INSIDE JEB

Perfume-blending orchid bee's sense of smell is fine-tuned

A male *Euglossa tridentata* collecting at an artificial scent source. Photo credit: Thomas Eltz.

The art of blending perfumes has been practised by humans for thousands of years, distilling essential oils and aromatic compounds to produce desirable fragrances. However, it appears that we are not the only species that indulges our olfactory senses: male orchid bees also gather scents from the environment and each species blends their own unique fragrance. 'The males expose them at the places where mating occurs, so the perfumes may be chemical signals to females', says Thomas Eltz, from the Ruhr-University Bochum, Germany, adding that the males could use the quality of their perfume blends to convey their superiority. Eltz also explains that many species of orchid bee live in close proximity, forcing species whose territories overlap to develop different blends that can be distinguished from those of their neighbours to prevent interbreeding. But it wasn't clear whether each of these species had also developed a sense of smell that is finely tuned to their own particular perfume – with close relatives barely responding to components from each other's scents – or whether differences in sensitivity had built up gradually over time as the species became more distantly related.

Working with Santiago Ramírez, from the University of California, Davis, USA, Eltz set out to determine how sensitive male orchid bees are to the constituent odours of their own unique perfume blend, but first he had to collect some bees. Eltz and his student, Lukasz Mitko, travelled to Panama, where they collected males from 15 species of *Euglossa* orchid bees by enticing them to trees with strips of filter paper dipped in attractive scents ranging from vanillin to methyl cinnamate. 'It is quite a spectacular sight to have dozens of green, blue or red metallic bees appear out of nowhere around a bait', says Eltz, who explains that Lukasz had to cycle to the forest collection site to collect the bees each day before rushing back to Bill Wcislo's Smithsonian Tropical Research Institute lab in Panama City. Having identified each species, Mitko then measured the response of their antennae to a puff of air containing one of 18 scents that the bees were known to gather for perfume production. 'Several of the test substances were not commercially available, so we had to isolate tiny quantities from bee extracts using preparative gas chromatography', says Eltz, adding that Erik Hedenström also synthesized one of the compounds, 6-(4-methylpent-3-enyl)-naphtalene-1,4-dione.

Fortunately, the males responded strongly to a selection of the odours that they incorporated into their own scents, 'confirming the idea of olfactory tuning to species-specific compounds,' says Eltz. However, when Ramírez and Marjorie Weber constructed a family tree of the relationships between the 15 species, instead of large differences in the sensitivities of closely related species – as the team had expected – the family tree showed that the most closely related species had the most similar odour sensitivities, while the most distantly related species showed the greatest differences. Instead of evolving dramatically different senses of smell to accompany their individual scents, the bees' senses of smell had diverged more gradually over time.

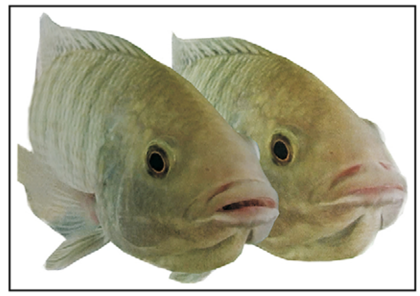
However, Eltz points out that despite the divergence in the bees' tastes over time, some distantly related species – the Panamanian *Euglossa mixta* and the Mexican *Euglossa dilemma* – are both sensitive to 2-hydroxy-6-nonadienyl benzaldehyde. He explains, 'This is a case of convergent sensory adaptation to a shared major perfume ingredient,' and is keen to track how these odorant receptors have evolved over the course of time through the orchid bee family tree.

10.1242/jeb.142497

Mitko, L., Weber, M. G., Ramírez, S. R., Hedenström, E., Wcislo, W. T. and Eltz, T. (2016). Olfactory specialization for perfume collection in male orchid bees. *J. Exp. Biol.* **219**, 1467-1475.

Kathryn Knight

Tilapia parents churn to cough kids clear



Mouthbrooding tilapia mother performing churning. Photo credit: Sam Van Wassenbergh.

Many egg-layers go to great lengths to produce cosy incubators for their families; they range from the sandy nests of ocean-going turtles to the immense leaf-litter compost heaps of Australian brush turkeys. But other species have opted for a less costly approach: some tilapia simply slurp up their eggs and hold them in their mouths until the larvae are ready to emerge. However, while the next generation is snuggled up safe and secure, their parents have to continue breathing through their heavily congested mouths. Sam Van Wassenbergh, from the University of Antwerp, Belgium, says, 'During mouthbrooding, cichlid fishes [tilapia] alternate the normal ventilation of their gills (breathing) with bouts of a sequence of movements of several head parts that is called "churning"'. But the jury was out as to why these parents appeared to chew on their charges: were they churning the eggs around to ensure that their offspring had sufficient access to oxygen or to ensure that they didn't choke on the kids? Intrigued by the bizarre parenting technique, Van Wassenbergh and Dominique Adriens, from Ghent University, decided to take a closer look inside the mouths of tilapia parents stuffed with eggs.

Inside JEB highlights the key developments in Journal of Experimental Biology. Written by science journalists, the short reports give the inside view of the science in JEB.

Journal of Experimental Biology (2016) 219, 1417-1419

Fortunately, Nile tilapia are content to breed in captivity, and can cram more than 1500 eggs in their mouths where the youngsters develop, so Gudrun De Boeck, Mathieu Desclée and Jung Liew filmed the expectant mums' head movements for 9 days to find out exactly how they moved their jaws as they churned their eggs. Comparing the jaw motions when the fish were 'churning' and breathing, Iris Joris, Van Wassenbergh and Peter Aerts saw that the churning parents' upper jaw always protruded several millimetres beyond the lower jaw; meanwhile, the mouth remained relatively static when the fish breathed normally. In addition, the churning fish expanded their mouth cavity significantly and the expansion rippled forwards from the back of the head as they depressed the mouth floor down, having pushed the gill covers (opercula) outwards. In addition, Joris and Van Wassenbergh realised that water was pulled into the mouth strongly through the gill slit during the first 100 ms of the churning action before jetting out of the gill slit behind the head. 'I was surprised to see such a strong inflow of water entering through the gill slits,' says Van Wassenbergh, who adds, 'during breathing or suction feeding, this is normally the place where water exits… it is markedly different in churning'.

However, the team was none the wiser about whether the eggs were moving inside the fish's heads until Van Wassenbergh cautiously popped a dummy egg carrying a steel pellet inside the mouth of an incubating mum. 'The X-ray video experiment to track the motion of a dummy egg was very challenging', he says, recalling that four of the mothers spat out their own eggs when he attempted to insert the ball into their mouths. However, Van Wassenbergh eventually succeeded, and saw the dummy egg pushed forward away from the gills as the water flowed into the mouth and the wave of expansion surged forward.

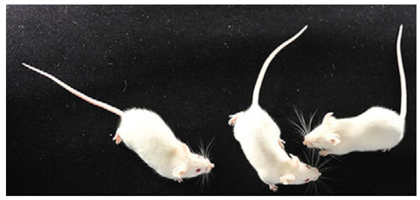
So, churning parents prevent themselves from asphyxiating on eggs that could clog their gills while mouth brooding by sucking water in through the gill slits and washing the eggs around in their mouths. 'In fact, churning can be regarded as a closed-mouth cough to avoid the parent choking', chuckles Van Wassenbergh.

10.1242/jeb.142513

Van Wassenbergh, S., Joris, I., Desclée, M., Liew, H. J., De Boeck, G., Adriaen, D. and Aerts, P. (2016). Kinematics of mouthbrooding in *Oreochromis niloticus* (Cichlidae). *J. Exp. Biol.* **216**, 1535-1541.

Kathryn Knight

Male mice chitchat more when there's an audience



Two males (left) and a female (right) SWR/J mouse. Photo credit: Matt Staley.

For centuries, mice have been the embodiment of silence, but now it seems that they could have more in common with a chattering flock of birds than with Trappist monks. 'In the 1950s and 1960s, following the development of ultrasonic microphones, several researchers showed that many rodents produce ultrasonic vocalizations,' says Roian Egnor, from the Janelia Research Campus, USA, although the function of the rodents' communications was poorly understood. Adding that mice often live in large colonies, Egnor's former graduate student, Kelly Seagraves, explains that other social species alter the ways in which they communicate depending on the presence of an audience: 'For example, people react differently to a situation depending on whether other people are around and who those people are,' she says. Could mice be the same? 'We were interested to see whether the vocalizations of mice indicated their awareness of the presence of an audience', says Egnor, and so the duo set about investigating how the presence of a male mouse altered the overtures of another male as he serenaded a female.

After recording the number of times that a male mouse called in response to the odour of an attractive female, Egnor and Seagraves saw that the male chirruped away contentedly as he wooed the lady. However, when they introduced an additional male into the arena, both males uttered more calls than they had when performing individually. The mice had altered their calling rate in the presence of an audience, in much the same way that other species do when there are onlookers. And when Seagraves analysed the structure of the males' overtures in the presence of other males with the help of acoustics expert Ben Arthur, she found that the calls became more sophisticated, including a wider variety of chirrups.

'After that, we wanted to know which aspects of the male's audience could also elicit changes in vocal behaviour', says Seagraves, who systematically tested how the males serenaded the females' odour in the presence of male urine, male odours on bedding and an anaesthetised male. However, the team was surprised to find that the odour of another male was not enough to trigger changes in the males' serenades: a male had to be physically present, even if he was anaesthetised, to alter the song's composition and the call rate.

'I was surprised that urine and odours from an unfamiliar male did not have an effect on male vocal behaviour', says Seagraves, adding that this was unexpected in the context that mice are thought to transmit social information about territory through scent marks. And when the duo analysed the males' interactions, it seemed that the presence of a female's odour was enough to prevent the males from fighting, encouraging them instead to sniff each other while calling.

Concluding, the team says, 'This suggests that when a male audience is present, males may be directing their vocalisations to the other male, instead of – or in addition to – the potential female partner'. And, having confirmed that males alter their performance in the presence of a male audience, Seagraves says, 'We now have sufficient evidence to begin investigating the neural mechanisms underlying this phenomenon in the mouse, where we have a much greater capacity for neural manipulations, and this in turn could lead us to a greater understanding of how the human brain is processing and using social cues from audiences'.

10.1242/jeb.142505

Seagraves, K. M., Arthur, B. J. and Egnor, S. E. R. (2016). Evidence for an audience effect in mice: male social partners alter the male vocal response to female cues. *J. Exp. Biol.* **219**, 1437-1448.

Kathryn Knight

Journal of Experimental Biology (2016) 219, 1417-1419

Turtle hatchlings pull together to push their way out

Being born is tough enough without having heavy sand piled on top of you and having to chip your way out of an egg, but this is the challenge faced by every hatchling turtle with its gaze firmly fixed on the wide blue yonder. Yet, each individual hatchling is not alone. Buried in clutches of up to 150 eggs, every youngster is accompanied by its siblings on its odyssey to the surface. Knowing that many creatures, such as fish and birds, move in unison to reduce energy consumption, Uzair Rusli and colleagues from The University of Queensland, Australia, and the Universiti Malaysia Terengganu, Malaysia, decided to measure the metabolic costs of burrowing to the surface for clutches of green turtle (*Chelonia mydas*) eggs ranging in size from 10 to 60 eggs.

However, Rusli and colleagues admit that designing the specially adapted respirometer required to measure the hatchlings' oxygen consumption while they laboured was particularly challenging. After months of trial and error, the team eventually discovered that the youngsters could only burrow to the surface successfully if they provided air gaps around the eggs and the nest was shrouded in darkness in a peaceful laboratory setting.

Having cracked the logistical issues, the team recorded that it took the hatchlings between 3.7 and 7.8 days to reach the surface through a 40-cm-thick layer of sand, with individual hatchlings from the largest groups using approximately 4.4 kJ of energy (11% of their egg yolk), while the hatchlings from the smallest clutches consumed as much as 28.3 kJ (68% of the egg yolk). The team calculated the amount of energy left over for the hatchlings after they made it to the surface, and suspects that the youngsters from large clutches benefit more from the combined efforts of their siblings. This leaves them with additional reserves to fuel their dash across the beach and final swimming frenzy in a bid to make it to the ocean, where they can dine on something other than egg yolk at last.

10.1242/jeb.142521

Rusli, M. U., Booth, D. T. and Joseph, J. (2016). Synchronous activity lowers the energetic cost of nest escape for sea turtle hatchlings. *J. Exp. Biol.* **219**, 1505-1513.

Kathryn Knight
kathryn.knight@biologists.com