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

CLICKING CATERPILLARS


Picture by Sarah Brown

Interested in how caterpillars use acoustics to communicate, Jayne Yack of Carleton University, Canada, noticed that the literature was littered with anecdotal reports of clicking caterpillars. But it wasn't until she was approached by George Boettner from the University of Massachusetts, who was rearing a species of clicking silk moth (*Antheraea polyphemus*) caterpillar, that Yack decided to explore further why and how caterpillars click (p. 993).

Yack and student Sarah Brown started out by pinching caterpillars' heads with forceps to induce clicking, while filming their movements and recording the clicks. They found that caterpillars click when they close their mandibles, which are covered with serrated, tooth-like ridges. Yack suspects that these toothed edges scraping over each other produce the distinctive clicks. After clicking, the caterpillars often regurgitated a dark brown liquid. 'The clicking is a warning signal, telling a would-be predator that the caterpillar is about to regurgitate a bitter liquid' says Yack. This response wasn't unique to the silk moth caterpillars: many other species silkmoths and hawkmoths tested clicked too.

When the team analysed the clicks in more detail, they found they lasted around 25 ms each, and were quite loud close up, between 58–79 dB. 'They were very short and don't carry long distances, ideally suited to a short-distance warning' says Yack, they 'wouldn't attract other predators'.

To further test their idea that clicking was a warning signal for regurgitation, the team monitored caterpillars' defensive behaviour after they had pinched them once, twice or five times. They found that more pinches caused more caterpillars to click, and to click for longer. More pinches increased regurgitation as well. 'Producing a chemical defence is costly' says Yack, 'so you'd give a warning first'. However if the pinches keep coming, the caterpillars will regurgitate as a last line of defence.

Having shown that caterpillars clicked and regurgitated when pinched with forceps, the team wanted to test whether they would behave in the same way when pecked by a predator, in this case a domestic chick. Chicks peck more forcefully than pinching with forceps and this affected the caterpillars' response. 'The caterpillars clicked more often' explains Yack, 'they also regurgitated more'. Many of the caterpillars regurgitated at the same time as clicking, and all of them survived the attacks. Although, researchers will need to do more experiments to determine how the caterpillars' defences are affecting the chicks, Yack explains.

Finally, to determine how palatable regurgitant was to invertebrates they soaked meal worm segments in the brown liquid and measured how long it took ants to eat or reject the worms. The ants took much longer to accept the food covered in regurgitant over uncoated food. When mice were given a choice between standard food and food soaked in regurgitant, they preferred the untainted food, showing that the regurgitant is unpalatable to vertebrates, too. 'If the regurgitant was lethal and completely disgusting, it's likely that the caterpillars would advertise this by using a long distance visual cue, like bright colouration' says Yack, who is planning to study the regurgitant's unpalatable qualities in more detail. These caterpillars are probably relying on cryptic colouring to keep them hidden, clicking and regurgitating only when they are discovered.

10.1242/jeb.02748

Brown, S. G., Boettner, G. H. and Yack, J. E. (2007). Clicking caterpillars: acoustic aposematism in *Antheraea polyphemus* and other Bombycoidea. *J. Exp. Biol.* **210**, 993-1005.

MODULATING LOBSTER LIMBS

The lobster is an ideal animal for researchers to study if they are interested in neural control: large and robust, these creatures have very few neurons controlling the muscles which move their limbs. The transmission of signals from neuron to neuron and from neuron to muscle is very dependent on temperature, so scientists are keen to know what happens to signal transmission at different temperatures. This is particularly relevant for the lobster, a cold-blooded crustacean that lives at temperatures ranging from a chilly 0°C to a

relatively balmy 20°C. But it's not just temperature that affects signal transmission: chemicals called neuromodulators also play a role and can alter muscle function as well. It is the combination of the effects of temperature and the neuromodulator serotonin that interested Mary Kate Worden and colleagues at the University of Virginia, who investigated the role that they play in affecting the function of a lobster's neurons and muscles (p. 1025).

The team chose to investigate one muscle in a lobster's walking leg, the dactyl opener muscle. This is controlled by only one excitatory neuron, which causes the muscle to contract, and two inhibitory neurons which cause the muscle to relax. To find out how temperature alone affected the transmission of neural signals to the muscle, and the muscle's contractions, the team extracted a dactyl closer muscle from a lobster's leg and placed it in a temperature controlled bath, which they could change in temperature from 2–20°C. Stimulating the excitatory neuron to make the muscle contract, the team used microelectrodes inserted into the muscle fibres to record the neural signals transmitted to the muscle. They found that these excitatory signals, called EJPs, were present at all temperatures but were particularly large at 2°C and between 14–16°C.

But what about the contractions caused by the EJPs: would they also differ in size according to the temperature? Using a force transducer attached to the muscle's end via a piece of thread, they found that the muscle contracted at all temperatures, but most strongly in the cold. When the team repeated their experiments by stimulating the inhibitory motor neuron to make the muscle relax, they found that temperature had a different effect. The neural signals, called IJPs, caused the muscle to relax at colder temperatures only, between 0–6°C, but not at warmer temperatures.

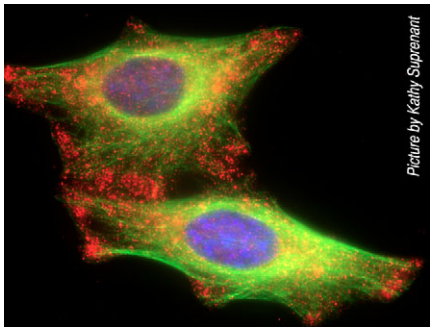
Having shown that temperature had a different affect on the excitatory and inhibitory neurons, and on the muscle, the team then added serotonin to the muscle's bath and repeated their measurements. They found that the neuromodulator changed the effect of temperature on the muscle. When they stimulated the excitatory neuron, the EJPs and the muscle contractions were bigger, especially at 18°C. Again, there was a different effect when they stimulated the inhibitory neuron. They found that serotonin both increased muscle relaxation, and also increased the temperature range over which IJPs relaxed the muscle to 0–12°C.

Because serotonin has such a wide range of different effects on muscles at different temperatures researchers should look at the influence of neuromodulators such as serotonin over a range of temperatures, Worden explains. One of the next challenges will be to unravel how temperature and neuromodulators affect lobsters in the wild, altering their behaviour and how their muscles and neurons function.

10.1242/jeb.02750

Hamilton, J. L., Edwards, C. R., Holt, S. R. and Worden, M. K. (2007). Temperature dependent modulation of lobster neuromuscular properties by serotonin. *J. Exp. Biol.* **210**, 1025-1035.

THE MYSTERY OF VAULTS



There is a mysterious structure lurking in your cells' cytoplasm. These giant ribonucleoprotein particles (RNA plus protein) are known as vaults because part of their structure, highlighted by staining, reminded researcher Nancy Kedersha of vaulted church ceilings. They are found in all eukaryotic cells and are very common in cancer cells and multi-drug resistant cancer cells, however researchers don't know much about their function. They are 'wonderful structures', says Kathy Suprenant at the University of Kansas, describing vaults as being barrel-shaped with a drawn-in waist, and with caps on the ends. Intrigued by vaults' presence in the cells she was studying, Suprenant set out to find out more about them (p. 946).

First Suprenant wanted to see if vaults had any molecular relatives. With bioinformatics experts Jianwen Fang and Gerald Lushington, Suprenant used a sequence alignment tool called BLAST which searches protein sequence databases for similarities or matches between sequences. She chose a repeated sequence of amino acids close to the end of the vault protein and found that this sequence in the vault protein was very similar to the sequence of TELA, a bacterial protein family.

The best match from their search was a TELA protein called TelA from the bacteria *Rhodobacter sphaeroides*. Since the structure of the vault protein had already been well described the team next characterised the structure of *Rhodobacter*'s TelA protein. Using a computer program which predicted how protein sequences might fold, they found that the TelA protein could fold in the same way as the vault protein, which suggested that they were related in function. The team knew that TelA proteins in *Rhodobacter* are involved in resistance to the environmental toxin tellurite, which is an oxyanion – a negatively charged ion containing oxygen – of the metalloid tellurium. Could vault proteins in mammalian cells also be involved in resistance to tellurite and similar toxins?

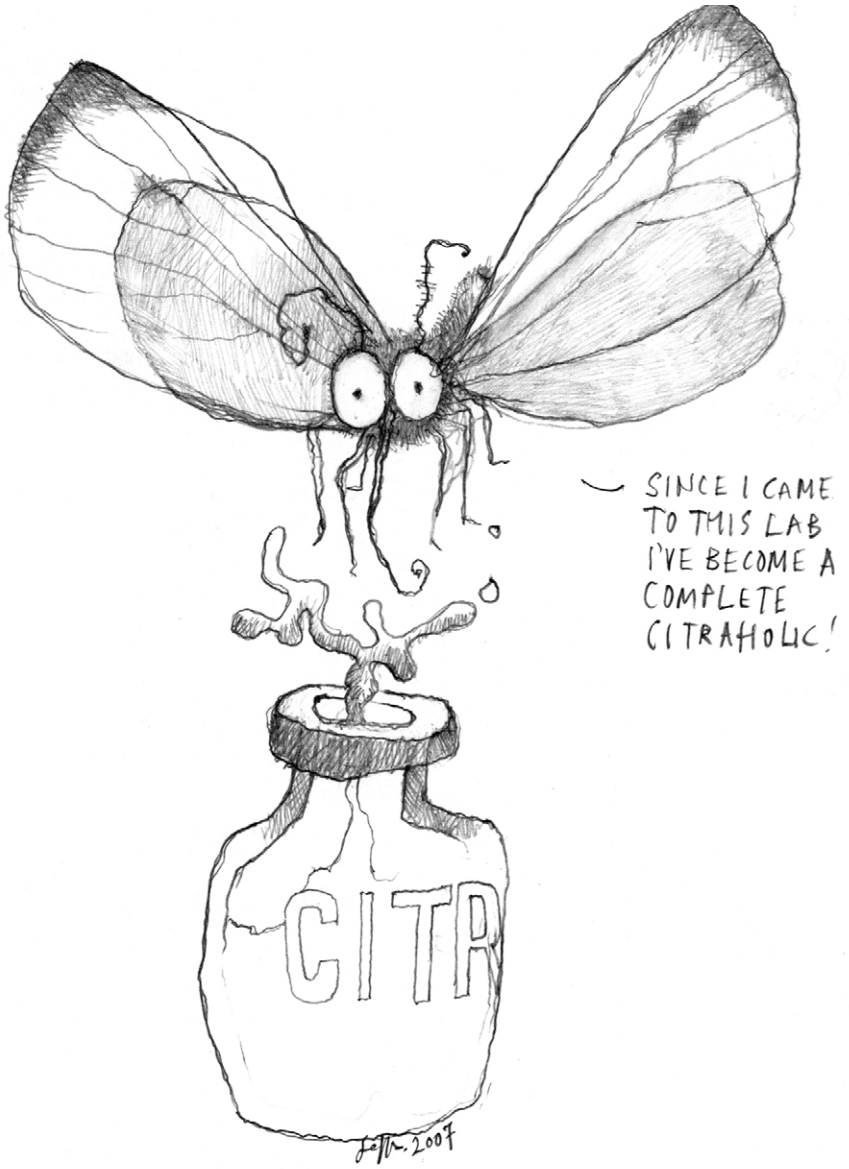
To find out, the team used human cells in culture and added tellurite to the culture medium. Using fluorescent antibodies that tagged the vault proteins, allowing them to see where they were in the cell, Suprenant and student Nathan Bloom found that adding tellurite caused vaults to migrate from their position in the cells' cytoplasm to the cell surface, collecting together and forming aggregates called vaultosomes. This response wasn't unique to tellurite, and also occurred in response to other related oxyanions such as arsenite and vanadate. Since vaultosomes were forming in response these toxic anions which stress the cell, the team wondered if vaultosomes formed independently of other known stress responses. Exposing the cells to arsenite, they found that vaultosomes formed independently of RNA-containing stress response particles and did not congregate with them. When they looked at aggresomes, complexes of proteins that form when the cell's ability to clean up misformed proteins is overwhelmed, the team found that vaultosomes did not congregate with aggresomes, either. These results suggested that vaultosome formation may be a unique response to stress.

The abundance of vaults in cancer cells and drug-resistant cancer cells may be part of this unique cellular stress response. In the future Suprenant hopes to determine whether vaults are necessary or sufficient for tellurite and toxic anion resistance. 'We need to know a million more things about them,' she says.

10.1242/jeb.02749

Suprenant, K. A., Bloom, N., Fang, J. and Lushington, G. (2007). The major vault protein is related to the toxic anion resistance protein (TelA) family. *J. Exp. Biol.* **210**, 946-955.

THE SWEET SMELL OF CITRAL



Pete Jeffs is an illustrator living in Paris

For a female butterfly, a male has to look and smell right before she'll choose him as a mate. Green-veined white butterfly males, *Pieris napi*, release a lemony smelling compound called citral, but up until now researchers didn't know what its role was. So Anna-Karin Borg-Karlson and her colleagues at KTH, Stockholm and Stockholm University, investigated whether citral would help males snare a mate (p. 964).

To find out when male butterflies release citral, the team measured how much they released as they flew in a sealed cylinder in the presence of male and female butterflies, of the same and different species. They found that males released citral whenever they flew, not specifically in the presence of females. But were females more responsive than males to citral? Next, the team measured the electrical response of male and female antennae as they wafted citral over them. They found that female antennae were 10 times more sensitive to the compound than males.

Finally, they tested the effect of citral on female mate acceptance behaviour. Virgin females accepted models of male butterflies with citral on their wings as potential mates, but paid no attention to citral free models. This behaviour, plus the sensitivity of females to citral, suggests that it functions as a sex pheromone in these butterflies, helping males attract a mate.

10.1242/jeb.02751

Andersson, J., Borg-Karlson, A.-K., Vongvanich, N. and Wiklund, C. (2007). Male sex pheromone release and female mate choice in a butterfly. *J. Exp. Biol.* **210**, 964-970.

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