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INSIDE JEB

Extreme excavation: fire ant style

Fire ant (*Solenopsis invicta*) excavating artificial soil. Photo credit: Daria Monaenkova.

Fans of *The Lord of the Rings* may disagree, but when it comes to exquisite excavation, the dwarves of Moria have nothing on the mighty fire ants of Georgia Tech. But Dan Goldman and Michael Goodisman aren't fascinated by the aesthetics of fire ant architecture alone. 'I have an interest in animals interacting with complex materials', explains Goldman, who has studied creatures such as sidewinder snakes and sandfish lizards moving through and across sand. With the ants on their doorstep, Goldman and Goodisman were intrigued to learn more about how the insects work together and the mechanical factors that affect ant nest construction in soils ranging from wet clay to coarse sand.

However, producing consistent simulated soil conditions for the ants to excavate was not as simple as stirring soil into water – 'Different people got different conditions', Goldman recalls – until Nick Gravish came up with the idea of sieving mixtures of sand and water to create uniform distributions of water through the soil. Then, everything was ready for Daria Monaenkova to take 14.5 cm long plugs of the uniform soil samples – ranging from minute clay-like particles to ant head-sized grains of sand with a moisture content ranging from complete saturation to perfectly dry – drop mini-colonies consisting of 100 ants on the top and leave them to their excavations for 20 h. The challenge then was to visualise the nests without destroying them, so Monaenkova meticulously scanned the plugs with X-rays – taking 400 shots per nest – and laboriously reconstructed the 3D structures with Greggory Rodriguez and Rachel Kutner to see what the industrious insects had achieved.

Analysing the structures, Monaenkova could see that no matter how large or small the grains of soil, the ants were able to excavate tunnels. However, the wetness of the soil dramatically affected their productivity. In completely dry soils, the ants couldn't dig at all, and Goldman says that they were only able to dig pitiful looking tunnels in slightly damp soils. But, when Monaenkova attempted to repeat the ants' efforts by driving a small steel rod into the relatively dry soils, they appreciated how impressive even these puny efforts were. 'Daria couldn't do it', says Goldman, adding that he suspects that the microengineers are building networks like Jenga puzzles to stabilise their structures. However, once the hydration reached an intermediate level, the ants were able to produce lengthy tunnels of a consistent diameter down through the soil. And, when the soil was completely saturated, the tiny engineers failed again: 'It's like trying to build a sand castle with sloppy sand', says Goldman. He suspects that the soils at intermediate levels of hydration yielded the most robust tunnels because the granules were bonded by strong capillary forces provided by minute droplets of water bridging the particles – although the stronger attachment did not impair the ants' excavations.

Next, Monaenkova investigated the mechanics of ant excavation by enticing them to burrow against a glass cylinder wall while she filmed their Herculean efforts, revealing that the animals used two tactics. When the soil was coarse, they grasped a single grain and shuffled backward up the tunnel, dragging it with them. However, when the soil was fine and the ants could grasp multiple grains, they gathered a pellet of grains and marched upward. Goldman was most surprised by the ants' inventiveness, moulding bulky pellets like snowballs with their forelimbs, mandibles and even their antennae.

Reflecting on the ants' engineering, Goldman says that he is impressed by their ability to work in a completely dark and confined environment while continually colliding with their nest mates. 'It is just mind blowing how they can dig so well', he says.

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Monaenkova, D., Gravish, N., Rodriguez, G., Kutner, R., Goodisman, M. A. D. and Goldman, D. I. (2015). Behavioral and mechanical determinants of collective subsurface nest excavation. *J. Exp. Biol.* **218**, 1295-1305.

Kathryn Knight

Beaked whales B-stroke for long dives

Mesoplodon densirostris swimming off the coast of Tenerife (Canary Islands). Photo credit: Teo Lucas – Gigante Azul.

Foraging animals tread a narrow metabolic tightrope, rationing the energy they expend in the pursuit of food to make the most of a catch. And marine mammals that dive on a single breath of air have to be even more frugal to eke out their meagre oxygen stores. Lucía Martín López and colleagues from the University of St Andrews, UK, and the University of La Laguna, Spain, explain that bigger diving mammals should be able to dive and forage for longer than more diminutive species with the same foraging style, as larger divers should be able to carry more oxygen on board: but the data didn't hold up. '25,000 kg sperm whales and 1000 kg beaked whales perform dives of comparable duration (30–50 min) and depth (600–1200 m),' says Martín López. Intrigued by the beaked whales' powers of endurance, Martín López and her colleagues, Mark Johnson, Patrick Miller and Natacha Aguilar de Soto, realised that they needed to know more about the diving styles of beaked whales – ranging in size from Blainville's beaked whales to Cuvier's beaked whales and northern bottlenose whales – to find out more about the impressive duration of their dives.

Tagging whales off the Canary Islands, Italy and Canada over a period of

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8 years, the team and a group of helpful volunteers collected 3D acceleration traces as the animals dived, in addition to recording their orientation in the earth's magnetic field, to learn more about the rotation and acceleration of their bodies as they swam. Recording almost 100 deep foraging dives – lasting on average between 49 and 59 min and plumbing depths of 844–1572 m – the team saw the whales continually beating their tails during the initial descent, before switching to prolonged glides. And during the whales' return to the surface, they often beat their tails continually. However, the team noticed a new style of tail beat interspersed between periods of the more conventional swimming style while they returned: a high acceleration tail beat produced by a faster and wider sweep of the tail followed by a brief glide. Dubbing the novel strokes 'B-strokes', because they are bigger (in amplitude and speed), the team realised that the ascending animals relied heavily on the powerful strokes during the early stages of the ascent, with B-strokes contributing 50% of the tail beats during the ascents of Cuvier's beaked whales.

Puzzled why the whales favoured this burst and glide technique over continual swimming during ascents, the team calculated the average speed over a B-stroke cycle, including the 3–5 s long glide, and discovered that even though the animals accelerated to 1.7 m s^{-1} during a B-stroke, the average speed over the entire cycle was similar to the speed achieved by animals that swam continually (1.3 m s^{-1}). The researchers suspect that the whales may be taking advantage of powerful Type II anaerobically fuelled muscle fibres, which surprisingly comprise 80% of their swimming muscles, to drive them on as their oxygen supplies dwindle toward the end of a dive. 'Similar fast-twitch recruitment when muscles are fatigued has been observed in exercising humans', they say. And the team adds that the short glides that follow each powerful burst could reduce the drag experienced by the animals to keep their costs down.

10.1242/jeb.123869

Martín López, L. M., Miller, P., Aguilar de Soto, N. and Johnson, M. (2015). Gait switches in deep-diving beaked whales: biomechanical strategies for long-duration dives. *J. Exp. Biol.* **218**, 1325-1338.

Kathryn Knight

Seminal plugs cost red-sided garter snakes dear

Female red-sided garter snake with seminal plug. Photo credit: Christopher Friesen.

Bubbling out of their hibernation burrows as the temperature begins to rise, male red-sided garter snakes only have one thing on their mind: mating. And with females in short supply, the pressure is on. But how much effort do these males invest in reproduction? The expense is clear for females, but how costly is seminal fluid production for males? Christopher Friesen from the University of Sydney, Australia, explains that male red-sided garter snakes are clearly exerting themselves as the seminal plugs left inside the females after copulation – to avoid sperm leakage and prevent the female from mating with other males – are massive. Also, the males' blood lactate levels soar, suggesting that seminal fluid production could be costly. Knowing that males produce and store their sperm in late summer, while the majority of the seminal fluid components are produced in spring, Friesen and his thesis advisor Robert Mason from Oregon State University, USA, realised that they could tease apart the males' investment in seminal fluid production from the cost of sperm production to begin understanding how costly reproduction is for red-sided garter snake males.

Collecting large and small males as they emerged from their Manitoba hibernation chamber, the duo then provided the males with a continual supply of fresh females, allowing half of the males to court and mate enthusiastically, while the attempts of the other group were thwarted by tape placed over the females' cloacae. Then they measured the snakes' energy consumption over the course of 9 days and found that it was around 50% higher (7.33 kJ day^{-1}) than that of males outside of the mating season.

Next, they calculated the energy consumption (per unit mass) for each of the snakes as they courted and mated with females and although they could see that the largest males invested little energy in seminal fluid production, the smallest snakes invested up to eight times more energy. And when the team tracked the snakes' mass loss relative to the number of times that they mated – the males do not feed during the mating season – the most successful males (that mated 5 times) lost as much as 8 g, while the least successful lovers (that only mated once) lost 4–6 g. In addition, Donald Powers and Paige Copenhaver measured the metabolic rates of males that had successfully mated and the males that had just lost out and found that the metabolic rates of the largest courting males were barely raised at all. However, the metabolic rates of the smallest males rose by approximately 30% during courting and rocketed by almost 50% when they mated successfully.

Finally, the team calculated the net cost of producing the seminal fluid's plug components, and they were impressed that the males were investing as much as 18% of their daily energy expenditure per ejaculation. They were also surprised that the resting metabolic rates of males after seminal fluid production ($\dot{V}_{O_2}$=0.0025 ml g^{-1} min^{-1}) were similar to the metabolic rates of pregnant female garter snakes ($\dot{V}_{O_2}$=0.0023 ml g^{-1} min^{-1}). However, the team were intrigued that sperm-free plugs produced by vasectomised males were 26% more energy dense than the plugs produced by fertile males, suggesting that sperm contain less energy than other seminal fluid components.

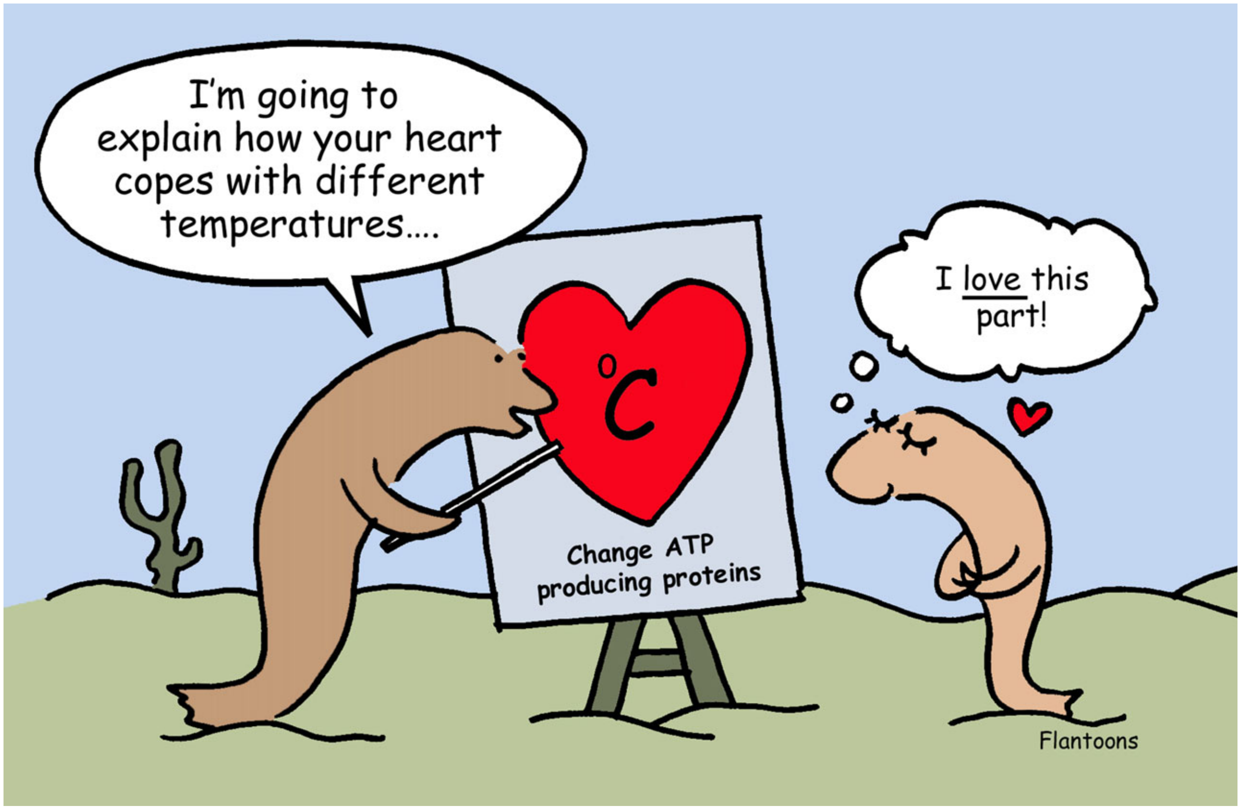
Reflecting on the smaller males' greater exertions, the team suspects that they throw everything they can into each mating opportunity as they may not survive the next harsh Manitoba winter to take advantage of the lower mating costs when older and larger.

10.1242/jeb.123836

Friesen, C. R., Powers, D. R., Copenhaver, P. E. and Mason, R. T. (2015). Size dependence in non-sperm ejaculate production is reflected in daily energy expenditure and resting metabolic rate. *J. Exp. Biol.* **218**, 1410-1418.

Kathryn Knight

Longjaw mudsuckers tune heart to temperature



Sitting in our nice warm bodies, it can be hard to appreciate the challenges faced by animals that live in environments with constantly fluctuating temperature, but one of the problems experienced by ectothermic animals is how to keep their hearts going across a wide range of temperatures. Nishad Jayasundara from Duke University, USA, explains that longjaw mudsuckers (*Gillichthys mirabilis*) reside along the Eastern Pacific coast from the cool northern Tomales Bay to the sizzling Baja peninsula in the south. While studying at Stanford, he wondered how the hearts of these animals adjust to life at 9, 19 and 26°C, so Jayasundara and his colleagues Lars Tomanek from California Polytechnic State University, USA, Wesley Dowd from Loyola Marymount University, USA, and George Somero from Stanford University, USA, decided to find out how the protein composition of the fish's hearts changed at the different temperatures.

Collecting the hearts of fish that had been acclimated to each of the three temperatures for 4 weeks, the team found 122 proteins that changed in abundance using 2D gel electrophoresis, 37 of which were identified using mass spectrometry. Cataloguing the functions of the 37 proteins, the team found that almost half (48%) are involved in energy metabolism, five are involved in cytoskeletal structure, three contribute to protein degradation and transport and two are associated with iron homeostasis. Focusing on the responses that are essential for survival at a cellular level at each temperature, the team found that the 19°C-acclimated fish had increased levels of proteins that are necessary for ATP production, while the hottest fish had reduced levels of the same proteins. Meanwhile, the coldest fish had an increased abundance of creatine kinase, an enzyme that is essential for energy supply to muscle fibres, and the hottest and coldest fish had increased levels of a protein called hexosaminidase, which has a role in stress protection.

The team says, 'The capacity to adjust ATP-generating processes is crucial to the thermal plasticity of cardiac function', and concludes that the mudsuckers optimise their cellular function at 19°C, which is within the range of temperatures at which they seem most comfortable.

10.1242/jeb.123844

Jayasundara, N., Tomanek, L., Dowd, W. W. and Somero, G. N. (2015). Proteomic analysis of cardiac response to thermal acclimation in the eurythermal goby fish *Gillichthys mirabilis*. *J. Exp. Biol.* **218**, 1359-1372.

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