Science 25 June 1999:
Vol. 284 no. 5423 p. 2108
DOI: 10.1126/science.284.5423.2108

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- NEWS

EVOLUTION

Test Tube Evolution Catches Time in a Bottle

Tim Appenzeller

By running experiments on microbes for thousands of generations, researchers are exploring the roles of chance and history in evolution

For most living things, 24,000 generations is a daunting span of time. Go back that many human generations, or about 500,000 years, and *Homo sapiens* had not yet evolved. Even for the fruit flies beloved of geneticists, 24,000 generations equals about 1500 years. But in Richard Lenski's laboratory at Michigan State University in East Lansing, 24,000 generations ago is a recent memory. The year was 1988, when he and his students first introduced 12 genetically identical populations of the bacterium *Escherichia coli*to their new homes: 50-milliliter flasks filled with sugary broth.

Since then, those bacteria have been clocking up the generations at a rate of about one every 3.5 hours, mutating and adapting right in front of Lenski's eyes. Lenski is a founding member of a subculture of evolutionary biologists—many of them his former students and colleagues—who are watching evolution unfold in laboratory cultures of microbes, where a single experiment can span enough generations for major evolutionary change. These laboratory microcosms, whether of bacteria, viruses, or yeast, can turn evolution into an experimental science, says Michael Travisano of the University of Houston. “You have the luxury of making a prediction, and then you can test it. It's almost like physics.”

Researchers can subject populations to the same environmental stresses again and again—a procedure that Paul Sniegowski of the University of Pennsylvania calls “analogous to being able to revive the fossils and rerun the evolutionary events.” They can thaw out ancestral forms, stored in laboratory freezers in what Lenski calls a “frozen fossil record,” and compare them to their descendants. And they can monitor the microbes' genomes as they evolve, tracking the ultimate roots of those changes in DNA or RNA. “It's some of the most exciting stuff in evolution,” says Stephen Jay Gould of Harvard University.

These laboratory microcosms are allowing researchers to address some of the field's biggest questions, such as how often the twists and turns of evolution are the result of chance rather than adaptation. Researchers can study how evolutionary baggage from one round of selection affects how an organism fares in the next, and how adaptive radiations can arise from a single organism. And they can address a question that has preoccupied evolutionary thinkers like Gould: How reproducible is evolution? If the history of life could be replayed from the same starting point, how differently would it unfold? So far they are finding that identical populations facing similar conditions can follow parallel courses, although the underlying genetic changes often differ. But over time, in new environments, the effects of those differences can grow, steering evolution into radically different courses and giving chance and history ever larger roles in a population's fate.

With the enormous complexity of nature reduced to test tube systems, researchers have to approach such questions with humility, Gould notes: “Of course, you're looking at a very different world at a different time scale.” Nor can researchers even be sure that what they see in one evolutionary microcosm will apply to any other, adds Holly Wichman of the University of Idaho, Moscow, who studies evolution in viruses. “One of the questions is how well [test tube findings] are going to generalize. … Is every case going to be a special story?”

Still, the granddaddy of these experiments—the 11-year, 24,000-generation *E. coli* cultures in

Lenski's laboratory—is telling stories about predictability, chance, and history that other experiments have echoed. All 12 of Lenski's cultures experience the same stresses: a daily boom-and-bust cycle, in which the bacteria are transferred to fresh glucose medium every 24 hours, then undergo 6 hours or so of plenty followed by 18 hours of starvation. All 12 lines have adapted to this regimen; when the researchers do a head-to-head comparison between the evolved bacteria and the ancestral strain, plucked from the freezer and revived, the descendants now grow about 60% faster in their standard glucose-containing medium. All 12 populations show other parallel changes, too—for example, a still-unexplained, twofold increase in cell size.

Yet underneath these consistent responses to selective pressure, says Lenski, "you see all this hidden variation." The fitness increases were almost identical in all of the populations, but not quite; the cell size expanded in all 12 lineages, but by different amounts. And when Lenski and his colleagues, including Michel Blot of the University of Grenoble in France and Werner Arber of the University of Basel in Switzerland, analyzed the genomes of their adapted bacteria, the similarities vanished. By chopping up the bacteria's DNA with enzymes and applying probes that home in on known sequences, they found that after thousands of generations, the populations' genomes were riddled with changes. The changes were different in each population and had accumulated at very different rates, the group reported in the March *Proceedings of the National Academy of Sciences*, even though the fitness increases were similar. That indicates what the authors called "conspicuous and significant discrepancies" between genomic evolution and its visible effects.

Lenski and graduate student Mark Stanek are now trying to pinpoint the particular beneficial mutations that boosted the bacteria's fitness. They've found one so far—and it is present in just one lineage, strengthening the idea that the others have found different paths to higher fitness. When it comes to organisms' adaptive performance, says Lenski, "evolution is remarkably reproducible. But as you move away from performance, to cell size or genes, things are less and less reproducible." Because all 12 populations started out genetically identical and have experienced the same selective pressures, the differences underscore the role of chance in setting evolution's course.

Evolutionary baggage

The role of chance becomes even more obvious over time, as those genetic differences become part of the baggage that organisms carry to their next evolutionary challenge—baggage that can dramatically affect how they fare, as Travisano and Lenski have shown. They took samples of the 12 *E. coli* populations after the bacteria had been growing in glucose for 2000 generations. By that point, all 12 populations had improved their ability to grow on glucose by about the same amount. But when they were put in a different sugar, maltose, some populations thrived while others languished. For each population adapting to limited glucose, says Travisano, "it seems likely that glucose uptake was tweaked in subtly different ways. And those subtly different tweaks had big effects in a different environment."

He and Lenski then allowed all 12 lineages of bacteria to evolve for another 1000 generations on their new staple, maltose. Evolution did its work, and after months of mutation and selection, all 12 could grow well on maltose. But the fitness improvement was not as consistent as it had been on glucose, where the starting genotype had been identical. Evolution was no longer as reproducible as before, because of chance variations in how the populations had adapted to their earlier environment. "Once we had diversity, we could prune it back tremendously with adaptation. But not completely. Once you are different, that difference tends to persist," says Travisano.

To Travisano, the results are a lesson in the importance of prior history in shaping the way organisms respond to an adaptive challenge. They "tell you that variation arises very easily … and it doesn't arise in ways that are easily predicted."

Other researchers are weighing the roles of predictability and chance in adaptive radiations, in which one form gives rise to many. Paul Rainey at the University of Oxford in England seeds vials of sugar water with cells of the common plant bacterium *Pseudomonas fluorescens*. He avoids shaking the containers, allowing the environment to stratify into regions that are chemically and physically different, with oxygen-rich layers near the surface and oxygen- depleted but nutrient-rich layers beneath. The result is a diverse array of ecological niches for the bacteria to fill—what an animal species newly arrived on an empty continent might find. He then follows their evolution for 10 days.

In his original work, done with Travisano and published in *Nature* last year (also see *Science*, 17 October 1997, p. 390), Rainey found that in virtually every one of these microcosms, the bacteria evolve into three major forms. He named them for the appearance of their colonies when he grows them on culture plates: wrinkly spreader, fuzzy spreader, and smooth morph, which is the unchanged ancestral form. Each has a taste for a particular niche, with the wrinkly spreader congregating at the surface of the broth, the smooth morph spreading through the liquid, and the fuzzy spreader hugging the bottom.

Rainey is now trying to account for these tastes. So far, he and his students have learned that wrinkly spreader overproduces a cellulose-based polymer, which helps glue the cells together into a mat. The mat supports them at the surface, where the wrinkly spreader cells benefit from the abundant air supply.

These miniature adaptive radiations unfold in the same way every time, governed by the available environmental niches. And Julian Adams, at the University of Michigan, Ann Arbor, saw some of the same repeatability in his experiments, where diversity arises seemingly out of nothing. Adams, with Frank Rosenzweig, now at the University of Idaho, Moscow, and their colleagues, grew genetically identical *E. coli* populations in a device called a chemostat, which kept conditions for the bacteria blissfully constant, except for a steady shortage of glucose. But in spite of this uniformity, two or more *E. coli* variants—an ecosystem in miniature—regularly made their appearance after around 200 generations, or about a month, says Adams.

The group first got a clue that one strain had turned into several when they extracted samples from their cultures, grew them on plates, and saw colonies of different sizes, rather than the uniform colony size expected of genetically uniform bacteria. “The differences were so dramatic that we thought we had contamination” and shut down the system, Adams recalls. “I don't want to tell you how many times we did that before we cottoned on to what was happening.” He and his colleagues went on to show that at least two strains had evolved in their chemostats.

Originally, Adams explains, natural selection favored mutants that had a souped-up appetite for glucose and so could outgrow its neighbors. But bacteria can metabolize only so much glucose; as their biochemistry got clogged with the sugar, the glucose-hogging mutants shunted the excess from aerobic metabolism to the less efficient anaerobic pathway, which generates a waste product, acetate. As Rosenzweig, Adams, and their colleagues described in the August 1994 issue of Genetics, the acetate buildup created a new ecological opportunity, and eventually a mutant emerged that could fill it: a new acetate-scavenging strain. Adams and his colleagues reported last summer in *Molecular Biology and Evolution* that the acetate scavengers appeared in six out of 12 populations they studied, and each time a mutation in the regulatory region of a gene that influences acetate uptake was responsible.

“It's the first stage in speciation,” says Adams. “Diversity can exist even if you don't seed it with something that can drive diversification.” And like other studies, this one shows that diversification is not only inevitable but also follows a predictable course.

But even if the general outline of such experiments is predictable, in many cases the genetic pathway they take depends on chance, as Travisano saw when he transferred glucose-adapted bacteria to maltose. That seems to be the case for Rainey's wrinkly spreader strains, too. When his group took 24 wrinkly spreader strains that had evolved independently and then forced them to evolve back into a smooth form by shaking their vials to keep the culture medium from becoming stratified, Rainey says, “some go back easily; some sort of struggle,” implying differences in their genetic makeup. Thus, Rainey concludes that “you can become wrinkly spreader by a variety of different paths.”

The influence of chance and history on how organisms diversify is still more vivid in Rainey and his students' new experiments, in which they introduce an additional evolutionary force: a predator. After allowing the microcosms to diversify, they infect them with a bacteriophage, a virus that kills bacteria. The population crashes, then rebounds as a resistant strain takes over. The resurgent strain diversifies again—but it does so differently within each microcosm, spawning odd new variants including a strain that secretes a mucoid slime.

“What it comes down to is just a chance thing,” Rainey says. “The phage puts the population through a bottleneck, which increases the role of chance. The reproducibility goes out the door.” Only individuals that happen to be resistant to the phage pass through the bottleneck, and the array of genes they carry varies from microcosm to microcosm. As a result, each miniature ecosystem rediversifies from a different starting point and reaches strange new adaptive peaks.

Carbon-copy evolution

In some experiments, however, evolution seems truly reproducible down to the level of genes—for example, Adams's work in which genetically similar acetate mutants appeared six times out of 12. Now researchers are trying to work out why. Travisano, for example, has reversed the experiment in which he switched glucose-adapted bacteria to a diet of maltose and saw a wide variety of responses. In work published in the June 1997 issue of *Genetics*, he adapted 12 identical populations of *E. coli* to a restricted diet of maltose. After 1000 generations, he switched them to glucose. But this time, every population responded to the diet switch in the same way, continuing to thrive. Apparently all 12 populations had evolved in the same way—perhaps, Travisano suggests, because bacterial physiology offers just one way to do better in maltose, forcing all of the populations down the same evolutionary path.

Similarly, Wichman, James Bull of the University of Texas, Austin, and their colleagues have found that the mutations underlying high-temperature adaptations in a particular bacteriophage are surprisingly reproducible, right down to the specific changes in the DNA sequence. Now Wichman, Bull, and their students are trying to identify the factors that favor this kind of predictability. “It's really too early to tell what the rules are,” she says, but she is enjoying her privileged view of evolution. “It's amazing to watch changes sweep through a population in a way we knew happened but had never seen before.”

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