested that this might be the target of a mammalian Hippo pathway. The team went on to dissect its possible role in mice and showed that the Hippo pathway leads to growth suppression caused by phosphorylation-dependent exclusion of YAP from the nucleus. Directly testing the role of Hippo signalling in mammalian organ size control, the scientists over-expressed YAP in the liver of a transgenic mouse line, where they could control gene expression by administering an antibiotic, and found a marked overgrowth of the liver tissue after inducing YAP expression. Interestingly, this effect was reversible, suggesting that the Hippo pathway not only controls organ size during development but also maintains the balance between growth and diminution in mature organs. In line with its role in size control, they finally found that misregulation of the Hippo pathway in transgenic mice leads to tumorgenesis.

Elucidating the Hippo pathway in *Drosophila* and mice has brought exciting insights into a universal mechanism controlling organ growth and size homeostasis. However, while many questions remain, the most important one is discovering the identity of the signal molecules that activate the Hippo pathway.

10.1242/jeb.011338

Dong, J., Feldman, G., Huang, J., Wu, S., Zhang, N., Comerford, S. A., Gayyed, M. F., Anders, R. A., Maitra, A. and Pan, D. (2007). Elucidation of a universal size-control mechanism in *Drosophila* and mammals. *Cell* **130**, 1120-1133.

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