INSIDE JEB

Parasitic fig wasps bore with zinc-tipped drill bit

Parasitoid wasp ovipositing on a fig. Photo credit: Lakshminath Kundanati.

Female insects have one goal in life: to find the best place to lay their eggs. For fig wasps, that is the developing fruit of the luscious fig plant. However, when the females of one particular species of parasitic fig wasp (*Apocryta westwoodi grandi*) descends onto a recently fertilised fruit, she has to bore her way through the tough unripe fig to find the larvae of other insects that are already developing within, which she will parasitize to give her own eggs the best start. Fortunately, the insect's immensely long (7–8 mm) and slender (15 µm) ovipositor – which injects eggs into the fig – is equipped with a sharp tip, ready to bore through the woody fruit. Namrata Gundiah from the Indian Institute of Science, Bangalore, was intrigued by the differences between the egg delivery systems of the boring parasitoid wasps and the wasps that pollinate the fig's flowers. 'Our first question was why don't we look at the different adaptations that these two species undergo?' says Gundiah (p. 1946).

Teaming up with graduate student Laksminath Kundanati, Gundiah used scanning electron microscopy to take a high resolution look at the tips of the insects' ovipositors and was amazed to see that the end of the boring wasp's ovipositor looked like a drill bit, complete with teeth to bore through the woody fruit. In contrast, the tip of the pollinator wasp's ovipositor closely resembled a spoon-like structure. And when they looked along the length of the borer's ovipositor, Kundanati and Gundiah noticed tiny pits in the shaft, roughly in the location where the structure bends as the female drives the tip into the fruit, to allow the ovipositor to flex without breaking. Gundiah could also clearly see sensory structures at the tip that could help guide the ovipositor to the best locations for the wasp to lay her eggs.

Next, the duo investigated the material from which the drill bit was made. 'We asked what could enhance the hardness of the structures', recalls Gundiah. Focusing a beam of electrons on the minute tip, Kundanati and Gundiah recorded the X-ray spectrum emitted by the material and discovered that the tooth structures were enriched with zinc. 'Zinc mainly increases the hardness, which will affect the wear resistance of the drill bits', explains Gundiah.

Kundanati and Gundiah then prodded the minute drill bit with an atomic force microscope (AFM) probe to indent it to find out how hard the zinc-enriched teeth were. Gundiah admits that pinpointing the tiny teeth on the miniscule curved structure was particularly challenging: 'Usually, AFM is done on relatively large surfaces and so it doesn't matter where you go and indent the material', chuckles Gundiah. But eventually the duo recorded the hardness of the teeth at 0.5 GPa: 'That is almost as hard as the acrylic cement used for dental implants', says Gundiah.

Finally, knowing that the females impale unripe figs with their ovipositors many times during the course of their lives, Gundiah decided to measure the buckling forces exerted on the slender structure as the female drives the ovipositor in. Kundanati filmed the tiny wasps on fig trees around the institute campus by attaching a microscope objective to a video camera. He clearly saw the slender structure bend and flex as the insect drove it in and calculated that the 15 µm diameter structure can tolerate buckling forces of almost 7 µN.

Having characterised the fig wasp's drill bit, Gundiah is keen to design a minute boring tool based on the lessons that she has learned from the insects.

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Kundanati, L. and Gundiah, N. (2014). Biomechanics of substrate boring by fig wasps. *J. Exp. Biol.* **217**, 1946-1954.

Kathryn Knight

Fish don't need eyes for numeracy

Blind cavefish (*Phreatichthys andruzzii*). Photo credit: Luca Scapoli.

Fish tend not to be renowned for their intellect. Their memories weren't thought to be great and until recently no one thought that they could assess quantity. But now we are having to reconsider these opinions. 'Fish are capable of processing both small and large numbers with a performance similar to that described in mammals and birds', says Christian Agrillo from the University of Padova, Italy. However, all of the fish species that have been tested to date were able to see. Could blind fish process numbers using other senses? Wondering whether vision is essential for fish to evaluate quantity, Agrillo and his colleagues from Padova and the University of Ferrara, Italy, set about testing the numeracy of blind cavefish (p. 1902).

According to Agrillo, Somalian cavefish (*Phreatichthys andruzzii*) are the ideal species to test as they have been deprived of light for two million years, leading to loss of eye function. However, the fish have compensated for the deficiency by increasing the sensitivity of their lateral line sensors, which allows them to discriminate between different 3D shapes.

Having trained the fish to associate groups of six objects with food until they automatically gravitated towards the groups – even when food was no longer present – the team then tested whether the fish could distinguish between clusters of six and two objects; which they did. However, the team needed convincing that the fish weren't relying on other cues (such as differences between the surface areas of the two clusters or the different volumes occupied) to differentiate between groups with different numbers of objects.

This time the team tested the fish's ability to discriminate between groups of two and four objects, but sometimes they made sure that the two groups had the same surface area, occupied the same volume or were distributed with the same density, while on other occasions they allowed the surface area, volume and density to differ between the two groups. As soon as the team removed the differences between the additional cues, the fish were no longer able to differentiate.

Puzzled, the team wondered whether changing the test conditions (groups of two objects versus groups of four) from the training conditions (groups of two objects versus six) had flummoxed the fish, or whether the fish may simply not have the processing power to differentiate between a small difference of two. The team trained the fish using clusters of two and four objects – where the two clusters had the same surface area, volume and density characteristics – and this time the fish successfully discriminated between the two clusters. 'This represents the first evidence of non-visual numerical abilities in fish', the team says. However, there is a limit to the blind fish's numerical abilities; they were unable to distinguish between groups of two and three objects, suggesting that vision may improve fish's numerical accuracy.

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Bisazza, A., Tagliapietra, C., Bertolucci, C., Foà, A. and Agrillo, C. (2014). Non-visual numerical discrimination in a blind cavefish (*Phreatichthys andruzzii*). *J. Exp. Biol.* **217**, 1902-1909.

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Earth worm proportions change as they grow

Juvenile and adult *Lumbricus terrestris* earthworms. Photo credit: William Kier.

Most animals' proportions change as they grow. Human babies are born with massive heads relative to their bodies that scale down as they grow and many young animals look as if they need to grow into their feet. But do earth worms' proportions change as they develop, or are the adults simply scaled up versions of youngsters? And, if the proportions of worms change as they grow, how does that affect their movements? Jessica Kurth and William Kier from the University of North Carolina, USA, explain that unlike most other animals, worms are supported by a hydrostatic skeleton – where a muscular body encases a liquid-filled internal cavity that extends when the muscles contract. They add that little was known about how the proportions of animals with hydrostatic skeletons change, or how growth might affect their ability to burrow. So, Kurth and Kier decided to find out more about how earth worms grow as they mature (p. 1860).

Measuring the vital statistics of the front, middle and rear portions of earth worms ranging from tiny (1–3 g) juveniles up to fully grown adults (3–10 g), the duo found that the worms' proportions did change as they grew. They became slimmer and the ratio of the worms' length to their diameter increased by as much as 128% at the front end and up to 163% at the rear. But how would the alteration in their physical proportions affect how far the animals could extend their bodies and the forces that they could exert as they burrowed?

Calculating the animals' stride length – worms stretch to move forward by contracting their circular muscles – Kurth and Kier were impressed to see that the longer, thinner adults could extend themselves 117% more than the stumpier juveniles: the adults were able to extend further than if they were simply youngsters scaled up to adult size. However, when they calculated how much force the largest worms could exert on the walls of their burrows compared with the thicker youngsters, the older worms' length did not improve their pushing power. In fact, the forces exerted on burrow walls by the adult worms were the same as those exerted by scaled-up youngsters.

But why don't larger earth worms take advantage of their altered proportions to amplify their burrowing power relative to that of younger worms? Kurth and Kier suspect that this could be due to differences in the way that soil behaves as small and large worms burrow. They explain that larger worms have to displace more soil radially while burrowing, increasing the stiffness of the surrounding soil, possibly making it advantageous for larger worms to be slender to reduce the effort required to heft soil aside. Alternatively, they suggest that smaller worms might be stockier to help them fracture compacted soils. They explain that small marine worms are known to force cracks through mud by expanding the front section of their bodies and they suggest that small earth worms may benefit from a similar advantage when boring through hard soils.

Having shown that older earthworms are not simply scaled up versions of their younger selves, Kurth and Kier are keen to find out how different soil types have affected the worms' burrowing technique.

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Kurth, J. A. and Kier, W. M. (2014). Scaling of the hydrostatic skeleton in the earthworm *Lumbricus terrestris*. *J. Exp. Biol.* **217**, 1860-1867.

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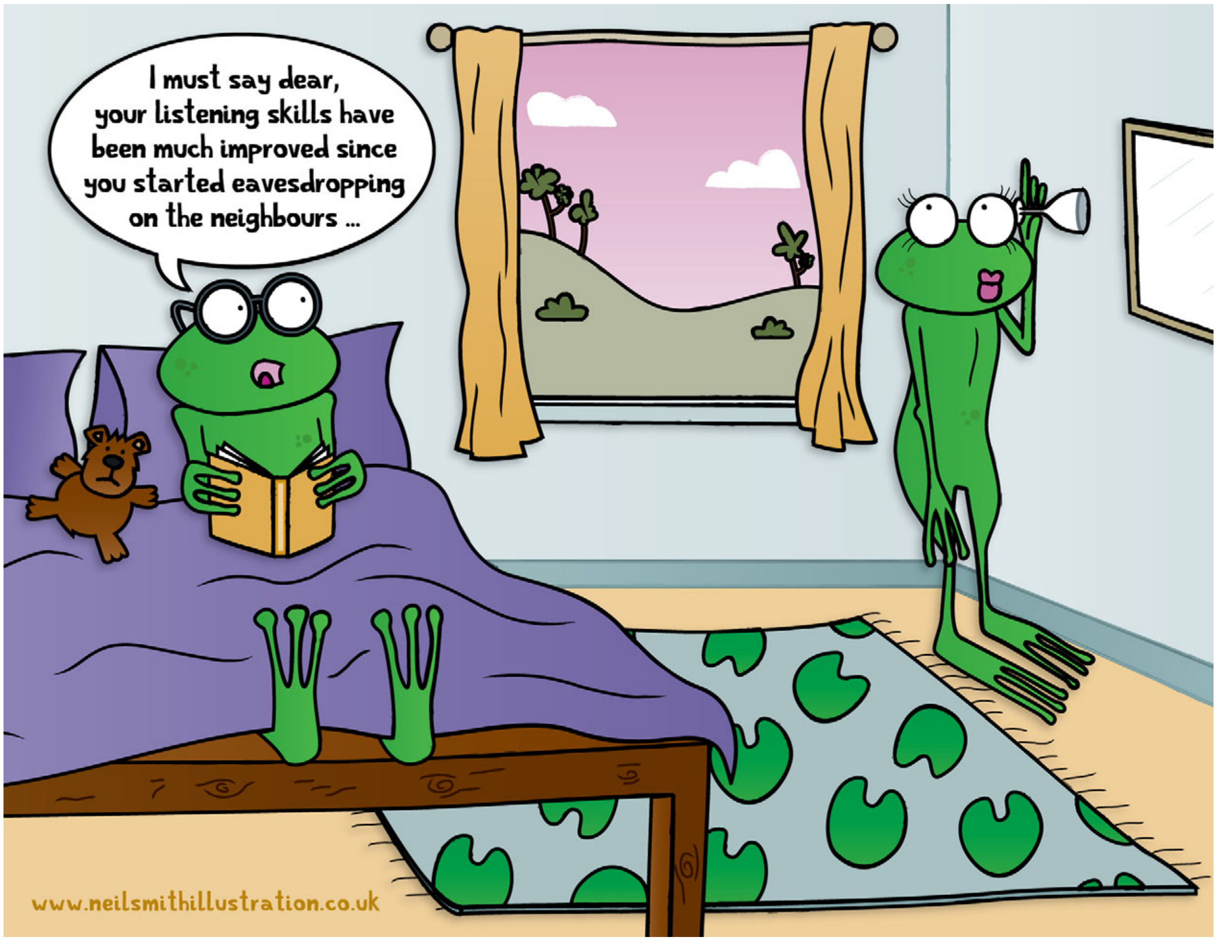
Listening to chorus sensitises tree frogs



When the breeding season comes around, American green tree frogs get calling, boasting to the ladies and letting other males know not to mess with them. But what effect does this racket have on their sensitivity to signals from other green tree frogs? 'Much of the previous work on the significance of repeated exposure to social signals has focused on neural habituation and diminished behavioural responses to individual signals', explain Megan Gall and Walter Wilczynski from Georgia State University, USA, adding that short-term exposure to calls clearly diminishes the responses of some species to subsequent communication signals. However, they explain that it is less clear how an animal's ability to respond to communication signals is affected by long-term exposure to social signals. Gall and Wilczynski decided to focus on a region of the brain – the midbrain – in American green tree frogs that is known to process social signals in order to find out how it responds to communication signals after lengthy exposures to either a chorus of tree frogs or random sounds (p. 1977).

Monitoring expression of a gene, the immediate early gene *egr-1* – which indicates cellular activity and is used as a proxy for neural excitation in brain tissue – the duo found that listening to sounds for 10 days increased the sensitivity of the amphibian's midbrain to calls from their own species. And when they compared the effects of the random sounds with those of the frog chorus, the tree frogs that had been listening to calls from their own species were more sensitive to the calls of other tree frogs than animals that had been listening to random sounds.

Gall and Wilczynski say, 'We believe that this is the first report of stimulation with an assemblage of mate attraction signals enhancing future sensory processing in anurans.' They also point out that the study raises various questions, including how the frogs alter their acoustic responses and the significance of this modification, which we hope to find out soon.

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Gall, M. D. and Wilczynski, W. (2014). Prior experience with conspecific signals enhances auditory midbrain responsiveness to conspecific vocalizations. *J. Exp. Biol.* **217**, 1977-1982.

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