Inside JEB is a twice monthly feature, which 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

CLOACAL COOLING

When Dale DeNardo saw a Gila monster wandering around the Sonoran Desert with its cloaca protruding from its body, the Arizona State University physiologist speculated that the animal might be shedding heat as well as waste from its cloaca. DeNardo went on to show that evaporative heat loss from a Gila monster's cloaca can cool the animal by 3°C. Ty Hoffman, working with DeNardo and Glenn Walsberg, immediately wondered if birds can also use their cloacae to cool off when the temperature soars (p. 741).

'For the last 50 years or so, physiologists have assumed that any evaporation that doesn't happen through a bird's mouth must happen through the skin,' Hoffman explains. He points out that the cloaca is a large, moist surface that's perfect for evaporative heat loss, yet nobody had measured how much heat birds lose through their cloacae. Hoffman decided to examine cloacal evaporation in Inca doves, which are known to have high rates of skin evaporation.

To determine how much water doves lose through their mouth, skin and cloacae, Hoffman placed each dove in a glass chamber separated into two compartments by a latex sheet with a hole for the bird's head to poke through, so that the top compartment captured water lost through the bird's mouth and the bottom captured water lost through the skin and cloaca. To measure water loss through the birds' skin only, Hoffman sealed the birds' cloacae with glue and measured the water content of air samples from the bottom compartment using a hygrometer. To measure water loss through the skin plus the cloaca, he removed the cloacal seals and repeated the water content measurement. 'Unsealed' birds had higher rates of water loss than 'sealed' birds, suggesting that cloacal evaporation was playing a large role in evaporative heat loss.

But can doves regulate how much heat they lose through their cloacae? To find out, Hoffman prevented evaporative heat loss from the birds' mouths by pumping humid air into the chamber's head compartment, and then cranked the temperature up. At 30°C, 35°C and 40°C, doves hardly relied on their cloacae. But at 42°C, cloacal evaporation accounted for a quarter of evaporative heat loss. 'This mirrors what we saw in the Gila monsters,' Hoffman says. Because cloacal evaporation only kicks in at extreme temperatures, it may be an emergency thermoregulatory tactic.

To show that this is not simply a 'by-product' of having a cloaca, Hoffman repeated the measurements for Eurasian quail, which have a large cloacal opening and may therefore be expected to use cloacal evaporation. Unfortunately, Hoffman could only test quail up to 32°C, because the birds became heat stressed above this temperature. Yet, despite the fact that the birds were showing obvious signs of heat stress, the evaporative heat loss from quail cloaca was negligible. This supports Hoffman's conclusion that doves actively regulate the contribution of their cloacae to heat loss.

Hoffman speculates that this ability could increase doves' fitness in hot places, as it may allow the animals to be active when predators aren't around, or increase the time they spend foraging at the hottest time of day. He would now like to establish whether other birds can also use their cloacae to cool off. 'It would be nice to show that I haven't simply stumbled across the only species to do this!' he laughs.

10.1242/jeb.02737

Hoffman, T. C. M., Walsberg, G. E. and DeNardo, D. F. (2007). Cloacal evaporation: an important and previously undescribed mechanism for avian thermoregulation. *J. Exp. Biol.* **210**, 741-749.

Yfke Hager

THE UPS AND DOWNS OF BEE NAVIGATION

Picture by Marie Dacke

Honeybees might have small brains, but their navigation skills are second to none. Marie Dacke and Mandyam Srinivasan's bees at the Australian National University, Canberra, could find a 4 cm wide feeder 500 m from their hive on many repeat visits, and like many honey bee researchers, Dacke wondered: 'how can an animal with such a small brain solve such incredibly complex tasks'? Dacke and Srinivasan specifically wanted to know how bees measure the distance that they travel using their internal 'step measurer', or odometer, when they fly three-dimensional trajectories (p. 845).

Bees calculate the distance they have flown using 'optic flow' which is how quickly, and in which direction, the image of the environment moves across their eyes. When they return to the hive they transmit this information to the other bees through the waggle dance. The duration of dance's waggle phase tells the other bees the distance they have to fly to the food, while the direction of the dance indicates their heading relative to the sun.

To find out how bees estimated distance in three dimensions, the team first trained bees to fly to a feeder inside a 11 cm by 20 cm by 6 m tunnel. They painted the inside of the tunnel with a black and white check pattern to provide optic flow to the bees as they flew along. After training, the team changed the orientation of the tunnel, and measured the bees' waggle dances back at the hive to see how far they thought they flew to reach the feeder. The team found that the bees danced to their nest mates that they had travelled 6 m to reach the feeder, regardless of whether the tunnel was horizontal, vertical, or tilted at an angle of 48°. The bees were signalling the total distance they had travelled, not the relative horizontal and vertical distances. This is unlike another great insect navigator, the desert ant, which measures the horizontal distance it travels even when on bumpy terrain, not the total distance travelled up and over the bumps.

Wondering if changing the direction of the optic flow half way through the bees' journey would change their dances, the team built an 'L' shaped tunnel, with a 2 m long vertical section attached to a 4 m horizontal section. Even though the bees changed direction on the way to the feeder, they still danced that they had travelled 6 m. To confirm that optic flow was necessary to estimate distance they painted the 2 m section of the tunnel with vertical stripes, which minimised optic flow. The bees 'missed out' this part of the tunnel when estimating distance, signalling that they had flown around 4 m, proving that they do need optic flow to calculate distance.

Finally, to test how reliably the bees were finding food in horizontal and vertical tunnels, the team trained bees to find food in the tunnels, before taking the food away for the experiment. Monitoring the bees' 'back and forth' searching behaviour as they tried to find the food again, the team found that bees were equally good at pinpointing where the food should have been in both horizontal and vertical tunnels, showing that the 3-D orientation they have to fly is not important when it comes to calculating distance.

'The biggest implication is the way the bees treat optic flow,' Dacke explains, 'the neurones in the bees' brains responsible for the distance calculation seem to be insensitive to the direction of optic flow.' 'Ultimately', she says, 'we want to understand the neural basis of how the odometer works'.

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Dacke, M. and Srinivasan, M. V. (2007). Honeybee navigation: distance estimation in the third dimension. *J. Exp. Biol.* **210**, 845-853.

EVOLVING FLIES' CIRCADIAN CLOCKS

It's not just human beings that divide into early rising 'larks' and late rising 'owls'. Flies too are governed by biological, or circadian, clocks that dictate when they emerge from the pupa and when they are active. But how did these clocks evolve? To investigate, Vijay Kumar Sharma and his colleagues at the Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India, imposed a selection pressure on flies (*Drosophila melanogaster*) as they emerged from the pupa. By selecting flies that emerged in the morning or evening, they wanted to see if they could change the timing of the flies' clocks over many generations (p. 906).

Keeping flies in a regime of 12 h dark then 12 h light every 24 h, the team chose larks that emerged from their pupae in the morning between 5 am and 9 am, or owls that emerged in the evening between 5 pm and 9 pm. The team kept these early and late risers separate, using them to breed the next generation, before selecting the early and late risers from the offspring for breeding, and so on for 55 generations.

Interested to know how the flies' clocks were changing through the generations, the team carried out a series of tests on the 5$^{\text{th}}$, 10$^{\text{th}}$, 25$^{\text{th}}$, 40$^{\text{th}}$ and 55$^{\text{th}}$ generations. Measuring when flies in the early or late groups emerged from their pupae, they found that the percentage of flies in the early group emerging in the morning increased, but evening risers decreased. In the late populations the reverse occurred; more flies emerged in the evening, and fewer in the morning. This showed that they were successfully breeding lark and owl populations of flies.

Next, the team looked at the pattern of emergence, noting how many flies emerged during set time periods, so that they could calculate the time when the greatest number of flies emerged relative to when the lights came on. They found that in early groups, the peak emergence occurred close to lights on, with more flies emerging at this peak through the generations. They observed a similar pattern in the late groups, only the peak occurred close to lights off.

While the team had selected flies according to when they emerged, they also wanted to see if any of the flies' other behaviours had changed. By measuring when the adult flies were active, they found that adults from early groups were more active in the morning, and those from late groups were more active in the evening, mirroring the emergence patterns. The implication of this result is that 'rhythmic processes in the body are controlled by multiple clocks, but the clocks talk to each other' says Sharma, so selecting for emergence also influences when flies are active.

Finally, to find out how the flies' clocks would operate without any light cues, they plunged the flies into darkness and measured their emergence patterns and activity. Previous studies had shown that animals with early clocks have a slightly faster circadian rhythm than animals with late clocks, and the team found the same with their flies. The flies' emergence patterns and activity followed the same patterns as when the lights were on and off, although the early flies had a slightly faster daily rhythm of 23.6 h compared to the late flies' rhythm of 24.3 h. This shows that 'clocks evolve through selection pressure on the timing of rhythmic behaviour,' Sharma says.

10.1242/jeb.02739

Kumar, S., Kumar, D., Paranjpe, D. A., Akarsh, C. R. and Sharma, V. K. (2007). Selection on the timing of adult emergence results in altered circadian clocks in fruit flies *Drosophila melanogaster*. *J. Exp. Biol.* **210**, 906-918.

FROG MUSCLES SURVIVE THE 'BIG SLEEP'

When the sun really starts to sizzle, most animals tough it out in the shade. But the Australian green-striped burrowing frog, *Cyclorana alboguttata*, avoids the sun and lethal dehydration altogether by retreating into the ground and undertaking a 'summer hibernation', or aestivation, for up to nine months. Long hibernations often cause havoc with mammals' muscles, which atrophy through misuse, however previous research on the green-striped burrowing frog showed that their leg muscles weren't affected by a three month aestivation. But, since the frogs are in the ground for up to nine months, Beth Symonds and her colleagues from the University of Queensland and Coventry University wanted to know if the frogs' muscles were still unaffected after a full aestivation. They chose to scrutinise the structure and the contractions of two frog 'thigh' muscles: the sartorius, which is a mostly fast twitch muscle at the front of the leg; and the iliofibularis, a slow twitch muscle found at the back of the leg (p. 825).

The team found that while the cross-sectional area of the iliofibularis and sartorius muscle fibre density decreased after aestivation, no other properties of the muscles such as muscle mass or the proportion of slow and fast fibres changed. Examining the muscles' contractions, they found that muscle contraction speed slowed down in the slower-twitch iliofibularis only, but despite these small changes the muscles still produced the same amount of power, showing that that they resisted atrophy during their subterranean break. The next challenge will be to work out how the frogs manage this feat.

10.1242/jeb.02740

Symonds, B. L., James, R. S. and Franklin, C. E. (2007). Getting the jump on skeletal muscle disuse atrophy: preservation of contractile performance in aestivating *Cyclorana alboguttata* (Günther 1867). *J. Exp. Biol.* **210**, 825-835.

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