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

TEMPERATURE EFFECTS

WARMER NURSERIES, FITTER FISH

Climate change is warming our world and is causing larger and more frequent temperature fluctuations. This could be a serious problem for fish and other ectothermic animals – which do not regulate their body temperature – because they are at the mercy of their variable environment. However, adult and juvenile fish have the capacity to acclimatize to challenging temperatures, altering their physiology to improve function and performance. Yet, relatively little is known about how being exposed to temperature extremes affects embryonic fish and how these effects might persist in later life. Graham Scott from McMaster University, Canada, in collaboration with Ian Johnston from the University of St Andrews, UK, set out to discover how fish are affected by their early thermal history and the duo's findings were published in a recent issue of *PNAS*.

First, Scott and Johnston wanted to find out how the temperature experienced by zebrafish embryos affected their swimming performance later in life. Swimming performance is an excellent measure of overall performance in fish because they swim to escape predators, and to find food, mates and suitable spawning grounds. The duo raised zebrafish embryos at three temperatures found in the wild during the breeding season (22, 27 or 32°C) until they hatched, then reared all of the fry to adulthood at 27°C. The researchers then tested the swimming performance of these adult fish after transferring them back to 22, 27 or 32°C and found that the animals performed better at the temperatures that they had been exposed to as embryos. In contrast, when the researchers acclimated fish for long periods at more extreme temperatures (16 or 34°C), the fish raised at the warmer temperature as embryos performed best at both extremes, indicating that they were overall hardier fish.

Next, the researchers determined whether the differences in swimming performance after acclimation to extreme temperatures were related to differences in muscle size and fibre type. Slow and intermediate muscle fibres are used for sustainable aerobic swimming, while fast muscle fibres are used for sprinting. Measuring the overall size of the fish's trunk muscles and using antibodies and histological stains to identify the slow and intermediate muscle fibre types, Scott and Johnston found that the fish that swum best at the hot extreme had remodelled their muscles to have a higher proportion of slow and intermediate muscle fibre types, while the fish that swum best at the cold extreme grew larger trunk muscles with more slow muscle fibres.

Lastly, the team wanted to determine which genes were responsible for the fish's enhanced swimming performance at extreme cold temperatures. They measured gene expression in the swimming muscles of adult zebrafish using whole-transcriptome shotgun sequencing (a novel technology for measuring gene expression). Whereas all of the zebrafish altered the expression of a large number of genes important for energy metabolism, oxygen supply and muscle remodelling after cold acclimation, the hardy fish raised at the warmest embryonic temperatures had an exaggerated response for several of these genes, explaining why these fish swum better and remodelled their muscle at extremely cold temperatures. The results also supported the researchers' previous finding that embryonic temperature can have long-lasting effects on physiology and swimming performance that persist into adult life.

This research suggests that the effects of environmental temperature in early life stages may differ from the effects on adult fish. Embryos raised in a warmed environment may sometimes grow to become hardier and better able to deal with both high and low temperatures – which is good news for fish facing an uncertain climate future!

10.1242/jeb.064428

Scott, G. R. and Johnston, I. A. (2012). Temperature during embryonic development has persistent effects on thermal acclimation capacity in zebrafish. *Proc. Natl. Acad. Sci. USA* **109**, 14247-14252.

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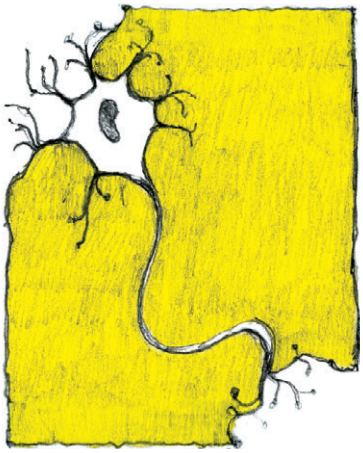
VISION



THE FLY EYE: A MODEL FOR RETINAL DEGENERATION

According to the World Health Organization, age-dependent macular degeneration is the most common cause of blindness in industrialized countries. Fortunately, the fruit fly's compound eyes, each containing ~800 eye units, offer an exceptional model in which to uncover the molecular basis of retinal degeneration. Housed within each of these units are the eight photoreceptor cells that serve as the functional core of photosensation. Each photoreceptor cell contains an array of densely stacked membranes called rhabdomeres – the light-gathering structures where the rhodopsin (Rh) proteins, responsible for visual signalling, reside. In addition to light detection, Rh1 has been shown to be crucial during development for morphogenesis and differentiation of photoreceptor cells. To better understand the role of rhodopsin in retinal degeneration, Inga Kristaponyte, Yuan Hong, Haiqin Lu and Bih-Hwa Shieh from Vanderbilt University, USA, used microscopy to monitor the turnover of Rh1 in live *Drosophila*. Using fluorescently tagged Rh1, the team found that mutant flies that were unable to produce sufficient Rh1 displayed age-dependent degeneration and had fewer rhabdomeres. Although initially present, the rhabdomeres in mutant flies became smaller and eventually disappeared, confirming that Rh1 is important for the survival of photoreceptor cells in adulthood.

Signaling proteins in photosensation are commonly modulated by phosphorylation, which allows protein partners to couple transiently and tags proteins for degradation or recycling. Mutations that result in retinal degeneration have been shown to arise from the impairment of reversible phosphorylation of rhodopsin. In the vertebrate visual system, members of the arrestin (*Arr*) gene family are known to interact with phosphorylated and photoactivated rhodopsin. In the *Drosophila* visual system, *Arr2* is crucial for the inactivation and degradation of photoactivated (phosphorylated) Rh1. Knowing that in vertebrates, arrestin binds phosphorylated rhodopsin to inactivate it and then targets it for internalization, recycling or degradation, Shieh's team wondered whether the same is true for the *Drosophila* visual system. Generating mutant flies with Rh1 proteins that either lacked the C-terminus or carried substitutions at the putative phosphorylation sites, the team tested whether phosphorylation is required for Arr2 to interact with rhodopsin. They found that, contrary to the situation in vertebrates, *Drosophila* Arr2 can still bind to unphosphorylated Rh1. Thus, phosphorylation of Rh1 at its C-terminus is not needed for the Arr2 interaction.

Given that flies with insufficient Rh1 display age-dependent retinal degeneration, the researchers decided to test whether they could protect photoreceptor cells from degeneration by expressing a phosphorylation-deficient form of Rh1, which cannot be degraded. As Arr2 and Rh1 interact, Shieh's team co-expressed fluorescently tagged arrestin and phosphorylation-deficient Rh1 in mutant flies that suffer the insect equivalent of macular degeneration. They then imaged the live retinas to find out where the proteins were located in the rhabdomere and whether they could protect the photoreceptors from damage. The team found that Arr2 moves from a uniformly cytoplasmic distribution in the photoreceptors to a subcellular localization, but only when bound to Rh1, and by co-expressing the two proteins, the researchers were able to completely block retinal degradation. This led them to conclude that the loss of Rh1 causes the loss of rhabdomeres, and the expression of Arr2 and Rh1 together protected the rhabdomeres from degradation.

Shieh's team propose that future therapies explore the use of kinase inhibitors that selectively reduce the phosphorylation of rhodopsin – and hope their work will offer novel alternatives in treating arrestin-dependent retinal degeneration.

10.1242/jeb.064469

Kristaponyte, I., Hong, Y., Lu, H. and Shieh, B.-H. (2012). A role of rhodopsin and arrestin phosphorylation in retinal degeneration of *Drosophila*. *J. Neurosci.* **32**, 10758-10766.

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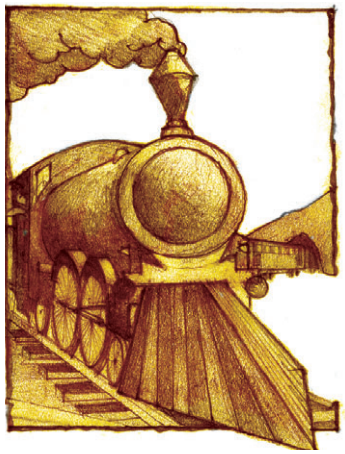
GAIT SELECTION



THE GENE THAT GATES HORSE GAITS

Over the course of this summer's Olympics, the full spectrum of human gaits has been on display, from Ding Chen's wiggly high speed walk to Mo Farrah's effortless lope and Usain Bolt's powerful sprint. All animals have evolved a spectrum of locomotion patterns, or gaits, in order to maximise their efficiency at different speeds. However, we know very little about how these different motor patterns are selected within the nervous system.

All horses have at least three gaits: walking, trotting and galloping, in order of increasing speed. However, some – including harness-racing horses and some Icelandic breeds – have gained additional gaits such as pacing, in which the two legs on one side of the body are moved in unison. What allows these animals to move differently from the rest? A collaborative study by teams in Sweden, Iceland and the USA, recently published in *Nature*, sheds light on this phenomenon by focusing on the genetic differences between various horse breeds with different numbers of gaits.

By comparing the genomes of hundreds of horses of various different breeds, they found that nearly all animals that have an additional gait ('gaited' horses) carry two copies of a mutation in a transcription factor gene, *DMRT3*. This mutation, which is never found in 'non-gaited' animals, results in the production of a truncated version of the transcription factor protein that presumably does not function normally, and allows the horse to perform additional gaits. How does the mutation lead to this ability?

The authors reasoned they would be better off looking at mice to address this question: the neurons that underlie their locomotion have been carefully described

and can be experimented on with relative ease. Mice that lack *DMRT3* altogether have largely normal motor coordination, but have problems running at high speeds. Electrophysiological recordings from their spinal cords showed that the neural circuit that is responsible for generating rhythmic muscle contractions during locomotion is disturbed, firing largely uncoordinated bursts of action potentials. This suggests that DMRT3 is required in mice for either the generation or the continued function of the circuitry that produces coordinated movements. The researchers needed to know more about the identity of the *DMRT3*-expressing cells in order to understand what might be going on.

Using different labelling techniques, the authors found that these cells make connections between the left and right halves of the spinal cord, and that they have an inhibitory function. In *DMRT3* mutants, the identity of these cells is altered, resulting in fewer connections between the left and right sides of the spinal cord being made. These results are consistent with the idea that DMRT3 is necessary for the coordination between limbs, although the exact role of the *DMRT3*-expressing neurons remains unclear.

This study has identified a gene that plays an interesting role in determining how different modes of locomotion are generated. It is involved in the ability to produce alternative gaits, possibly by regulating the development of the circuitry in the spinal cord that generates these movements. It will be very interesting to see how the neurons that express this gene interact with the rest of the locomotor network in order to coordinate gait.

10.1242/jeb.064444

Andersson, L. S., Larhammar, M., Memic, F., Wootz, H., Schwochow, D., Rubin, C.-J., Patra, K., Arnason, T., Wellbring, L., Hjälm, G. et al. (2012). Mutations in *DMRT3* affect locomotion in horses and spinal circuit function in mice. *Nature* **488**, 642-646.

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BEETLES USE BUBBLES TO STICK UNDERWATER

Geckos have attracted attention for their sticky-but-dry toe-pads, capable of clinging to smooth surfaces in the air or under water. But who would have guessed that land-dwelling beetles are also remarkably good at sticking underwater? Terrestrial beetles' feet are a model of excellent wet adhesion, using a combination of tiny projections, called setae, and oily secretions to adhere to dry surfaces *via* capillary action. Naoe Hosoda of Japan's National Institute for Materials Science, and Stanislav Gorb of the University of Kiel, Germany, were curious as to how land-dwelling beetles might also be able to adhere to submerged surfaces, such as leaves after a heavy rain.

One of the main challenges to oil-based underwater adhesion is that water itself is sticky and once a structure has got wet, there is no air to generate surface tension at the oil–surface interface. Hosoda and Gorb thought that leaf beetles' setae might trap air bubbles around their feet, effectively keeping them dry and maintaining the ability of oily setae to stick to a submerged surface.

To see just how well beetle feet could adhere when underwater, the authors tethered specimens of *Gastrophysa viridula*, the green dock beetle, to small force transducers and submerged them in a tank. As the beetles crawled underwater, the authors observed both the force with which the beetles could pull and the appearance of bubbles beneath the beetles' feet.

Microscopic observation revealed structured air bubbles trapped around the beetles' setae and the smooth substrate. These bubbles moved with the foot while the beetle walked underwater. Hosoda and Gorb then added surfactant to the water to prevent bubbles from forming. With no bubble to hold them down, the leaf beetles floated to the surface, demonstrating that the bubble was essential for underwater adhesion.

Inspired by the beetles' adhesion, Hosoda and Gorb built a underwater adhesive surface, inspired by the beetle's sticky feet, covered with pillar-like outgrowths resembling the beetles' setae. Using the manufactured surface, the authors demonstrated that outgrowths are necessary to trap the air that supports bubble adhesion. Furthermore, the structured material required a substantial pull-off force to be separated from the submerged surface. In fact, the bio-inspired polymer's attachment to submerged hydrophobic surfaces was as good as its adhesion to the same surfaces in air. Though the size of this adhesive force changed depending on the contact angle of the water meeting the foot, and the properties of the substrate, the beetle-mimicking structure generated at least some adhesion under many different conditions.

The authors are hopeful that the beetle's approach to underwater adhesion may lead to new bubble-based bio-inspired adhesives. Indeed, the authors' own biomimetic adhesive shows promise for engineering applications. Terrestrial leaf beetles may have a lot more to teach us about being sticky when wet.

10.1242/jeb.064477

Hosoda, N. and Gorb, S. N. (2012). Underwater locomotion in a terrestrial beetle: combination of surface de-wetting and capillary forces. *Proc. R. Soc. Lond. B* doi: 10.1098/rspb.2012.1297.

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