OUTSIDE JEB

When gills became gills

Gills are often thought of as a fish's lungs. They pump oxygen in and carbon dioxide out. But that's not all gills do. They also balance out a fish's electrolytes – the ions that we lose when we sweat. The gills of modern fish evolved from structures that their ancestors – small, worm-like animals – used to pull little specks of food from the ocean. It's not entirely clear when that change occurred, but it's believed to have happened no earlier than when the first fish appeared. Michael Sackville and Colin Brauner from the University of British Columbia, Canada, teamed up with colleagues from the University of Montreal, Canada, and the University of Cambridge, UK, to test this. They looked at the gills of Pacific lamprey (*Entosphenus tridentatus*) larvae, Florida lancelets (*Branchiostoma floridae*) and acorn worms (*Saccoglossus kowalevskii*) to figure out when fish gills took on their modern role.

The team selected these animals because lampreys are an ancient group of fish and, although the gills of adults behave like other fish gills, their larvae use gills for eating. In contrast, lancelets and acorn worms aren't fish. They look more like how we picture the ancestors of fish; even the adults use their gills for eating. But just because they use their gills to eat doesn't mean they can't also use their gills to breathe and restore electrolytes. If this is the case for all three species, Sackville and the rest of the team reasoned, then gills started to take on the characteristics of modern fish gills before a modern fish ever existed. If, on the other hand, only the gills of lamprey larvae breathe and restore electrolytes, then these functions of the modern fish gills would be entirely a modern fish adaptation.

The researchers started by looking at the lamprey larvae, which they placed in divided chambers such that each half of the chamber contained one half of the lamprey. They measured how much oxygen and electrolytes each lamprey half took in and how much carbon dioxide each half released. Because the gills are located near the head, the scientists were able to compare what happened in each chamber and determine whether more movement occurred in the half with the gills. They found that gills always played an important role in balancing electrolytes, but only really started to play a big role in breathing as the larvae got bigger.

Studying the function of gills in lancelets and acorn worms was a bigger challenge, so the researchers had to get creative. The acorn worms were too fragile for the team to put them in the divided chamber, so instead they cut the acorn worms inhalf to determine whether the half with gills played a larger role in breathing. However, it was impossible to divide the lancelet in such a way that they could isolate the role of the gills. It was also impossible to measure the amount of electrolytes that lancelets and acorn worms took in or released, because the animals live in such salty water that measuring small changes in the electrolytes leaving and entering their bodies was impossible. Instead, the scientists looked for cells in the gills with the same features that fish cells use to move electrolytes across the skin and other body surfaces. They found those features in the gills of both lancelets and acorn worms, even though the acorn worms didn't use their gills to breathe.

Based on these observations, the team offer an exciting conclusion. While breathing through the gills seems to be a large fish adaptation, balancing the electrolytes in the body may have been an older trick of the gills.

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Sackville, M. A., Cameron, C. B., Gillis, J. A. and Brauner, C. J. (2022). Ion regulation at gills precedes gas exchange and the origins of vertebrates. *Nature* **610**, 699-703. doi:10.1038/s41586-022-05331-7

Andrew Saintsing (0000-0003-0934-7652)
University of California, Berkeley
andrew_saintsing@berkeley.edu

Fight, flight or bright(en)

When you think of colour-changing animals, what comes to mind? A slow-moving chameleon barely distinguishable from its spot in a tree? Or maybe an octopus camouflaging itself on the ocean floor? What about hundreds of bright yellow male Asian common toads (*Duttaphrynus melanostictus*) gathering to fight each other for the company of their somewhat drab, brown-coloured mates? During monsoon rains, the toads gather in groups of over 200 individuals at breeding sites throughout South and Southeast Asia. Before congregating, the male toads swiftly change colour to a conspicuous yellow, signalling to other males that they are ready to fight – and to distinguish themselves to potential female mates. Unsurprisingly, hormones associated with the 'fight or flight' response are high during these 'explosive breeding' events thanks to the stressful monsoon rains that coincide with the mating season and fighting for partners. Could these hormones have something to do with the toads' striking colour change? Susanne Stückler and Doris Preininger from the University of Vienna, Austria, and their colleague Matthew Fuxjager from Brown University, USA, decided to investigate.

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The trio turned to a group of toads housed in Vienna Zoo, Austria, to see which hormones were responsible for the colour change. First, they measured the colour of the toads' backs – specifically, the hue (what we normally think of when we describe colour), brightness (lightness or darkness) and chroma (colour saturation). Having established the toads' baseline colour, the researchers tested whether a dose of the reproductive hormone human chorionic gonadotropin (hCG) could trick the toads into thinking that it was breeding season and trigger a rapid colour change. Surprisingly, the toads did not turn bright yellow when they experienced an increase in their reproductive hormone levels. If reproductive hormones such as hCG aren't triggering a change in colour, could the hormones associated with a stressful experience be driving the colourful transformation instead?

To test this, the scientists gave the toads one of the stress hormones, either adrenaline (epinephrine) or noradrenaline (norepinephrine), and again recorded the toads' hue, brightness and colour saturation. With either stress hormone, the toads became brighter and their shade of yellow became more saturated. Interestingly, for these toads, only a grape-sized patch of skin changed colour (about half the size of the body), and a few did not change colour at all.

To make sure any colour changes the scientists saw in these treatments could actually be seen by potential mates (or sparring partners), Stückler and colleagues also calculated how noticeable the colour changes would be to the eyes of a toad. Using the colour perception data of a close relative, the common toad (*Bufo bufo*), the scientists were able to estimate just how different this stress-induced yellow would be from the typical brown colour of females or males – and indeed, the yellow stood out! The scientists suggest that to trigger the whole-body colour change, perhaps there are other hormones at play that they haven't tested yet. Or perhaps physical signals such as monsoon rains and social cues such as the company of other toads work together with stress hormones during explosive breeding events in the wild.

This is the first time that stress hormones alone have been shown to cause animals to change colour rapidly without the help of other hormones that signal breeding.

For Asian common toads, being a brighter, flashier yellow might make them more successful during these explosive breeding events, so it would make sense that they have embraced another means to quickly change colour. So, next time you think of colour-changing animals, perhaps you'll picture the bright yellow Asian common toad – and how their 'fight or flight' hormones have now been adopted for flashiness.

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Stückler, S., Fuxjager, M. J. and Preininger, D. (2022). Evidence that catecholaminergic systems mediate dynamic colour change during explosive breeding events in toads. *Biol. Lett.* **18**, 20220337. doi:10.1098/rsbl.2022.0337

Angelina Dichiera (0000-0002-9635-0229)
The University of British Columbia
dichiera@zoology.ubc.ca

Great gray owls overcome sound illusion to hunt

Have you ever heard how quiet owls are when they fly? They are very stealthy, giving them a distinct advantage over their prey. Owls can also pick up sounds that are almost inaudible to the human ear while hunting. However, great gray owls (*Strix nebulosa*) live in cold environments, often with snow on the ground, which can make hunting by ear more difficult, because their prey, such as voles (*Cricetidae*), hide beneath the snow and the sounds they produce could be dampened by the icy covering. Christopher Clark from University of California, Riverside, USA, James Duncan from Discover Owls, Canada, and Robert Dougherty from University of Washington, USA, wanted to know how the sounds produced by prey are affected by the presence of snow and how the owl overcomes these acoustic challenges to hunt.

First, the team wanted to find out how snow affects how sound carries. They went out into the field during the winter (February) in Manitoba, Canada, to identify locations where the owls had been hunting, and found seven holes in the snow produced by owls as they retrieved their food. Then, they dug 40 cm deep holes near the owls' hunting sites and placed a waterproof loudspeaker at the bottom; they also placed an acoustic camera 1–1.5 m above the snowpack and 1.2–6 m from the loudspeaker. The team then played a recording of the sounds produced by a meadow vole (*Microtus pennsylvanicus*) digging beneath the snow through the loudspeaker, while gradually scraping the snow away in layers, recording the volume and location of the sound relative to the speaker at six snow depths. They used this information to simulate how the owl would perceive the sound after it traveled through the snow.

They found that snow does in fact act as a muffler for sound produced by rodents buried beneath it, especially for high-pitched sounds, such as when the voles are communicating with one another. As the snow was removed, the sound level increased and the location of the sound also appeared to move, with the sound source appearing to be displaced farthest to one side of the speaker when the snow was deepest, moving closer to the speaker as the snow was removed until it appeared to come directly from the speaker when all the snow was gone. The team suggests that owls could overcome this challenge by positioning themselves well above the snow, either on a perch or flying high, to reduce the likelihood of being misled by the distorted sound position. And it seems that the great gray owls have already come to the same conclusion as they often hover directly above their prey before plunging into the snow, to increase their accuracy.

The work done by Clark and colleagues highlights how the snow creates a sound illusion by bending the path of the sound – much like light is bent when passing through a glass of water, making a straw appear bent – directly affecting how great gray owls target food beneath snow cover. The birds have also evolved to fly extremely silently, diminishing the noise produced by their own flight, allowing them to overcome this sound illusion and hear voles digging beneath the snow while they hover above. One could say that these birds are the ninjas of the sky.

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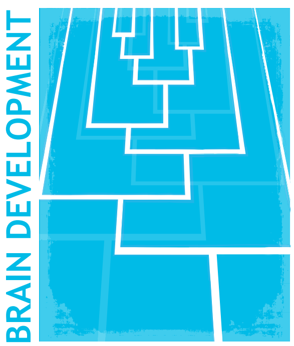
Clark, C. J., Duncan, J. and Dougherty, R. (2022). Great gray owls hunting voles under snow hover to defeat an acoustic mirage. *Proc. R. Soc. B* **289**, 20221164. doi:10.1098/rspb.2022.1164

Jonaz Moreno Jaramillo (0000-0003-4640-8573)
University of Massachusetts, Amherst
jmorenoj@umass.edu

Did microRNAs make octopuses smart?



Octopuses are geniuses: they can unscrew jars from the inside, solve mazes and pull off Houdini-like feats of disguise and escape. Bizarrely, there aren't many compelling explanations for how octopuses are so smart; although their brains are large, the genes and proteins used to build them are similar to those of less intelligent invertebrates such as oysters. Fascinated by the mysterious 'how' of the cephalopod nervous system, an international team of researchers led by Grygoriy Zolotarov, affiliated with the Max Delbrück Center for Molecular Medicine, Germany, used bioinformatics to delve into the mind of the octopus. Their work suggests that the brilliance of the octopus lies not in what they have but in how they use it.

All of the information required to build cells is stored within the nucleus in the DNA, and this is translated into messenger RNA (mRNA) for protein synthesis. Perhaps, reasoned the researchers, octopuses were modifying their mRNA in some way to use their genes in unorthodox ways, allowing them to construct their bizarre nervous system. But when they tested their hypothesis on the common octopus, *Octopus vulgaris*, it fell apart. After characterizing the amount and types of mRNA in 18 different tissues, including areas of the brain and a cluster of nerve cells in the gut, the researchers realized that the octopus's mRNA didn't have many unusual features that made the molecules stand out from the mRNA of other invertebrates – except for one thing. Many of the octopus mRNAs had unusually long tails compared with those of other invertebrates. Tails are an important part of mRNA, as proteins and other molecules use them as handles to grab onto the molecules. A longer tail could change how that process works and add an extra layer of control to the mRNA.

The researchers turned their attention to one well-known type of tail-grabber: microRNA (miRNA). These are tiny, hairpin-shaped molecules that can mark mRNA for disposal or prevent the message from being translated in protein by cellular machines. By halting protein production, miRNAs control the kinds and amounts of proteins that cells make. Octopuses have an extraordinary repertoire of miRNAs with at least 164 miRNA genes belonging to 138 families at their disposal, compared with oysters, which have only 20, putting octopuses on a par with zebrafish and chickens. miRNA expansions of this degree are exceedingly rare, having only been observed in the intellectual rivals of octopuses: vertebrates.

How much of this expanded miRNA repertoire is concerned with brain power? A lot. Thirty-four of the 43 miRNA families only found in octopuses (and not in close relatives such as squids) are concentrated in neural tissue. Many of these miRNAs were super active in embryos and hatchlings: exactly as expected if they were crucial for the development of a complex nervous system. And, when the team compared the common octopus's miRNAs with those of a far-flung relative, the California two-spot octopus (*Octopus bimaculoides*), they were quite similar, despite 50 million years of evolution between them, suggesting that they were useful enough to keep (or at least not worth throwing out). Overall, the new miRNAs showed up in the right places and at the right times to imply that they played key roles in nervous system development and evolution.

In short, it seems that great minds think alike. While we can't know what chickens, zebrafish or octopuses are really thinking, it seems that they all owe some of their brainpower to vast repertories of miRNAs. The alien brain of the octopus is perhaps not so alien after all.

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Brittney G. Borowiec (0000-0003-0087-7577)
University of Waterloo
bborowiec@uwaterloo.ca