OUTSIDE JEB

Fearful fairy-wrens have the blues

Fear of death is a fairly strong motivator. And yet, animals get eaten all the time despite constant vigilance for predators in their midst. The problem is that animals are multi-purpose machines: they eat, they mate, they hunt for food and mates, and so on, and these activities can get in the way of spying for predators. This trade-off is particularly acute for males with conspicuous sexual signals. But as shown by Alexandra McQueen and her colleagues from Monash University in Victoria and the Australian National University in Canberra, male birds can adapt their behaviors to mitigate the risks of their flashy sexuality. Even more striking, so too can their drab neighbors.

Superb fairy-wrens are small Australian birds that live in social groups composed of a mating pair and a handful of male helpers. While females and non-mating males are an inconspicuous dull brown, mating males sport a plumage of pure blue brilliance. This matters, because not only do these fancy guys get the girl they're with (at least socially), but their blueness also attracts females from other territories as extra-pair mates. But being attractive is a double-edged sword. On the one hand, greater visibility leads to more offspring sired. On the other hand, a bright blue male is an easier target to visual predators than a dull brown one. And the blue males seem to be aware of this.

McQueen and her colleagues found striking differences in the behaviors of blue and brown birds. While brown birds spent most of their time foraging in open landscape, their blue counterparts eschewed the open space and opted instead to forage in or near shrubbery. In addition, blue birds spent considerably more time scanning the landscape, presumably to aid in predator detection. And when the team spooked the birds by playing a recording that signaled an alarm, the blue birds were far more likely to flee into cover and they remained hidden for more than twice as long as brown birds.

These results suggest that blue birds assess their own predation risk and act to reduce it. What's more surprising is that brown birds nearby also take advantage of the blue bird's caution. They spent less time searching for predators if a blue bird was around and also spent less time hiding in the bushes after they were spooked by the alarm.

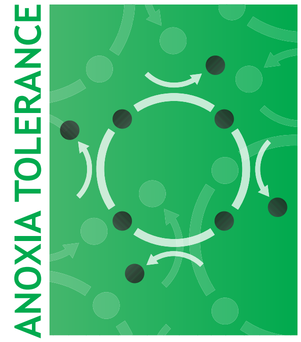
I'm reminded of the old joke about a pair of campers and an attacking bear. While one camper laces up his running shoes, his friend questions why, as he'll be outrun by the bear anyway. The first responds that he doesn't need to outrun the bear, just him. Just like this camper, brown birds seem to distinguish absolute and relative risks. When on their own, they flee from perceived danger. But when in the presence of more conspicuous bait, brown birds can be more relaxed. They seem to understand that they don't need to outfly the predator, just the snazzy player in the blue suit.

10.1242/jeb.147579

McQueen, A., Naimo, A. C., Teunissen, N., Magrath, R. D., Delhey, K. and Peters, A. (2017). Bright birds are cautious: seasonally conspicuous plumage prompts risk avoidance by male superb fairy-wrens. *Proc. R. Soc. B* **284**, 20170446.

Daniel E. Rozen
University of Leiden
d.e.rozen@biology.leidenuniv.nl

A new PDH leaves *Carassius* drunk and breathless



Think you can survive anoxia? Don't hold your breath! Without oxygen, your cells – like those in most vertebrates – are unable to produce enough energy to supply even basic cellular demands and they quickly die. But certain members of the *Carassius* genus, such as crucian carp, are able to withstand long periods with little to no oxygen at low temperatures, conditions they encounter during winter beneath frozen lakes. Their remarkable anoxia tolerance is thanks to a unique metabolic pathway in their muscle cells that allows pyruvate, an intermediate breakdown product of glucose, to be processed by the cells in a way that still produces energy but does not require oxygen. This anaerobic energy-producing process involves the conversion of pyruvate into ethanol, and this is something that other vertebrates cannot do. However, despite the passage of nearly 4 decades since the discovery of ethanol production by *Carassius* muscle cells, a team of researchers headed by Norwegian scientists Göran Nilsson and Stian Ellefsen has only just elucidated the molecular mechanisms underlying this metabolic novelty for extreme anoxia tolerance.

When they started their research, the team knew that in the presence of oxygen,

Outside JEB is a monthly feature that reports the most exciting developments in experimental biology. Articles that have been selected and written by a team of active research scientists highlight the papers that JEB readers can't afford to miss.

Journal of Experimental Biology (2017) 220, 3402-3405

pyruvate enters the aerobic metabolic pathway after being converted in a series of steps to acetyl-CoA by the pyruvate dehydrogenase enzyme (PDH) complex. But in the absence of oxygen, *Carassius* muscle cells instead convert pyruvate into acetaldehyde, which is then converted to ethanol by another enzyme. The researchers wanted to figure out how pyruvate is converted into acetaldehyde, so they began by sequencing every gene component of the PDH complex in *Carassius*. They soon realized that *Carassius* are endowed with extra copies of the PDH complex enzymes thanks to a genome duplication event in *Carassius* ancestors long ago. The team also noticed that some of these extra copies, in particular the first enzymes in the complex (E1α and E1β), which are responsible for initiating pyruvate conversion, now contained important sequence differences that would change their function. Importantly, these sequence modifications were not present in the anoxia-intolerant *Carassius* cousin the common carp, which further implicated these new genes in bestowing anoxia tolerance.

To test whether the new E1α and E1β were responsible for driving pyruvate towards ethanol synthesis under anoxic conditions, the research team kept *Carassius* in either fully oxygenated water or oxygen-depleted water for up to 1 week and then quantified and compared the expressions of the old and newly identified E1α and E1β genes. As suspected, the researchers found that *Carassius* muscle, the major ethanol-producing tissue during anoxia, expressed considerably more of the new E1α and E1β genes than the old ones (about 100 times more). They also found that brain, which does not produce ethanol during anoxia, only expressed the old E1α and E1β genes. Interestingly, the team showed that this expression pattern was the same irrespective of the oxygen content in the fish's aquaria; instead, anoxia changes the phosphorylation state of E1α and E1β in *Carassius* muscle. As regulating phosphorylation takes a fraction of the time that it takes to transcribe and translate new proteins from genes, this means that *Carassius* are primed for anoxia and can rapidly switch between the aerobic and anaerobic pyruvate pathways.

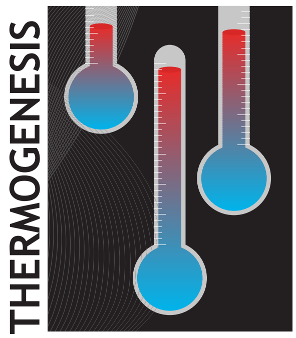
Now you can breathe easy – the mystery of ethanol production in *Carassius* muscle cells is solved!

10.1242/jeb.147611

Fagernes, C. E., Stensløkken, K., Røhr, A. K., Berenbrink, M., Ellefsen, S. and Nilsson, G. E. (2017). Extreme anoxia tolerance in crucian carp and goldfish through neofunctionalization of duplicated genes creating a new ethanol-producing pyruvate decarboxylase pathway. *Sci. Rep.* doi:10.1038/s41598-017-07385-4.

Sarah Alderman
University of Guelph
alderman@uoguelph.ca

Fighting the cold as pigs do



Cold temperatures during winter are not only uncomfortable for us – depending on how we dress – but also challenging for animals. Whereas large mammals usually just grow thicker hair during winter for protection from the cold, many small mammals, as well as the newborns of larger species, typically use a more complex mechanism to stay warm in winter: they produce heat in a specialized organ, the brown adipose tissue (also often called brown fat), in a process involving the thermogenic protein UCP1. But this mechanism has been lost in some species. Pigs, for example, lost this mechanism about 20 million years ago. As a result, many pig species are very sensitive to cold and early death of piglets is a major problem in the swine industry. But not all pig breeds are sensitive to cold, suggesting that other mechanisms of heat production must have evolved in those breeds.

Jun Lin from the Chinese Academy of Science and colleagues from different institutes speculated that another protein called UCP3, basically a relative of UCP1, could play a role in heat production in pigs. The team focused on the Tibetan pig, a breed that occurs at high altitudes and is quite tolerant to cold exposure. The researchers first confirmed that Tibetan pigs do not have brown adipose tissue or functional UCP1. They then placed the piglets in a cold room and compared the response of the cold-tolerant Tibetan pigs with the response of Bama pigs, a species that is known to be very sensitive to cold exposure. In addition to looking out for a drop in body temperature in both breeds, the researchers also compared the structure of fat cells at body locations in which brown fat occurs in other species, as well as expression patterns of the genes that are active after cold exposure.

The study found that both breeds shivered when exposed to the cold and were able to maintain a normal body temperature thanks to the muscle contractions. However, treating the animals with a drug that inhibits shivering led to a marked decrease of body temperature in the Bama pigs, whereas the Tibetan pigs were still able to keep warm. This indicated that Tibetan pigs must have an alternative heat production mechanism. Furthermore, when the team compared the structure of fat cells from the two breeds after they had been kept in the cold room for a few hours, they found evidence of the appearance of beige fat cells, which have similar heat production potential to that of brown adipose tissue, in Tibetan pigs but not in Bama pigs.

Looking into the genes that were upregulated in both breeds after cold exposure, the team again found marked differences. While the genes that were upregulated in the cold-sensitive Bama pigs had nothing to do with heat production, the genes that showed increased expression in Tibetan pigs did. And one of them was the gene coding for the UCP3 protein, which occurred primarily in the beige fat cells. Further analyses then revealed similarities in the protein structure between pig UCP3 and UCP1 in rodents, the protein that is involved in heat production in brown adipose tissue. Importantly, when Lin and colleagues extended the analysis to other breeds, they found the same results in Min pigs – another breed that can tolerate cold temperatures – but not in breeds that have trouble coping in the cold.

Although it is not yet clear how UCP3 works, this study suggests that it is involved in heat generation and contributes to the cold tolerance of pig species living in cold habitats.

10.1242/jeb.147587

Lin, J., Cao, C., Tao, C., Ye, R., Dong, M., Zheng, Q., Wang, C., Jiang, X., Qin, G., Yan, C. et al. (2017). Cold adaptation in pigs depends on UCP3 in beige adipocytes. *J. Mol. Cell. Biol.* doi: 10.1093/jmcb/mjx018.

Julia Nowack
University of Veterinary Medicine Vienna
julia.nowack@vetmeduni.ac.at

People power reveals starling secrets

Before coming down to roost, starlings regularly flock together in murmurations of thousands of individual birds moving in synchrony, creating one of nature's most impressive aerial displays. This sight has piqued the curiosity of many scientists across different fields and, as a result, we know how these murmurations function, with each bird following the movements of their six or seven nearest neighbours. Despite this, we still don't know why starlings come together in such large numbers.

There are two hypotheses that attempt to explain this behaviour: the 'warmer together' hypothesis, which suggests that starlings gather to advertise a roost site, with the roost becoming warmer as more birds join; and the 'safer together' hypotheses, suggesting that large murmurations confuse predators, make individual birds harder to pinpoint and attack, and simply reduce the chances of any one individual being preyed upon.

A team of researchers, headed by Anne Goodenough at the University of Gloucestershire, UK, harnessed the power of citizen science to try and determine which of these is the case. They sent out surveys to members of the public asking for details of murmuration sightings from across the UK, as well as several other countries. In addition to the location, respondents were asked to estimate the number of birds present and the duration of the murmuration event, to log how it ended (did the birds go to roost or disperse?), the temperature and the presence of other birds, namely predatory species – such as kestrels, sparrowhawks and buzzards – and corvids and gulls, which when silhouetted could be mistaken for predatory species.

From these surveys, over 3200 records of starling murmurations were collected. By putting the different variables together in a model, the team could analyse which were correlated with murmuration size. They found that the temperature was not significantly linked to the number of starlings in a murmuration; however, the presence of predatory birds was positively associated with larger murmurations, providing evidence for the safer together hypothesis. Interestingly, murmuration size was not linked to the presence of gulls or corvids, suggesting that starlings may be able to distinguish avian predators from other visually similar species.

However, this link isn't as simple as it sounds. With these data it is difficult to tease apart cause and effect; so, do large murmurations congregate because predators are close by, or are the predators in fact simply attracted to a bigger group of birds? Despite this, the power of citizen science has, for the first time, enabled a large enough dataset to be assembled for a robust analysis of starling murmuration behaviour to be undertaken.

Citizen science is a powerful and growing tool for researchers to draw upon. Although it is not without limitations – such as potential recording errors from untrained participants – it is an extremely effective way of generating large datasets from which to test or generate hypotheses, as well as increasing public interest and involvement in science. Just like the starlings, when many individuals come together to work towards a common goal, great things can be achieved.

10.1242/jeb.147595

Goodenough, A. E., Little, N., Carpenter, W. and Hart, A. G. (2017). Birds of a feather flock together: Insights into starling murmuration revealed using citizen science. *PLoS ONE* **12**, e0179277.

Michelle A. Reeve
michelle.a.reeve@gmail.com

Honeybees remember that size matters

Honeybees likely use relational information to decide which flowers are optimal for foraging nectar. For instance, laboratory research has shown that the bees are able to discriminate between sizes of stimuli as well as their relative positions and can apply learned rules to make decisions. Such experiments have typically presented bees with a choice among simultaneously encountered images or objects. Scarlett Howard at RMIT University in Australia, in partnership with colleagues at Australia's University of Melbourne and Monash University as well as the Université de Toulouse in France, wanted to find out whether honeybees trained to prefer a certain relative size of artificial flower (larger or smaller) could apply those preferences to flowers presented one after another – in other words, whether the bees could remember the flowers they saw previously in making decisions about subsequent flowers. The research team also wanted to investigate whether honeybees could exercise their size preferences when encountering flowers of a new size outside of their training range.

To address their research questions, the scientists first trained two groups of honeybees to prefer either larger or smaller artificial flowers presented in a viewing chamber that only allowed the bees to view one flower at a time (to prevent simultaneous size comparisons). Offering the bees a choice of four flowers – two identical larger flowers and two identical smaller flowers – the team allowed the bees to select a flower by landing on a small platform beneath. The bees were rewarded with a sugary substance when they landed by the flower of the relative size that they were being trained to prefer, or were discouraged from landing with a foul-tasting substance

Journal of Experimental Biology

Journal of Experimental Biology (2017) 220, 3402-3405

when they selected the wrong flower. After training, the bees were reintroduced into the chamber for the first test, in which they were asked to identify the relative flower size (larger or smaller) that they had just been trained to choose, without the benefit of a reward, to prove that they could select the correct flower size. Then, in a separate trial, the bees were given choices between flowers that were completely outside of the size ranges they had seen before, to assess whether they could apply their learned size preferences to unfamiliar flowers and select either the larger or the smaller of the two flower sizes according to the size they had learned to prefer during training.

Howard and her colleagues discovered that the bees were able to learn to choose flowers based on their relative sizes, and they were also able to correctly choose between flower sizes that were outside of the size range they had encountered previously. These results indicate that the honeybees are able to remember what previously viewed flowers looked like, and can compare the remembered images with successive flowers in order to make informed choices. The ability of the bees to discriminate between flowers of new sizes also shows that they are able to extrapolate learned preferences to novel situations.

In all, the study suggests that honeybees have considerable cognitive ability and are able to hold information in their working memory to aid in decision-making processes. Clearly, honeybees are not simply bumbling about when foraging for food; rather, their brains are buzzing as they choose the perfect flower.

10.1242/jeb.147603

Howard, S. R., Avarguès-Weber, A., Garcia, J. and Dyer, A. G. (2017). Free-flying honeybees extrapolate relational size rules to sort successively visited artificial flowers in a realistic foraging situation. *Anim. Cogn.* **20**, 627-638.

Molly H. B. Amador
University of Miami
m.broome@umiami.edu