Inside JEB, formerly known as 'In this issue', 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

HOT ORYX OPTS FOR HETEROTHERMY

By desert standards, 2001 was a wet year in Saudi Arabia, with almost 14 cm of rain falling. But even in the wettest years, water is scarce, and mostly locked up in the few hardy plants that tolerate the harsh conditions. Which is why the Arabian oryx never drinks! It extracts every drop of water that it needs from its diet of grass and shrubs. But as the daily temperatures rise, how can a large mammal protect itself from the threat of dehydration? Under some conditions, camels and other ungulates seem to prevent themselves from dehydrating by simply absorbing heat, rather than losing precious fluid by sweating. But no one had ever seen a case of heterothermy outside the lab in a large endotherm. Knowing that the Arabian oryx had adapted to survive one of the planet's driest environments, Stephane Ostrowski and his colleagues took to the desert to track the oryxes' body temperature as the animals roamed the desert, gathering the first clear evidence that a large mammal resorts to heterothermy in the wild (p. 1471).

The Arabian oryx is one of those rare animals that returned from the brink of extinction when it was successfully reintroduced into the Arabian Desert in the 1980s. By 1996, when Ostrowski joined the team of conservators at the National Wildlife Center, the population had reached almost 500 in the wild. As the oryxes' survival was relatively assured, Ostrowski decided that it was time to learn more about this remarkable animal's physiology.

Working with Joseph Williams and Khairi Ismael, he fitted temperature sensitive radio transmitters and tracking devices to six young adults. But tracking the oryx over more than 2000 km^2 of desert was far from straight forward! With the help of a team of local rangers, Ostrowski and Ismael followed the animals' progress over a two year period, collecting over 800 hours of temperature data and recording the animals' behaviour throughout the day.

Not surprisingly, during the summer the animals spent a large part of the day sheltering from the heat, and sure enough, as each day wore on, the animal's body temperature gradually rose from 36°C in the morning to over 40°C at sunset! But could its ability to store heat really protect the oryx from dehydration? Ostrowski calculated how much energy the animal stored during the day, and then calculated how much water the animal would have lost if it had stayed cool by either sweating or panting; the oryx had saved 0.5 l, almost one third of the animal's daily water requirements!

The team then compared the animals' temperature fluctuation in the winter, and were astonished when they realised that the oryxes' minimal body temperature remained higher than in the summer, despite the cooler weather. But Ostrowski can explain this apparent paradox. Even an oryx begins losing water when its temperature reaches 41°C, so in summer it could be in serious danger of overheating, unless it could drop it's body temperature low enough at the start of the day, to store the extra summer heat. So in winter, when the heat is less threatening the animal never needs to go as low as it does in the summer.

10.1242/jeb.00290

Ostrowski, S., Williams, J. B. and Ismael, K. (2003). Heterothermy and the water economy of free-living Arabian oryx (*Oryx leucoryx*). *J. Exp. Biol.* **206**, 1471-1477.

FISH FIND A FROG IN THE FAMILY

As amino acids go, glutamine is much more than a simple protein building block. Functioning either in the brain during recycling of the neurotransmitter glutamate, or as a key step in the metabolic pathway that produces urea to detoxify ammonia, the enzyme that produces glutamine is essential for most creatures' well being. Consequently glutamine synthetase has 'an extraordinarily long evolutionary history' explains Patricia Wright. Most mammals only have a single glutamine synthetase gene, but as there are two copies of each chromosome in mammals' cells, there are always two almost indistinguishable copies of the gene, one on each half of a chromosome pair, called alleles. However, sometime during the last 100 million years, some fish species broke away from the rest of the fish evolutionary tree, and duplicated their diploid chromosomes,

resulting in an animal with four sets of chromosomes. So when Wright and Tom Mommsen set out independently in their laboratories to investigate the tetraploid rainbow trout's glutamine synthetase gene, they fully expected to find that the fish had doubled up a single glutamine synthetase gene when they duplicated their chromosomes, resulting in two different glutamine synthetase genes. But after months of patient cloning and sequencing, both scientists began to realise that the sequences just didn't match up! Instead of having two alleles of each gene, the 'alleles' were too different to code for the same protein, so the rainbow trout must have four glutamine synthetase genes (p. 1511)! Somewhere along the line, the rainbow trout's diploid ancestor must have already duplicated its glutamine synthetase gene before it went tetraploid.

But if the rainbow trout had four glutamine synthetase genes, other fish that had stuck firmly to the diploid branch of the evolutionary branch might have a duplicated gene too, just like the trout's ancestor. Brent Murray dived into the zebra fish and fugu genomes, and after searching through thousands of unnamed genes in the enormous databases, he discovered that both fish had two glutamine synthetase genes. And when Wright and Mommsen reconstructed the enzyme's phylogentic tree, they realised that the fugu and zebra fish's ancestors had both duplicated the gene, but at different times. All of which made Pat Walsh wonder whether his favourite, the toadfish, might also have multiple copies of the gene.

He began scrutinising mRNA throughout the fish's body, to see if the glutamine synthetase from different tissues had been produced by a single gene. But when he analysed the nucleic acid that produced the gill's glutamine synthetase, the gene was completely different from the glutamine synthetase produced in the fish's brain and liver. Instead of having a single glutamine synthetase gene, the toadfish had also duplicated the enzyme, but only used the second gene to produce glutamine synthetase in its gills (p. 1523).

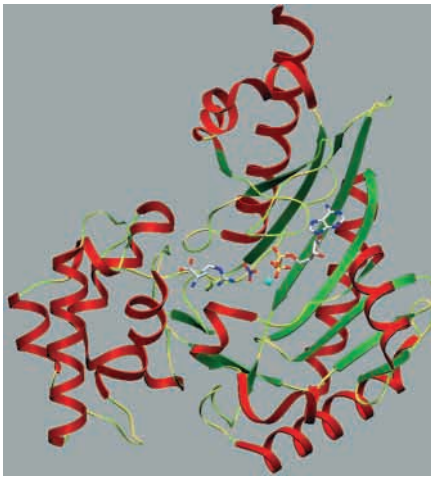
Walsh was intrigued to find out when the toadfish had duplicated the gene, and constructed another phylogenetic tree, which he adds agrees well with Wright's and Mommsen's phylogeny. He looked for the new gene's closest relative. But instead of resembling other fish glutamine synthetases, the new gene was right out on a limb, and looked more like the *Xenopus* enzyme!

Why would a fish enzyme look so much like an amphibian's when the kinetics of urea production are so different on land and in the water? Walsh explains that for some unknown reason, toadfish suddenly stop excreting ammonia. This should cause the toxic waste product to accumulate in the gill, unless the fish could suddenly switch on urea production. By ramping up glutamine synthetase production, the enzyme could mop up the stray ammonia and convert it into non-toxic glutamine, but he explains that the enzyme in the toadfish's gill would have to a high affinity for ammonia, just like a terrestrial animal's glutamine synthetase. Walsh thinks that this 'ammonia trapping' mechanism could explain the similarity between the fish and the frog genes, but he knows that there are more questions to be answered before this theory becomes more than speculation.

10.1242/jeb.00291

Murray, B. W., Busby, E. R., Mommsen T. P. and Wright, P. A. (2003). Evolution of glutamine synthetase in vertebrates: multiple glutamine synthetase genes expressed in rainbow trout (*Oncorhynchus mykiss). J. Exp. Biol.* **206**, 1511-1521.
Walsh, P. J., Mayer, G. D., Medina, M., Bernstein, M. L., Barimo, J. F. and Mommsen, T. P. (2003). A second glutamine synthetase gene with expression in the gills of the gulf toadfish (*Opsanus beta*). *J. Exp. Biol.* **206**, 1523-1533.

EXTRA DOMAIN KEEPS ENZYME IN THE FOLD



Picture provided by Michael Chapman, Institute of Molecular Biophysics, Florida State University

Every day, our bodies consume kilograms of ATP, which drives almost all our metabolic processes from opening our eyes in the morning, to keeping our brains functioning every moment of our lives. But making sure that a cell has enough ATP ready for the moment when it leaps into action is the job of a group of enzymes known as the phosphagen kinases, such as creatine kinase and arginine kinase. When energy demands are low they effectively store energy by transferring the terminal phosphate group from ATP to either creatine or arginine to form creatine phosphate and arginine phosphate. But when a cell needs a sudden burst of energy, the kinases retrieve the phosphate group, restoring it to an ADP molecule to produce ATP. Most arginine kinases function as a single protein molecule, but some invertebrates evolved novel forms of the protein, with two copies of the arginine kinase enzyme linked together in a single polypeptide. Ross Ellington explains that having two copies of the enzyme makes understanding the catalytic mechanism of the enzyme an intriguing problem. Working with Deanne Compaan, he began looking at the doubled-up enzyme produced by razor clams to find out how both domains function when linked together in a single enzyme (p. 1545).

Compaan and Ellington set about cloning the long enzyme. Ellington explains that the razor clam foot muscle is a 'burst type muscle', so the muscle has high levels of the enzyme and the associated mRNA, 'which makes our task easier' adds Ellington. Within a matter of weeks, the team had cloned the enzyme and when he looked at the enzyme's sequence, both domains had all of the amino acids that are essential for them to catalyse the energy storing reaction. But then the team hit a snag; they could make large amounts of the protein in *E. coli*, but only the full length enzyme behaved like a properly folded protein. When they tried expressing each domain separately, they only found unfolded protein in the *E. coli* cells. Fortunately Ellington had experience of rescuing denatured proteins, and after successfully refolding both domains, Campaan and Ellington began testing the enzymes' kinetics.

Sure enough, the full length enzyme, equipped with both domains, catalysed the reaction between arginine phosphate and ADP to produce ATP, and did it at twice the rate of a monomeric arginine kinase from the horseshoe crab. But when they looked at the individual domains, the team was in for a shock; the first domain didn't function at all! However, when Ellington began looking at the second domain he says that 'everything fell into place'; the

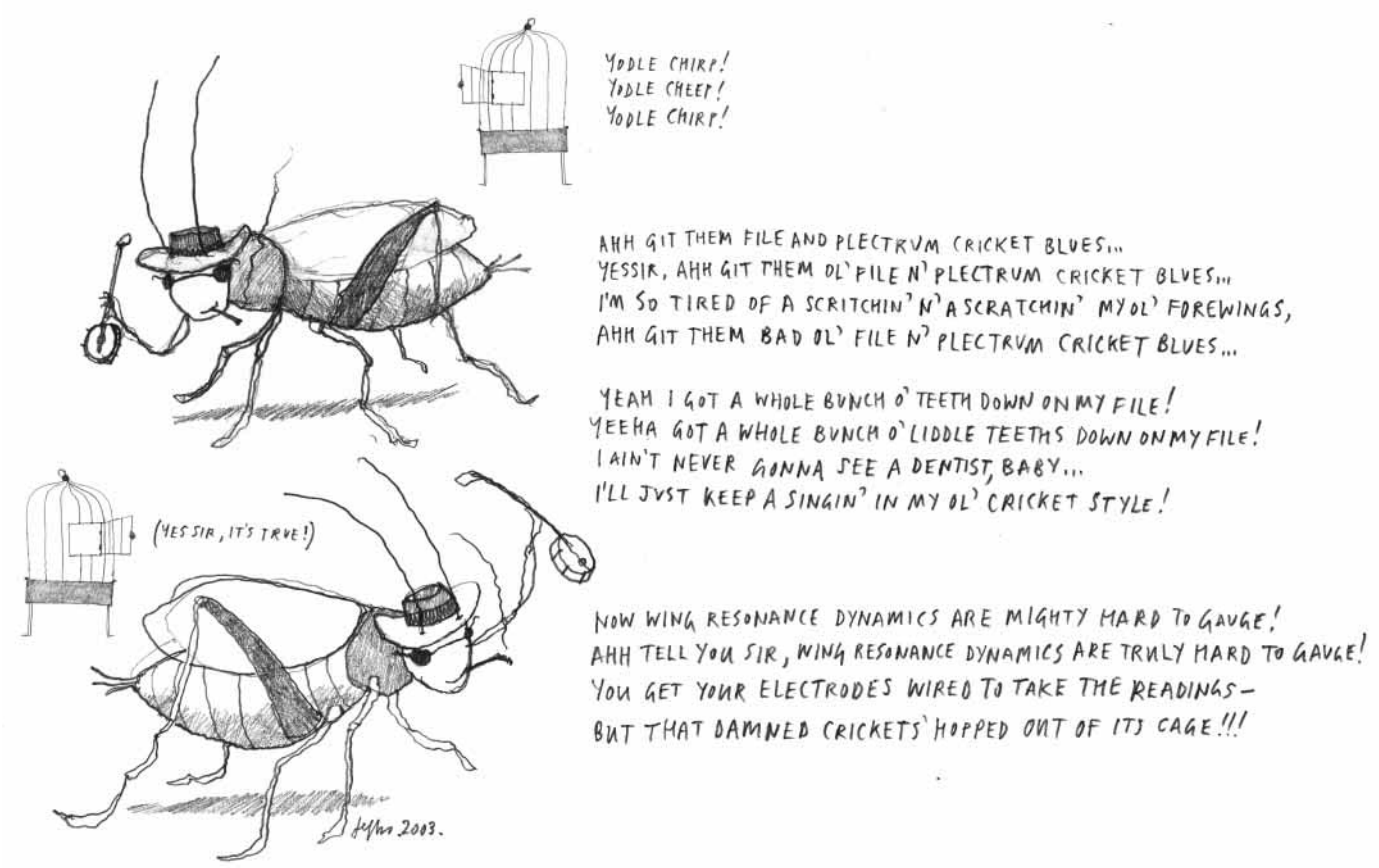
second domain alone accounted for all of the full-length enzyme's activity.

Why has the enzyme retained a domain that doesn't seem to be doing much, after all it costs the mollusc twice as much effort to synthesise the larger enzyme, so the extra domains must be doing something important? Ellington suspects that the extra domain comes into its own before the enzyme sees arginine phosphate or ADP; he thinks that it behaves as a chaperone, helping the full-length enzyme to fold correctly, producing a highly active enzyme, despite a 'rather cumbersome evolutionary accident'.

10.1242/jeb.00340

Compaan, D. M. and Ellington, W. R. (2003). Functional consequences of a gene duplication and fusion event in an arginine kinase. *J. Exp. Biol.* **206**, 1545-1556.

CRICKET'S WINGS DOUBLE UP



Pete Jeffs is a cartoonist living in Paris

Like a violin player that picks up their instrument with the left hand, the Australian cricket always closes the right wing over the left when it begins to call. The insect begins with a chirrup, followed by a train of trilling notes as it makes successive wing movements and draws a plectrum on the upper side of the left wing along a serrated file structure on the bottom of the right. Henry Bennet-Clark is fascinated by the loud sounds that these tiny insects make, so he began analysing how the crickets produce their distinctive call.

First he tested the wings' acoustics, and discovered that a system of veins in the wing begins resonating as the insect pushes the plectrum over the upper wing's file. This was unexpected, as the harp membrane that lies between the resonating veins had been thought to be the main resonant structure, but it seems that it simply helps to radiate the sound.

He also tested each wing's resonance to see whether the low pitched Australian cricket sang like its higher pitched bush cricket relatives; a bush cricket's file wing remains silent when it begins chirruping. When he analysed the Australian cricket's wings' resonances he found that the left wing's resonant frequency was at the same pitch as the insect's song, but the right wing's resonant frequency was much lower than the left's. However when the right wing was played by driving the stiff plectrum along the file, the right wing's pitch rose, to match the left wing. So when the cricket sings, both wings vibrate at the same frequency. Bennet-Clark explains that the low pitched Australian crickets are 'under selection pressure to maximise the size of the [sound] source and achieve this by using both wings'.

10.1242/jeb.00303

Bennet-Clark, H. C. (2003). Wing resonances in the Australian field cricket *Teleogryllus oceanicus*. *J. Exp. Biol.* **206**, 1479-1496.

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