OUTSIDE JEB

The bond between tropical forests and orangutans

Plants often need some assistance when it comes to moving their progeny away from home. Employing animals to disperse their seeds is a great way for plants to achieve this. Rodents and birds often come to mind, but would you think of orangutans? Yet, these remarkable great apes are actually the largest tree-dwelling fruit-eating animal in the world. Wondering how these charismatic creatures may impact the distribution of plant species in their habitat, Esther Tarszisz, a researcher with the Bornean Nature Foundation, supervised by Adam Munn from the University of New South Wales, Australia, in collaboration with a team of international researchers, set out on a mission to untangle the relationship between orangutans and their forests.

The first step was to time how long it takes a seed to travel through the entire digestive system of an orangutan and to show up in its poop. This is pretty important information if you want to know where seeds are likely to turn up after being eaten. The team chose different coloured beads ranging from 2 to 6 mm in diameter, similar to the size of seeds found in the faeces of wild orangutans, and added the seed mimics into the diets of six female and male orangutans in zoos in Australia. Tarszisz then had the job of spying on the orangutans throughout the day to catch them in the act of pooping, at which point she noted the time, collected the deposit and counted the plastic seeds that it contained. With these data, the team was able to calculate that all of the bead seeds took an average of 76 h to pass through the gut, regardless of their size.

Once the researchers had this figure, they ventured into the wilderness of peat swamp forests in Central Kalimantan, Indonesia, where they followed four females and three males around the forest. They documented the daily lives of these animals, including where they moved through the forest, why they moved, where they ate, how long they foraged and what they consumed. Orangutans definitely have a sweet tooth for fruit – this made up the chief component of their diet. The team also discovered that, perhaps not surprisingly, a day in the life of a female orangutan is quite different to that of their male counterparts. While females devoted their time to eating, displaying quite predictable movement patterns in their home range, males roamed much more haphazardly and over greater distances; they seemed to have other things on their minds than just eating, such as mating and fighting or avoiding confrontations.

Combining the information on seed transit time with details of the orangutans' movements, Tarszisz and her colleagues realised that the orangutan population can influence the distribution of plant species in the peat swamp forest. It seems that within the 76 h that it takes for seeds to pass through the orangutan's digestive system, the females have often returned to the core area of their home range, so the seeds that they deposit in their faeces will probably grow into trees not far from the parent plants upon which the females dined. In contrast, males would disperse seeds further and more randomly. This combination of movement patterns suggests that the gene flow of plant populations will occur over a wide area.

Tarszisz and her colleagues warn that any changes to the Indonesian environment are likely to cause orangutans to alter their behaviour, thus influencing their vital role as seed dispersers. Habitat loss in particular may cause populations of both orangutans and plant species to become genetically isolated, especially as there are no other dispersers of large seeds in this ecosystem. Genetic diversity is vital if orangutans and the forest are to survive.

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Tarszisz, E., Tomlinson, S., Harrison, M. E., Morrough-Bernard, H. C. and Munn, A. J. (2018). An ecophysiologically informed model of seed dispersal by orangutans: linking animal movement with gut passage across time and space. *Conservation Physiol.* **6**, coy013. doi:10.1093/conphys/coy013.

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In frogs, even leptin function doesn't escape metamorphosis

If you think your pubescent years were incommodious, you should talk to a frog. We humans see some changes here and there, sure. But for a tadpole metamorphosing into a frog, so many profound changes occur that, were it not known to science, this developmental process would probably defy belief. It has not escaped the notice of Robert Denver, however, a developmental neuro-endocrinologist at the University of Michigan, USA. He and his team recently turned their attention to the many changes that occur in the anuran digestive system during the transition from tadpole to frog. While these changes may be less familiar than the gross anatomical changes that occur during metamorphosis, they are no less

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extraordinary, and they impact the animal at various levels of organization.

Underlying these changes are the anuran's evolving culinary preferences, from vegan tadpole to carnivorous frog. A taste for hemolymph requires different gastrointestinal architecture, and the frog remodels during metamorphosis by shortening the intestines and overhauling the epithelial lining. Remodeling in this way requires a halt to feeding, and what intrigued Denver and his colleagues were the signals that trigger this halt and how they may change with development.

Using *Xenopus laevis* as their representative anuran, the team first confirmed that food intake increased steadily throughout tadpole development before falling precipitously at metamorphic climax. The point in development at which this occurred coincided with a shortening of the intestines and an emptying of the intestinal contents, supporting the idea that feeding is arrested to enable gastrointestinal remodeling. The team then pursued the hypothesized involvement of leptin, which is the body's primary satiety hormone that makes frogs (and us) feel full. They searched for both leptin and the leptin receptor throughout the tadpole's development, finding that that initial appearance of the hormone and receptor occurred slightly out of step with each other: leptin levels increased just prior to metamorphosis, while hypothalamic leptin receptors (and their functionality) increased at metamorphic climax.

Denver and his colleagues further investigated leptin functionality by manipulating their frogs in two ways. First, the team injected pre-metamorphic tadpoles with recombinant frog leptin, finding it did not suppress feeding until just prior to metamorphic climax. Next, the investigators neutralized the leptin of tadpoles just before metamorphic climax, and found that the lack of functional leptin caused the tadpoles to resume feeding when they would normally cease.

Together, the team's results revealed two things. First, pre-metamorphic tadpoles lack central feeding control. This allows them to eat insatiably and maximize growth rate, which is important because metamorphosis from a highly vulnerable tadpole into a less vulnerable frog occurs only once sufficient weight has been packed on. Second, leptin's role evolves throughout development. Initially, leptin functions as an adiposity signal to the hypothalamus, effectively telling it that the body is ready for the hypothalamus to begin secreting metamorphosis-inducing hormones. As metamorphosis progresses, the hypothalamus eventually produces leptin receptors, which, by metamorphic climax, alter leptin function from adiposity signal to appetite suppressant. With feeding halted and the intestines emptied of their contents, the gastrointestinal tract is free to remodel for its upcoming carnivorous diet.

In the wild, *Xenopus* parents hit the road immediately after the female lays her hundreds of fertilized eggs. Most humans would probably frown upon this hands-off parental approach, but I implore those humans to recall their own pubescent years – better yet, those of their children should they have any – and then ponder the many-fold greater hormone-driven changes that occur during anuran development. Now multiply that by several hundred offspring. Still frowning?

10.1242/jeb.170043

Melissa Cui Bender, Caroline Hu, Chris Pelletier and Robert J. Denver. (2018). To eat or not to eat: ontogeny of hypothalamic feeding controls and a role for leptin in modulating life-history transition in amphibian tadpoles. *Proc. R. Soc. B,* **285**, 20172784.

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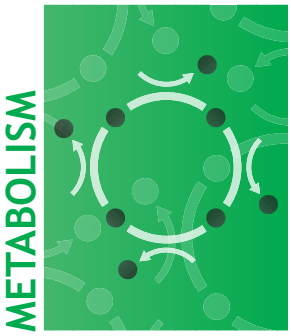
Insulin-resistant cavefish avoid diabetes



When digesting a meal, blood glucose surges and ordinarily prompts the release of insulin from the pancreas, which causes tissues throughout the body to take up and store the sugar. In type II diabetes, the body becomes insensitive to insulin, meaning that blood glucose stays elevated and eventually becomes harmful. Whilst humans eat frequently throughout the day, many animals are adapted to survive for weeks without food. An extreme example can be found in Mexican caves, where some populations of Mexican tetras (*Astyanax mexicanus*) have independently invaded the subterranean habitat and thus rely on unpredictable seasonal floods to provision food.

The cavefish are clearly more tolerant of starvation than their surface relatives. For example, when food deprived, they remain larger than surface fish. In order to understand how cavefish are adapted to their life of feast and famine, Misty Riddle and Ariel Aspiras, from the Harvard Medical School, USA, and their colleagues investigated glucose metabolism in both cave- and surface-dwelling populations of the Mexican tetra.

The team observed that the cavefish had extremely high blood glucose levels, both immediately after feeding and after a day of fasting. In one population, blood glucose remained high after 3 weeks of starvation. However, the elevated blood glucose was not due to an inability to make insulin; the pancreas of the cavefish developed normally, and the cavefish were producing insulin as their circulating insulin levels were similar to those of their surface relatives. When the researchers injected synthetic insulin into surface fish to see how they responded to the hormone, their blood glucose dropped; however, no such change occurred in the cavefish. In addition, cavefish muscle incubated with insulin also lacked the molecular response that normally triggers glucose uptake. In effect, the cavefish state is reminiscent of type II diabetes: the pancreas can produce insulin, but the cells don't listen.

In order to identify why the cavefish were insulin resistant, the team scoured the genome sequences of the different tetra populations. They identified a mutation in the cavefish insulin receptor, which sits on cell membranes, that prevents the hormone from binding normally. To prove this was the critical mutation, they next used the fashionable gene-editing technology CRISPR to induce the cavefish mutation in a popular lab species, zebrafish. This rendered the zebrafish insulin resistant and also made them larger. Conversely, the equivalent

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mutation in the same receptor in humans results in diminished growth, but why fish and mammals differ so much in their response remains to be established.

The most pertinent finding of the study, however, may be peculiar to the cavefish. Despite their high blood glucose, the cavefish live healthy lives – the damage that excessive blood glucose normally wreaks couldn't be detected in the cavefish blood – and they even appear to age more slowly than their relatives that inhabit the surface. The team therefore concludes that, to compensate for their insulin resistance, cavefish must have evolved compensatory adaptations. The specific nature of these adaptations is as yet unknown, but understanding them could eventually have major implications for diabetes research.

10.1242/jeb.170050

Riddle, M. R., Aspiras, A. C., Gaudenz, K., Peuß, R., Sung, J. Y., Martineau, B., Peavey, M., Box, A. C., Tabin, J. A., McGaugh, S., et al. (2018). Insulin resistance in cavefish as an adaptation to a nutrient-limited environment. *Nature* **555**, 647-651.

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Midbrain puts the pace in mice

Once in a while, I spot a mouse on a trail eagerly tiptoeing by before fleeing as I get closer. Sometimes, as the episode unfolds, I wonder how animals, including humans, control whether to walk in a leisurely fashion or to run for it. This question has also occurred to Nicolas Josset and his colleagues at Université Laval, Canada, and so the team decided to study a midbrain region in mice that sits atop the spinal cord and is highly conserved from fish to humans. When the region is electrically stimulated in animals, it sets the pace at which the animal moves like a volume knob: ramping up the intensity speeds up the animal so that it goes from a walking pattern into a run. While recent work in mice has clarified how neurons in the spinal cord set the speed and pattern of locomotion, the neural input from the brain remains poorly understood. To clarify how this region sets the pace, the team studied neurons in two separate areas of the region: the cuneiform nucleus and the pedunculopontine nucleus.

To isolate each area's contribution to locomotor control, the team genetically modified neurons in mice to turn on when blue light was shone on them or to turn off with yellow light. To target each area's neurons, the team surgically inserted a 0.2 mm light probe into either area. In a series of experiments, Josset and his colleagues watched the response of the mice as they shone light in the areas – thereby turning on or off the neurons – for either short 10 ms periods or longer 1 s periods, while the mice sat still or matched the speed of a treadmill. To assess the responses of the mice, the team put reflective markers on the leg joints and filmed the mice on the treadmill to track their speed and whether they walked or ran.

When the team stimulated the cuneiform neurons of a stationary mouse with a sequence of 10 ms bursts of blue light for a total of 1 s, the mouse set off on a short bout of running. However, when the team activated pedunculopontine neurons, the mouse remained at rest. This shows that neurons in the cuneiform nucleus are important for initiating locomotion.

Next, the team tested how each area modifies locomotion. They activated neurons for 50 ms while the mice were running at a set speed on a treadmill and found that cuneiform neurons briefly sped up the mouse, while pedunculopontine neurons briefly slowed it down. When the team prolonged the activation to a sequence of 10 ms intervals for 1 s, the cuneiform neurons set the mouse into a fast running pattern, while the pedunculopontine neurons halted the mouse altogether. In a final experiment, the team tested whether the areas are necessary for locomotion by turning off the neurons with yellow light for 1 s. Turning off either cuneiform or pedunculopontine neurons slowed down or stopped the mice. This suggests that the cuneiform and pedunculopontine neurons have two different roles: cuneiform neurons initiate locomotion, increase speed and induce running, while pedunculopontine neurons decrease speed and regulate slow walking.

Josset and colleagues' results demonstrate that the midbrain comprises two areas that control either walking or running. As the midbrain region is conserved in species from lamprey to salamander, rabbit and monkeys, the two areas might apply their distinct functions similarly across vertebrates. Also, the team's results suggest that targeting neurons in the cuneiform nucleus, which speeds up locomotion, could help boost the effects of electrical brain stimulation in people with Parkinson's disease, which is used to alleviate the slowed movements that are a common symptom of the disease.

10.1242/jeb.169995

Josset, N., Roussel, M., Lemieux, M., LaFrance-Zougba, D., Rastqar, A. and Bretzner, F. (2018). Distinct contributions of mesencephalic locomotor region nuclei to locomotor control in the freely behaving mouse. *Curr. Biol.* **28**, 884-901.

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