Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

MINIMISING THE COST TO GET BY


Picture by John Bertram

No matter what the terrain, we usually adjust our gait to stagger on. Even if it means adopting some rather strange styles, we adjust speed, step length and stride frequency to overcome most obstacles. John Bertram began investigating the mechanics of walking several years ago. Controlling stride length, Bertram was surprised to realise that the athletes he was investigating adapted spontaneously to relatively unnatural gaits, adjusting their speed and frequency in unexpected ways. 'If we'd seen this in animals' says Bertram' we'd have thought we'd interfered with them'. But the athletes seemed completely unaware of their adjustment. Bertram realised that the walking gait is very plastic and that he could predict these strange behaviours by minimising the energetic cost of locomotion. But was this optimisation an artefact of walking, or a general characteristic of human locomotion? Bertram needed to investigate another gait, running (p. 622).

Based at Florida State University, Bertram had no trouble finding outstanding athletes to volunteer for running practice and a convenient track to test them on. Having found 5 willing participants, Bertram, Brian Jacobi and Michael Butcher set the athletes three tasks; running at set speeds on a treadmill, running at set frequencies striding in time to a metronome, and running with fixed stride lengths by stepping on markers on a field. Needing spontaneous responses to the running conditions, Bertram only allowed the runners two practice runs to find their rhythm, before recording the performance and monitoring how the runners adjusted to the constrained conditions.

Just as Bertram had found with the walkers, the Florida runners adjusted naturally to the unusual running styles and came up with some unexpected, but natural, solutions. Bertram realised that speed, stride frequency and length were linked; adjusting one forced the runner to modify the other two to compensate. The runners must be adapting to optimise some aspect of their performance. Bertram turned to the metabolic cost.

But measuring the volunteer's metabolic costs as they repeated the constrained running tasks was going to be too tricky. Bertram realised that the metabolic data might already be out there in the literature. At this point Anne Gutmann, a Cornell graduate student visiting Bertram's lab for a year, joined the team. She began trawling through journals, and identified 4 well documented running studies that she could extract reliable, constrained metabolic cost data from. Using sophisticated mathematics Gutmann constructed a 'cost of transport' surface as a function of the runners' speed, step length and running frequency, and used this to predict how a runner's gait would respond if any of these parameters were constrained. Gutmann also compared how Bertram's runners faired on the Florida running track with the metabolic data she'd extracted from the literature. The running behaviours agreed remarkably well, although the behaviours weren't completely identical.

So, constrained optimisation of the metabolic cost of movement allows the prediction of both walking and running gaits because we always get by for the least possible cost. And the relationship between our speed, stride length and frequency as we walk or run 'is not an accident of the mechanics; the body is monitoring gait all the time' says Bertram.

10.1242/jeb.02110

Gutmann, A. K., Jacobi, B., Butcher, M. T. and Bertram, J. E. A. (2006). Constrained optimization in human running. *J. Exp. Biol.* **209**, 622-632.

MUTATION GIVES INVADER THE UPPER HAND

No one knows when the European invader arrived, probably in the early twentieth century, but by the time anyone noticed in the 1980s, it was too late. The Mediterranean blue mussel (*Mytilus galloprovincialis*) had already driven its close relative, *Mytilus trossulus*, from its warm home in Southern California, leaving *trossulus* the cooler waters it occupied in Northern California and around the Pacific basin to Japan. George Somero has been fascinated by the physiological adaptations that organisms make to their environments and was intrigued by the *galloprovincialis* invasion. He knew

that *galloprovincialis* had left the Pacific for the warmer Mediterranean a mere 3.5 million years ago, but what adaptations had the mussel made during its brief European sojourn that allowed it to thrive and drive *trossulus* out? Peter Fields, returning to Somero's lab for a brief sabbatical period, decided to take up the challenge by investigating one of the mussel's key metabolic enzymes: cytosolic malate dehydrogenase (p. 656).

According to Fields, malate dehydrogenase is a well-characterised, and essential, component of several metabolic pathways, but the enzyme is also temperature sensitive, making it an ideal candidate for adaptation to warmer conditions. However, sequencing the gene from mussels proved trickier than Fields had hoped. Little is known about mollusc genomes, so designing the DNA primers essential for the sequencing process proved challenging. Fortunately Somero's colleague, Andy Gracey, was on hand to guide Fields through the complex sequence databases needed to design the primers, before Fields ran the sequencing reactions on both blue mussels and another California local, *Mytilus californianus*, that lives in the mild waters where *galloprovincialis*'s and *trossulus*'s territories overlap.

Having sequenced the three genes, Fields realised that one position in the gene lit up as a mutation hot spot; all three species had completely different amino acids at position 114, the hinge of a loop region essential for the enzyme's catalytic activity. Fields was astonished. He explains that he'd investigated related enzymes in cold adapted species and never seen mutations at this location before. What effect would this mutation have on the enzyme's function?

Instead of extracting the enzyme directly from each of the three mussels, Fields' student, Emily Rudomin, expressed all three proteins in bacteria, allowing her to produce larger quantities of each enzyme than she could extract from the mussels. Measuring each enzyme's kinetics, Fields realised that each of the enzymes were well adapted to the water temperature where the mussels lived. He explains that enzymes that function well in warm conditions have low substrate turnover rates and bind their substrate more tightly than enzymes adapted to cold conditions, which process their substrate faster and bind substrate more loosely. Sure enough, the warm adapted *galloprovincialis*' malate dehydrogenase behaved like a warm adapted enzyme, while the cold adapted *trossulus* enzyme functioned best at low temperatures. *Mytilus californianus*, found on the beach outside Somero's Pacific Grove lab, was optimised for a mild, intermediate temperature.

The mutated amino acid located in the hinge region had dramatically affected the enzyme's function, and is one of a raft of adaptations giving the impostor, *Mytilus galloprovincialis*, the upper hand over *Mytilus trossulus* in hot water.

10.1242/jeb.02107

Fields, P. A., Rudomin, E. L. and Somero, G. N. (2006). Temperature sensitivities of cytosolic malate dehydrogenases from native and invasive species of marine mussels (genus *Mytilus*): sequence-function linkages and correlations with biogeographic distribution. *J. Exp. Biol.* **209**, 656-667.

TUNICATES SET TREND FOR POTASSIUM CHANNEL

Ion channels are proteins that form miniscule pores in nerve cell membranes, and when open they generate currents across the membrane to propagate nerve signals. Andrew Spencer from the University of Alberta, Canada, explains that one class of ion channels, the potassium channels, play a major role in shaping the action potentials in many tissues, including heart, and as a consequence have been subject to strong natural selection resulting in a wide variety of potassium channels, each finely tuned to a particular function. One family of potassium ion channels found in vertebrate hearts, the Kv4 voltage-gated potassium channels, comprises three individual members (Kv4.1, Kv4.2 and Kv4.3) that evolved from one of our ancestor's early Kv4 channels. However, the hearts of simpler invertebrates only carry a single Kv4 channel, like our ancestors. Knowing that sea squirts (tunicates) are our most ancient living relatives, Vicenta Salvador-Recatalà and Andrew Spencer wondered whether our tunicate ancestors are more like invertebrates, with only one Kv4 channel, or whether the *Kv4* gene had been duplicated early enough in evolutionary history for tunicates to carry multiple Kv4 ion channels. Salvador-Recatalà decided to investigate how many copies of the gene the tunicate *Ciona interstinalis* possesses, and whether the ion channel plays a role in the tunicate's simple heart (p. 731).

Searching through the *C. intestinalis* genome, Salvador-Recatalà, Warren Gallin and Spencer identified a single *Kv4* gene; the vertebrate channel must have become duplicated after tunicates diverged from our family tree. Salvador-Recatalà also knew that an accessory protein, KChIP, modulates the function of modern Kv4 channels, and wondered whether our tunicate predecessors still carry this gene? Trawling through the genome again, the team was delighted to discover and clone the first non-vertebrate *KChIP* subunit gene.

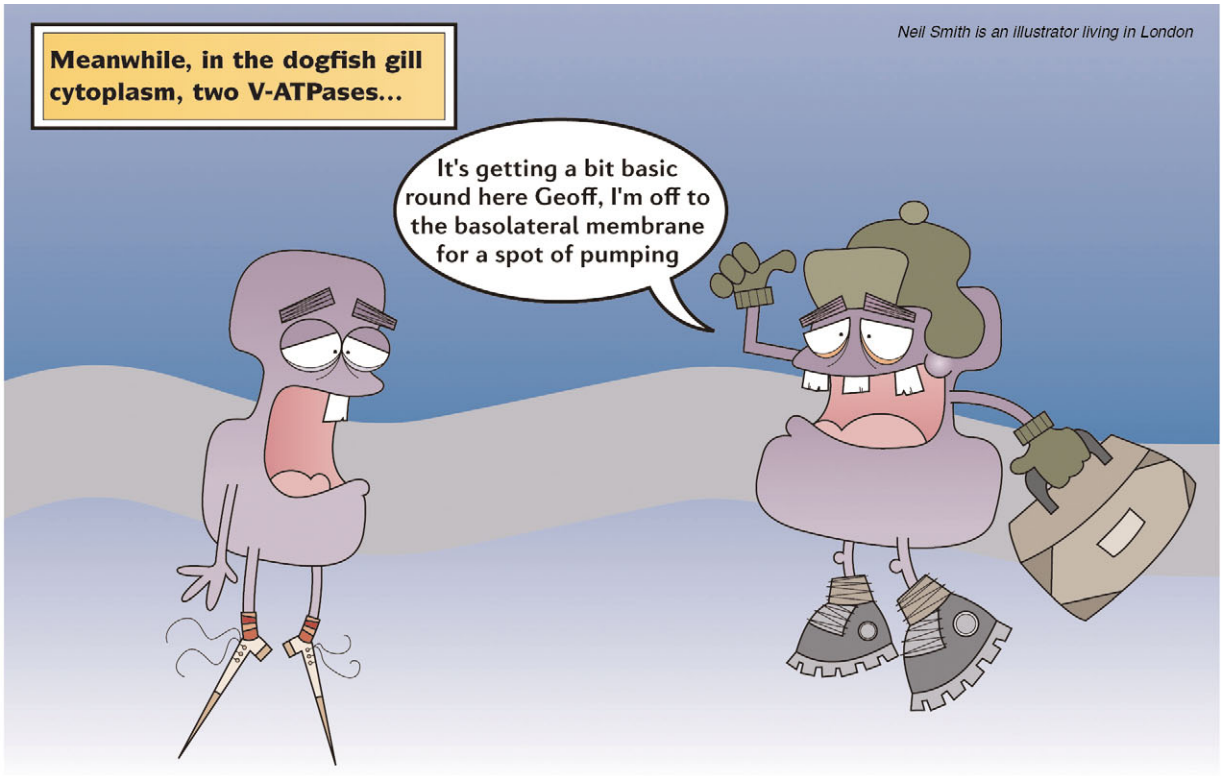
Curious to know how the tunicate's potassium channel functions, Salvador-Recatalà, Peter Ruben and Jennifer Abbruzzese began investigating the ion channel's electrical properties by injecting *Xenopus* egg cells with different combinations of Kv4 and KChIP RNA. Knowing that the cells would use the RNA to produce the ion channel and modulating protein, the team monitored the electrical properties of the modified cells and found that when they introduced the channel alone, the egg cells generated a significant potassium current. However, when the team generated both Kv4 and KChIP proteins in the cells, they saw a dramatic shift in the Kv4 channel's electrical properties; KChIP increased the strength of the egg cell's potassium current as well as prolonging the current's duration, suggesting that the ancient channel contributes to tunicate heart function. Spencer suspects that KChIP probably aids insertion of the ion channel in the cell's membrane, increasing the number of ion channels in the membrane, and the potassium current in turn.

Probing the ion channel's function further, the team removed the first 32 amino acids of the Kv4 protein, which interact with KChIP in vertebrates, to see whether this affected the tunicate channel's function; KChIP no longer modulated the truncated ion channel's function, suggesting that KChIP interacts with tunicate Kv4 through the channel's N terminus, just like modern KChIP. So tunicates invented the blueprint for Kv4 ion channel modulation while vertebrates have refined ion channel function further by expanding their repertoire of *Kv4* genes.

10.1242/jeb.02109

Salvador-Recatalà, V., Gallin, W. J., Abbruzzese, J., Ruben, P. C. and Spencer, A. N. (2006). A potassium channel (Kv4) cloned from the heart of the tunicate *Ciona intestinalis* and its modulation by a KChIP subunit. *J. Exp. Biol.* **209**, 731-747.

RELOCATING PUMPS



Life in the ocean is a constant battle to maintain a healthy ionic balance. Fish excrete acid and base, such as bicarbonate, across their gills to stay in equilibrium with their environment. While disposing of unwanted bicarbonate ions, gill cells in turn generate hydrogen ions that are subsequently carried away in the blood. Martin Tresguerres explains that the H^+ transporting V-H^+-ATPase proteins are an essential component of the base excretion process, but earlier work had suggested that the protein was located in the cell's cytoplasm, well away from the membrane that the hydrogen ions must cross for removal in the blood.

Wondering whether the gill cell's protein trafficking machinery may relocate V-H^+-ATPases from the cytoplasm to the cell membrane as part of the excretion process, Tresguerres and his colleagues at the University of Alberta disrupted the dogfish protein trafficking machinery while infusing the fish with bicarbonate to see if they were still able to excrete the excess base (p. 599). Monitoring levels of carbonate in the fish's blood, the team found that the carbonate levels rose; disrupting the protein trafficking system had interrupted the ion pumping process. And when they looked for the V-H^+-ATPase proteins in gill cells after the fish experienced a high dose of bicarbonate, the team found that the protein was situated in the gill cell membrane, where they expected to find it if it contributed to base excretion. 'Our results strongly suggest that cellular relocation of V-H^+-ATPase is necessary for enhanced HCO_3^- secretion' says Tresguerres.

10.1242/jeb.02108

Tresguerres, M., Parks, S. K., Katoh, F. and Goss, G. G. (2006). Microtubule-dependent relocation of branchial V-H^+-ATPase to the basolateral membrane in the Pacific spiny dogfish (*Squalus acanthias*): a role in base secretion. *J. Exp. Biol.* **209**, 599-609.