

Modern genetics vs. ancient frog-killing fungus

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Scientists at the University of Idaho currently are involved in a CSI-like investigation of a killer known to have been running rampant for the past decade. But the killer's name can't be found on the FBI's Most Wanted list. Instead, it's on the minds of ecologists on every continent in the world.

Its name is *Batrachochytrium dendrobatidis* (Bd). It is a "chytrid" fungus that lives on keratin, a type of protein found in the skin of amphibians, and is particularly deadly for certain species of frogs. A summary of key findings from the 2004 Global Amphibian Assessment states that 43 percent of all frog species are declining in population, with less than 1 percent showing increases. Although there are many reasons for frog decline, including climate change and habitat loss, Bd seriously is affecting a growing number of species.

"This fungus is really bizarre," said Erica Bree Rosenblum, assistant professor of biological sciences at the University of Idaho and lead author of the study published this week in the *Proceedings of the National Academy of Sciences* (PNAS). "It's a member of an group of ancient fungi that are at least a half billion years old. But it only recently began killing amphibians and unequivocally is responsible for a lot of the catastrophic frog die-offs during the past decade."

Previous studies have shown that once Bd is introduced to a habitat, up to 50 percent of amphibian species and 80 percent of individuals may die within one year. The fungus has been studied for the past decade, yet



scientists still do not know much about how Bd kills its host.

However, Rosenblum's new paper brings scientists one step closer to solving the mystery. The study uses some of the most advanced genetic technology available in an attempt to understand how the fungus works at the most basic level. It identifies several gene families for future study, including one strong candidate that may be a key element in the killing process.

Because the fungus is so ancient, it differs wildly from most species scientists study, and many of its genes have unknown functions. To combat these unknowns, Rosenblum and her colleagues sequenced Bd's entire genome and compared the expression of genes in two phases of the fungus's life - the zoospore and sporangia stages.

The zoospore stage is the earliest form of the fungus when it is just a single cell swimming around looking for a host on which to grow. Once it embeds itself into an amphibian's skin, it grows into a more complex form called the sporangia stage. In this stage, Bd grows on the keratin in the frog's skin, creating more zoospores to spread the disease and often killing the host.

By looking at which genes are turned on when the fungus actively is destroying the skin, but are turned off when the fungus is doing little more than swimming around, scientists hoped to find candidates for genes responsible for both spreading the fungus and killing the frogs.

"We care about the zoospores because that's the stage it is swimming around and finding frogs to infect," said Rosenblum. "And we care about the sporangia stage because that's when Bd is actually killing the frogs."

The study flags many genes as potentially important, but Rosenblum identifies one family as particularly interesting. The family of genes in



question, known as fungalysin metallopeptidase, has only one or few representative in similar fungi that do not kill frogs. But in this deadly fungus, genes in the family appear 29 times. Additionally, the genes generally are turned on when the fungus is infecting frogs, but turned off in the zoospore stage.

Although this gene family is an excellent candidate for the pathogen's killing ability, it is not certain. Discovering for sure which genes raise or lower the fungi's killing ability is a long process, partly because the fungus is so far removed from other organisms in the evolutionary tree.

"This fungus is strange and different, partly because it is so ancient," said Rosenblum. "One of the really amazing and wonderful things about this genetic technology is that we can take something we don't know anything about, sequence its whole genome, look at what each gene is doing in different life stages, and learn a tremendous amount about the organism."

Rosenblum and her team will continue their quest to stop Bd from killing off frog species in several ways. They currently are comparing active genes in Bd grown on frog skin to Bd grown in a test tube without exposure to keratin. Also, they plan to sequence genomes from different strains of Bd that kill less efficiently, or other, similar fungi that don't kill amphibians at all.

They also will study the parasite from the other side of the coin – the frog's point of view. By comparing different species of frogs, some of which are not killed by Bd, they hope to discover what genes make different species more or less susceptible to the fungus.

"The strength of these studies is the collaboration of ecologists and disease biologists," said Rosenblum. "We are not just choosing one factor to study. Looking at absolutely every gene in the genome is now a



financially and practically feasible thing to do."

Rosenblum's research is featured in the October 13-17 edition of PNAS Online Early Edition, article #08-04173. Read it online at <u>www.pnas.org/early/recent</u>.

Source: University of Idaho

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