Skin Fight: Could Bacteria Carried by Amphibians Save Them from Extinction?

Symbiotic bacteria may help frogs and other amphibians ward off the chytrid fungus plague wiping out populations worldwide

By Erica Rex

HELP FOR FROGS: Wild mountain yellow-legged frogs (*Rana muscosa*) host naturally occurring bacteria whose metabolites are toxic to *Batrachochytrium dendrobatidis,* or Bd, the fungus that has been wiping out frog populations worldwide. The *R. muscosa* shown is infected with Bd.
Vance Vredenburg

As many as one third of the world's 6,260 known amphibian species are in danger of going extinct. The main killer—outside of ongoing destruction of habitat—is a fungal disease known as chytrid (*Batrachochytrium dendrobatidis*). Now researchers in California and Virginia have identified symbiotic bacteria living on amphibians' skins that protects them from the deadly fungal disease, and later this summer the scientists will collect some of the microbial samples, culture them in the lab, and use the product to inoculate some frogs in California's Sierra Nevada to see if the approach stops chytrid in the wild. If a management plan can be developed, "creating a self-disseminating system [to fight chytrid] will be revolutionary," says Reid Harris, a biologist at James Madison University (J.M.U.) in Harrisonburg, Va., and one of the scientists whose research led to isolating and identifying this bacteria group in a telephone interview.

First identified in 1999, *B. dendrobatidis*, or Bd, a fungal zoospore, has been named as a leading cause of a global amphibian population decline, including frogs and salmanders. Scientists estimate it accounts for the extirpation or extinction of 200 species, including the disappearance of mountain yellow-legged frogs from several watersheds in the Sierras since the mid-1990s. Once plentiful, both species have now disappeared from 93 percent of their historic range. In fact, biologist Vance Vredenburg at San Francisco State University likens chytrid's devastating arrival to the emergence of HIV in humans. He thinks that, like HIV, the disease was at some point in the past endemic to a circumscribed population. The African clawed frog, for example, is known to carry Bd, the fungus that causes chytridiomycosis—commonly known as chytrid—but not die of it. Once used for pregnancy tests, African clawed frogs were raised in captivity in the 1940s. After they were supplanted by modern technology, they were simply released. Now, there are feral populations of African clawed frogs everywhere in the United States—even in San Francisco's Golden Gate Park National Park.

But there is hope in the fight against the deadly amphibian disease. Wild mountain yellow-legged frogs (*Rana muscosa*) and redback salamanders (*Plethodon cinereus*) host naturally occurring bacteria whose metabolites are toxic to Bd, the fungus that causes chytrid. Metabolites are the by-products of life-supporting chemical reactions taking place continuously in living organisms.

In lab experiments using captive-bred mountain yellow-legged frogs from eggs collected in the wild, Harris and his colleagues determined that the bacterium *Janthinobacterium lividum*, cultured from wild frogs, was protective against chytrid.

Harris found that if frogs were inoculated with a solution containing *J. lividum* before they were infected with Bd, they survived. The frog experiment used an initial concentration of 26.25 x 106 *J. lividum* cells per milliliter and an initial Bd zoospore concentration of 20 per milliliter. Bd numbers present increased exponentially as the 20-week experiment progressed until their populations tapered in response to the effects of the bacteria. A similar experiment with salamanders started with 6.7 x 107 *J. lividum* cells per milliliter and the same concentration of Bd zoospores.

The presence of *J. lividum* in any concentration was the key factor in determining survival. Neither the concentration of Bd zoospores nor the concentration of *J. lividum* nor their ratio, per se, correlated with survival. Frogs that hadn't been inoculated all died once they were exposed to chytrid. The one factor that appeared to be the most important was the concentration of a *J. lividum* metabolite, the peptide violacein. Harris found that the quantity of violacein on the frogs' skins was proportional to the population density of *J. lividum*. The salamander experiment also yielded a direct correlation between survival and violacein concentration. The amount of violacein produced by *J. lividum* varies widely between bacterial colonies. When it came to protection against chytrid, a study published by Harris, Vredenburg and colleagues in 2009 in *ISME Journal* (International Society for Microbial Ecology) showed that more violacein was definitely better: frogs with higher concentrations of the peptide did not succumb.

In the field results of a study by Brianna Lam, a graduate student in the J.M.U. Department of Biology, published in the journal *Biological Conservation* in 2009 (as well results published by Vredenburg and Harris in 2008 in *Abstracts of the General Meeting of the American Society for Microbiology*), revealed that wild mountain yellow-legged frog populations in the Sierras differed greatly in their amount of cutaneous *J. lividum*. The presence and quantity of the bacteria was key to their survival following a Bd outbreak. Chytrid's arrival in ponds lacking *J. lividum* resulted in rapid annihilation of the frogs.

This kind of mutualism among species, Harris points out, occurs everywhere in the natural world. "Beneficial relationships like this are found in fluorescent pseudomonad species that protect plant roots from pathogenic fungi, for example. In sessile, [or immobile] marine organisms, epiphytic bacteria in sea lettuce prevent fouling," he notes.

Bioaugmentation—taking existing beneficial bacteria, culturing them, and then adding more of them back into a biological system (not too different from eating probiotic yogurt for its purported beneficial effects on the human gut)—is the key to restoring amphibian populations.

Yet the real test of the bioaugmentation approach using *J. lividum* is going to depend on its safe and effective use in nature. Fortunately for the frogs, work starting in California's Sequoia and Kings Canyon National Park in the Sierras under the auspices of the National Park Service may add microbial oomph into the ecosystem. Vredenburg and his colleagues are carrying out a test this summer that involves applying *J. lividum* to populations of mountain yellow-legged frogs in an area where chytrid is just now showing up.

Harris points out that the existing management tools out there for addressing the chytrid problem are limited. Collecting individual frogs from ponds with dip nets and applying antifungal medications to individuals simply is not practical.

As promising as bioaugmentation may be, it's an area where research must proceed with caution, says Louise Rollins-Smith at Vanderbilt University Medical Center's Department of Microbiology and Immunology. She points out that bioaugmentation is routinely carried out in agriculture: "*Bacillus thuringiensis* was introduced to control caterpillars. It affects other butterflies but it continues to be used commercially. It's considered safe—an 'environmentally friendly' solution to a pest problem"—mostly because it obviates the need for the application of chemicals.

J. lividum, she notes, is a naturally occurring organism on frogs' skins. The unanticipated, negative outcomes that can follow when non-native species enter naive populations—such as the now pestilential Kudzu (*Pueraria lobata*), a legume native to Japan that was introduced into the southern U.S. during the late 19th century—might not apply in the same way with a bioaugmentation approach to the chytrid problem. The microbe and the frog already exist as natural symbionts in nature. And, in any case, reintroducing frogs whose bacterial communities have been altered should be carried out initially in protected ponds located in isolated areas, Rollins-Smith says.

Vredenburg is developing a project to do just this. Starting in three weeks, he and his colleagues will collect cutaneous bacteria from mountain yellow-legged frogs in the isolated Dusy basin area of the Sierras: "We'll go in with skin swabs, take samples, culture bacteria, grow it in the lab at San Francisco State, then wait a week, go back out and inoculate a bunch of frogs," Vredenburg says. It is impossible to speculate on the outcomes, he adds, because research on protection has been carried out only in the lab, so far.

"The goal is for scientists to augment naturally occurring bacteria" so that these helpful symbiants can spread to even more frogs and other amphibians, Harris says.