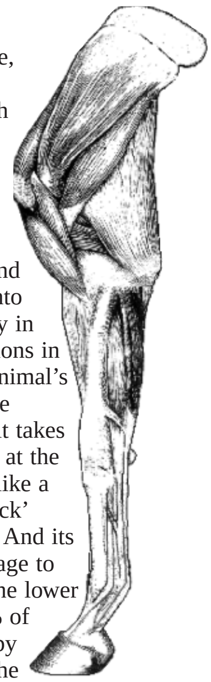
Inside JEB, formerly known as 'In this issue', is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

HORSE'S LEG SET TO SPRING



Watching a pack of racehorses thundering towards a finishing line, you're probably concentrating on which animal gets its nose across the line first. But the real action is going on closer to the turf. As the horse's hoof hits the ground and transfers the weight onto the leg, it stores energy in the springy flexor tendons in the lower half of the animal's leg, ready to launch the animal forward when it takes the weight off its hoof at the end of the stride; just like a like a 'child's pogo stick' explains Alan Wilson. And its little wonder that damage to the flexor tendons in the lower limb accounts for 50% of the injuries sustained by race horses; strain in the long tendons can be three times greater than the strain on a running human's tendon! But even if we can't 'pogo' along on our less elastic limbs, we can change the relative stiffness of our legs to bounce us from one step into the next as we speed up from a walk to a run. Could horses vary their limb stiffness too? Wilson, and his student Polly McGuigan, began putting horses through their paces (p. 1325).

Although there was little question that the horse's lower leg was powered by elastic energy, any doubts the team might have had were dismissed when they began testing the way the limb responded as they compressed the leg and stretched the tendons. They applied a force to the top of an animal's leg and amazingly, the relaxed leg suddenly sprung into shape as the tendons stretched! And as they varied the force on the limb, the tendons lengthened, just like a spring.

Wilson explains that there are three springy tendons in the lower half of the horse's limb. But only one of the tendons has enough associated muscle to possibly adjust the tendon's stiffness, the deep digital flexor, which stretches down the back of the leg. McGuigan and Wilson wanted to know if the small muscle was powerful enough to change the leg's stiffness as the horse gears up from a walk to a gallop. First they monitored the animals' leg movements as they walked, trotted and cantered on a treadmill, measuring how the lower leg's length varied during a stride. Amazingly, when the horse galloped, it compressed the leg by 12 cm, tensioning the legs' tendons just like a pogo stick's springs.

McGuigan then videoed horses as they walked, trotted and cantered across a force plate, recording the compression in the leg and correlating it with the force on the limb. If horses could adjust the stiffness of the deep digital flexor, then the relationship would vary as the animal changed gait. But no matter what speed the horse hit the force plate, the leg's springiness remained unchanged; the horse couldn't alter the deep digital flexor's stiffness. Which doesn't bode well for horses trotting on the 21st Century's hard road surfaces.

So what the horse has gained in efficiency from its incredibly elastic lower leg, it has lost in flexibility to roam over different surfaces. But Wilson is optimistic that his work will eventually pay off, if he can design a cushioned racing surface that will make getting to the finishing line a less jarring experience.

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McGuigan, M. P. and Wilson, A. M. (2003). The effect of gait and digital flexor muscle activation on limb compliance in the forelimb of the horse *Equus caballus*. *J. Exp. Biol.* **206**, 1325-1336.

DUNG BEETLES TURN IN WINGS FOR A LONG, DRY WALK

For most dung beetles, wings are pretty handy when they need to get to a steaming pile of dung in a hurry. But not having wings has allowed one species that specialises in elephant dung to evolve a pretty nifty respiration system that might help it survive in arid environments. Lacking wings, the flightless dung beetle that lives in the Addo Elephant Park has a tightly sealed elytra (wing case) that forms a cavity where the wings would normally be. According to Marcus Byrne and Frances Duncan of the University of Witwatersrand, South Africa, this cavity may help the beetle conserve water (p. 1309).

This isn't a new theory. Beetles breathe by pumping air in and out of their bodies through small valves called spiracles. The problem is that when these valves are open, the beetle can loose valuable water by evaporation. All flightless beetles were thought to tackle this problem by breathing in through the spiracles at the front of their bodies, and out through the ones in the subelytral cavity at the back, lifting the elytra to release the carbon dioxide waste. 'This is a bit like breathing into your cupped hands' explains Byrne, because as the cavity becomes humid it helps prevent water diffusing out of the beetle.

But when the team looked to see whether the beetles exhaled through their rear ends 'instead of air coming in at the front and out at the back, it came in and out at the front' says Duncan! Maybe the insects didn't use the subelytral cavity during respiration at all. The team decided to see by monitoring the gases inside the subelytral cavity as the beetle breathed.

First they fitted a rubber skirt around the beetle's waist so that they could monitor the gases released from the insect's front and back ends. Then they drilled tiny holes in the beetle's elytral case, to monitor carbon dioxide and oxygen levels inside the cavity, and to see how the spiracles below were involved in breathing.

What they found was a 'rather weird respiratory cycle'. The spiracles within the subelytral cavity respired cyclically, keeping oxygen below normal atmospheric levels, but raising the level of trapped CO_2 until the beetle pumped the gas out of the elytral cavity and back through its body to be released at the front end. So the subelytral cavity behaves like a CO_2 store, but why might this help the beetle?

Byrne and Duncan suggest that by storing CO_2 outside its respiratory system, beneath the elytra, the beetle might be able to exhale less often, reducing the time that its spiracles are open to the environment and minimising water loss. And the low concentration of oxygen inside the insect's CO_2 tank may also help to draw oxygen in around the edges of the elytra, allowing the insect to hold its breath for longer.

Winged beetles have the advantage over flightless beetles in searching for dung, but the remarkable respiratory system of the flightless dung beetle may give it the upper hand in arid areas. Nevertheless, it's still a long walk to the elephant takeaway when you've got no wings!

10.1242/jeb.00269

Byrne, M. J. and Duncan, F. D. (2003). The role of the subelytral spiracles in respiration in the flightless dung beetle *Circellium bacchus*. *J. Exp. Biol.* **206**, 1309-1318.

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HOW HEARTLESS LARVAE HANDLE HYPOXIA

For most creatures, life without a heart is completely unthinkable. Once an animal develops beyond a certain size, it's impossible for oxygen to reach every cell in the body simply by diffusion. But some water-born larvae are tiny enough to survive perfectly without a cardiovascular system for almost two weeks before their gills begin collecting oxygen for distribution through the body. Which makes these tiny larvae the perfect animals to study if you want to untangle the physiology behind cardiovascular development. Thorsten Schwerte is fascinated by cardiac development and is systematically teasing apart the processes of development, but he adds that the system is so complex that he can only focus on one effect at a time. In the current issue of *J. Exp. Biol.*, he reports how the tiny larvae's developing cardiovascular system responds to hypoxia during the early stages of life (p. 1299).

However, before he could begin following the youngsters' progress, Schwerte decided to design a completely non-invasive technique for visualising blood flow; he turned to the human brain for inspiration. Schwerte explains that we simply can't see some stationary objects, but they instantly become visible when they move. He decided to use microscopic digital imaging to track blood cells moving through the tiny fish's bodies, and to measure blood cell concentrations and locations in the larvae's tissue. First, he anaesthetised the fish, and immobilised individuals in agarose where they rested peacefully as he collected video images of the larvae's cardiovascular system. By collecting enough images, he knew that he could build up a complete view of the fish's vasculature, as well as tracing the path of individual blood cells, to measure their velocity and the total number of cells moving through the tiny blood vessels. With all of these measurements Schwerte, could accurately measure the tiny creatures haematocrit level directly! And he is delighted that after their incarceration in agarose the fish recover quickly, before retiring and passing out the rest of their days in local aquaria.

After months of writing software to interpret the thousands of digital images that he collected, Schwerte could reconstruct the larvae's cardiovascular system, and he began looking for differences between the hyopxic fish's cardiovascular system and fish reared under normoxia. He was amazed to see that the tiny larvae respond to the hypoxic conditions when they were only 5 days old, almost 5 days earlier then oxygen begins to be limited by diffusion. Schwerte thinks that this head start probably gives the youngsters an advantage; he explains that this could be a case of 'opening the parachute before you hit the ground'. And when he looked at the distribution of blood cells through the larvae's bodies, not only had the larvae increased their haematocrit levels but they had also shunted their blood supply from the gut to the muscles. Schwerte thinks that this might be a form of the 'fight or flight' response, diverting oxygen-carrying cells to the tissue that might help the tiny larvae escape and catch their breath.

Schwerte admits that 'it's astonishing what happens, these processes happen at very early stages in development', and he believes that 'the zebra fish could form a vital bridge between molecular biology and physiology'. After all, it's not enough to know that a gene plays a role in a disease, it's important to know what the physiological consequences are, and with thousands of zebra fish mutants to study, Schwerte is optimistic that he could soon begin to understand the physiology behind some human cardiac disorders.

10.1242/jeb.00268

Schwerte, T., Überbacher, D. and Pelster, B. (2003). Non-invasive imaging of blood cell concentration and blood distribution in zebrafish *Danio rerio* incubated in hypoxic conditions *in vivo*. *J. Exp. Biol.* **206**, 1299-1307.

INKA CELLS GET INSECTS OUT OF A TIGHT SPOT

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When a growing insect shrugs off its restrictive outer layer, ready to replace it with a looser fitting cuticle, the process is tightly choreographed by hormone peptides that trigger a set of pre-programmed manoeuvres to rid the insect of the unwanted layer. Dusan Žitňan and colleagues had already studied ecdysis in fruit flies and two moth species, and discovered that two of the key hormones involved in the three insects' life changes are released by a group of cells in their tracheas, called Inka cells. But would other insects use the same mechanism for triggering their own instar-to-instar transition?

Working with an international team of researchers, Žitňan began testing 26 species, ranging from primitive silverfish and dragonflies up to relatively advanced insects such as ants, for evidence of peptides that might trigger ecdysis (p. 1275). They tested Inka cells from each insect's tracheae, and found that the cells produced hormone peptides that were structurally similar to the moth and fruit fly ecdysis hormones. The team also injected silk moth larvae with tracheal extracts from several different insects to see whether the peptides could produce ecdysis; the silk moths began shedding their skins, proving that the extracts contained ecdysis-triggering hormones. But when the team looked at the number, size and distributions of Inka cells across the species, each insect had its own unique cell pattern and distribution, even though the hormones they produce are closely related.

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Žitňan, D., Žitňanová, I., Spalovská, I., Takác, P., Park, Y. and Adams, M. E. (2003). Conservation of ecdysis-triggering hormone signalling in insects. *J. Exp. Biol.* **206**, 1275-1289.

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