

INSIDE JEB

Nepalese porters do it the hard way



A Nepalese porter carrying a typical load. Photo credit: Norman Heglund.

If you've ever found yourself struggling under a heavy pile of suitcases at the end of a holiday, spare a thought for the porters in some of the planet's most remote locations. 'When you start looking around, professional porters all around the world carry loads supported by their heads', says Norman Heglund, from the Université catholique de Louvain, Belgium, who is fascinated by the techniques that allow people to carry heavy burdens. Previously, Heglund had investigated how the Kikuyu and Luo women in Kenya carry water on their heads, and he discovered that they have a unique trick where they transfer their weight from one foot to the other extremely effectively. This allows them to conserve up to 80% of the energy from the previous step, enabling them to carry loads of up to 20% of their body weight with no additional effort. 'Then we were thinking, who are the most famous porters in the world... and the Nepalese are reputed to be the best load carriers', says Heglund, adding, 'so we decided to go look at them'.

Shipping one tonne of equipment from Belgium to Nepal and selecting Phakding in the Everest valley as his base, Heglund recalls hiring about 30 porters and a dozen yaks to haul the equipment half a day's hike up to the settlement. As Phakding is on the path to Namche – which hosts a weekly bazaar that supplies the local population – Heglund knew that there would be a steady stream of male and female porters hauling cargo on the 10-day trek from

which he could recruit volunteers. 'The first thing we did was put a bathroom scale out with the equivalent of a lemonade stand and we said, "If you stop, let us weigh you, weigh your loads, we will give you a glass of lemonade", and virtually everybody did', recalls Heglund. Then he and his colleagues Guillaume Bastien, Patrick Willems and Benedicte Schepens asked some of the volunteers to walk back and forth across a force plate at various speeds, carrying loads ranging from 0 to 154% of their weight in baskets suspended from straps across their foreheads. The team also filmed the porters' movements, and Bastien later used the footage to calculate the amount of mechanical work performed by each of the participants' limbs as they carried their burdens.

However, when the team compared the energy transferred between steps when the Nepalese porters were loaded and unloaded with the steps of European volunteers, they were surprised that the Nepalese were not using any energyconserving tricks to help them carry their extraordinary loads. 'They are doing nothing special', says Heglund. Instead, they found that the porters were moving at a relatively slow speed of around 3-4 km h⁻¹ and took frequent breaks, walking sometimes for as little as 15 s before resting for another 45 s, to ensure that they never had to revert to costly anaerobic metabolism. Even when the porters were in danger of missing their Saturday market deadline, they never walked faster, preferring instead to walk late into the night to make up time.

Admitting that he was initially disappointed that the Nepalese porters did not use any special adaptations to conserve energy, Heglund explains that in retrospect this may not be so surprising. 'These guys don't ever take two steps on the same level', he says, which prevents them from moving economically. And he concludes that this makes it 'all the more awe-inspiring that they carry these heavy loads up and down hills at high altitude as they do it the hard way'. 10.1242/jeb.151951

Bastien, G. J., Willems, P. A., Schepens, B. and Heglund, N. C. (2016). The mechanics of headsupported load carriage by Nepalese porters. *J. Exp. Biol.* 219, 3626-3634.

Kathryn Knight

Dynamic filter keeps sandfish lungs sand-free



Sandfish lizards (*Scincus scincus*). Photo credit: Anna Stadler.

Creatures that make their homes in the desert have to be tough and adaptable. To overcome the searing heat and lack of water, many have developed strategies that help them to avoid the sun at critical times. Sandfish lizards (Scincus scincus) from the North African desert resolve the challenge by plunging beneath the surface of the dunes and burrowing down into sand at a comfortable temperature. Yet how these reptiles breathe in an environment where most would suffocate was a mystery. Not only must they breathe in sufficient oxygen while entombed in sand, but they must also avoid inhaling grains into their delicate lungs. According to Anna Stadler, from Johannes Kepler University Linz, Austria, another burrowing species - the Colorado Desert fringe-toed lizard (*Uma notata*) – is equipped with a U-shaped kink in its respiratory tract, which traps sand particles that are then exhaled. Might subterranean sandfish use a similar twist to keep their lungs sand-free?

First, Stadler, Werner Baumgartner and Wolfgang Böhme needed to find out how well the lithe creatures protect their delicate respiratory tissue from the abrasive grains, and when they dissected animals that had died from natural

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causes, they were impressed to find that the sandfish's airways and lungs were entirely sand-free. However, when they reconstructed the 11-mm-long airways from 3D images of the respiratory tract, there was no evidence of a sand trap kink. And although they found tissue that could swell and trap particles, it was not large enough to close the airways and account for the lizard's sand-free lungs. 'In general, the only thing that was interesting is that there is mucus and cilia and there is a narrow part that gets wider and then it gets narrow again', says Stadler.

With no evident filtration system, Stadler and her PI, Werner Baumgartner, decided to build a model of the minute respiratory system to try to discover how the tiny animals were excluding sand from their lungs. Realising that she would have to scale up the model if they were to have any hope of making meaningful measurements, Stadler teamed up with Michaela Huemer, Martin Riedl, Stephanie Shamiyeh and Bernhard Mayrhofer to print a 3D model that was 7.8 times larger; 'It was not that easy', says Stadler. The team also had to switch to using a helium atmosphere – to ensure that the gas flows in and out of the model respiratory system were realistic – in addition to replacing the fine sand with coarser grains. Meanwhile, Boštjan Vihar and Mathias Günther attached a tiny sensor to the lizards' chest to measure their breathing while submerged, and the team was surprised to find that although the lizards inhaled slowly when buried, they seemed to 'cough' the air out of their lungs in just 40 ms. The team then buried the respiratory system model in sand, simulated the lizard's breathing pattern with a pump and saw that the model remained sand-free. Finally, Stadler calculated the flow pattern through the airways and realised that the air speed decreases dramatically at the point at which the airway widens above the section coated in mucus and cilia when the animals inhale.

Stadler suspects that this drop in air speed allows the sand particles to fall and become trapped in the mucus before the lizard exhales hard to effectively cough the sand out of its airways. So instead of using a physical obstruction to protect themselves from suffocation, sandfish appear to use a dynamic filter to prevent their lungs from becoming clogged with sand.

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Stadler, A. T., Vihar, B., Günther, M., Huemer, M., Riedl, M., Shamiyeh, S., Mayrhofer, B., Böhme W. and Baumgartner, W. (2016). Adaptation to life in aeolian sand: how the sandfish lizard, *Scincus scincus*, prevents sand particles from entering its lungs. *J. Exp. Biol.* **219**, 3597-3604.

Kathryn Knight

Bees rewarded by instinctive and learned behaviours



Honey bee. Photo credit: Paul Stein, New Jersey, USA [CC BY-SA 2.0], via Wikimedia Commons.

From the instant that a newly hatched turtle begins heading toward the sea to the irresistible urge that salmon have to return to the stream of their birth, many animal behaviours are driven by hard-wired instincts that are impossible to resist. However, animals are also capable of learning a wide range of activities, and it is the similarities and differences between the mechanisms that underlie these two types of behaviour - instinctive and learned - that intrigues Gene Robinson, from the University of Illinois at Urbana-Champaign, USA. 'Little is known about the degree to which related instinctive and learned behaviours rely upon similar molecular mechanisms', says Robinson, who wanted to investigate the expression patterns of genes in the brains of honey bees embarking on instinctive searches for mates and those that have learned to forage for food to find out how they compare.

Knowing that male drones at the bee research facility in Urbana favour searching for mates in the afternoon, Robinson and Nicholas Naeger trained female foragers to seek out food at a nearby feeder at the same time of day. The duo then collected the brains of bees from both groups when on the verge of embarking on their afternoon flights and compared the gene expression patterns with those in the brains of bees collected the following morning.

Focusing on one region of the brain – the mushroom body - that is known to participate in learning and memory, Robinson and Naeger began analysing the differences in the gene expression patterns. Not surprisingly, a large number of the differences in gene expression - 5680 were due to the sex difference between the male drones and the female workers. However, after filtering those differences out, the team identified 623 specific expression changes in genes associated with the drones' instinctive mating flight behaviour, in contrast with the 473 gene expression changes that the duo found in the female workers that had learned to forage. And when Naeger and Robinson compared the gene expression patterns of both castes of bee just before initiating their flights with those of inactive bees the following morning, they identified 166 genes that were critical for both flight behaviours.

Classifying the genes that were common to both types of flight, the duo identified members of the family of 'unfolded protein binders' and heat shock proteins, which are usually expressed in response to stressful situations. In addition, genes that are involved in reward-conveying dopamine neurotransmission also contributed to both flight behaviours, and the duo comments, 'The similarities suggest that reward-related instinctive and learned behaviours share common molecular architecture'.

However, the drones that were embarking on instinctive mating flights seemed to have primed transcription factors that would prepare the brain for navigational learning. Meanwhile, the foraging females that had already learned their flight path were modifying the expression of genes that contribute to brain remodelling and protein modification, which contribute to memory formation.

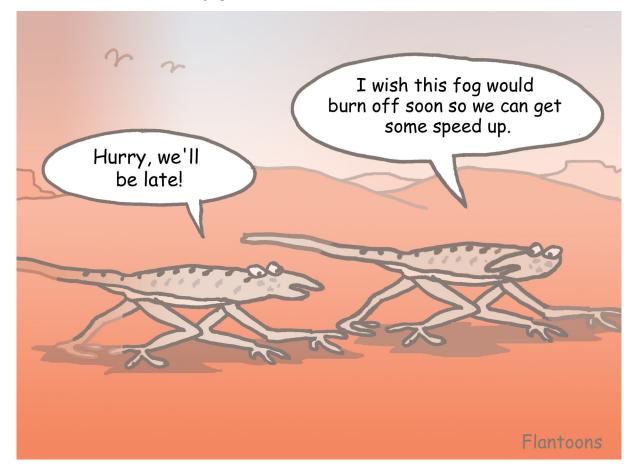
Pointing out that this is only the second study that has investigated similarities and differences between instinctive and learned behaviours, Naeger and Robinson say that these studies 'show that there are common molecular substrates for both instinctive and learned behaviours'. Having identified that genes that contribute to reward (dopaminergic) sensations are activated in the altruistic behaviours of foragers gathering food on behalf of the entire colony, they also say, 'These findings suggest that social evolution has relied on elements of reward processing, involving both innate and learned behaviours, which function at the level of the individual in order to build a social reward system'.

10.1242/jeb.151936

Naeger, N. L. and Robinson, G. E. (2016). Transcriptomic analysis of instinctive and learned reward-related behaviors in honey bees. J. Exp. Biol. 219, 3554-3561.

Kathryn Knight

Namib day geckos less stable in dim conditions



'Hanging out' takes on a new dimension when you're a gecko; clinging onto smooth surfaces with gravity-defying toes, many gecko species are perfectly content to perch at mind-bogglingly steep angles. However, Namib day geckos have taken a different path from their cousins. Preferring to sprint by day at high speeds through the relatively level desert terrain in their foggy coastal home, Rhoptropus afer geckos have lost many of the adhesive features that keep their nocturnal relatives firmly anchored. Explaining that night active geckos rely heavily on vision when navigating their surroundings in the dark, Timothy Higham and Aleksandra Birn-Jeffery at University of California, Riverside, USA, wondered how much of an impact varying light levels might have on R. afer activity when the day

begins shrouded in fog that burns off later.

After filming the nimble lizards as they scampered across a sandpaper runway at simulated light levels ranging from complete darkness to a foggy morning to the equivalent of full daylight, Higham and Birn-Jeffery saw the animals sprinting fast (1.4 m s^{-1}) on relatively erect legs in the brightest conditions; 'Sprinting fast is likely very important for the survival of this species', the duo says. However, when Higham and Birn-Jeffery turned the lights off, the animals slowed to a more sedate speed (0.6 m s^{-1}) , sprawled their legs wide apart and dropped their bodies closer to the ground, leading the duo to suggest that 'stability may be a significant problem during times when visual input is sub-optimal'. And when they consider the impact that foggy conditions might have on the animals' mobility, the duo suspects that the geckos have lost the adaptations that benefited the vision of nocturnal ancestors and say, 'The negative impact of thick fog on light level might cause a corresponding reduction in activity'. They also warn that climate change, which may increase the occurrence of fog, could limit the ability of the fleet-footed geckos to forage in the dimmer conditions.

10.1242/jeb.151944

Birn-Jeffery, A. V. and Higham, T. E. (2016). Light level impacts locomotor biomechanics in a secondarily diurnal gecko, *Rhoptropus afer*. *J. Exp. Biol.* **219**, 3649-3655.

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