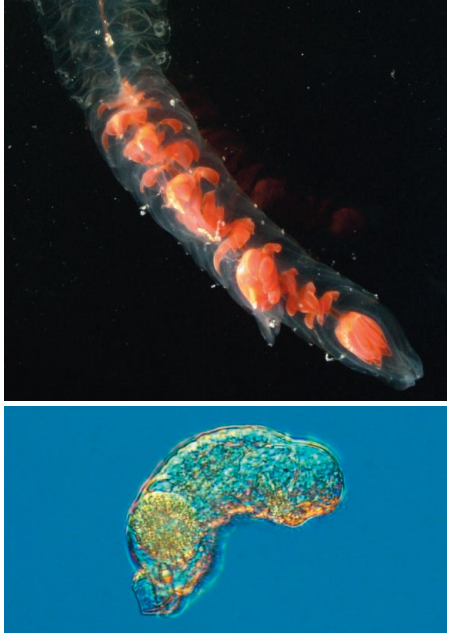
among organisms, identifying differences within equivalent pieces of DNA in various species. Controversy often ensued when those early results didn't agree with more traditional classification schemes, such as those based on fossils or morphology. To add clarity, researchers have, over time, increased the amount of genetic material they compare, creating a field called phylogenomics in which many hundreds of genes are evaluated in each analysis (*Science*, 27 June 2008, p. 1716).

A few years ago, it cost about $12,000 per animal to sequence 1000 or so genes, says Dunn. Now, a few thousand dollars delivers many more genes. "We can do now what we couldn't do before," Dunn says. That includes sequencing little-studied organisms, such as micrognathozoans, so accurately that scientists may resolve the relationships of invertebrates whose lineages split off from a common ancestor 500 million years ago.

There are often challenges to sequencing unusual organisms. Sometimes researchers don't have enough DNA to work with; other times the organism has odd ratios of DNA's four bases that make decoding samples difficult. But Giribet has already sequenced



Odd creatures. Siphonophores (*top*) and micrognathozoans may clarify animal evolution.

and analyzed 20 of these animals, including a whip scorpion, a ribbon worm, and several mollusks. Dunn is excited about the prospect of resolving the animal tree as never before: "It's clear we are going to be able to base our tree on lots of data from lots of species."

Next-generation sequencing technologies are also allowing Dunn to explore the evolution of animals by documenting differences in gene expression patterns across closely related species. The goal is to find out how these changes influence shifts in traits and behaviors across the tree of life. To do this, Dunn and his colleagues have turned to a new technique, known as RNA-Seq, that can gauge genetic activity in a sample by sequencing the complementary DNAs (cDNAs) that represent specific genes. The busier a gene is in a sample, the more times its cDNA will be sequenced.

Dunn and Stefan Siebert, one of his postdocs, have already compared the gene activity of the swimming and feeding forms of a siphonophore, a marine colonial organism. That analysis yielded thousands of genes potentially responsible for the differences in the animal's two structures. By repeating this experiment with multiple related siphonophore species, Dunn hopes to home in on those key to, say, the swimmer's development. "This will allow us to identify which genes have changes in expression that are associated with evolutionary changes," he says. **–E.P.**

Using DNA to Reveal a Mosquito's History

Ten years ago, the mosquito *Wyeomyia smithii* lived a largely anonymous life inside the "pitchers" of the purple pitcher plant common in bogs along the eastern United States, the Great Lakes, and southeastern Canada. Unlike some of its nastier relatives, the insect isn't known to transmit diseases to people or livestock. Larvae feast on microbes and detritus inside the pitcher plant, and adults sip on nectar, not blood, for the most part. Then in 2001, husband-and-wife evolutionary geneticists Christina Holzapfel and William Bradshaw of the University of Oregon (UO), Eugene, made the mosquito a poster child for climate change when they demonstrated for the first time that an animal had evolved in response to global warming.

Now the same researchers are applying next-generation DNA sequencing tools to probe further details of this species' evolutionary history—tools that have become so cheap and widely available that they can be applied to other poorly studied organisms as well. It's a "transformative technology," says Mark Blaxter of the University of Edinburgh in the United Kingdom.

Mosquito hunters. Christina Holzapfel and William Bradshaw embraced next-generation sequencing last year.

Holzapfel and Bradshaw began studying *W. smithii* 30 years ago, curious about how the mosquito had made its way so far north, because its relatives tend to reside in the tropics. In the course of their studies, they found that from 1972 to 1996, the mosquito's larvae in Maine had gradually delayed the start of hibernation by a week. Mosquitoes from farther north had postponed hibernation even later, whereas those in Florida had stuck to the same schedule as 25 years earlier. The pair concluded that the change in this genetically controlled trait was triggered by the longer growing season that resulted from gradual warming in the northern United States (*Science*, 23 November 2001, p. 1649).

Although the finding drew headlines, it still didn't explain how the mosquitoes had ended up in the north. To address that,

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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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