

Inside JEB 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

## HYGIENIC BEES CLEANSE DAMAGED BROOD



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It's a beekeeper's worst nightmare: to find their bee colonies decimated by *Varroa* mites. Hopping into brood cells just before the pupae are sealed in during metamorphosis, the mites reproduce and feed on the developing bees before hitching a lift on their emerging cellmates when development is complete. Initially a natural parasite of Asian honeybees, *Varroa destructor* began wreaking havoc among European bees during the last century. And the situation escalated when the parasites picked up naturally benign viruses, which had previously lived in harmony with their European hosts, transmitting them back in a sometimes-lethal form to the vulnerable bees. Fortunately though, all was not lost. Elke Genersch from the Institute for Bee Research, Germany, explains that nurse bees naturally weed out and dispose of damaged or deformed pupae while developing in the brood comb to protect the colony from infection.

Yet, attentive nurse bees face a dilemma when presented with mite-infested brood. According to Genersch, honeybee viruses – such as deformed wing virus – mutate continually, so some mite populations carry virulent and crippling forms of the disease whereas the viruses transmitted by other mites are practically harmless. However, it was not clear whether the *Varroa* mites carrying harmless virus or no virus might also damage the developing pupae sufficiently to trigger removal. So, would bee nurses dispose of all developing pupae that are infected with *Varroa* mites, or dispose only of those pupae where the mites are carrying damaging forms of deformed wing virus (p. 264)?

Teaming up with Sebastian Gisder, Genersch screened honeybee colonies infested with *Varroa* mites to identify colonies living with mites carrying virulent deformed wing virus and other colonies infested with mites carrying less-virulent – or even no – virus. Having isolated two mite-source colonies, the team then looked for colonies that were completely free of *Varroa* infestation – to provide clean brood – and identified hygienic colonies with

good housekeepers, which had been selectively bred by Kaspar Bienefeld as part of a *Varroa* control strategy. Then Caspar Schöning and Ivonne Kretschmann tested how the good housekeepers would respond to virulent and non-virulent *Varroa* infestations to find out whether the bees respond to mite-induced damage or deformed wing disease.

Taking clean brood combs from the uninfested colonies, the duo added virulent and less-virulent mites from the mite-source colonies to the comb's clean cells. Then, the team transferred the newly infested combs to the hygienic colonies and waited to see how the good housekeepers dealt with the contaminated combs. Would they dispose of all *Varroa*-infected pupae, regardless of deformed wing virus virulence, or would the nurses select out the pupae at risk from the most virulent virus, leaving the pupae infested with the less-virulent mites to develop?

Amazingly, the bees homed in on the pupae that were infested with mites carrying the most virulent deformed wing virus, leaving the pupae that had been infested with less-virulent mites to develop to maturity. And when Sven Geiselhardt and Monika Hilker analysed the odours produced by pupae infested with virulent mites and pupae infested with the less-virulent mites, they found distinct differences between the odours emanating from both groups.

'The bees reacted towards the damage done to the brood by the virus rather than to the *Varroa* mite parasitisation *per se*', says Genersch. She adds, 'There is a certain combination of deformed wing virus and *Varroa* mite that is fatal for the bees and beekeepers need to treat against mites or breed for hygienic bees to get rid of this virulent combination.'

10.1242/jeb.069096

Schöning, C., Gisder, S., Geiselhardt, S., Kretschmann, I., Bienefeld, K., Hilker, M. and Genersch, E. (2012). Evidence for damage-dependent hygienic behaviour towards *Varroa destructor*-parasitised brood in the Western honey bee, *Apis mellifera*. *J. Exp. Biol.* 215, 264-271.

Kathryn Knight

## SQUID MUSCLE MYOSIN HERESY

Equipped with a pair of rapidly deployable tentacles, little will escape the squid's grip once a victim has wandered within range. Justin Shaffer from the University of North Carolina, USA, explains that a hunting squid can double the length of its lethal tentacles in as little as 15 ms, whereas the eight arms are used for slower more

mundane tasks, such as eating and swimming. So how have squid modified their tentacles to contract so fast?

Shaffer explains that muscles contract when thick filaments of myosin hydrolyse ATP to slide past thin filaments of another muscle protein, actin. The smallest contractile unit is known as the sarcomere and when Bill Kier looked at the arrangement of sarcomeres in the muscle fibres in squid arms and tentacles, he realised that the short sarcomere structures in the tentacles' transverse muscle fibres could account for the muscle's rapid contraction. However, the speed of most muscular contractions is regulated by the rate at which myosin hydrolyses ATP. 'Every other animal uses different myosin isoforms to control speed', explains Shaffer. Suspecting that squid also use specifically tailored myosin molecules to fine-tune the speed of muscular contraction, Shaffer and Kier decided to sequence myosin mRNA – which is later translated into protein – from various squid muscle tissues to find out whether squid express specialised myosins in their fast-contracting tentacles (p. 239).

Coming from a cardiac biochemistry background, Shaffer was well prepared to begin searching for distinct myosin mRNA molecules in the tentacle, arms, mantle, fin and funnel retractor muscles of the squid, *Doryteuthis pealeii*. Dividing the colossal myosin transcript (about 6600 base pairs long) into six regions, cloning each region and sequencing it, Shaffer reconstructed the mRNA sequences of each myosin isoform from each tissue and was amazed to find the same three myosin transcripts turning up in all five tissues. 'I was doing a lot of these samples side by side', recalls Shaffer. 'We had this hypothesis that there should be different myosins and I kept getting all this sequence data showing the same sequence, whether it was in the tentacle or the mantle, and I thought, "there is no way this is right", but I kept doing it, I ran all my controls and it kept coming out.'

So the squid were not producing a uniquely tailored high-speed myosin in the tentacle to account for its unusually fast contraction; instead they had modified the muscular structure with their short sarcomeres to catch prey fast. 'I was surprised', admits Shaffer. He explains that invertebrates such as lobsters and scallops produce so-called 'fast' myosin isoforms in combination with short sarcomeres to produce fast-contracting muscle, and says, 'This squid is really unique because so far, it is the only animal we have encountered that uses only

ultrastructural differences to alter contractile performance.'

However, Shaffer points out that even though each tissue produces all three myosin isoforms, it is possible that the isoforms are specifically produced in the different muscle fibre types (transverse vs longitudinal vs helical) that construct a muscle to fine tune contractile performance. He is also keen to investigate myosin isoform expression in the muscles of other cephalopods to find out whether they too use muscle ultrastructure modification to speed up muscle contraction.

10.1242/jeb.069161

**Shaffer, J. F. and Kier, W. M. (2012).** Muscular tissues of the squid *Doryteuthis pealeii* express identical myosin heavy chain isoforms: an alternative mechanism for tuning contractile speed. *J. Exp. Biol.* **215**, 239-246.

**Kathryn Knight**

## SNAKES HEAR THROUGH SKULL VIBRATION



Christian Christensen

Snakes are remarkably sensitive to most stimuli, but there is one sense that they seem to have almost done away with: hearing. They have no visible means for detecting airborne sound, having lost the tympanum and the external ear. Equipped with only an inner ear linked to the jaw apparatus by a single middle ear bone, the columella auris, it would seem that snakes have little hope of hearing the world as we know it. They would have to rely on sensing vibrations transmitted through the jaw. Yet the debate about whether snakes hear airborne sounds raged, with evidence stacking up on both sides of the argument. Intrigued by the mystery, Christian Christensen, Jakob Christensen-Dalsgaard, Christian Brandt and Peter Madsen decided to find out whether snakes detect sound *via* sound pressure or sound-induced mechanical vibrations through the body (p. 331).

Playing sounds ranging in pitch from 80 to 1000 Hz at volumes between 50 and 110 dB re. 20  $\mu$ Pa to 11 royal pythons, Christensen recorded electrical responses in one of the snakes' cranial nerves and their brain stems. Increasing the sound volume until he

recorded a measurable electrical signal in the brain stem, Christensen found that the snakes could hear very loud airborne sound (10,000 times louder than the softest sounds heard by people). They were also most sensitive to low frequencies between 80 to 160 Hz and their sensitivity decreased at higher frequencies, falling from 78 dB re. 20  $\mu$ Pa at 160 Hz to 96 dB re. 20  $\mu$ Pa at 800 Hz.

But how were the sounds transmitted to the snake's vibration-sensitive inner ear? As low-frequency sounds are efficiently carried by solid materials, the team wondered whether sound vibrations might be transmitted from the ground into the snake's body.

Christensen measured vibrations generated in the surface upon which the snakes were lying by a loudspeaker suspended above the platform. Meanwhile, he recorded the animals' auditory electrical response to the vibrations. He found that the animals responded well to 80 Hz vibrations, but at higher frequencies, the vibrations produced in the surface by the airborne sound were too weak for the snake to respond.

So, how were the snakes able to sense the higher pitched sounds that they hear? 'Some suggested that they could use the lung as fish use the swim bladder. Also, we humans still hear by bone conduction in water, that would be another way of sending the sound', says Christensen. So the team decided to test whether the animals could sense their own skulls' vibrating in response to airborne sounds.

Attaching minute vibrometers to the snakes' heads, Christensen measured the mechanical vibrations induced in the head by loud airborne sounds that were just above the snakes' hearing thresholds. He found that these skull vibrations were the same intensity as the minimum mechanical vibrations that the animals could sense. So instead of responding to sound pressure, snakes respond to vibrations transmitted directly from the air to the skeleton.

Having shown that snakes are sensitive to sound-induced vibrations rather than sound pressure, the team is keen to investigate the hearing of other earless animals.

10.1242/jeb.069104

**Christensen, C. B., Christensen-Dalsgaard, J., Brandt, C. and Madsen, P. T. (2012).** Hearing with an atympanic ear: good vibration and poor sound-pressure detection in the royal python, *Python regius*. *J. Exp. Biol.* **215**, 331-342.

**Kathryn Knight**

SLIPPERINESS AFFECTS STICKY FEET SELF-CLEANING



Sticky feet are ideal for clinging to vertical surfaces and ceilings, but not so good when it comes to staying clean. ‘Contamination of the adhesive surfaces by loose material can seriously compromise attachment ability’, says Michael Orchard and colleagues from the University of Hull, UK, and the Australian National University. Yet, the sticky feet of ladybirds and ants self clean routinely, so how do they do it? The Anglo-Australian team suspected that the surface upon which an insect is walking could have something to do with their ability to shuck off dirt. ‘We hypothesised that... cleaning would depend on the free surface energy [FSE – a measurement of a surface’s slipperiness] of both the contaminating particles and the surfaces, with surfaces with high FSE (glass) predicted to be better cleaning surfaces than

those with low values of FSE’, they say (p. 279).

Coating glass vials with slippery silicon, the team then systematically contaminated the hairy feet of large and small species of ladybird and the smooth sticky pads of ants with large and small PTFE (slippery) and glass microspheres (less slippery). Next, the team monitored the insects’ behaviour and recorded how long it took them to regain their footing on the vertical walls of the untreated and siliconised glass vials.

The team report that all of the insects found it easiest to remove glass spheres on a glass surface and it always took them longer to clean feet contaminated with slippery PTFE particles. They also found that small contaminants were the most difficult to

dislodge, especially for the smallest insects. Considering the slipperiness (FSE) of the vial surfaces, the team found that the ladybird’s hairy adhesive pads shed dirt more easily on the non-slippery (high FSE) glass than on the slippery (low FSE) siliconised vials. However, the slipperiness of the surface did not affect the smooth-padded ants. So the slipperiness of the surface can affect an insect’s ability to self-clean, but this depends on which attachment mechanism the insect uses.

10.1242/jeb.069112

Orchard, M. J., Kohonen, M. and Humphries, S. (2012). The influence of surface energy on the self-cleaning of insect adhesive devices. *J. Exp. Biol.* **215**, 279-286.

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