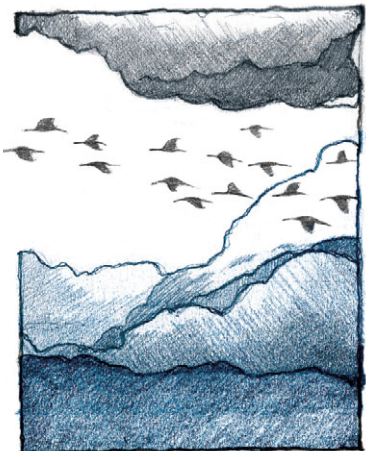
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

FORAGING



ALBATROSSES FOLLOW THEIR NOSES

The wandering albatross (*Diomedea exulans*) is an amazing seabird capable of flying for thousands of kilometres, usually gliding a few metres above sea level, on wings that span up to 340 cm. Although we have been fascinated by these huge birds for centuries, not much is known about how the animals locate their prey, especially squid, over the oceans. The study by Gabrielle Nevitt and coworkers published in the *Proceedings of the National Academy of Sciences* shows that individual wandering albatrosses can find food using their sense of smell; in fact, the birds can pick up a scent from up to 20 km away.

Nevitt and her team set out to investigate to what degree the wandering albatrosses rely on olfactory *versus* visual cues when foraging over thousands of square kilometres of open sea. The team tagged 19 wandering albatrosses during brief nesting periods on Possession Island in the southwestern Indian Ocean. The animals were equipped with a small global positioning system (GPS) sensor that recorded their exact position every 10 s. In addition, a small thermometer was fed to the animals to measure stomach temperature, indicating feeding bouts. In this way, the researchers could, very elegantly, record flight behaviour (flight path and prey capture) and compare the data with the wind direction.

The hypothesis was that an olfactory search would be facilitated by cross-wind flight to optimize the probability of encountering scent from a prey item. Thereafter, the animals would fly along an upwind zig-zag flight path to localize the prey. Birds predominantly using their sight to capture prey would be expected to fly more directly towards a prey item, independent of the wind direction.

The researchers analysed 55 track segments and observed five distinct foraging behaviours. The authors termed the first flight pattern 'direct', when there was no change in overall flight direction and the flight did not depend on wind direction, indicating that the bird was responding to visual cues. The second flight pattern was referred to as 'turn', where the birds stopped flying across the wind path and turned upwind, indicating that they had caught the scent of prey. In the third flight pattern, referred to as 'zig-zag', the animals zig-zagged upwind in the direction of prey, much like a dog following a trail, indicating that the birds used their sense of smell to follow the direction of a faint scent signal. The team also identified a flight pattern that they called 'circle', where the bird circles the prey and finally captures it. And in the final flight pattern, which the team called 'water', the animals adopted a sit-and-wait strategy, where they remained on the water's surface between prey captures.

When adding up both the weight and number of fish caught using the different strategies, Nevitt and coworkers concluded that the wandering albatross uses its sense of smell to locate prey on almost 50% of occasions.

Thanks to a well-designed experiment using high-precision GPS, the authors succeeded in unfolding the feeding behaviour of freely ranging wandering albatrosses – a truly outstanding achievement. As it turned out, the wondering albatrosses were much better at detecting scent than anticipated, and followed their noses 50% of the time.

10.1242/jeb.011593

Nevitt, G. A., Losekoot, M. and Weimerskirch, H. (2008). Evidence for olfactory search in wandering albatross, *Diomedea exulans*. *Proc. Natl. Acad. Sci. USA* **105**, 4576-4581.

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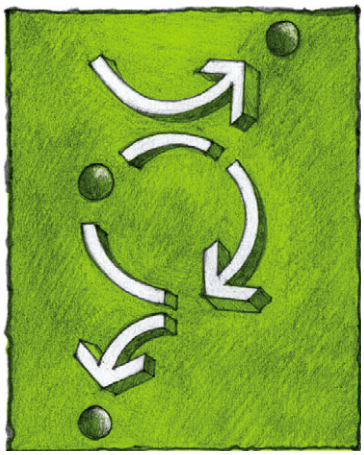
TRANSPORT



BATS ABSORB NUTRIENTS LIKE A BIRD

If you've ever had to pay excess baggage on an aircraft, you've probably thought about the costs of flight when carrying additional loads. Another flying mammal, the bat, faces the same problem and seems to get round this problem by having less intestinal tissue than comparably sized but non-flying mammals. With this refinement bats manage to reduce the mass of material carried by the gut to improve their take-off and manoeuvrability during flapping flight. However, small bats have very high energy demands when flying and thus need to extract energy efficiently from food at high rates, despite their smaller intestines. So how do flying mammals like bats efficiently absorb energy rich carbohydrates, given their smaller intestinal surface area and high energy demands? Birds meet their high energy demands by absorbing nutrients by passive diffusion across the gut lumen into the blood (paracellular uptake). However, transporter proteins actively transport solutes across the gut lumen, *via* the transcellular uptake mechanism, in mammals. So is nutrient uptake in the bat's digestive system more bird like or mammal like?

To answer this question, Enrique Caviedes-Vidal from Argentina and his international team of colleagues measured intestinal carbohydrate uptake in the great fruit-eating bat (*Artibeus literatus*). First they fed the bats, and then they injected the animals intraperitoneally with two metabolically inert, water soluble carbohydrates (L-rhamnose and cellobiose) and compared the carbohydrate concentrations in the blood plasma over 3 h after intake. As the molecular weight of cellobiose is twice that of L-rhamnose, L-rhamnose will pass into the bird's bloodstream faster than cellobiose if the uptake mechanism is paracellular, while the two carbohydrates will be absorbed at the same rate if uptake is mediated by transcellular uptake.

The researchers found that intestinal uptake of L-rhamnose was much higher than the absorption of the large disaccharide cellobiose. And when the team repeated experiments with another carbohydrate, 3-*O*-methyl-D-glucose, which is both actively and passively transported, they found out that even 3-*O*-methyl-D-glucose was predominantly absorbed by the paracellular pathway. The bats absorb nutrients *via* passive paracellular uptake. When comparing the bats' absorption rates with the data previously measured in other mammals, it turned out that passive absorption is significantly higher in great fruit-eating bats than in non-flying mammals and can amount to 70% of total glucose absorption. The observed uptake rates in the bats were more similar to those of birds, rather than mammals, and thus the authors conclude that paracellular absorption by passive diffusion may serve small bats as well as birds to compensate for their relatively small intestinal surface area.

The team also propose several mechanisms that might account for high passive carbohydrate absorption including increased microvilli surface areas and more cell junctions, a higher pore radius to facilitate the sieving effect and an increase in water flux to elevated the carriage of solute across the junctions.

Together, the study on intestinal absorption in the great fruit-eating bat provides evidence that small flying bats and birds compensate for their relatively small guts and reduce the surface area for absorption with paracellular carbohydrate absorption. However, the team point out that there are risks associated with paracellular absorption. High intestinal permeability might permit toxins to be absorbed in the intestinal lumen, leaving bats vulnerable to toxins that other mammals would not fall prey to.

10.1242/jeb.011585

Caviedes-Vidal, E., Karasov, W. H., Chediak, J. G., Fasulo, V., Cruz-Neto, A. P. and Otani, L. (2008). Paracellular absorption: a bat breaks the mammal paradigm. *PLoS One* **3**, e1425.

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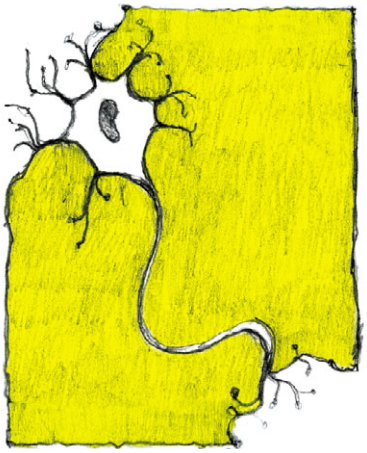
THERMOREGULATION



A COLD RESPONSE TO WARM BLOOD

Living organisms face a constant battle to maintain a stable internal environment, known as homeostasis. As part of the homeostatic system, warm-blooded animals, including humans, have developed a thermoregulatory response to counteract changes in temperature. Variations in the ambient temperature initiate various behavioural and physiological responses that are necessary to maintain an internal temperature within a narrow range for the proper functioning of many cellular and molecular mechanisms. The pre-optic area in the hypothalamus is the thermosensory command centre for thermoregulation. It elicits a barrage of physiological responses in the periphery before a significant impact on core temperature can occur. However, it is unknown how signals from skin thermoreceptors are relayed through the spinal cord to eventually terminate in the pre-optic area. Kazuhiro Nakamura and Shaun Morrison from the Neurological Sciences Institute at the Oregon Health and Science University have identified a novel pathway in rats that responds to cold and stimulates the pre-optic area to initiate a thermoregulatory response.

Neurons transmit signals in a linear fashion from one neuron to the next, requiring physical connections called synapses. The authors set out to identify the specific brain region where thermal signals from the spinal cord are transmitted before terminating on neurons in the pre-optic area. First, they injected a fluorescent tracer into the pre-optic area. This tracer migrated across synapses into regions directly connected with the pre-optic area. Then, when the temperature was changed from 24°C to 4°C, a subset of neurons in a region called the parabrachial nucleus were 'switched on' by the cold stimulus. The parabrachial nucleus receives a variety of signals involved in homeostatic responses but had not previously been shown to

communicate directly with the pre-optic area.

Next the team showed that cold-responsive neurons in the parabrachial nucleus play a functional role in modulating thermoregulatory responses in the pre-optic area. The authors measured physiological parameters that are known to increase in response to skin cooling. This included measurements of metabolic activity in heat-generating brown adipose tissue, expired carbon dioxide, heart rate and blood pressure. They then used pharmacological inhibitors of neurotransmission within the parabrachial nucleus to see whether switching off the pathway affected the animal's physiological response to cold. They found that in response to cooling there was no longer an increase in these measured parameters. This implies that neurons in the lateral parabrachial nucleus play a crucial role in mediating the thermoregulatory response to a cold challenge.

Finally, the authors investigated whether the pathway responsible for the conscious perception of temperature, the spinothalamocortical pathway, which is relayed through the thalamus to the cortex, is also involved in the unconscious thermoregulatory response. By preventing neuronal signalling in areas of the thalamus that receive an abundance of spinothalamic projections, and measuring the same parameters as above, the authors established that the temperature perception and the thermoregulatory signalling pathways are distinct.

While cold-blooded organisms must acquire heat from the sun, an internal thermoregulatory response is a fundamental characteristic of warm-blooded animals. However, there are days when sunbathing seems a pleasant alternative even for the most warm blooded.

10.1242/jeb.011627

Nakamura, K. and Morrison, S. F. (2008). A thermosensory pathway that controls body temperature. *Nat. Neurosci.* **11**, 62-71.

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EVOLUTION

HAEMOGLOBIN EVOLUTION IN MAMMALS

Haemoglobin is one of the most studied proteins in biology. It binds and transports oxygen in our blood, and releases it at our cells to fuel metabolism. Adult haemoglobin is composed of four subunits, two α-globin subunits and two β-globin subunits, and the interaction between these subunits dictates many oxygen binding characteristics of the protein. Unlike adults, however, embryos and fetuses cannot breathe for themselves, and so have very different oxygen transport requirements. To accommodate this, the haemoglobin of placental mammals has a high affinity for oxygen before birth, which is helpful for loading oxygen into the blood. After birth and the development of the cardiorespiratory system, haemoglobin oxygen affinity decreases. This change in haemoglobin function occurs because there are different forms of the haemoglobin subunits, which arose from duplications of ancestral genes, and their expression changes throughout development. Early and late expression β-globins exist in all mammalian groups, not just in placental mammals, so Juan Opazo and colleagues from the University of Nebraska wondered how these genes evolved.

The authors used phylogenetic techniques to determine the evolutionary relationships between 168 β-globin genes from across the vertebrates. This approach takes advantage of the fact that closely related genes are often more similar in DNA sequence than more distantly related genes. The authors were particularly interested in comparing the three mammalian subclasses: placental mammals, which includes humans, elephants and mice; marsupials, which includes kangaroos and opossums; and monotremes, which includes platypuses and echidnas. Their simplest prediction was that the β-globin gene was duplicated in the common ancestor of all mammals, and if this were so, the early and late expressed β-globins would form separate clades in the phylogeny. In other words, all of the early expressed genes would be more closely related to each other than to any of the late expressed genes, and *vice versa*.

However, the researchers did not see two separate clades, but observed something very different: early and late β-globins from monotremes were more similar to each other than to any of the β-globins from other mammals. This observation made Opazo and colleagues suspect that the early and late forms of monotreme β-globins arose from a gene duplication that occurred after this subclass split from other mammals. The presence of different β-globins in multiple mammalian lineages therefore seemed to arise from two distinct duplication events.

But the coding sequence of duplicated genes can sometimes evolve in concert if they have similar functions, and thus become similar in sequence. The β-globin genes of monotremes could therefore appear to be more closely related than they actually are, so Opazo and colleagues decided to test this possibility and analysed the non-coding DNA sequences flanking each gene. In doing so they found that the flanking regions of the early and late genes in monotremes were also more similar to each other than to the β-globins from other mammals. In addition, when they looked at all the DNA sequence around the globin genes, they saw that monotremes had a unique arrangement of duplicated DNA sequences, different from other mammals. These observations affirmed the authors' conclusion that independent duplication events in monotremes and other mammals led to the occurrence of both early and late β-globin genes in all mammals. These probably evolved later in both lineages to deal with the differing oxygen transport requirements before and after birth.

Despite being one of the best studied proteins in biology, studies of haemoglobin are still providing insight into how physiological systems evolve. Natural selection has formed complex physiological systems, and Opazo and colleagues have brought us one step closer to understanding how the evolution of genes can make this happen.

10.1242/jeb.011601

Opazo, J. C., Hoffmann, F. G. and Storz, J. F. (2008). Genomic evidence for independent origins of β-like globin genes in monotremes and therian mammals. *Proc. Natl. Acad. Sci. USA* **105**, 1590-1595.

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