

INSIDE JEB

Bull ants fine-tune ocelli to their lifestyle



Ocelli on top of the head of a nocturnal male *Myrmecia nigriceps*. Photo credit: Ajay Narendra.

Aeronautical engineers would love to know more about how flying insects control their manoeuvres. While human pilots are equipped with an artificial horizon for orientation, flying insects depend on three simple eyes (ocelli) set in the tops of their heads, which probably track the position of the horizon for stability. Intriguingly, some walking insects, such as members of the *Myrmecia* bull ant family, are also equipped with these unusual mini-eyes, even though they are probably of most use when members of a nest take to the wing to establish a new colony. Knowing that different members of the *Myrmecia* family, which often share the same territory, tend to be active at different times of day, Ajay Narendra wondered whether the insects have fine-tuned the specialised minute eyes to their different lifestyles.

Armed with boots for protection from the ants' fearsome stings, Narendra and Willi Ribi ventured out into the mountains and national parks in the vicinity of Canberra to collect workers of the walking day-active *M. croslandi* and dawn/day-active *M. tarsata* bull ants, while the dusk-active *M. nigriceps* and the nocturnal *M. pyriformis* ants had to be collected by infrared light at night, so that the pair could 'spot them before they spotted us'. However, collecting flying members of each family was more challenging: 'They

fly out of the nest only once a year and were produced by only a few of the nests', recalls Narendra, who eventually gathered night-flying *M. nigriceps* males and day-flying *M. pyriformis* males.

Back in the lab, the pair took detailed images of the ants' mini eye structures using light and electron microscopes. 'The ocelli of the [walking] worker ants were surprisingly small compared to those of the flying ants', says Narendra, who had to take great care handling the delicate structures. The day-active *M. croslandi* had the smallest ocelli lenses (76 μm), the species that were active in dim light and during the night had larger (129–201 μm) lenses, and the lenses of the flying ants were at least 10 times larger than those of the walking ants. The duo then compared the size of the light-sensitive portion of the eye, the rhabdom, across the four species of walking ants and found that the rhabdoms were widest in the ants that are active in dim-light conditions, probably to increase their light sensitivity, while the eyes of the flying ants were packed with 3 times as many rhabdoms as the eyes of the walking workers.

Knowing that many insects are capable of sensing polarised skylight with their ocelli for navigation, Narendra and Ribi looked for structures in the ant ocelli that might detect light polarisation and were impressed to see that the two walking day-active species (*M. croslandi* and *M. tarsata*) had the structures, while the species that were active toward the dim end of the day lacked them. They also found that the ocelli of both species of flying ants were equipped with structures that may be sensitive to light polarisation, suggesting that the mini-eyes may be polarisation detectors, even though *M. nigriceps* flies at night.

In addition, the two scientists was surprised when they discovered a reflective crystal structure in the ocelli of the flying night-active *M. nigriceps* males, toward the top of the rhabdom. Explaining that other animals use similar reflective eye coatings to enhance their sensitivity to light in dim conditions, Narendra suspects that the nocturnal fliers take advantage of the increased light

sensitivity to make the most of what little light there is when they take to the wing.

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Narendra, A. and Ribi, W. (2017). Ocellar structure is driven by the mode of locomotion and activity time in *Myrmecia* ants. *J. Exp. Biol.* **220**, 4383–4390.

Kathryn Knight

Fruit fly mum's gift protects larvae through development



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

As if life wasn't hard enough for insect-kind, with intensive agriculture and insecticides obliterating their numbers, the planet is also warming at an unprecedented rate and it is not clear how well these ectotherms will fare in a warmer future. Brent Lockwood from the University of Vermont, USA, explains that the majority of our understanding of how these animals will adapt is based on research carried out on adult insects, with little account taken of the effects that higher temperatures may have on earlier life stages. 'For terrestrial insects, this oversight has been particularly problematic', he says, explaining that, unlike their parents, developing insects are essentially immobile during the earliest life stages and unable to evade the hottest conditions. In addition, recently fertilised eggs are unable to activate their own protective genes following a sudden heat wave, depending instead on the pool of protective RNA molecules bequeathed by their mothers to them. Lockwood and

Kristi Montooth from the University of Nebraska, USA, suspected that one such maternal molecule – Hsp23 mRNA – might encode a protein that protects against cellular damage caused by overheating, so they decided to investigate how well *Drosophila* eggs that were well provisioned by their mothers with Hsp23 mRNA survived during a heat wave.

Stimulating fruit fly mums to produce eggs with higher levels of the protective Hsp23 mRNA, Lockwood and Cole Julick then allowed the females to mate with males and lay their eggs on a nutritious fruit gel before heating the eggs to temperatures ranging from 22 to 40°C for 45 min. ‘Our temperature treatments mimic the sudden temperature changes that frequently occur in nature where the temperature of rotting fruit can increase rapidly on a hot day’, says Lockwood. They then transferred the eggs to a comfortable 22°C before monitoring their survival and development over a 15 day period.

Impressively, the eggs that had received a larger dose of Hsp23 mRNA from their mothers were significantly more robust than eggs that had just received the regular dose. ‘For example, a brief exposure to 34°C killed approximately 50% of normal embryos, but killed only 10% of embryos with higher levels of the Hsp23 mRNA provided by their mothers. This is a huge effect caused by the action of only a single gene’, says Lockwood. And when the team of three tracked the development of the normal eggs, which had low levels of the protective molecule, through the larval stage to pupation, they were surprised to see how seriously the brief heat wave affected their development many days later: larvae from the overheated normal eggs were unable to climb as well as larvae from overheated eggs that were packed with their mother’s additional supply of Hsp23 mRNA.

‘These results demonstrate that single genes can have big effects on the whole organism, and maternal effects are not only important for the physiology of early embryos but also have effects that last through larval development’, says Lockwood, who is keen to find out whether populations of fruit flies that experience warmer climates have developed specialised strategies to tolerate toasty conditions compared with populations from cooler climes.

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Lockwood, B. L., Julick, C. R. and Montooth, K. L. (2017). Maternal loading of a small heat shock protein increases embryo thermal tolerance in *Drosophila melanogaster*. *J. Exp. Biol.* **220**, 4492-4501.

Kathryn Knight

Locust oxygen delivery matched to souped-up muscle



A locust in flight. Photo credit: Edward Snelling.

Most insects have pretty sluggish metabolisms until they jump into the air; but when they take off, these animals are virtually on fire. Boosting their metabolic rates by as much as 100 times, fliers have to ensure that they can keep the furnace that powers flight well supplied with oxygen. Roger Seymour from the University of Adelaide, Australia, explains that insect bodies are plumbed with air capillaries – trachea – that supply oxygen directly to every tissue and this system is matched to the tissues that they supply: the tracheal system of the locust flight muscles is 6 times larger than that of the leg muscles, in line with the flight muscle’s high energy consumption. However, the flight muscle contains 20 times more mitochondria – which consume oxygen during ATP production – than the leg muscles; could the muscle consume even more oxygen and if so could the tracheal system meet the demand? Seymour and graduate student Edward Snelling wondered how well the flight muscle’s oxygen delivery system was matched to its oxygen demands.

Reasoning that the insects would be very sensitive to the amount of oxygen in the air at low concentrations if their oxygen delivery system and oxygen consumption were matched, Seymour realised that he could measure the locusts’ sensitivity by creating a variety of different atmospheres – oxygen mixed in different ratios with either nitrogen, light helium or heavy sulphur hexafluoride – and recording the insects’ oxygen consumption while they flew in the gases. If the insects’ performance dropped

off below a specific oxygen concentration, then their respiratory system and muscle would be well matched.

Seymour assigned the task of designing the atmospheres to Honours student Rebecca Duncker and worked with her and Edward Snelling to figure out how to attach insects to a tether during flight via a tiny magnet glued to the insect’s back. However, he recalls that the locusts weren’t always happy to fly in the unfamiliar atmospheres. ‘The difference in resistance of their wings... apparently stopped them from flapping’, he says.

Finally, Duncker plotted the metabolic rate of the insects against the fraction of oxygen in the air and realised that the metabolic rate of the insects plummeted below ~21% oxygen: the oxygen delivery system was perfectly matched to the flight muscle. ‘The flight motor of locust seems to be either on or off, and it has only one gear. No excess oxygen supply is required’, says Seymour, who is keen to find out whether species that carry cargo can turn up oxygen delivery to power flight in a way that unburdened locusts cannot.

Having developed the novel atmospheres, the trio realised that altering the main gas component would also change how fast oxygen diffuses into the tissue and this could allow them to test whether oxygen was delivered to the flight muscle by diffusion through the trachea or by the contraction of the flight muscles pumping air through. Duncker scoured the literature to find out how to combine oxygen, helium and sulphur hexafluoride to create one atmosphere that had a normal density where oxygen diffused fast and a second where oxygen diffused at the normal rate but the atmosphere was relatively dense in order to find out whether increasing the diffusion rate of oxygen in the flight muscle trachea permitted the insects to increase their metabolic rate. However, when she compared the metabolic rates of the locusts flying in the two atmospheres, they were essentially identical, confirming that oxygen is pumped into the flight muscle by the muscle’s own contraction.

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Snelling, E. P., Duncker, R., Jones, K. K., Fagan-Jeffries, E. P. and Seymour, R. S. (2017). Flight metabolic rate of *Locusta migratoria* in relation to oxygen partial pressure in atmospheres of varying diffusivity and density. *J. Exp. Biol.* **220**, 4432-4439.

Kathryn Knight

Divided swim bladder maintains brooding cichlid mum's balance



When *Astatotilapia burtoni* females lay eggs, they don't simply turn their backs and make a quick exit. The soon-to-be mothers vacuum the eggs up into their mouths, where the youngsters develop and grow before they are ready to depart 14 days later. But the sudden weight shift, amounting to as much as 30% of the female's weight, could seriously destabilise brooding mums and the situation could become even more perilous as the larvae and fry become heavier and more negatively buoyant during development. Yet, the mothers seem to take the inconvenience in their stride and never topple forward. Knowing that the fish's swim bladder is divided into two compartments, Julie Butler, Prosanta Chakrabarty and Karen Maruska from Louisiana State University, USA, wondered whether the fish may adjust the distribution of gas between the two parts of the swim bladder to compensate when they become front heavy.

Butler, Sarah Whitlow and Polly Gwan collected female *A. burtoni* that were ready to spawn large eggs, at various stages over the 14 day brooding period, and 1 and 3 weeks after the fry sallied forth, and then

X-rayed the fish. Comparing the X-ray images, the team could see that the overall area of the swim bladder did not alter during the development of the offspring; however, the distribution of gas between the two compartments did shift. The front section expanded while the rear section deflated when the females slurped up their eggs and remained so until the youngsters left their mother's mouths 2 weeks later. Then, the front section of the swim bladder deflated and the rear section reinflated. The team also monitored the fish's swimming posture when the fry were liberated, and found that the unencumbered females' heads bob upwards, so that the fish swam at an angle of 7 deg for 5 min until they resumed their pre-release almost-horizontal posture. And when the team attached a small bead weighing roughly the same as a mouthful of 7-day-old fry to the underside of the jaws of females that were not brooding, they saw the same shift in the swim bladder shape 4 h later.

Considering the cichlid's rapid reaction to their sudden weight redistribution, the team suspects that the fish redistribute gas between the two parts of the swim bladder

by opening and closing a ring of muscle in the diaphragm that separates the two. As tissue in the front section of the swim bladder produces gas, while tissue in the rear section removes gas from the swim bladder, the team suggests that the sphincter in the diaphragm closes when the fish suck the eggs into their mouths, allowing gas to inflate the front section to counteract the weight of the eggs. When the mothers release the fry, they can then restabilise their posture by rapidly opening the sphincter to drain gas into the rear compartment of the swim bladder. And the team suggests that a compartmentalised swim bladder could be an essential adaptation for fish that prefer to give their young a head start in their mouths.

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Butler, J. M., Whitlow, S. M., Gwan, A. P., Chakrabarty, P. and Maruska, K. P. (2017). Swim bladder morphology changes with female reproductive state in the mouth-brooding African cichlid *Astatotilapia burtoni*. *J. Exp. Biol.* **220**, 4463-4470.

Kathryn Knight
kathryn.knight@biologists.com