EVOLUTIONARY BIOLOGY

Have 25-Million-Year-Old Bacteria Returned to Life?

The first clue that there's something unusual about the colony of bacteria in Raúl Cano's freezer is the lock on the freezer door. It's not that the bacteria are dangerous—they're members of a harmless species found in the bellies of Dominican bees. What makes them worth safeguarding is their age: They've apparently been alive for more than 25 million years.

Researchers have been struggling for years to extract ancient DNA from extinct creatures, but few of their results have been replicated and many apparently "ancient" DNA stretches may actually belong to modern molecules contaminating the experiments. Now Cano, a microbiologist at California State Polytechnic University, San Luis Obispo, and graduate student Monica Borucki claim to have retrieved the old molecules by a new route—bringing the entire organism containing them back to life.

On page 1060 of this issue, they report they have dissected a Dominican stingless bee trapped in 25- to 40-million-year-old amber, found bacterial spores (a dormant state of bacteria), grown them, analyzed some of their DNA, and found that it closely matches that of *Bacillus sphaericus*—the same bacteria found in modern Dominican bees. "When you look at them they don't look any different from the modern ones," Cano says, but these bacteria are ancient and "they're alive!"

In a field haunted by skeptics, Cano and Borucki's claim is being greeted by amazement, grudging acceptance—and calls for other labs to repeat the findings. "The data are consistent with it being ancient. And they've done just a huge number of controls to ensure there was no contamination," says Peter Setlow, a biochemist and specialist in bacterial spores at the University of Connecticut, Farmington. "But they can't actually prove it—no one saw the insects go into the amber." Svante Pääbo, an expert on ancient DNA at the University of Munich, says the claim is "an extraordinary one ... [but] the ultimate acid test of any scientific discovery is if others can reproduce it." In this case, he adds, the work should have been replicated, using the same bee, before these results were published.

Yet if the findings do hold up, scientists will be able to study evolution through a true living fossil. And if anything merits that moniker, chances are it would be a spore-forming organism. "Spores are nature's ultimate survival package," says Setlow. Starvation prompts many bacteria, including *B. sphaericus*, to don a thick protective protein shell. Within this shell, the organism's metabolism slows to an undetectable rate, and its single chromosome becomes dehydrated, which stabilizes the molecule. Setlow has shown that the chromosome is also saturated with proteins known as SASPs, or specialized acid-soluble spore proteins. SASPs change the structure of the DNA when they bind to it, preventing it from reacting with damaging molecules such as oxygen radicals. The spores are so hardy, he says, that they're even used to test the sterilizing power of the superheated steam in autoclaves: "If spores die, everything dies." But in contact with nutrients, such as glucose and amino acids, the bacteria come out of their shells and start growing again.

The previous best documented record for spore survival, says Setlow, is about 70 years: Louis Pasteur put some in ampules a century ago, and scientists found them still alive in 1956. "But that's a huge jump to millions of years," he says.

GEORGE POINAR

Living relic. From an ancient, amber-encased Dominican bee such as this, researchers claim to have restored living, 25-million-year-old microbes.

Amber, says Cano, seems to make that jump possible. The hardened, clear tree resin provides a sealed environment, keeping water out and exquisitely preserving insects for millions—sometimes hundreds of millions—of years. Researchers have long wondered whether they could extract bacteria from these ancient insects, and *Proplebeia dominicana*, a stingless bee whose descendants today have a symbiotic relationship with *B. sphaericus*, seemed like a good candidate. But earlier efforts had proved frustrating: George Poinar, a University of California, Berkeley, entomologist and Cano collaborator, had tried to isolate bacteria from these ancient bees, but had not been able to prove that he was working with an old organism rather than a modern contaminant.

So Cano and Borucki proceeded very carefully. In a clean lab hood, they sterilized the amber containing the bee, cracked it open, and dissected out the stomach contents, placing some in a culture medium. Within 2 weeks, microbes flourished. The researchers checked for contamination by trying to grow bacteria from cultures of the pieces of amber as well as the various sterilizing solutions, and they performed a variety of other tests. Nothing happened.

Under the microscope the bacteria looked very much like modern *B. sphaericus*; many of their enzymes also matched those of the modern counterpart. But the crucial test was to determine whether the likeness extended to DNA. Cano and Borucki used bacillus-specific primers to fish out a 530–base pair fragment of ribosomal DNA (rDNA) called S16 from both the microbes and the bee abdomen. The sequences were quite similar, strengthening the chain of circumstantial evidence connecting the microbes to the bee. And when the researchers compared both sequences with a GenBank database of bacterial rDNA genes, including about 50 different bacillus species—and seven strains of *B. sphaericus*—Cano and Borucki found that modern *B. sphaericus* was the closest match. "This," Cano says, "is real."

While other researchers wait for a similar affirmation to come from another lab, they are beginning to debate the scientific value of such an ancient creature. Says evolutionary biologist Blair Hedges of Pennsylvania State University, "One of the most useful pieces of information that could be gained from a revived 25-million-year-old microbe would be knowledge of rates of evolutionary change in its genes." It could, in essence, be used to measure the pace of evolution. But Norman Pace, a microbiologist at Indiana University and expert in rDNA phylogeny, emphatically disagrees. The ancient and modern microbes could belong to different strains of *B. sphaericus*, he says, so you can't claim that the modern gene derived from the ancient one, much less conclude anything about the time it takes for such changes to occur. "Unless you know this organism is a specific ancestor, you can't say anything. This is a wonderful testament to longevity, but any estimate of evolutionary rates is ill-conceived."

Pääbo still thinks such comparisons are possible with longer DNA sequences and more genes, which would give scientists a better idea of the familial relationship between ancient and current creatures. Cano is already trying for a complete version of the S16 gene—and waiting to see whether this discovery will withstand the test of time.

–Joshua Fischman