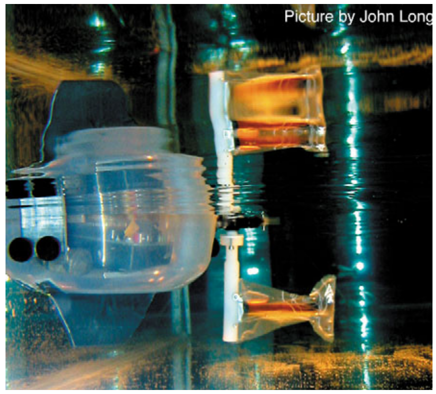
Inside JEB is a twice monthly feature, which highlights the key developments in the *Journal of Experimental Biology*. Written by science journalists, the short reports give the inside view of the science in JEB.

Inside JEB

ROBOTS' SWIMMING TEST



Picture by John Long

Most vertebrates' early ancestors were a lot squashier than they are today – they had a primitive backbone, called a notochord, but no vertebrae. Since the 1930s scientists have thought that vertebrae evolved to make bodies and tails stiffer, which would have helped these ancient predecessors swim more powerfully by attaching muscles to solid vertebrae to produce more force. John Long at Vassar College and his research team are interested in vertebrae evolution, so they came up with a novel way to test this idea using both robots and computer simulations (p. 4732).

First, the team built experimental robots, three one-eyed light-sensitive 'tadpoles' called Tadros, complete with a mini-computerised brain that controls their movement. The team then modified Tom Koob's method of making artificial tendons out of gelatine to manufacture tails of different lengths and stiffnesses. Wondering how these tails would help the Tadros to swim, they entered the robots in a swimming gala. Placing the three Tadros on one side of a tank, they trained their cyclops eyes on the target: a light suspended above the tank on the other side. The team measured the Tadros' speed and time as they raced toward the light, and their ability to maintain position under it, to rank their overall swimming performance.

Next, the team wondered what would happen if the three Tadros could mate with each other and produce the next generation, complete with new tails; would better swimmers have offspring with stiffer tails? They turned to an algorithm in a computer to model the process of genetic mixing that occurs during mating, to produce the next generation of Tadro tails, allocating the most successful tail the greatest mating success and poorer swimmers less luck. The final product of the algorithm was the stiffness and length values for the second generation of tails, which the team made and attached to the Tadros for the next swimming gala, starting the evolutionary cycle all over again.

The team showed that over 10 generation cycles, stiffer tails evolved as swimming performances improved. In generations where one or two Tadros did very well and were successful in mating, there was a big improvement in the next generation's swimming performance. They swam faster to the light target, maintained position more reliably and wobbled less. Tail stiffness went up too, showing that a stiffer tail is important for improving swimming performance.

However, Long says, their analysis shows that increasing tail stiffness only accounts for 40% of the improvement in swimming performance, meaning that other factors are involved. The next step, he adds, will be to find out what these factors are. He's planning a nasty surprise for the next generation of Tadros – adding a predator to the tank during the swimming competition, to see how this affects their swimming performance, and their tail evolution.

10.1242/jeb.02621

Long, J. H., Jr, Koob, T. J., Irving, K., Combie, K., Engel, V., Livingston, N., Lammert, A. and Schumacher, J. (2006). Biomimetic evolutionary analysis: testing the adaptive value of vertebrate tail stiffness in autonomous swimming robots. *J. Exp. Biol.* **209**, 4732-4746.

FLIES ON THE TURN

Fruit flies rely on a plethora of sense organs to keep them airborne and prevent crashes: if they see an object looming in their visual field on a collision course, they execute a 90° turn, called a saccade, in a lightning fast 70 ms to get themselves out of trouble. They also depend on short, stubby, modified hind wings called halteres to detect rapid changes in their body's orientation in space. Researchers already know that feedback from the eyes and halteres complement each other to keep a fly aloft – the eyes respond to slower and smaller changes in a fly's body orientation while the halteres respond rapidly to larger and quicker changes. John Bender and Michael Dickinson at Caltech want to know more about how feedback from sense organs affects a fly's saccades. Knowing that halteres respond to rapid changes, they set out to discover whether feedback from the halteres affects the size and speed of fly's saccades, and whether vision also plays a role (p. 4597).

First the team had to create an apparatus that would allow flies to perform saccades freely. Modifying an idea to tether a fly attached to a wire filament with magnets, first tried out by Martin Heisenberg and Reinhard Wolf, Dickinson and Bender built a simpler magnetic tether. Each fly had a steel pin attached to its back, and the pin's end sat in a small depression in a minute block attached to a magnet fixed directly above the fly. Flies could turn freely in the low friction set-up, and their every move was captured by a camera filming at over 500 frames s^{-1}.

Before testing the feedback from halteres, Bender says, the team had to confirm that visual feedback did not affect the speed and size of saccades. They placed tethered flies inside a cylinder, lined with light-emitting diodes (LEDs) displaying a striped pattern on the inside. Each time a fly turned, the team moved the stripes by turning LEDs on and off in a specific sequence. The stripes were moved either in the same direction of the turn or in the opposite direction, to trick the flies into thinking they had turned more or less than they really had, and adjust their saccades accordingly. They found that the flies made no adjustment to their saccades when the stripes were rotated in either direction. This confirmed that visual feedback did not play a role, so the team then turned to the halteres.

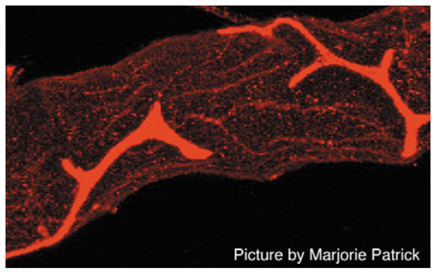
To change feedback from the halteres, they stuck a small blob of glue on the end of each one, which approximately doubled their weight. 'This was pretty tricky', says Bender, as the tip of each haltere is similar in size to the sharp end of a pin. When the halteres were heavier, the angle of saccades to the left and right were smaller, and saccades were a slower speed. Removing the left haltere to reduce its mass increased the angle and speed of saccades, both to the left and right, showing that feedback from the halteres does play a role in controlling a fly's saccades. Although, Bender adds, they're not sure of the exact mechanism yet.

Finally, Bender and Dickinson wondered if flies' halteres could compensate for changes to saccades caused by clipping the wings, affecting flight aerodynamics. While tethered flies with clipped wings altered their wing movements in order to maintain a stable heading, saccades were smaller. When they plunged flies with clipped wings into darkness, their flying got a lot worse, which also occurs in flies with intact wings. The team suspect that in this situation feedback from the eyes is needed to keep flies on the straight and narrow, and that feedback from the halteres is not precise enough to compensate for the effects that changes to the wings have on saccades, as they can't respond to slow changes in body orientation.

10.1242/jeb.02622

Bender, J. A. and Dickinson, M. H. (2006). A comparison of visual and haltere-mediated feedback in the control of body saccades in *Drosophila melanogaster*. *J. Exp. Biol.* **209**, 4597-4606.

MOSQUITOES KEEP THE BALANCE



Picture by Marjorie Patrick

A larval mosquito floating around in its watery home has a tough job to do, sucking up yummy nutrients from the rotting plant material it feeds on, while maintaining just the right balance of body water and salts. Adult females face a different problem: they must extract nutrients from blood meals, yet rid themselves of excess salts and water. Both adults and larvae rely on ion pumps called ATPases in the inner and outer membranes of the alimentary canal to help maintain the water and salt balance in their bodies. However, scientists aren't sure how important two types of ATPase (Na^+/K^+ and H^+-ATPase) are in this process. Marjorie Patrick and her colleagues at the University of California, Riverside, wanted to understand more about the roles of ATPases in larval and adult mosquitoes, but first they needed to know exactly where the ATPases are located. Using gene and protein expression techniques, they set out to describe where the two ATPases are found in the alimentary canal in larval and adult mosquitoes (p. 4638).

First, the team identified where Na^+/K^+-ATPase and H^+-ATPase genes were being expressed, using a technique known as RT-PCR. 'RT-PCR asks a simple question: is a gene on, yes or no?', says Patrick. They found that both ATPase genes were transcribed, producing mRNA in all the alimentary canal tissues involved in maintaining water and salt balance, in both larvae and adults. Having shown that the genes were being transcribed in all the tissues, the team needed to show where the mRNA is translated into functional protein, in this case the membrane pumps. To do this they turned to tissue-staining techniques and confocal microscopy, which show where proteins are in the cell. Having delicately extracted the alimentary canal tissue from larvae and adults, or sectioned whole larvae and adults, the team bathed the tissue samples in solution containing fluorescently labelled antibodies, designed to stick to their target ATPase. They then examined the colourfully glowing tissues under the microscope, which lit up where Na^+/K^+-ATPase and H^+-ATPase were pumping away in the tract's inner and outer epithelial membranes.

In both larvae and adults, Patrick says the team found a surprising result, which hadn't been seen before, in the two cell types that contribute to ion secretion in the Malpighian tubules, the insect equivalent of the kidney. They found Na^+/K^+-ATPase in cells called stellate cells: the staining revealed the cells had extensive finger-like projections wedged between the other cell type, the principal cells. These glowed with H^+-ATPase. Also, there was a difference in ATPase position in the larvae and adult midguts. Both ATPases in larval midgut were found in the inner and outer membranes, but the position of both switched between inner and outer membrane down the whole midgut's length. In the adults, this switching didn't happen, and both ATPases were distributed evenly.

Patrick speculates that these differences are due to the different lifestyles of larval and adult mosquitoes, but needs to do more experiments to be sure. The next step, she says, is to work out what the ATPases do in their different positions along the alimentary canal. She is planning to challenge mosquitoes' ability to maintain water and salt balance by giving adults a slap-up meal and changing the chemical composition of the larvae's aquatic environment to see how different parts of the alimentary canal help maintain the status quo.

10.1242/jeb.02623

Patrick, M. L., Aimanova, K., Sanders, H. R. and Gill, S. S. (2006). P-type Na^+/K^+-ATPase and V-type H^+-ATPase expression patterns in the osmoregulatory organs of larval and adult mosquito *Aedes aegypti*. *J. Exp. Biol.* **209**, 4638-4651.

LOCUSTS FEEL THE HEAT

LOOKS LIKE THOSE OVER-ACHIEVING 16-HOUR A DAY LOCUSTS JUST CAN'T STAND THE HEAT!

Locusts are used to sweltering temperatures, but sometimes their desert home is just too hot to handle. Corinne Rodgers and her colleagues at Queen's University, Ontario, are keen to know more about how locusts cope when the temperature rockets. They already knew that locusts handle extreme temperatures better when they've had a heat shock (a blast of higher temperatures) beforehand, and that daylength also influences how insects respond to heat. So, they wondered, how will locusts raised under two different daylengths respond to increased temperatures after a heat shock? To find out, the team first heat shocked locusts raised under 12 hours or 16 hours of daylight per day, then examined their ability to maintain a breathing rhythm as the temperature rose to a sizzling 45°C. 12 h locusts kept their cool: they maintained a stable breathing rhythm at higher temperatures than 16 h locusts, and when the rhythm broke down in extreme heat, 12 h locusts recovered quicker when the temperatures dropped again (p. 4690). Daylength, and heat shock, are important in helping locusts cope when the temperature soars.

10.1242/jeb.02624

Rodgers, C. I., Shoemaker, K. L. and Robertson, R. M. (2006). Photoperiod-induced plasticity of thermosensitivity and acquired thermotolerance in *Locusta migratoria*. *J. Exp. Biol.* **209**, 4690-4700.

Laura Blackburn
laura@biologists.com