EXTREME ECOSYSTEMS

Biological Dark Matter Exerts Irresistible Pull in Yunnan

TENGCHONG, CHINA—A sulfurous stench wafts from the hot spring. "You wouldn't want to dip your toes there," warns Brian Hedlund, an ecologist at the University of Nevada, Las Vegas. The simmering acidic broth, a few degrees shy of 100°C, is eating away the clayey ground. But Hedlund isn't worried solely about our welfare: He doesn't want anyone trampling on scientific terra nova. Beyond the fact that this pool is dominated by archaea, the domain of microbes famed for their love of extreme environments, little is known about this otherworldly ecosystem.

The Rehai hot springs in Tengchong, a volcanic landscape in southwest China's Yunnan Province, are presumed to harbor hundreds of unknown organisms. DNA fragments gathered by Hedlund and others betray the existence of these shadowy microbes, but the organisms have eluded capture or defied cultivation. Most of the genes seen so far "are unrecognizable," Hedlund says. "This biological dark matter is a profound mystery." He hopes it will yield under a 5-year study launched here this summer.

Extremophiles have been isolated from some of the most forbidding corners of the planet. But, like zoo animals, many specimens do not reveal a full range of behavior in captivity. Hedlund's 20-member team from 14 Chinese and U.S. institutions intends to penetrate the rhythms of life in Rehai's alien environment. "We want to understand what it means to live at high temperature," Hedlund says.

Researchers have accumulated a wealth of data on extremophiles in diverse settings, from deep-sea vents to the picturesque hot springs of Yellowstone National Park. But they know little about how extreme ecosystems function. "We don't have a meaningful story," Hedlund says. He and his colleagues set their sights on Tengchong, home to China's biggest geothermal system. During World War II, this area near the Myanmar border was a critical base for the Flying Tigers, American pilots who helped China repel Japanese forces advancing from Southeast Asia. Its geological history has been hard to pin down. Evidence suggests that Tengchong was part of a large island in the ancient Bangong-Nujiang Ocean that joined the Eurasian plate 150 million years ago, at the end of the Jurassic Period. Much of the geothermal heat may be left over from the Pleistocene, when the region's now-dormant volcanoes were active.

Little was known about Rehai's life forms until Li Wen-Jun, a microbiologist at Yunnan University in Kunming, came on the scene. Over the past few years, Li says, his team has "established many new cultivation techniques." Thus armed, they have already discovered here four genera of Actinobacteria and two major groups of Bacteroidetes. "Wen-Jun is just an amazing force," says Hedlund, who recruited him to the project. With a $3.75 million grant from the U.S. National Science Foundation (http://faculty.unlv.edu/pire), the team expects to unmask dozens more species and higher taxonomic groups. They are applying new techniques such as single-cell genomics, in which DNA is sequenced from meticulously isolated cells of unknown organisms. "That gives us access to the microbes we can't grow," Hedlund says. With as many as 75% of bacterial phyla impervious to cultivation, Rehai will offer only a glimpse into a hidden world. Still, Hedlund says, "this will be one of the most comprehensive studies of any geothermal habitat."

Archaea are a particularly enticing target. "Certain springs at Tengchong are almost 100% archaea," says project co-leader Hailiang Dong, a geomicrobiologist at Miami University of Ohio in Oxford. These microbes are equipped to withstand the harshest conditions on Earth. Some species can thrive at temperatures above 120°C—some 20° hotter than the hardiest bacteria can tolerate. Their cell membranes are composed of lipids unlike any seen in other life forms, extraordinarily long chains with branched methyl groups and ring structures. "These lipids are tough at high temperatures," says Zhang Chuanlun, a geomicrobiologist at the University of Georgia, Athens, and Tongji University in Shanghai. Even by the exotic standards of archaea, the lipids of organisms found at Rehai, Zhang says, are "very unusual," featuring H-shaped bridge-like structures that make them extra stable.

A big challenge will be to unravel how archaea and other extremophiles interact and form food webs in which elements such as arsenic that are inimical to higher life can serve as fuel. As a research site, Rehai offers key advantages over its much-probed analog, Yellowstone. Managers here permit the sorts of long-term experiments and monitoring that are tightly restricted at Yellowstone. That should enable researchers to get at fundamental issues, such as how extremophiles, although lacking certain metabolic processes of temperate organisms, maintain a community. "We really don't have a handle on that," Hedlund says.

Acid test. Li Wen-Jun (*far right*) looks on as students probe Pearl Spring. Brian Hedlund (*left*) hopes Rehai will inspire a global geothermal census.

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Not far from Rehai's majestic frog's mouth geysers, two graduate students ease over a carved stone railing and delicately draw samples from a bubbling pool in a hydrothermal outbreak, an area prone to violent steam explosions. Hedlund and his team are hoping the life forms revealed in Rehai's 90-odd springs will inspire a global geothermal census. "We want to do genetic fingerprinting of all the hot springs in the world," he says. In China, a second microbial wonderland lies an hour's drive north of here. At Ruidian hot springs, the dominant microbes, according to DNA fragments and lipid profiles the researchers have collected in preliminary forays, are "completely different" from Rehai's and have not been cultivated, Hedlund says with a glint in his eye. Another terra nova waiting to be explored. **–RICHARD STONE**

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