Genes of the world's coolest fish

Antarctica is almost the last place on Earth that you would expect to be teaming with life. Survival below zero should be impossible, especially for ectothermic creatures that depend on their environment for warmth. But for the enterprising icefishes, Antarctica represented bountiful evolutionary and ecological opportunities. The icefish lineage dominates the modern Southern Ocean and its members famously sport colourless blood, huge hearts and ample mitochondria, the cell's power packs. Charmed by these unusual fish, a multinational team led by H. William Detrich III of Northeastern University, USA, John H. Postlethwait of the University of Oregon, USA, and Hyun Park from the Korea Polar Research Institute and the University of Science and Technology, Korea, investigated the genetic building blocks of extreme evolutionary adaptation in icefish.

A single adult *Chaenocephalus aceratus*, angled from the Antarctic Peninsula, provided the raw genetic material to sequence 30,773 protein-coding genes. When comparing the new genome with those of other bony fish, the researchers found 373 gene families that were larger than expected, and 346 gene families that looked too small, revealing icefish-specific genetic quirks. Nearly 40% of these quirks appeared within the last 7 million years, coinciding with plummeting temperatures and rising oxygen levels in the ocean surrounding Antarctica. How did these 719 gene families relate to adaptation to the world's coldest marine environment?

Antifreeze glycoproteins prevent ice crystals from expanding and tearing tissues. Unsurprisingly, icefish have a respectable 23 genes for antifreeze glycoproteins and their evolutionary precursors, trypsinogen and trypsinogen-like proteases. Yet strangely, icefish embryos do not express antifreeze glycoproteins, raising the question of how these animals develop at sub-zero temperatures. The authors hypothesized that embryos rely on other glycoproteins, such as those in the zona pellucida layer around their eggs, to fulfil the antifreeze role; and the genome supported their hunch. Icefish have 131 zona pellucida genes, far more than the 16 to 35 genes found in other fishes.

Another piece of the icefish adaptation puzzle is their huge stock of mitochondria and polyunsaturated fats in their muscles, to counteract the effects of cold on the fish's metabolism and biological membranes. Mitochondria naturally produce small amounts of toxic molecules that can damage DNA, fats and other cell components. The high mitochondria and polyunsaturated fat content of icefish tissues makes them extra sensitive to oxidative stress, and this shows in their genes. Icefish expanded the gene families that prevent oxidative damage, including antioxidant proteins superoxide dismutase (icefish have three extra copies) and NAD(P)H-quinone dehydrogenase (there are 33 copies in icefish, compared with between 2 and 10 in other fish). Icefish are also the only vertebrates to double up on their 8-oxoguanine DNA glycosylase gene, which produces a protein that removes damaged pieces of DNA.

Beyond the challenges of living in the cold, making their homes so far south means icefish experience the summer 'midnight sun' and prolonged winter darkness. Day length guides the rhythm and expression of hundreds of genes through the circadian clock pathways. How do icefish keep time when the sun is so unreliable? Their genome suggests that they simply don't bother. The icefish genome is missing many (though not all) of the time-keeping 'period' and cryptochrome genes found in other fish. Apparently, such extreme fluctuations in day length made light-dependent timekeeping less useful and the genes disappeared from the genome.

Somehow, 10 to 14 million years ago, life found a way. Although we have known about the peculiarities of icefish since the earliest expeditions to the remote continent, we are just starting to understand the complex genetic machinery underlying their physiology. With over 30,000 genes to mine for interesting patterns, this new high-quality genome is only the tip of the iceberg in our understanding of adaptation to extreme environments.

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Are fish self-aware?

Looking in the mirror and understanding that the person staring back at you is indeed yourself and not another person entirely is something that humans do without effort. But this understanding has long been used as a hallmark of advanced thinking and self-awareness for other species in the animal kingdom. Very few animals are considered self-aware, and this exclusive club is currently limited to mammals including chimpanzees, elephants and dolphins, as well as one bird species. However, researchers from universities in Japan, Switzerland and Germany have suggested that a small tropical fish – the cleaner wrasse – might also be a part of this elite group of thinkers, or perhaps the mirror test is somewhat flawed…

The mirror test typically works like this. First, an animal is shown a mirror. Next, a researcher removes the mirror and places a distinct mark somewhere on the animal's body that could only be seen with the help of a mirror. The mirror is then returned, and the researcher observes whether the animal attempts to remove the mark. Seems simple enough. Passing this test tells us that the animal understands that the reflection is itself, and no fish has ever passed it. But Masanori Kohda and colleagues thought that the cleaner wrasse might be able to. The fish are called cleaner wrasse because they munch parasites from the skin of other fish, or clients, and they are known for having the social intelligence to navigate their cleaner–client relationships. Kohda wondered whether this social intelligence might extend to self-recognition.

The researchers tested the wrasse in several phases. First, they left the fish unmarked and recorded how the individuals responded to a mirror. Having never seen a mirror before, the fish attacked – how did another wrasse get in my tank?! But this anger quickly subsided and shifted to something stranger. The fish began moving in ways that had never before been recorded for this species. For instance, they would swim upside-down towards the mirror or rush at it, only to stop right before crashing into it. The team of scientists interpreted these oddities as 'contingency behaviours', meaning the fish were exploring their bodies and may be testing whether their reflection would follow suit if they behaved weirdly.

Finally, the researchers marked the fish. They started by adding a small injection of clear paint under the scales (a common method for marking fish) to make sure the marking procedure itself did not indicate to the fish they had been marked; it didn't. The fish only inspected the mark in the mirror when the coloured paint was added. Amazingly, the fish would then leave the mirror and try to scrape off the mark by brushing themselves against the sand in their tanks. According to the rules of the mirror test, the wrasses passed.

However, the team has not been quick to declare that cleaner wrasses are self-aware. Instead, they conclude that these fish must have some level of self-recognition; whether this extends to a fully realized 'sense of self' remains to be seen. Perhaps self-awareness exists on a continuum, instead of being either present or absent, and the mirror test may not capture these nuances. The researchers know that their findings are controversial; the prevailing view among biologists has long been that fish have few advanced cognitive abilities. Kohda's team hopes that their work will encourage others to think carefully about the methods we use to compare intelligence across species. They also point out that we need to re-evaluate our preconceived notions about fish: they may be much smarter than we give them credit for.

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It's a small world on a hot leaf

Human-induced climate change is warming our world, delivering wide-ranging impacts to all animals and plants and the environments they live in. But what if our world were a single leaf on a single apple tree? Would a heat wave affect us in the same way that it would affect bigger animals and their ecosystems? Sylvain Pincebourde and Jérôme Casas from Université de Tours, France, had an inkling that for itsy bitsy beasts, not all microclimates are created equal. To find out, they delved into the diminutive world of plant-eating mites and insects to see whether these critters can beat the heat.

How does one study the microcosm of animals that are only a few millimetres long? Very carefully, taking all environmental factors into account. Pincebourde and Casas chose six species with three different methods of feeding on leaves: two aphids (phloem feeding; phloem is a sugar- and mineral-rich form of sap), a leaf miner (leaf mining), and two spider mites and a lace bug (tissue piercing). First, the authors determined the thermal tolerance limits of each species – or how much heat the critters can handle – by finding their upper lethal temperatures, the temperature at which 50% of the individuals die. They then studied the microclimate on the surface of the leaves by measuring the carbon dioxide intake, water loss and temperature of intact leaves and leaves that had been fed upon by the tiny herbivores. Finally, the authors combined their data on the species' temperature limits and the leaves' surface environment to predict just how much heat each species can handle in the wild.

Each species had a different thermal tolerance and, incredibly, the upper temperatures that creatures living on the same plant could withstand varied by 8.5°C. The tissue piercers (spider mites and lace bug) could endure higher temperatures than the phloem feeders (aphids), and the leaf miner sat in between. The phloem feeders increased the leaf transpiration rate – that is, they increased the amount of water vapour leaving microscopic holes in the leaf called stomata – while the tissue piercers decreased transpiration a little and the leaf miner was once again in the middle. Importantly, the duo found that the creatures' ability to withstand heat is directly related to leaf transpiration.

By increasing leaf transpiration rates, the phloem feeders, which have the lowest thermal tolerance, were able to minimise the temperature increase of the microclimate surrounding the leaf.

This means that they can stay cooler than the species that reduce leaf transpiration and allow leaves to warm. The problem with this strategy is that the phloem feeders cannot handle high temperatures because they have adapted to living in a relatively cool environment, so heat waves may spell more trouble for them than tissue piercers, which live on a relatively hot leaves. The authors' take-home message is that after taking leaf microclimate into account, spider mites, lace bugs, aphids and leaf miners only have a safety margin of 2–3°C, as half the species were already at risk of overheating at the highest temperature (37°C) tested by the scientists.

Mites and insects are likely to be more vulnerable to extreme temperatures than previously thought, and species living in temperate areas may find themselves in serious thermal strife during heat waves. Pincebourde and Casas reveal that current warming tolerances could be overestimated by up to 12°C – an enormous problem for accurately predicting future survival. Additionally, animals with different feeding apparatuses may be at different risks from climate change. It appears that creatures that turn on the air conditioning might not survive as well those that like to live with the windows open.

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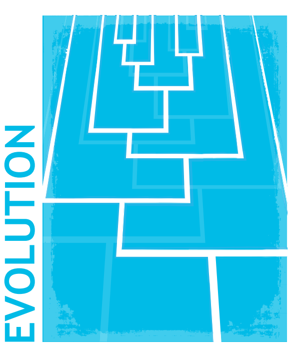
The vertebrate story lies in the fossil of a slimy fish



Physiologists are fascinated by the link between animal form and function. Some try to understand how animals use this link to adapt to their changing environments, while others have made it their career goal to uncover how an animal has evolved a specific function. But what if that evolutionary road is not as clear, perhaps because there are missing links in the evolutionary tree and competing theories about how that animal has come to be the way it is today? Or, what if an animal is so different from the rest that it is difficult to tell how it relates to other groups of animals?

Undeterred by such challenges, Tetsuto Miyashita, working out of University of Chicago, Illinois, USA, and University of Alberta, Alberta, Canada, along with an international group of researchers from two different continents, set out to resolve the evolutionary history of a very peculiar creature: the hagfish. It is the only animal to have a skull, but no vertebral column or spine – even though it is classified as a vertebrate – and it produces slime when threatened. Miyashita and his colleagues investigated the creature from a unique perspective by studying the features of a hagfish fossil (*Tethymixine tapirostrum*) from the Late Cretaceous period found in Lebanon, which they obtained from the Black Hills Institute of Geological Research in South Dakota, USA. To add to their challenge, the team tried to reconcile two competing theories about the place of the hagfish in the evolutionary history of the vertebrates: (1) that hagfish are a primitive group of vertebrates, or (2) that they are a separate, specialized group of vertebrates all-together.

Using a sophisticated technique for studying minerals in soft tissues, called synchrotron rapid-scanning X-ray fluorescence (SRS XRF), the authors examined the gill and mouth structures of the fossil. Because the fossil lacked paired fins, jaws, a mineral skeleton and features such as an oral sucker, cartilage and dorsal fin, the researchers concluded that it was not an ancestor of either the eel or the lamprey, which are similar; it was certainly a hagfish fossil. Next, the authors examined the fossil's slime glands, which are unique to hagfish. The group determined that it had 133 glands on the right side, which is more than most living hagfishes, but the animal was smaller in size. Using complex statistical analysis and historical data on the structure and evolution of lampreys and eels, the team was then able to assign the hagfish fossil to its rightful place in the evolutionary history of vertebrates. They classified it as the missing link between the last common ancestor of all vertebrates and the living soft-bodied round-mouthed fishes, thus filling a 100 million year gap in evolution.

Miyashita and his colleagues' study is revolutionary, because it shows that features such as lack of vertebral column and the round mouth of living hagfishes, which were previously believed to be primitive, are actually quite specialized, and these ancient animals seem to have evolved new adaptive features, such as their slime glands. The team's findings appear to agree with the second theory about the location of the hagfishes in the tree of life, classifying them as a specialized group rather than a primitive vertebrate. In addition, the team of researchers propose that the last common ancestor of all vertebrates is not a soft-bodied hagfish-like animal, as is the current theory. However, what that animal is, what it looked like and how closely it relates to the modern animals we see today awaits discovery.

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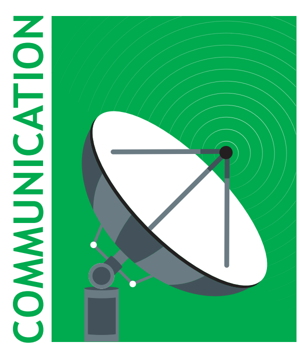
Singing mice take turns talking



As the northern winter silence melts, spring greets us with its familiar choir of birds chirruping in the tree tops. Yet, beneath the trees on the floor of Costa Rica's tropical cloud forests, the year-round sound of animal chatter never dies down. Alston's singing mice (*Scotinomys teguina*) carry out raucous conversations with one another as they defend territory or court a mate. Beyond that, how their back-and-forth communication operates is largely unknown. In a new study published in *Science*, Daniel Okobi Jr and Arkarup Banerjee in the lab of Michael Long at the New York University School of Medicine, USA, describe how male Alston's singing mice avoid interrupting each other and the brain circuit responsible for their chitchat etiquette.

To test how the mice responded when a conversation started up, the team initially kept some of the rodents in isolation while recording their solo serenades. Then the scientists relocated the loners to a new home, adjacent to another mouse. However, neither mouse could see or smell the other; they could only communicate by singing. After settling in to his new abode, the team noticed that the incomer became more chatty, singing three times more than he had when there was no audience. What was more striking was how polite the mice were when exchanging words: the mouse that had just moved into the neighbourhood waited patiently until after his new neighbour finished singing and then, within a half a second, rapidly responded with his own tune. This precisely choreographed duetting suggests that singing mice actively track conversations and respond only when the social situation is appropriate.

To figure out how singing mice coordinate their colloquy, the researchers investigated the motor cortex, a brain area they suspected to be responsible for polite conversation in mice, and uniquely required for speech production in humans (but not for generating other mammals' sounds). Knowing that mice become gradually louder and produce notes of longer duration when singing, the team cooled down the motor cortex – to slow down the neural activity. This caused the mice to take longer to spit out what they had to say, but all of the notes were articulated just fine. In contrast, stimulating the motor cortex momentarily interrupted a vocalizing mouse, after which the rodent picked up right where it left off. Lastly, when the team silenced brain activity in the motor cortex with nerve-blocking drugs, the mice still sang as often as before; however, they did not sing back in response to hearing another mouse's song. Taken together, the motor cortex in singing mice seems to be a hub for rapidly perceiving song and precisely orchestrating a vocal reply.

Many creatures across the animal kingdom rely on making sounds to communicate with one another: people use language; birds twitter; Alston's mice sing. These findings from Okobi and his colleagues help extend our understanding of how vocal communication evolved in animals and the shared brain areas that enable animals to chatter, in addition to raising the question of just how quiet mice really are.

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