Evolution of behaviour in Gif-sur-Yvette, France

Coping With the Dark

The Blind Cave Tetra, *Astyanax mexicanus*, likes peace and solitude

Photo: H. Zell, State Museum of Natural History Karlsruhe, Germany

Life in a jet-black cave caused some fish populations to evolve specific phenotypic and behavioural features. Sylvie Rétaux's research group is exploring the genetic, neural and developmental basis of these traits.

Imagine life inside a cave. For organisms coming from brighter environments, the perpetual darkness must be one of the biggest challenges. And the darkness brings more calamities: there is only little food available and spatial orientation is limited. But as life has done in the strangest and more challenging corners on Earth, it has also found its way into caves. Many of the cave animals we know today derive from surface-dwelling ancestors, who evolved convergent traits that allowed them to survive in such habitats. Their 'cave-specific' characters have therefore fascinated evolutionary biologists since Darwin's times.

The Mexican tetra fish *Astyanax mexicanus* is such an example of a fascinating natural history. It comprises two forms that diverged one million years ago: the surface fish or sighted river morphs and the cavefish, which are blind and depigmented morphs distributed in at least 29 populations in north-eastern Mexico. In the last couple of decades, these two morphs have been established as successful animal models for evolutionary research and the scientific community studying it, though small, is growing. A research group in Gif-sur-Yvette, 30 km south of Paris, led by Sylvie Rétaux, has fruitfully used *Astyanax* to study the evolution of brain development.

Fresh from the cave: Sylvie Rétaux (front row, 2nd from left) and Yannik Elipot (back row, 3rd from left)

Switching mouse for fish

Initially trained as a biochemist, Sylvie Rétaux, professor at the Institut Alfred Fessard at the CNRS, was introduced to the neuroscience world during her PhD. "From there, I became quite fascinated by the cerebral cortex of mammals," she relates to *Lab Times*. Today, she leads a research group focused on the evolution of development of the forebrain, the prosencephalon that has gone through an extraordinary diversification among vertebrates. For many years, Rétaux tackled the problem by comparing embryonic brains of various classic animal models, including frog and mouse. But eventually, she discovered *Astyanax mexicanus*.

Rétaux remembers that in 2004, she found a paper showing that, in cavefish, sonic hedgehog (*shh*) gene expression is expanded along the midline centre. This signalling centre, at very early stages of brain development, was already known to play an important role in the final neuronal anatomy of this organ and the protein Shh is one of the key players in this process. "When I read it, I thought that this would be a wonderful and ideal model to study the impact of midline signalling on forebrain evolution," says Rétaux, who has been working with *A. mexicanus* for eight years now. During those eight years, she learnt more about the fish's embryonic development, the evolutionary aspects related to the loss of eyes and the differences between surface and cavefish brain and body morphology.

Reasons to attack

One of the differences between surface and cavefish that Rétaux's lab wanted to understand is the loss of aggressive behaviour in the latter. To compare aggressiveness, the group initially paired up either two surface fish or two cavefish and counted the number of attacks, explains Yannick Elipot, who is currently a PhD in Rétaux's group and first author of their latest paper (*Curr Biol*, 23(1), 1-10). They found that the surface fish were ten times more aggressive than their cave cousin. Moreover, the attacks of the surface fish increased over time, while the cavefish attacked mainly at the beginning of the experiment, decreasing their strikes as time passed.

To test their hypothesis, they repeated the experiments with groups of fish that were raised under different diets (overfed, starved and normal) and confirmed the prediction: "When the cavefish were overfed, they behaved less aggressive, while under starvation they increased their number of attacks at the beginning of the experiments." They probably interpreted the other fish vibrations as potential food. This negative correlation between the amount of food and aggressiveness was not found in surface fish.

But Elipot explains that they also wanted to find the link between neuroanatomy and behaviour. "We know that in mammals and in other fish there is a link between serotonin and aggressiveness," he says. Their experiments suggested the same for *Astyanax*: a negative correlation between the level of serotonin and aggressiveness. Anatomically, there is a similar pattern of the serotonergic system between surface and cavefish, but one of the three hypothalamic nuclei is larger in cavefish. They found that the same shh gene expression expansion during cavefish brain development played a role in this size difference.

The basis of aggressiveness

The results altogether have led Rétaux and colleagues to propose that aggressiveness in surface fish is triggered by a down-regulation of serotonin levels in the raphe neurons, located in the posterior part of the brain, taking place while the fish establish dominance in fish schools. The attacks in cavefish, however, are rather food-seeking attempts driven by an increased number of serotonergic hypothalamic neurons – in the anterior part of the brain – that result from increased Shh signalling during early embryogenesis.

Rétaux and Elipot believe that cavefish might have abandoned aggressive behaviour for different reasons. Their first hypothesis is related to the fact that these blind fish have lost schooling behaviour. As food is scarce inside caves, living in schools is not an advantage for cavefish. And as aggressiveness in surface fish is known to be induced by schooling, loss of aggressiveness in cavefish probably came with the loss of this social lifestyle.

On the other hand, the Shh morphogen might as well be playing a role. "We know that Shh signalling in the midline increases the serotonin level and probably decreases aggressiveness in the same way," Elipot explains. "So for us, aggressive behaviour might also be a side effect of the Shh expansion in the midline centre," he concludes.

Losing sight

Rétaux is also interested in characterising, at the genetic level, other traits evolved during *Astyanax*' transition from surface to cave, such as the loss of eyes. There are different hypotheses to explain this degeneration of the visual system. One of them is genetic drift, "This means that there are mutations accumulating in eye genes, which are not counter-selective in the darkness," she explains. The second hypothesis involves selection. This means either that eyes are somehow deleterious in these environments, due to energy consumption, for example, or, indirect selection, as a consequence of increasing other sensory systems that are advantageous in caves.

Developmental biology data supports the latter, indirect selection hypothesis: due to an expansion of Shh signalling during early embryogenesis, cavefish increase their number of taste buds, have larger jaws and probably improve their olfactory system. At the same time, studies have shown that this increase in Shh signalling might also induce the loss of eyes.

On the other hand, Rétaux's research group has recently published new and contradictory insights on this subject through a transcriptome sequencing project, done in collaboration with Genoscope, one of the big sequencing centres in France (*PLoS ONE*, 8(1):e53553). "Looking at the transcriptome and studying the mutation patterns in various genes, we found that there is an increased number of mutations in cavefish eye genes. This suggests that, indeed, there is an accumulation of mutations in eye genes in cavefish, supporting the drift mechanisms," says Rétaux.

"At the end, we end up with both mechanisms playing a role in the loss of eyes. What we don't know is which one was first but we may have a hint by comparing old and younger cavefish populations," Rétaux affirms.

Difficult times for French scientists

When your *LT* reporter asks Sylvie Rétaux about the current situation of science in France, she says these are difficult times – an answer that, unfortunately, can increasingly be heard when European scientists are interviewed. "I don't know if it is better or worse than in the surrounding countries but the situation here is critical at several levels," she explains. This year, for example, the success rate for getting grants for fundamental scientific research was around 18.5%. She adds that there is also a lot of pressure to get positions, as there are very few of them offered per year. "Another problem is the fact that more people are dealing with short term contracts getting really financially insecure," she concludes.

But difficult times do not stop Rétaux and her colleagues from making interesting discoveries. She mentions that she was lucky this year to get some of her exciting projects financed. She plans to keep on concentrating on *Astyanax* research and, with this integrative approach, get a better picture of the enigmatic evolution of the forebrain.

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