

## INSIDE JEB

## Parasitic wasp hypodermic needle sniffs out larvae to lay eggs



*Apocrypta westwoodi* on an unripe fig. Photo credit: Pratibha Yadav.

It takes a special kind of insect to pollinate an inside-out flower, which is exactly what the wasps that pollinate figs do. Crawling inside the firm swelling lined with microscopic flowers that will eventually ripen into a fleshy fig, tiny fig wasps pollinate the flowers within while laying their own eggs. However, the fig wasps' robust nursery is not entirely secure. Renee Borges from the Indian Institute of Science explains that some parasitic wasp species seek fig wasp larvae to nurture their own young, and these and other parasitic species that lay their eggs directly in the fruit are equipped with a sharp hypodermic needle (ovipositor) that drills into the hard fig before the wasp injects its eggs. However, it is not sufficient for these parasitic interlopers to inject their eggs at random. Parasitic wasps that target fig wasp larvae must locate the developing youngsters within the flesh of the fruit. Knowing that pockets of gas could indicate the presence of fig wasp larvae developing within ripening figs, Borges and her student Pratibha Yadav wondered whether the tip of the parasitic wasp's egg injector could be guided by odour sensors that could literally sniff out potential host larvae within.

But before Yadav could test the hypothesis, she had to obtain elusive *Apocrypta westwoodi* parasitic wasps. 'We collected figs that are about to release wasps from trees on our campus', says

Borges, who kept the fruit in the lab until the male wasps gnawed the holes in the fruit that would allow the females to emerge. Then, Yadav released the wasp's ovipositor from within its protective sheath. 'The hardest thing was to obtain electrophysiological recordings from the extremely thin ovipositor', says Borges, who had to experiment with different techniques to amplify the faint electrical signals produced by the ovipositor in response to odours.

Knowing that the wasp larvae that were already developing in the fruit exhale carbon dioxide, Yadav blew a dilute stream of carbon dioxide over the tip of the exposed ovipositor and was impressed to see that the gas triggered nerve signals. And when she tethered the insects at the tip of a hypodermic syringe and blew a thin stream of air over the wasps, the ovipositor only twitched in the direction of the jet of air when she added a puff of carbon dioxide.

*Apocrypta*'s ovipositors were capable of sniffing out carbon dioxide, which could help guide the ovipositors to their larval egg-laying sites. And when Yadav photographed the tip of the ovipositor using a scanning electron microscope, she could clearly identify sensory structures that resembled the carbon dioxide sensors found on the antennae of other insects.

Curious to find out how the ovipositors of other parasitic fig wasps would respond to odours, Yadav extracted scents from developing figs that were at the stage when *Sycophaga fusca* fig wasps – a parasitic species that injects its eggs directly into the fig – make a visit. Blowing the fruit scent over *Sycophaga*'s ovipositor, Yadav saw it twitch strongly when it caught a whiff of the fruit. But how would *Apocrypta*'s ovipositor respond to the fruity odours? Yadav blew the same scents over the *Apocrypta* ovipositors and this time they were unresponsive; *Apocrypta* attend the fruit later, when the fig smells different.

Having confirmed that the ovipositors of *Apocrypta* parasitic wasps are capable of sniffing out wasp larvae upon which to

lay their eggs, while *Sycophaga*'s ovipositors can find the ideal locations for their eggs inside fleshy fruit, Borges is keen to learn more about how other parasitic wasps select their egg-laying locations, site unseen.

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Yadav, P. and Borges, R. M. (2017). The insect ovipositor as a volatile sensor within a closed microcosm. *J. Exp. Biol.* **220**, 1554-1557.

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## Do flies over-generalise memories like PTSD sufferers?

No two cakes smell identical, yet we are still able to recognise the general aroma that promises pleasure. And Ayse Yarali from the Leibniz Institute for Neurobiology, Germany, explains that this ability to generalise a previously learned link between a cue – such as an odour – and an experience – such as a pleasant taste or the attack of a predator – to a range of similar cues can help us to ensure that we repeatedly encounter pleasant situations while avoiding risky ones. As the ability to generalise what is learned about a scent to a wide range of similar scents is an essential life skill for many species – including fruit flies – Yarali wondered whether the humble insect's ability to generalise changes over time. Teaming up with Christian König, Yarali decided to investigate how flies that had learned to associate an electric shock with a specific fruity odour reacted later when they experienced other fruity scents.

König and his colleagues Emmanuel Antwi-Adjei and Mathangi Ganesan identified three odours (3-octanol, *n*-amylacetate and 1-octen-3-ol) that could be distinguished by the fruit flies and used a Teflon tube lined with a pair of tightly wrapped copper coils attached to a power supply to shock the insects. 'If a fly happens to touch both of the coils with its legs – six legs give many possibilities – it gets an electric shock', explains Yarali. König then wafted one of the odours



A small male *Drosophila melanogaster* fly. By André Karwath aka Aka (Own work) [CC BY-SA 2.5], via Wikimedia Commons.

through the tube while the flies received a series of shocks in the hope that they would learn to avoid the odour in future. Twenty minutes later, König tested the insects' responses to the odour by placing them in a T-shaped maze where one arm was scented. Sure enough, the majority of flies avoided that region of the maze; they also recalled the memory 24 h later. But would the flies generalise the shocking experience and recall it when they encountered a similar, but different, odour?

König replaced the odour in the T-maze with a new scent and, although some of the shocked flies avoided the novel scent 20 min later, it was a different matter after 24 h. This time, the flies' aversion to the new odour was as strong as their desire to avoid the odour that was directly associated with the shock. They had generalised their memory of the shock so that they recalled the memory even when the scent that they encountered was only vaguely familiar.

So, flies can generalise a troubling memory across odours that smell similar. However, Yarali explains that even though the ability to generalise a memory can be beneficial, it can also become an impediment if it is exaggerated. 'Overly generalised memories of a traumatic experience in humans are one of the key behavioural hallmarks of post-traumatic stress disorder [PTSD]', explains Yarali; and the effects of human PTSD are often delayed, in much the same way that flies did not develop their general aversion to similar odours until several hours later. 'We cannot say that we have a fully fledged fly model of human PTSD', says Yarali, as the flies are unlikely to be suffering the same horrifying experiences that victims of PTSD endure, but they do appear to have at least one of the behavioural hallmarks of the human condition. And she is eager to

discover whether the shocked flies exhibit some of the other behaviours that are indicative of a 'disturbed state of mind' with the hope that this research may one day help us to develop new therapeutic strategies for human sufferers of PTSD.

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**König, C., Antwi-Adjei, E., Ganesan, M., Kilonzo, K., Viswanathan, V., Durairaja, A., Voigt, A. and Yarali, A.** (2017). Aversive olfactory associative memory loses odor specificity over time. *J. Exp. Biol.* **220**, 1548-1553.

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## Impulse gives best bounce cost

Hopping is more than just a child's game for John Bertram. When Bertram sees a person bouncing around on one or two legs, he sees part of a running step. 'Hopping and running are bouncing gaits where kinetic and potential energy are in sync and involve the same bending and straightening motion of the leg', says Bertram. As hopping or bouncing on two feet offers a mechanical analogy for running, Bertram and his colleague Anne Gutmann decided to measure the metabolic cost of bouncing – as if rope skipping on two feet – and then compare the values with predictions from calculations simulating different aspects of how the leg functions during a bounce to learn more about the energetics of the movement.

In order to collect the colossal quantity of measurements – which added up to a total of 3–4 h of bouncing on a force plate while measuring the oxygen consumption for each individual – Gutmann recruited six fit young athletes who could bounce for extended periods without developing the burning muscle sensation associated with the switch to anaerobic respiration. 'If they exceeded their aerobic threshold, the oxygen consumption would not be a valid measure of total energy use', explains Bertram. In addition, Gutmann required the athletes to bounce in time with a metronome at different rates while adjusting the height of each two-legged hop in response to computer feedback, which Bertram admits was 'a bit like training people to rub their belly and pat their head at the same time while doing a workout'. However, after months of dedicated exertion from the athletes,

Gutmann eventually had over 250 force and oxygen consumption measurements from bounces ranging in height from 7 to 25 cm at speeds of 1.5 to 3.7 bounces  $s^{-1}$  from which to calculate metabolic costs per time over the entire range of bouncing performances.

Gutmann then built a series of mathematical simulations – taking into account the athletes' muscle force, muscle force rate, muscle impulse (which is the total force generated over the duration of the bounce) and mechanical work done as the bent leg propelled the body upward – and then used each model to calculate the metabolic cost per bounce, metabolic cost per time and metabolic cost per height for each bounce combination. Comparing the results of the calculations with the genuine physical costs, the duo was impressed to find that the simulation that best mimicked the athlete's performance was based on the total force exerted over the course of the bounce – the impulse.

'We were surprised that a work-based model did not do a better job of predicating metabolic cost per [two-footed] hop', admits Bertram, who was also intrigued that the other muscle-based models performed poorly, even though they appear to accurately predict the metabolic costs of running. However, when Gutmann revisited the force rate model of running, she realised that it only predicted the metabolic cost of running accurately over the narrow set of stride frequencies that runners naturally select when running on a treadmill.

Considering the implications of this study for our understanding of human running, Gutmann says, 'We need to base our metabolic cost models [for running] on data collected across a wide range of conditions to create more generally applicable models, which is what we have attempted to do in this study'. And the duo hopes that this investigation will inspire future studies to better understand the role of the mechanics of the musculoskeletal system and energy use in running.

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**Gutmann, A. K. and Bertram, J. E. A.** (2017). Metabolic cost of human hopping. *J. Exp. Biol.* **220**, 1654-1662.

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