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Fast-Reproducing Microbes Provide a Window on Natural Selection

By CARL ZIMMER

In the corner of a laboratory at Michigan State University, one of the longest-running experiments in evolution is quietly unfolding. A dozen flasks of sugary broth swirl on a gently rocking table. Each is home to hundreds of millions of Escherichia coli, the common gut microbe. These 12 lines of bacteria have been reproducing since 1989, when the biologist Richard E. Lenski bred them from a single E. coli. “I originally thought it might go a couple thousand generations, but it’s kept going and stayed interesting,” Dr. Lenski said. He is up to 40,000 generations now, and counting.

In that time, the bacteria have changed significantly. For one thing, they are bigger — twice as big on average as their common ancestor. They are also far better at reproducing in these flasks, dividing 70 percent faster than their ancestor. These changes have emerged through spontaneous mutations and natural selection, and Dr. Lenski and his colleagues have been able to watch them unfold.

When Dr. Lenski began his experiment 18 years ago, only a few scientists believed they could observe evolution so closely. Today evolutionary experiments on microbes are under way in many laboratories. And thanks to the falling price of genome-sequencing technology, scientists can now zero in on the precise genetic changes that unfold during evolution, a power previous generations of researchers only dreamed of.

“It’s fun for us, because we can watch the game of life at the molecular level,” said Bernhard Palsson of the University of California, San Diego. “Many features of evolutionary theory are showing up in these experiments, and that’s why people are so excited by them.”

In the past century scientists have gathered a wealth of evidence about the power of natural selection. But much of that evidence has been indirect. Natural selection is a process that takes place over many generations, that may affect thousands or millions of individuals, and that may be shaped by many different conditions. To document it scientists have searched for historical fingerprints. They study fossils, for example, or compare the DNA of related species.

In the late 1980s a few scientists began experimenting with microbes, hoping to observe natural selection in something closer to real time. Microbes can reproduce several times a day, and a billion of them can fit comfortably in a flask. Scientists can carefully control the conditions in which the microbes live, setting up different kinds of evolutionary pressures.

While working at the University of California, Irvine, Dr. Lenski decided to set up a straightforward experiment: he made life miserable for some bacteria. He created 12 identical lines of E. coli and then fed them a meager diet of glucose. The bacteria would run out of sugar by the afternoon, and the following morning Dr. Lenski would transfer a few of the survivors to a freshly supplied flask.

From time to time Dr. Lenski also froze some of the bacteria from each of the 12 lines. It became what he likes to call a "frozen fossil record." By thawing them out later, Dr. Lenski could directly compare them with younger bacteria.

Within a few hundred generations, Dr. Lenski was seeing changes, and the bacteria have been changing ever since. The microbes have adapted to their environment, reproducing faster and faster over the years. One striking lesson of the experiment is that evolution often follows the same path. "We've found a lot of parallel changes," Dr. Lenski said.

In all 12 lines the speed of adaptation was greatest in the first few months of the experiment and has since been tapering off. The bacteria have all become larger as well, although Dr. Lenski is not sure what kind of adaptation this represents. When other scientists saw these sorts of results begin to emerge, they set up their own experiments with microbes. Today they are observing bacteria, viruses and even yeast as they adapt to challenges as diverse as infections, antibiotics and cold and heat.

Albert F. Bennett, a physiologist at the University of California, Irvine, is an expert on temperature adaptation. He started out studying animals like reptiles and fish, but he seized on bacteria after hearing about Dr. Lenski's experiments. "It was one of those 'Star Trek' moments," he said. "I was looking out the window, and for about 10 minutes my mind was going into hyperdrive."

Dr. Bennett was particularly curious about how organisms adapt to different temperatures. He wondered if adapting to low temperatures meant organisms would fare worse at higher ones, a long-standing question. Working with Dr. Lenski, Dr. Bennett allowed 24 lines of E. coli to adapt to a relatively chilly 68 degrees for 2,000 generations. They then measured how quickly these cold-adapted microbes reproduced at a simmering 104 degrees.

Two-thirds of the lines did worse at high temperatures than their ancestors, experiencing the expected trade-off. "If you're a betting person, that's the way you'd better bet," Dr. Bennett said. But the pattern was not universal. The bacteria that reproduced fastest in the cold did not do the worst job of breeding in the heat. A third of the cold-adapted lines did as well or better in the heat than the ancestor. Dr. Bennett and Dr. Lenski published their latest findings last month in The Proceedings of the National Academy of Sciences.

Other scientists are watching individual microbes evolve into entire ecosystems. Paul Rainey, a biologist at the New Zealand Institute for Advanced Study at Massey University, has observed this evolution in bacteria, called Pseudomonas fluorescens, that live on plants. When he put a single Pseudomonas in a flask, it produced descendants that floated in the broth, feeding on nutrients. But within a few hundred generations, some of its descendants mutated and took up new ways of life. One strain began to form fuzzy carpets on the bottom of the flask. Another formed a mat of cellulose, where it could take in oxygen from above and food from below.

But Dr. Rainey is only beginning to decipher the complexity that evolves in his flasks. The different types of Pseudomonas interact with one another in intricate ways. The bottom-growers somehow kill off most of the ancestral free-floating microbes. But they in turn are wiped out by the mat-builders, which cut off oxygen to the rest of the flask. In time, however, cheaters appear in the mat. They do not produce their own cellulose, instead depending on other bacteria to hold them up. Eventually the mat collapses. The other types of

Pseudomonas recover, and the cycle begins again, with hundreds of other forms appearing over time. “The interactions are everything you’d expect in a rain forest,” Dr. Rainey said.

Scientists have long known that underlying these visible changes were genetic ones. But only now are they documenting the mutations that allow this evolution to happen in the first place.

Dr. Palsson has been running experiments in which E. coli must adapt to a diet of glycerol, an ingredient in soap. He found that within a few hundred generations, the bacteria could grow two to three times as fast as their ancestor. He then selected some of the evolved microbes and sequenced their genome. He compared their DNA with that of their common ancestor and pinpointed a few mutations that each line had acquired.

Dr. Palsson then inserted copies of these mutated genes into the ancestor and found that it now could thrive on glycerol as well. But the order in which he inserted the genes made a big difference to the bacteria.

Some mutations were beneficial only if the bacteria already carried other mutations. On their own, the mutations could even be harmful. Dr. Palsson’s results offer a detailed picture of what biologists call epistasis — the intimate ways in which mutations can influence the effects of other mutations during evolution.

As Dr. Palsson and other scientists have pinpointed mutations in microbes, they have been surprised by how mysterious the mutations are. They are struggling to find out how the mutations benefit the organisms. And in some cases, they do not even know what the mutated genes did before they mutated.

“It just makes you ask, ‘What on earth is that doing?’ ” said Gregory J. Velicer, a former student of Dr. Lenski’s who is now an associate professor at Indiana University. Dr. Velicer experienced this bafflement firsthand while watching the evolution of a predatory microbe called Myxococcus xanthus. Myxococcus swarms lash their tails together and hunt in a pack, releasing enzymes to kill their prey and feasting on the remains. If the bacteria starve, they come together to form a mound of spores. It is a cooperative effort. Only a few percent of the bacteria end up forming spores, while the rest face almost certain death.

This social behavior costs Myxococcus energy that it could otherwise use to grow, Dr. Velicer discovered. He and his colleagues allowed the bacteria to evolve for 1,000 generations in a rich broth. Most of the lines of bacteria lost the ability to swarm or form spores, or both.

Dr. Velicer discovered that some of the newly evolved bacteria were not just asocial — they were positively antisocial. These mutant cheaters could no longer make mounds of spores on their own. But if they were mixed with ordinary Myxococcus, they could make spores. In fact, they were 10 times as likely to form a spore as normal microbes.

Dr. Velicer set up a new experiment in which the bacteria alternated between a rich broth and a dish with no food. Over the generations, the cheaters became more common because of their advantage at making spores. But if the cheaters became too common, the entire population died out, because there were not enough ordinary Myxococcus left to make the spore mounds in the times of famine.

During this experiment, one of Dr. Velicer’s colleagues, Francesca Fiegna of the Max Planck Institute for Developmental Biology, discovered something strange. She had just transferred a population of cheaters to a dish, expecting them to die out. But the cheaters were making seven times as many spores as their normal ancestors. “It just made no sense,” Dr. Velicer said. “I asked her I don’t know how many times, ‘Are you sure you marked the plates correctly?’ ”

She had. It turned out that a single Myxococcus cheater had mutated into a cooperator. In fact, it had evolved into a cooperator far superior to its cooperative ancestors. Dr. Velicer and his colleagues sequenced the genome of the new cooperator and discovered a single mutation. The new mutation did not simply reverse the mutation that had originally turned the microbe’s ancestors into cheaters. Instead, it struck a new part of the genome.

But Dr. Velicer has no idea at the moment how the mutation brought about the remarkable transformation in behavior. The mutated segment of DNA actually lies near, but not inside, a gene. It is possible that proteins latch on to this region and switch the nearby gene on or off. But no one actually knows what the gene normally does.

Mutations like this one, Dr. Velicer said, “make for a much more complicated story.” It is a story he and other scientists are looking forward to revealing.