Genetic analysis finds greater threat in frog-killing fungus

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Shown is a mountain yellow-legged frog (Rana muscosa) at Milestone Basin in Sequoia National Park. Credit: Photo by Vance T. Vredenburg, UC Berkeley

A deadly fungus that has decimated populations of mountain yellow-legged frogs in the Sierra Nevada can likely be spread by sexual reproduction, seriously complicating efforts to save the frogs from extinction, according to a new genetic analysis led by researchers at the University of California, Berkeley.

The dramatic decline of the mountain yellow-legged frog over the past several decades has been attributed to the introduction of non-native predatory fish in some areas and to chytridiomycosis, a quickly spreading disease caused by this waterborne fungus, Batrachochytrium dendrobatidis.

The study, to appear in next week's edition of the journal *Proceedings of the National Academy of Sciences*, suggests that the frog-killing fungus may end up playing the bigger role in the frog's demise because of the pathogen's ability to spread over long distances and possibly persist in the environment as a consequence of sexual reproduction, according to the researchers.

"This group of fungi, when it reproduces sexually, can create spores that can last for a decade," said John Taylor, UC Berkeley professor of plant and microbial biology and principal investigator of the study. "That could make this pathogen a harder problem to defeat. As a resistant spore, the fungus could be transported by animals, including humans or birds, or lay dormant in an infected area until a new host comes along."

Biologists are still determining exactly how this fungus, first identified in 1998, kills the amphibians it infects, but most believe that the pathogen disrupts the frogs' ability to absorb water through its skin.

In the western United States, the fungus has been spreading quickly, moving west to east across the Sierra Nevada at a pace of about a mile per year, according to the researchers. Tens of thousands of mountain yellow-legged frogs in hundreds of sites have virtually disappeared in the wake of the pathogen's emergence in the area.

The researchers set out to determine which of two competing hypotheses for the origin of chytridiomycosis was more plausible. If the fungus was recently introduced to an area, the researchers would expect to find a single genotype that had spread by clonal reproduction. If, however, the fungus is endemic to a region, they would expect to find diverse genotypes resulting from a long history of association that provides enough time for isolates to diverge through mutation and genetic recombination.

If the fungus is endemic to a region, the animals in the area would normally be resistant to its destructive effects because they would have co-evolved together. However, biologists theorize that changes to the environment - from global warming to pollution from agricultural chemicals - could make native frog populations susceptible to a pathogen with which they've previously co-existed.

According to the study, neither epidemic spread nor endemism alone explains the decline of these frogs.

"We found sites dominated by a single fungal genotype, which suggests a recent spread of the pathogen through clonal reproduction," said lead author Jess Morgan, who was a UC Berkeley post-doctoral researcher working with Taylor at the time the study was conducted. "But this study also provides the first evidence of genetic recombination in B. dendrobatidis, which results in multiple, related genotypes and signals that sexual reproduction is occurring."

The findings could help explain the global spread of this pathogen, which has also been found in South America, Australia, Europe and Africa, said the researchers. While human-assisted spread is possible, the fungus has infected amphibians in pristine areas too remote for human activity.

"Up until now, people thought the movement of this pathogen was mainly via infected frogs, so such measures as restrictions on the pet trade were put in place," said Morgan, who is now a research scientist at the Department of Primary Industries and Fisheries in Queensland, Australia. "If, in fact, this fungus produces resistant spores, people could be unwittingly transferring this pathogen around the world from dirt on our shoes or car tires. But spores could also hitchhike on the feathers of birds for quick transport across mountain ranges."

Moreover, if resistant spores remain in lakes where previous populations of frogs have succumbed to chytridiomycosis, attempts to repopulate the lakes with healthy frogs will likely fail.

Study co-authors Roland Knapp, ecologist at UC Santa Barbara's Sierra Nevada Aquatic Research Laboratory, and Vance Vredenburg, a post-doctoral scholar in integrative biology at UC Berkeley, have led a number of such efforts to reintroduce mountain yellow-legged frogs in remote lakes in the Sequoia and Kings Canyon national parks and the John Muir Wilderness. The sites were areas where previous frog populations had been wiped out by chytridiomycosis.

Out of 10 reintroduction attempts over the past four years, seven have failed, the authors said.

"Within two years, the healthy frogs we introduced would become infected with the fungus and die," said Knapp. "It's a stunning thing to see. One year, there is no obvious evidence of the disease, the next year, we'd come back to see hundreds of dead or dying frogs, and then the following year, they'd all be gone."

Although genetic testing should be able to detect the spores, scientists do not know where to look for them. In addition, blindly testing environmental samples has thus far failed to yield evidence of the spores.

Infected frogs can be treated with fungicides to remove the pathogen, but researchers say it is not a practical long-term solution since they would remain susceptible to re-infection if returned to the same lake.

To conduct the study, researchers collected two species of mountain yellow-legged frogs from six sites in the Sierra Nevada. Rana sierrae is found in northern Sierra Nevada and represented in the study by frogs at the sites at Little Indian Valley, Summit Meadow and Mono Pass, while Rana muscosa is found in southern Sierra Nevada, where study sites Laurel Lake, Hitchcock Lakes and Woods Lake are located.

The sites at Mono Pass and Summit Meadow are both easily accessible to humans by Tioga Pass Road, a popular thoroughfare in Yosemite National Park. Accessing the other four sites, however, was decidedly more difficult, necessitating hikes of two to three days or, on several occasions, the use of helicopters.

The researchers cultured the fungi samples obtained from the 100 frogs collected and had the pathogen's genome sequenced by the U.S. Department of Energy's Joint Genome Institute in Walnut Creek, Calif. They compared genetic markers for the Sierra Nevada B. dendrobatidis samples with fungi collected from other regions around the world.

"The genotypes of our fungi in the Sierra are not that different from genotypes found around the world," said Taylor. "That means there must be someplace else on earth where this fungus is endemic. One would guess that the frogs living where the ancestral population of this fungus is located would not be affected that badly. We could then try to determine the mechanisms those frogs use to resist the pathogen."

The study also found that the sites near Tioga Pass Road contained two similar populations of fungi. Because the lakes are 40 kilometers apart, the evidence is strong that movement of the fungi between the two locations was somehow assisted by humans, said the researchers.

"If we confirm that spores are a factor, then there may be precautions we can take to contain their spread," said Morgan. "This could involve cleaning shoes before moving from one infected site to another. Some fungi produce spores during certain times of the year. If that is the case with this fungus, we could consider restricting public access to infected sites during those times."

As the U.S. Fish and Wildlife Service considers listing the mountain yellow-legged frog as an endangered species, biologists are racing to find ways to staunch the spread of the frog-killing fungus.

"This frog used to be the most abundant amphibian, and perhaps the most abundant vertebrate, in the whole Sierra Nevada," said Knapp. "Over the past 30 years, it has disappeared from up to 95 percent of its historic range, and its absence is impacting other organisms. Garter snakes that used to prey on these frogs are now declining. The frog's decline is leading to an unraveling of a high-elevation ecosystem."

Source: University of California - Berkeley