

How a frog's molecules 'leaped,' and 'crawled,' to evolve violet vision

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The African clawed frog took a strange evolutionary path to change from ultraviolet to violet vision. Credit: Photo by Brian Gratwicke

The African clawed frog is tongue-less, has long, curvy toes and eyes that are perched on top of its head, but that's not all that's odd about it.

This species of frog also took a strange evolutionary path to change from ultraviolet to violet vision: Some of its visual pigment molecules kept trying to leap ahead, but other molecules shut them down and kept the process moving at a crawl.

Science Advances published the complete molecular interactions involved in the pathway, as detailed in a study led by Shozo Yokoyama, a biologist at Emory University who specializes in adaptive evolution of vision.

"It's the most bizarre, and sophisticated, case of color vision evolution that I've ever encountered," says Yokoyama, who previously headed up efforts to construct the most extensive evolutionary tree for vision, including 500 species of animals, from eels to humans.

"This frog had these quirks for rapid molecular change, but it also had something to control these quirks," he says. "In fact, it had triple protection."

Five classes of opsin genes encode visual pigments for dim-light and color vision. Bits and pieces of the opsin genes change and vision adapts as the environment of a species changes.

Ultraviolet (UV) vision gives a bi-chromatic, high-contrast view of the world that can be useful for many basic behaviors. Mice, for instance, are mainly nocturnal and mark their territory with urine and feces that reflects UV light for other mice. Unfortunately for mice, however, many of their predators are also UV sensitive so they, too, can spot these signs of mice more easily.

Violet vision, or the ability to see blue light, provides better resolution and detail for colors in a scene. Among the possible reasons that frogs evolved from UV to violet sensitivity may have been to give them a

better view of potential mates. It may also have improved their ability to pick out predators - such as a green snake amid green leaves.

In previous research on the African clawed frog, Yokoyama and collaborators had identified some of the genetic mutations involved in the process of the frog's switch from UV vision to its current function of violet vision. They also noticed that amino acid site 113 on this pigment of the African clawed frog had changed from glutamic acid to aspartic acid.

"Out of all the species in the animal kingdom that have been studied, site 113 is made up of glutamic acid, but this frog had changed site 113 to aspartic acid," Yokoyama says. "Why did it do that? This question was very mysterious and interesting to me. What is so special about this frog?"

Yokoyama studies ancestral molecules to tease out secrets of [adaptive evolution](#). The lengthy process involves teams of collaborators to first estimate and synthesize ancestral proteins and pigments of a species, then conduct experiments on them. The technique combines microbiology with theoretical computation, biophysics, quantum chemistry and genetic engineering.

For the current paper, he and his co-authors found that 12 mutations were involved in the frog's vision shift. These 12 molecular changes could have 500 million possible combinations of pathways that connect the ancestral UV vision and the frog's violet vision. The researchers narrowed the problem down and focused on changes in the six layers of transmembranes where the 12 molecules in the process are located. That focus reduced the number of possible evolutionary pathways to 720.

They then assembled molecular "chimeras" between the ancestral and frog pigments for all of these pathways. They tested how the molecules

functioned in all the different combinations, to hone in on the right pathway.

The results showed that the mutations that occurred on transmembranes four, five and six happened early during the evolutionary process. It was not until eons later, however, that these mutations came into play.

The mutations occurring on transmembrane two caused small shifts in the range of the light spectrum that the pigment detected. The mutations occurring early in evolution on transmembrane three, however, where site 113 resides, caused a big jump in the light-wave range - from 400 nanometers to 600 nanometers.

"Rapid change is not convenient evolutionarily," Yokoyama says. "In fact, it can be a disaster."

He uses the example of emerging from a darkened movie theater on a sunny day, and being temporarily blinded until your eyes adjust to the new environment.

Three times, molecules on transmembrane three mutated to cause a big jump toward violet sensitivity. The first time it happened, transmembrane five came into play, shrinking the molecular structure of the pigment and making it non-functional.

The second time that transmembrane three mutated, launching another jump, transmembrane six sprang into action, again shrinking the molecular structure. The third time transmembrane three tried to make the evolutionary leap, number four shut it down by destroying a critical chemical structure of the pigment.

The frog pigment essentially put on the brakes early during the evolutionary process for the mutations from [glutamic acid](#) to aspartic

acid at site 113. Only towards the end of the process did the pigment accept the site 113 shifts. By then, Yokoyama explains, the changes to the frog's light spectrum were no longer a big jump. Instead, they were just 15 nanometers.

"The human process for evolving from UV to violet [vision](#) was far more simple and straight-forward," Yokoyama says. "The story of this frog is full of mysterious twists and turns. A series of strange coincidences happened at the right time, at the right spot, for the right species."

More information: *Science Advances*,
advances.sciencemag.org/content/1/8/e1500162

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