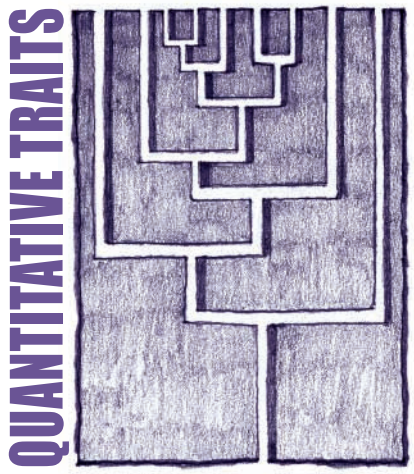
Keeping track of the literature isn't easy, so Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Short articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Outside JEB




## MUTATION MAKES PIGS PORKY

Fashions change all the time, and food is no exception. What's 'in' one year is 'out' another. Fifty years ago, a nice piece of pork came with a rich coating of fat. But in the 21st century, fat is out of favour, and now pigs are bred for pork muscle rather than lard. Muscle mass is an example of a measurable physical characteristic that varies significantly between individuals: it is a 'quantitative trait'. Quantitative traits are produced by intricate interactions between environmental factors and regions in genes, known as 'quantitative trait loci'. In the case of muscle mass, it seems that a single quantitative trait locus in the insulin growth factor 2 (IGF2) gene accounts for a significant proportion of muscle mass and back-fat variations amongst pigs. Nonetheless, a major question remained: what are the differences in DNA sequence in this quantitative gene locus that could explain the change in muscle mass between pigs? Puzzled by the problem, Anne-Sophie Van Laere and colleagues decided to track down the elusive mutations and found that a single nucleotide mutation in the IGF2 gene is responsible for making porky pigs!

Identifying the mutations underlying quantitative trait loci is not a trivial task, since each locus is usually only responsible for a fractional change in the quantitative trait. Knowing that IGF2 stimulates myogenesis, the authors started by sequencing the genomic region corresponding to the IGF2 quantitative trait locus. Working with crossbred piglets from wild-type and muscly parents, the team analysed the parents' and youngsters' DNA sequence polymorphisms and found that the muscly pigs had a guanine base where less well built pigs had an adenine. The team had found a needle in the haystack: this single mutation is the quantitative trait nucleotide that contributes to a 3–4% increase in muscle mass.

When the team took a closer look at the position of the mutation in the gene, they noticed that the causative mutation does not affect the IGF2 protein sequence; instead, it is in a non-coding region of the gene that might control its expression. Whilst the wild-type allele with the adenine base was able to bind a nuclear protein that repressed IGF2 transcription, the muscly pig's guanine mutation inhibits this interaction, which might affect the expression of IGF2. The team went on to investigate the effect of the causative mutation in the regulation of IGF2 expression. They monitored the levels of IGF2 transcripts from pigs at different growth stages and found that the paternally inherited mutant allele induced a three-fold increase of post-natal IGF2 expression in skeletal muscle, without affecting its expression in the liver. These results confirm that this non-coding region has an important regulatory role in IGF2 expression in muscle. And when the team mapped the mutant allele's distribution in farmed animal populations, they found high frequencies of the mutation in leaner breeds, matching the modern preference for lean cuts. Artificial selection pressure imposed on commercial pig populations has caused the mutant allele to spread between breeds.

In general terms, this outstanding work establishes a direct relationship between a single nucleotide substitution in a non-coding region and the expression of quantitative trait in a domestic animal. It is yet another example of the importance of introns and untranslated regions in regulation of gene expression, and it also shows that farm animals can help us to unveil the molecular basis of complex traits.

10.1242/jeb.00879

**Van Laere, A., Nguyen, M., Braunschweig, M., Nezer, C., Collette, C., Moreau, L., Archibald, A., Haley, C., Buys, N., Tally, M. et al.** (2003). A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. *Nature* **425**, 832-836.

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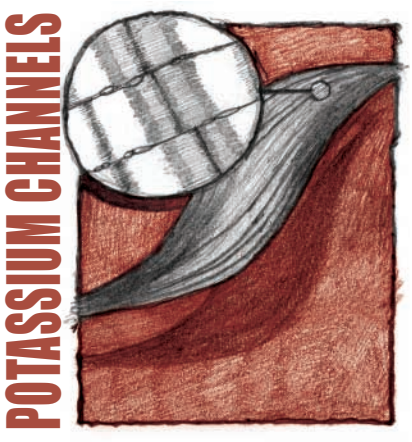


## POTASSIUM CHANNELS – WHY SO MANY?

With the sequencing and annotation of multiple genomes now complete, it is possible to survey whole families of genes at once *in silico*. One of the more remarkable findings is the sheer size of the potassium channel family – or families, because they are also remarkably diverse. This is in contrast to the channel families for the other major ions (sodium, chloride and calcium), each of which only have a few dozen representatives.

Why then are there so many potassium channels? In this paper, we begin to get an inkling. Salkoff and a large team of collaborators use reverse genetic techniques to dissect the potassium channels in the body wall muscle of the genetic model worm *Caenorhabditis elegans*. This may not seem like a smart choice for physiology, when the whole organism is only a millimeter long and has only a thousand cells! Nonetheless, there are several advantages. Firstly, each worm conforms to an invariant developmental plan, so every cell in the organism is known, and its cell lineage mapped. This allows 'identified cell' physiology; repeat experiments can be performed on the identical cell (rather than similar cells) in multiple individuals, so reducing experimental variability. Secondly, the genome is sequenced, so all potential genes (in this case, for the >70 potassium channels) are already known. Thirdly, the experimenters already knew, from systematic studies with worms transgenic for green fluorescent protein fused to promoters for different potassium channel genes, that only nine genes were likely to be of interest to them, with detectable expression in muscle. Fourthly, there is a wealth of existing mutants for various genes. And fifthly, it is possible to knock down the activity of individual genes by RNA interference, a technique that works nowhere better than in *C. elegans*. In fact, it is necessary merely to feed the worms with *E. coli* bacteria expressing RNAi from plasmids, and there are systematic panels of such *E. coli* freely available.

Using these techniques, it was possible to show that, of the channels known to be expressed in muscle, only SHAL and SHAKER normally carried significant current. So what were the others there for? The authors' answer is based on a single channel, SLO-2. This is normally not functional under physiological conditions. However, when the worm is exposed to hypoxic conditions (something that could be expected to happen frequently in the worm's natural soil-living habitat), the SLO-2 channel becomes the major potassium carrier in the muscle. The authors' plausible hypothesis is thus that the other, 'reserve', channels are also present to be invoked under different, exceptional physiological conditions, and that this conveys a sufficient selective advantage to justify the organism's investment in making channel proteins that are not normally used. Consistent with this, *slo* mutants are known to be hypersensitive to hypoxia. By extension, this argument can be extended to other gene families and to other organisms.

10.1242/jeb.00877

**Santi, C. M., Yuan, A., Fawcett, G., Wang, Z.-W., Butler, A., Nonet, M. L., Wei, A., Rojas, P. and Salkoff, L.** (2003). Dissection of $K^+$ currents in *Caenorhabditis elegans* muscle cells by genetics and RNA interference. *Proc. Natl. Acad. Sci. USA* **100**, 14391-14396.

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## STRESS UNDERLIES POPULATION DIFFERENCES IN KILLIFISH

Killifish habitats extend from the cold waters of Newfoundland to the relative warmth of Florida, and so individual populations inhabit waters with very different temperatures. However, killifish appear to migrate very little, so individual populations have probably adapted to their local thermal regime. Adapting to a new thermal environment can be stressful. While investigating genetic differences between populations, Patricia Schulte and others discovered an intriguing trend; many genes that differed between these populations in laboratory-acclimated fish were also genes that respond to stress. Yet, the physiological, biochemical and genetic mechanisms by which this species has adapted to its local habitat are unknown. Identifying relationships between genes that vary in expression between populations and the gene's physiological function may aid in understanding how these fish adapt to new environments. Therefore, in their current study, Daniel Picard and Schulte test the hypothesis that stress-responsive genes also differed in their expression between killifish populations.

Using molecular techniques, Picard and Schulte first looked at gene expression in the livers of killifish from northern and southern populations, both at rest and after chronic handling stress. Five putative stress-regulated genes were then used to test the relationship between the stress response and differences in gene expression in killifish populations associated with different thermal environments. These were glucokinase, glycogen synthase kinase, phosphoenolpyruvate carboxykinase, cRAF and serine threonine kinase, all of which are

important in carbohydrate metabolism and cell signalling. They also measured expression of warm-acclimation-related-protein, which has been implicated in the thermal acclimation of killifish and should therefore vary between cold and warm populations.

The team looked at gene expression patterns in response to stress and found that glucokinase, serine threonine kinase and cRAF were stress regulated in the southern population of killifish. But the genes were not affected by stress in northern killifish.

The expression patterns of these genes also differed between both populations in unstressed fish, supporting the idea that there is a relationship between the stress response and inter-population differences in liver gene expression. Although only 2–3% of genes had previously been found to vary in expression pattern between populations, by selecting for specific genes that are stress regulated, Picard and Schulte have increased this to 60%. Selecting for stress-regulated genes appears to enhance for genes that differ between populations.

Not surprisingly, warm-acclimation-related-protein varied in unstressed fish between both warm and cold populations. Warm-acclimation-related-protein expression was eight fold higher in southern killifish, helping to confirm its role in adaptation of fish to warmer waters.

This study demonstrates that differences in liver gene expression between populations of killifish may be related to the stress response, which may indicate a physiological mechanism by which these populations have diverged. Not all of the genes that responded to stress were different between populations, indicating a complex regulatory pattern underlying the link between stress and inter-population gene expression, rather than a simple correlation. Nevertheless, this study clearly highlights the importance of considering the role of stress in population genetic differences.

10.1242/jeb.00876

**Picard, D. J. and Schulte, P. M.** (2003). Variation in gene expression in response to stress in two populations of *Fundulus heteroclitus*. *Comp. Biochem. Physiol. A* **doi: 10.1016/51095-6433(03)00292-7**.

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## HARES STOCK UP ON FATTY ACIDS

Did your parents ever tell you that eating oily fish would make you clever? If so, you are probably aware that this particular old wives tale has now come true. And what magical ingredients do oils contain? The simple answer is fatty acids – fat molecules that are either saturated or unsaturated. Of the unsaturated fats, long-chain polyunsaturated fatty acids (PUFAs) are the most magical of all. Essential to human health, PUFAs are needed for building cell membranes, regulating the body's response to pain and maintaining a strong immune system. However, our bodies are unable to make these essential nutrients and we have to eat the right foods in order to obtain them. By regularly forking down fish, for example, we gain enough PUFAs to promote eye and skin health, reduce the risk of cardio-vascular disease and improve long-term memory. Your parents were right.

But what does this have to do with hares? As fellow mammals, hares have a similar need for PUFAs and a similar inability to synthesise them. Diet has a big influence on the fatty acid composition of their cell membranes. Recent studies on rats and humans suggest that muscle performance is reduced if muscle membranes don't contain enough fatty acids. Quite a serious issue for hares, which avoid predators by sprinting at speeds of up to 80 km $h^{-1}$.

Teresa Valencak and colleagues at the University of Veterinary Medicine in Vienna were intrigued by the idea that hares might safeguard their athleticism by keeping their muscles enriched with fatty acids. The researchers were particularly eager to discover if leg muscles contained especially high levels of PUFAs, and if PUFA contents varied with seasonal differences in diet.

Valencak and the team collected over 100 hares during one summer and two winters in Austria. The scientists dissected muscles from various locations in the hares' bodies and measured fatty acid contents in cell membranes. To their surprise, they discovered that cell membranes from both skeletal and heart muscles had a high degree of unsaturation with 66% PUFAs. Results from studies on other mammals report PUFA proportions between 36% and 54%. Valencak's work therefore reveals that hares boast the highest proportion of PUFAs reported in any mammalian tissue. The team also discovered remarkable consistency in the PUFA contents of different muscles and suggest that, by maintaining record levels of PUFAs in muscle cell membranes, hares ensure that PUFAs are available for all other body parts.

The team's work also revealed that the fatty acid composition of hare cell membranes changes with season. The proportion of PUFAs increased markedly in hares during winter months, largely at the expense of saturated fatty acids. The changes in the types of fats present at different times of the year indicate that hares have a specific winter need for certain types of PUFAs. The researchers suggest that high levels of these PUFAs may help the animals to keep warm in the winter cold.

Taken as a package, Valencak's study points to PUFAs as having a critical role to play in the functioning of hare muscle cells. In the knowledge that hares can run four times faster than similarly sized rodents, it will be fascinating to learn if their record PUFA content is linked to their swift speed. Could it be that fatty acids maketh the hare?

10.1242/jeb.00878

**Valencak, T. G., Arnold, W., Tataruch, F. and Ruf, T.** (2003). High content of polyunsaturated fatty acids in muscle phospholipids of a fast runner, the European brown hare (*Lepus europaeus*). *J Comp. Physiol. B* **173**, 695-702.

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MOTOR LEARNING

## GETTING TO THE HEART OF MOTOR LEARNING

For most animals, whether it's molluscs or mice, practice makes perfect. Learning to ride a bike (if you're human) or to catch a grasshopper mid-jump (if you're a robber fly) might not work perfectly the first time around but with a little perseverance the goal (and in some cases the meal!) might just be reached. This motor learning is central to performing the complex sets of co-ordinated movements that make up animal behaviour, and the 'memory' associated with motor learning seems to be stored in changes in the strength of connections between neurons. Although this idea appears to underlie motor learning in all animals, Kandel and co-workers now suggest that as well as knowing about connections between neurons it is also important to understand the intrinsic properties of the neurons themselves. Can these intrinsic properties affect animal's motor learning? To find the answer to this question we must look in an unlikely place: the heart.

At first, the heart may not appear to have much relevance to motor learning, but the electrical properties of neurons are not so different from those of the heart muscle cells (myocytes). In fact, many of the ion channels that allow ions in and out of heart myocytes are also found in neurons. Over 25 years ago, the hyperpolarization-activated, cyclic nucleotide-regulated nonselective cation (HCN) channels were discovered in heart myocytes, but more recent work has shown that they are also expressed in the mouse brain. One of these channels, HCN1, was expressed in one of the key structures involved in motor learning – the Purkinje cells of the cerebellum, suggesting a possible role in motor learning. But what are the effects of HCN1 on behaviours in which motor learning is essential?

Answering this question is no easy task. Kandel and co-workers not only made mutant mice with a deletion of the HCN1 channel but also targeted this deletion to different regions of the mouse brain. They then put the mutant and wild-type mice through a series of behavioural tests worthy of an assault course! These tests included swimming through a water maze and balancing on an accelerating rod that required complex repeated co-ordination of motor output as well as discrete behaviours such as an eye blink conditioning. When expression of the deleted HCN1 channel was targeted to the cerebellar Purkinje cells, the discrete eye blink conditioning was unaffected but the more complex behaviours, such as swimming and balancing, were impaired. Targeting the deleted HCN1 channels to the forebrain and not the cerebellum caused no impairment in these complex behaviours. This evidence suggests that HCN1 channels in the Purkinje cells play a vital role in motor learning.

Not content with their behavioural evidence for the role of HCN1 channels in motor learning, Kandel and co-workers extended the study to reveal the specific effects of these channels on the Purkinje cells themselves. Purkinje cells were injected with negative current pulses. Those cells lacking HCN1 channels took longer to recover to their previous activity level than their wild-type counterparts. This suggested that the HCN1 channels allow Purkinje cell activity to be independent of previous activity. Such a function would be critically important during complex repeated behaviours when Purkinje cells receive repetitive bursts of inputs from other neurons; without HCN1 channels, the Purkinje cells would still be recovering from one burst when the next one arrived.

This new study from Kandel and co-workers takes an unusual approach to motor learning in mice. It is likely that it will provoke many researchers to examine intrinsic neuronal properties and relate them to learning where previously they focussed on the relationship between neural connections and learning.

10.1242/jeb.00875

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