Holzapfel and Bradshaw wanted to know where the mosquitoes were in the past, particularly following a glacial period 20,000 years ago, when a warming trend had allowed them to migrate to new habitats. And to trace the migratory history of the species, the couple needed to establish the relatedness of populations from across the mosquito's range.

For years, they had tried to do this, but existing techniques were not able to resolve the differences between populations clearly enough. The mosquitoes from the various populations look too much alike to be distinguished morphologically, for example. In the 1990s, they tried in vain to reconstruct the biogeographical record by comparing proteins called allozymes among populations. Later, they fruitlessly looked at population differences in the insect's mitochondrial DNA. Even microsatellites, short stretches of DNA used in constructing genetic fingerprints, weren't up to the task. "We needed a better tagging or sorting system," Holzapfel recalls.

In 2009, they found one down the hall. UO colleague William Cresko had just teamed up with UO molecular biologist Eric Johnson to study the evolution of sticklebacks. They had genetically characterized populations of this fish by developing a catalog of single-nucleotide polymorphisms (SNPs), individual bases that vary frequently within a species. That work was made possible because a year earlier, Johnson's and Cresko's labs had developed a shortcut SNP-discovery method known as restriction-site-associated DNA sequencing (RADSeq).

This approach takes advantage of the speed and low cost of next-generation sequencing to quickly generate thousands of SNPs that distinguish populations and individuals. Researchers start by taking animals from multiple populations of a species and using so-called restriction enzymes to, at specific DNA sequences, chop up the genomes of each one into short fragments. Each animal's DNA fragments are then joined to a unique "bar code," a synthetic five-base strand of DNA whose sequence reveals which animal the non-bar-code DNA came from. All the fragments are then pooled together for mass processing by a next-generation sequencing machine. Because the bar codes allow the resulting sequences to be associated with specific animals, researchers aided by bioinformatics software can quickly identify genetic differences among individuals or populations.

**Test case.** Researchers didn't need a sequenced genome to make a dense genetic map of the pitcher plant mosquito.

For the mosquitoes, the researchers found 13,000 SNPs, 3700 of which helped to finally determine the relatedness of various populations of *W. smithii*. "This gave us the resolution to discriminate between postglacial populations," says Bradshaw. Based on that information, the researchers deduced that after glaciation, a remnant population of the pitcher plant mosquitoes gradually expanded out of the mountains of North Carolina—not out of the Gulf Coast, as some had presumed. The expansion proceeded gradually northward, then westward, they reported online 26 August 2010 in the *Proceedings of the National Academy of Sciences*.

When Cresko and Johnson's team tested RADSeq on the stickleback, they were able to match the fish's already sequenced genome to the newly generated sequence to help look for differences. No one had the resources to sequence the genome of *W. smithii*, and yet RADseq still worked effectively on the mosquito, demonstrating that the technique could be useful for a variety of organisms, even those for which little is known about their genetics. "This tagging system is definitely the wave of the future," says Holzapfel.

Furthermore, the cost for the entire mosquito study—examining all 23 populations of *W. smithii*—was just $3000. "The RADSeq method is cheaper, faster, and delivers thousands of markers," says Blaxter. He and his collaborators now have 18 RADSeq projects under way in snails, moths, nematodes, butterflies, salmon, ryegrass, sturgeon, beavers, beetles, oaks, elms, and spruce. Already for the diamondback moth, a crop pest, they have used newfound DNA markers to help pinpoint a gene that makes this moth resistant to a certain insecticide. Says Bradshaw, "This is an awesome technique." **–E.P.**



CREDITS (TOP TO BOTTOM): CHRISTINA HOLZAPFEL AND WILLIAM BRADSHAW; ROLAND KNAPP

# Tackling the Mystery of The Disappearing Frogs

For more than a decade, Roland Knapp has watched and agonized as the mountain yellow-legged frog, which normally thrives in high-altitude lakes and ponds too cold for other amphibians, disappears from the Sierra Nevada. In 1997, Knapp counted 10,000 tadpoles in a single mountain lake—the frogs seemed to "occupy every possible bit of water," he recently recalled on his blog. This past summer there were almost none. Surveys of 15,000 sites by Knapp, a field ecologist at the Sierra Nevada Aquatic Research Laboratory in Mammoth Lakes, California, and others have shown that this frog—which is actually two species—

**Going, going.** The mountain yellow-legged frog has disappeared from 90% of its Sierra Nevada habitat.



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is now missing from more than 90% of its former habitat.

There are multiple explanations for the frog's disappearing act, but a key one is the chytrid fungus, *Batrachochytrium dendrobatidis*, which has wiped out amphibians around the globe, including many populations of the mountain yellow-legged frogs. Yet every so often, some of these frogs survive the fungus, and Knapp has been unable to discern whether the amphibian's immune response or some environmental factor made the difference. "It's been pretty clear that our field experiments and observations only take us so far," he explains. "We needed to go to an entire new level of investigation."

So he was thrilled when Erica Bree Rosenblum, an evolutionary biologist now at the University of Idaho, Moscow, approached his team about collaborating on the endangered amphibian. In the past, Rosenblum, who studies the genetic basis of animal traits such as color or limb length, had been limited to what she calls "spearfishing": sequencing specific genes already suspected of influencing the trait. But about 5 years ago, she realized that new sequencing technologies would make it affordable to directly decipher all the active genes of a species without doing the extensive, and expensive, presequencing legwork required in the past. Thus, she could try "net-fishing," casting a net that could ensnare more than just suspected genes.

Rosenblum, Knapp, Cherie Briggs of the University of California, Santa Barbara, and ecologist Vance Vredenburg of San Francisco State University are now using this approach on wild populations of the frogs, comparing ones that persist despite exposure to the fungus to nonexposed ones that ultimately prove susceptible to it. The key step, which next-generation sequencing greatly facilitated, was elaborating the frog's transcriptome, its full repertoire of expressed genes, by sequencing the so-called complementary DNAs (cDNAs) that represent each gene. With these cDNAs in hand, the researchers could construct a device known as a microarray to assess which genes were active in various organs of exposed and unexposed frogs. Results so far suggest that in susceptible frogs, the immune system doesn't go on the defensive, says Rosenblum; the fungi somehow evades the body's defenses.

The researchers are also using the same microarray to evaluate gene activity in the amphibian's skin to understand why it degrades during infection. And by sequencing microbial DNA swabbed from frog skin, they are examining whether resistant frogs have an unusual repertoire of surface bacteria, as some microbes have been found to make an effective antifungal compound. Such genomic insights are much welcomed, says Vredenburg; the sequencing projects have "affected my work immensely."

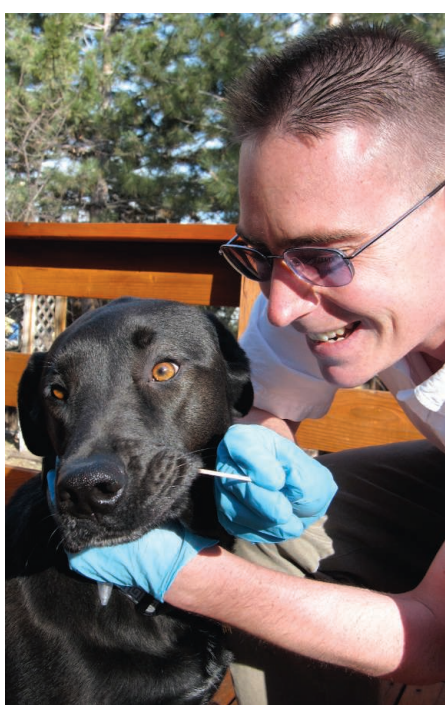
**–E.P.**

**Gone.** Roland Knapp's genomic studies may help explain the mountain yellow-legged frog's die-off.

## Digging Deep Into The Microbiome

It isn't only animal studies that have benefited from the explosion in genomics tools. Next-generation DNA sequencing has transformed microbial ecology studies as well. The past decade has seen the growth of metagenomics, in which researchers sequence DNA from a soil sample, the gut, even a computer keyboard, to learn what bacteria live there. With the new technologies, "you can sequence at a level deep enough that you can understand what's going on in the community," says Rob Knight, a microbiologist at the University of Colorado, Boulder.

The microbial makeup of our gut is a case in point. In the past decade, scientists have come to realize that animal intestines naturally harbor diverse microbial communities that help provide nutrients and sustain good health. A landmark 2005 study by Stanford University's David Relman and colleagues (*Science*, 10 June 2005, p. 1635) concluded that the bacterial communities in the human gut vary tremendously from one individual to the next. But that work looked at the guts of just three people, using traditional sequencing technology to probe for different variants of ribosomal RNA genes, each of which represented a different microbe.



A new analysis of 146 people, made possible by the lower cost and higher efficiency of DNA sequencing, is now telling a much more detailed story. Junjie Qin of BGI Shenzhen in China and colleagues recently collected fecal samples from 124 Europeans, some healthy, some obese, and a few with inflammatory bowel disease. They not only identified and sequenced all available ribosomal RNA genes in the samples but also deciphered more than 3 million other genes from the bacteria in the people's guts. (The 576.7 gigabases of DNA sequence data was

**Bug hunt.** Rob Knight studies the microbiomes of humans, dogs, and other animals.

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