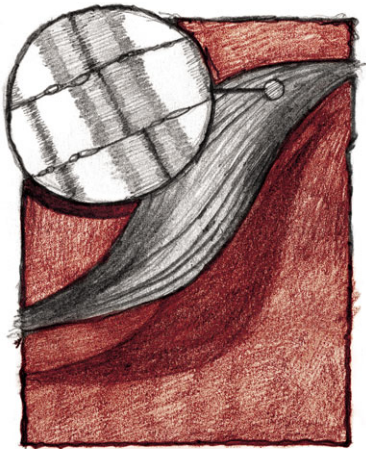
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB

PENNATE MUSCLES



## CONSEQUENCES OF BULGING

Nicolaus Steno, famous today as the founder of modern geology, was better known in his own day as an anatomist. His public dissections attracted crowds across Europe in the 17th century. Undergraduates today still know his anatomical work – even if they may not know its source – because Steno provided a description of the structure and function of pennate muscles. Pennate muscles look a bit like feathers, or '*pennae*' in Latin; many short muscle fibers insert at an angle on a long central tendon, called an aponeurosis, like the vanes of a feather attach to the central shaft. Surrounding the feather-like structure is another tendonous aponeurosis.

Steno understood that this feather-like structure allowed more muscle fibers to be packed into a given volume of muscle – and more muscle fibers means more force. To analyze the function, he assumed that the aponeuroses stay the same distance apart at all times, which means that as the muscle fibers shorten they rotate. And because of the rotation, the velocity of the central aponeurosis can exceed the fibers' own shortening velocity. However, the fibers trade this increased shortening velocity for decreased force because they insert at an angle on the central aponeurosis. This tradeoff is called the architectural gear ratio.

However, Steno's analysis was two dimensional, and neglected one important constraint: muscles cannot change volume. As muscle fibers shorten, they also bulge out. To examine the consequences of bulging, Brown University researchers Emmanuel Azizi, Elizabeth Brainerd and Thomas Roberts constructed a three-dimensional mathematical model of a pennate muscle, essentially stacking multiple 'feathers' on top of each other. Then they constrained how the fibers could bulge. According to the calculations, if the fibers bulge out perpendicular to the flat surface of the 'feather', then the aponeuroses can stay the same distance apart or even get closer together. If they get closer, then the fibers don't need to rotate as far, which reduces the gear ratio, resulting in stronger but slower contractions. Alternatively, the fibers could bulge in the plane of the feather, which causes the aponeuroses to get further apart even as the fibers themselves get shorter, resulting in even larger angular changes and a higher gear ratio for fast but weak contractions.

To test how real pennate muscles change shape, the researchers examined the lateral gastrocnemius muscle from wild turkeys. They stimulated the muscle to contract and used a servomotor to vary the shortening velocity so that the force remained fixed at certain levels. Throughout the contraction, they measured the angle and length of the muscle fibers and the width and thickness of the whole muscle.

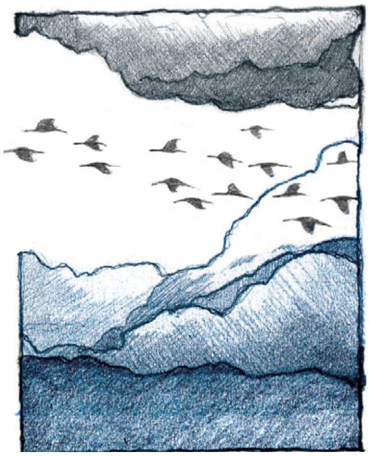
They found that the gear ratio changed automatically, depending on the total force. For high forces, the fibers tended to rotate relatively little, pulling the aponeuroses together and resulting in a slow whole-muscle contraction and a low gear ratio. For low forces, the fibers rotated much more, and the bulging fibers pushed the aponeuroses apart, causing a fast whole-muscle contraction and a high gear ratio. This automatic change in gear ratio with varying load expands the range of possible operating conditions for the muscle, increasing both the maximum force at low speeds and the maximum speed at low forces.

10.1242/jeb.011379

**Azizi, E., Brainerd, E. L. and Roberts, T. J.** (2008). Variable gearing in pennate muscles. *Proc. Natl. Acad. Sci. USA* **105**, 1745-1750.

**Eric Tytell**
**University of Maryland**
**tytell@umd.edu**

## METABOLIC RATE



## LIFE IN THE SLOW LANE

Even in the animal world, there are high rollers and those with cheaper lifestyles. The links between the lifestyle of an animal and the associated costs has long fascinated both ecologists and physiologists. One environment that is associated with a slower lifestyle is the tropics. In a recent study published in the *Proceedings of the National Academy of Sciences*, Popko Wiersma, Mark Chappell and Joseph Williams looked at the energy expenditure in tropical birds during expensive activities such as exercise and keeping warm. They hypothesized that rainforest birds have a reduced capacity to stay warm when exposed to cold, but also a lower endurance in flight. In other words, is it cheaper to live in the tropics?

The team headed down to the Smithsonian Tropical Research Institute in Panama, where they captured species of birds living in the rainforest. One test they performed consisted of placing the birds in a chamber where the rate of oxygen consumption was measured as the temperature in the chamber was lowered. This measurement allowed them to assess the peak metabolic rate of 19 species during exposure to the cold, as well as finding the lowest temperature at which the birds could maintain this peak metabolic rate. The researchers also measured peak metabolic rate during exercise; this was not a simple task but their flight wheel successfully motivated 45 species to take to the wing.

Ultimately the team compiled an impressive data set of physiological readings, across 14 species, of measurements of individual's cold temperature limit, basal metabolic rate, peak metabolic rate during exercise and peak metabolic rate in the cold. Having analysed the data, taking into account phylogenetic relatedness, the team confirmed their first hypothesis when they found that birds living in the tropics have a lower ability to stay warm; their peak metabolic rate when exposed to the cold is lower than the peak metabolic rate of temperate species when conditions get chilly. They also showed that the lowest ambient temperature at which the tropical birds could maintain their own body temperature is more than 8°C higher than for temperate species. Tropical birds do not do as well in the cold, maybe not so surprisingly, but they also appear to have a lower peak metabolic rate during exercise. The authors suspect that this is because tropical birds commute less or rely less on endurance; their comings and goings can be sustained on lower metabolic rates, so it is cheaper to live in the tropics.

Having gathered so much metabolic data on individual birds, the group were also able to tackle the issue of links between different species' energy expenditures in various situations. The team looked at whether a species that idles at a high basal metabolic rate does well in the cold or has a greater exercise capacity, and whether doing well in the cold is related to exercise ability. It turns out that idling rate and exercise ability are related, but not the ability to tolerate cold temperature. It begs the question, what is the mechanism that links metabolic rate at rest and during exercise? – a well-known old animal physiology riddle.

In this study, Wiersma, Chappell and Williams have highlighted the ecological causes and consequences of physiological diversity, as well as providing the raw material for further investigation of the mechanisms that connect metabolic rates of animals in different situations.

10.1242/jeb.011411

**Wiersma, P., Chappell, M. A. and Williams, J. B.** (2007). Cold- and exercise-induced peak metabolic rates in tropical birds. *Proc. Natl. Acad. Sci. USA* **104**, 20866-20871.

**Charles Darveau**
**University of Ottawa**
**cdarveau@uottawa.ca**

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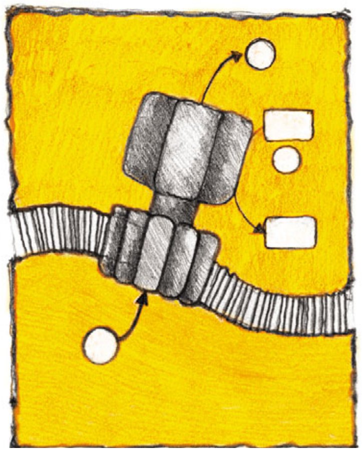
## ION DISCRIMINATION



## SUBTLE DIFFERENCES

Transporting ions across cellular membranes depends on the activity of little pumps that are of fundamental importance to life. They utilize the energy derived from ATP hydrolysis to establish and maintain electrochemical ion gradients across membranes that are the basis of vital processes such as nutrient uptake, pH homeostasis or electrical excitability. A significant number of them belong to the family of P-type ATPases, named for the formation of a phosphorylated intermediate in the reaction cycle. Although closely related, different P-type ATPases transport different ions. An unsolved question so far is how these pumps discriminate between different ions, and a recent *Nature* paper describing the atomic structure of the Na$^+$,K$^+$-ATPase, determined by a team of Danish scientists led by Bente Vilsen and Poul Nissen, sheds new light on ATPase ion discrimination.

The Na$^+$,K$^+$-ATPase is a transmembrane enzyme that transports three sodium ions for every two potassium ions pumped in the opposite direction. In contrast to more simple P-type ATPases, such as SERCA (a Ca$^{2+}$ pump composed of a single subunit), the Na$^+$,K$^+$-pump is made of three subunits. Its $\alpha$-subunit shares significant homology with SERCA, while the $\beta$- and $\gamma$-subunits are unique to the Na$^+$,K$^+$-ATPase. According to the classical model of the ion pump reaction cycle, P-type ATPases adopt two conformations, known as E1 and E2. During the reaction cycle the transported sodium and potassium ions are temporarily trapped in the protein's E1 and E2 state, respectively, ensuring that the entry gate closes before the exit gate opens, a mechanism that prevents leakiness. But does this classical model really explain how the Na$^+$,K$^+$-ATPase functions?

To get an initial snapshot of the Na$^+$,K$^+$-ATPase, the Danish scientists purified the enzyme from pig kidneys and crystallized it in the presence of rubidium ions, which behave like potassium ions but are large enough to be visualized in the crystal structure. The team expected that their snapshot would provide a view of the Na$^+$,K$^+$-ATPase with two rubidium ions trapped in the same location as the potassium ions during the pumping process.

Analysis of the Na$^+$,K$^+$-ATPase structure shows that the β- and γ-subunits interact with three of the α-subunit's helices in the transmembrane region. Interestingly, the carboxy-terminal end of the α-subunit contains positively charged arginine residues, which may act as membrane voltage sensors. As expected, the two rubidium ions were trapped in the transmembrane part of the α-subunit, confirming the classical model of the reaction cycle. Surprisingly, the α-subunit had an unexpectedly high structural similarity to SERCA, even in the binding pocket, posing the question of how such similar proteins distinguish between the different ions that they pump. The team concluded that subtle differences in side chain and water molecule positions must be sufficient to determine ion selectivity.

By providing the first structural model of the Na$^+$,K$^+$-ATPase, Vilsen and Nissen's team are following in the footsteps of Jens Christian Skou, who ultimately earned the Nobel Prize after discovering this fundamental pump. However, to completely understand how Na$^+$,K$^+$-ATPases work, we will need further structures showing the enzyme in different states to give us a detailed picture of this remarkable protein's function.

10.1242/jeb.011387

**Morth, J. P., Pedersen, B. P., Toustrup-Jensen, M. S., Sørensen, T. L.-M., Petersen, J., Andersen, J. P., Vilsen, B. and Nissen, P.** (2007). Crystal structure of the sodium–potassium pump. *Nature* **450**, 1043-1049.

**Hans Merzendorfer**
**University of Osnabrueck**
**merzendorfer@biologie.uni-osnabrueck.de**

## JEKYLL AND HYDE SQUID

Most of us think that our pet cats and dogs have personalities, but is this term really appropriate? The most widely studied dimension of animal personality is shy/bold behaviour. It is assumed that 'personality' should be constant across time in any given individual. However, organisms also develop, and it is quite possible that a shy child can grow into a bold and extrovert adult.

Studying simple animals with short life cycles makes it easy to investigate consistency in 'personality' and in principle makes it possible to investigate the neural bases of the behaviours involved. David Sinn and his colleagues from the Universities of Texas and Tasmania used such an animal for their investigation of animal 'personality' – the 7cm-long dumpling squid.

It is known that a dumpling squid that is shy in a predator-risk situation may be bold in a feeding situation. Sinn and his team repeatedly measured the behaviour of squid over a 16 week period, as the squid grew to maturity. They measured the personalities of juvenile and adult squid by poking them (a threat test) and by giving them shrimp to eat (a feeding test). They scored each animal on their personalities by grouping a range of behaviours, including speed of response and the frequency with which squid displayed certain behaviours in the test.

As in previous studies, squid could be bold in one test and shy in the other. This may be because the expression of any underlying 'personality' is altered by the developmental and behavioural context: squid were found to be boldest in the feeding test shortly before sexual maturity, while boldness in the predation test increased after maturity.

This change associated with development was not consistent for shy and bold squid, and changed with the experimental situation. In the predation test, shy squid tended to remain shy, while squid that were initially classified as bold showed a wide range of behaviour as they grew from juveniles into adults.

Once sexual maturity had occurred, behavioural responses to a predation threat tended to be consistent: shy squid remained shy, while bold squid became bolder. The opposite effect was found in the feeding test: squid classed as 'bold' as juveniles showed the greatest consistency in their personality, while individuals that were 'shy' at sexual maturity tended to become shyer as they grew older.

These findings in squid support previous studies on great tits and sticklebacks, and suggest that animal personality is not fixed but is 'plastic' – it changes depending on the environment and the developmental stage of the organism. In particular, it can be affected by key phases in the animal's physiology, such as sexual maturity.

However, the squid study also raises the question of whether the 'shy/bold' dimension is really measuring something consistent in the animal. If this behaviour can show such variability across time, and can be altered by major developmental changes, it may be something that exists in experimental situations but has no consistent reality in natural conditions.

The solution probably lies in a richer description of behaviour: although it is tempting and convenient to reduce animal 'personality' to a single dimension, this may obscure important parts of what the animals actually do. Humans are not simply 'shy' or 'bold' – why should other animals be?

10.1242/jeb.011395

**Sinn, D. L., Gosling, S. D. and Moltschaniwskyj, N. A.** (2008). Development of shy/bold behaviour in squid: context-specific phenotypes associated with developmental plasticity. *Anim. Behav.* **75**, 433-442.

**Matthew Cobb**
**University of Manchester**
**cobb@manchester.ac.uk**