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# The Evolution of this Eyeless Fish Helps us Pinpoint When Caves Started to Form

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Beneath the surface of eastern North America are countless freshwater caves. Deep, dark, and closed off from the surface, these landforms are difficult to study and difficult to date, with their ages being especially tough to determine through the traditional techniques of geochronology.

But biologists have discovered a surprising new method for dating the formation of these cave systems. According to a new study in *Molecular Biology and Evolution*, the evolution of the amblyopsid cavefishes — the eyeless fishes that inhabit these subterranean environments — provides important insights into the origins of these landforms, millions and millions of years ago. In fact, by studying when these fish started to lose their eyes, biologists can determine when these cave systems first took shape.

“The ancient subterranean ecosystems of eastern North America are very challenging to date using traditional geochronological cave-dating techniques, which are unreliable beyond an upper limit of about 3 to 5 million years,” said Chase Brownstein, a study author and a student at Yale University’s Department of Ecology & Evolutionary Biology, according to a press release. “Determining the ages of cave-adapted fish lineages allows us to infer the minimum age of the caves they inhabit.”

## Cavefish Lost Vision, Then Eyes

Amblyopsid cavefishes are small freshwater fishes that swim through the low-light environments of caves — including underground lakes, ponds, rivers, and streams — in eastern North America. And like the other organisms that live underground, they have a handful of adaptations that they’ve acquired over time, including their lack of color, their lack of vision, and even their lack of eyes.

To learn more about these visionless, eyeless creatures, Brownstein and colleagues set out to create a chronologic family tree of the amblyopsid cavefishes through morphological and genetic study. While their morphological analyses revealed that the amblyopsid cavefishes likely evolved from a common ancestor that had already acquired adaptations for low-light living above the surface, their genetic analyses revealed something much more intriguing.

Comparing the genetic mutations in 88 genes that are tied to vision, the team used the fishes’ genomes to show that the loss of sight in different cavefish lineages involved different combinations of genetic mutations. According to the team, this suggests that different cavefish lines colonized their own cave systems and then evolved their own additional low-light adaptations — their lack of vision and their lack of eyes — independently as they lived in their unique environments.

Because each lineage seems to have occupied its own cave system before losing its ability to see, this analysis meant that Brownstein and colleagues could date each cave system that each cavefish lineage had colonized, by identifying the age of the genetic mutations that had caused its vision to degrade.

"The fishes wouldn't have started losing their eyes while living in broad daylight," Brownstein said in the release. "In this case, we estimate a minimum age of some caves of over 11 million years."

## Evolutionary Loss and Geologic Gain

To date the genetic mutations behind the fishes' degenerating vision, Brownstein and colleagues used the cavefishes' genomes to gauge how many generations had passed since each cavefish line had gained the genetic mutations that had deteriorated its vision.

The mutations arose around 11.3 million to 2.25 million years ago in the oldest cavefish species, the Ozark cavefish (*Troglichthys rosae*), and around 8.7 million to 0.3 million years ago in other cavefish lineages, with four or more amblyopsid lines gaining their genetic mutations in their own cave ecosystems.

Ultimately, the age of these genetic mutations is much older than the age of the oldest cave systems that the traditional geochronologic techniques can correctly or consistently date, suggesting the potential of this method, and similar methods, for dating difficult-to-date caves and cave systems.

"An emerging field, geogenomics, seeks to test hypotheses about landform evolution by studying the history of biological diversification recorded in genomic data," Brownstein and colleagues added in their study. "Our results show the promise for the application of evolutionary histories to resolve ongoing questions in the earth sciences."