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- NEWS FOCUS

The Man Who Bottled Evolution

Elizabeth Pennisi

Richard Lenski's 25-year experiment in bacterial evolution shows no signs of running out of surprises about how mutation and selection shape living things.

EAST LANSING, MICHIGAN—When most biologists want to understand how evolution unfolds, they look for clues in the fossil record or the natural world. Richard Lenski simply walks across his Michigan State University lab to his freezers. There, stored in 4000 vials, are bacteria dating back to 1988. That was the year Lenski started a simple but radical experiment. He put samples of *Escherichia coli* into a sugar solution, stoppered the flasks, and waited to see what would happen. It was a study with no defined endpoint, so risky that he didn't try very hard to get outside funding for it.

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REPORT

Long-Term Dynamics of Adaptation in Asexual Populations
Michael J. Wiser et al.
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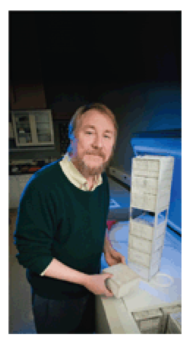
After 25 years and 58,000 bacterial generations, Lenski's bacteria are still growing, mutating, and evolving. They are proving as critical to understanding the workings of evolution as classic paleontology studies such as Stephen Jay Gould's research on the pace of change in mollusks. Lenski's humble *E. coli* have shown, among other things, how multiple small mutations can prepare the ground for a major change; how new species can arise and diverge; and that Gould was mistaken when he claimed that, given a second chance, evolution would likely take a completely different course. Most recently, the colonies have demonstrated that, contrary to what many biologists thought, evolution never comes to a stop, even in an unchanging environment. The work is "an absolutely magnificent achievement," says Douglas Futuyma, an evolutionary biologist at Stony Brook University in New York.



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Time traveler. To turn back evolution's clock, Richard Lenski dips into his freezer.

CREDIT: G. L. KOHUTH/MICHIGAN STATE UNIVERSITY (2009)

Other researchers have done experimental evolution, setting up populations of insects, yeast, and even fish in the lab and in controlled field conditions, and subjecting the organisms to a particular environmental stress for relatively short periods. But Lenski's long-term experiment "is just orders of magnitude beyond what anyone else has done," Futuyma says.

The project's quarter-century has witnessed the rise of bioinformatics and the birth of whole-genome sequencing, and Lenski has taken advantage of both technologies to glean new insights. Generations of students have tended and analyzed the microbes, and the project sparked a memorable conflict between Lenski and creationists. Fifteen years ago, he almost abandoned it for digital models of evolution, then reconsidered—and was vindicated when his bacteria took one of their most dramatic evolutionary leaps. As Scott Edwards, an evolutionary biologist at Harvard University, said at a June evolution meeting,* "the principles of evolution that Rich has uncovered have touched all of us and caused us to look at evolution in a new way."

Hard data

Lenski, 57, has been a MacArthur Fellow, a member of several editorial boards, and a society president; he belongs to the National Academy of Sciences and served on committees that evaluated genetically modified organisms and the investigation of the 2001 anthrax attacks. In August, he became an avid tweeter and started his own blog, Telliamed Revisited, named after an 18th century book that stressed the importance of understanding the world through observations and not religious dogma. He has collected old scientific texts and baseball statistics, although these days, he says, "my granddaughter is my current hobby."

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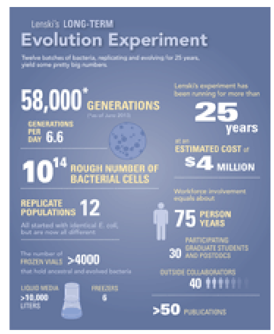
Evolution on display. A mutation led to a population boom in one of 12 cultures, turning the medium turbid (third flask from left). Investigators can compare two strains' fitness by culturing them together and then counting colonies (petri dish). One strain carries a mutation that makes its colonies red.

CREDITS: BRIAN BAER AND NEERJA HAJELA/MSU (2)

But one thing will always capture his attention. Show up at his door with new data, and he's "like a 5-year-old at Christmas," says graduate student Michael Wiser. That hunger for hard data about evolution was what drew him to bacteria in the first place. Lenski never had a course in microbiology. As a graduate student at the University of North Carolina, Chapel Hill, he studied the ecology of ground beetles—work he found interesting but limited. "[I]t was difficult to imagine feasible experiments that would really test the scientific ideas that most excited me," he wrote in an August blog. For his postdoctoral training in 1982, he switched gears and joined the lab of Bruce Levin, one of the few researchers doing experimental evolution in microbes. They studied the interactions between bacteria and viruses called bacteriophages. But their systems were still too complex to get at the question Lenski most wanted to answer: Was Gould right about evolution's irreproducible nature, or would evolution often repeat itself if given a second chance?



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DATA SOURCE: R. LENSKI

So after starting his own lab at the University of California, Irvine, Lenski used some of his own money, from a National Science Foundation (NSF) Presidential Young Investigator Award, to set up a much simpler system using just bacteria. It was "like a physics experiment [where] you try to strip [out] all the complications so you can isolate the phenomenon that you want to describe," explains Lenski postdoc Noah Ribeck, a physicist.

In 1988, Lenski placed identical *E. coli* populations into 12 flasks filled with a liquid containing nutrients and 25 milligrams of glucose per liter. Every 24 hours, with constant stirring and a comfortable 37°C environment, the bacteria multiplied explosively, depleting the sugar. Each day, 1% of the bacteria were transferred to a new glucose-laden flask. Every 75 days—about 500 generations for *E. coli*—Lenski's team froze some of the bacteria for future studies and as backups should the experiment become contaminated. This protocol has continued day in and day out, weekends and holidays, virtually uninterrupted for 25 years. (The cultures were temporarily frozen during one winter break when the campus was deserted and when Lenski moved his lab from California to Michigan.)

Setting up the protocol and sticking with it "took a certain spirit and vision," Levin says. Early on, skeptics argued that evolution in bacteria, particularly bacteria growing in a stable, comfortable environment, wouldn't reveal much about how the process unfolds in nature. Yet, as they monitored their cultures, Lenski and his colleagues saw what looked very much like an accelerated process of evolutionary change. By using bacteria instead of a slower-growing organism, Lenski compressed time: The *E. coli* go through 6.6 generations in a day, compared with more than a year for the same number of generations in mice. In 25 years, he's seen the equivalent of a million years of evolution in humans. With that span, "you can examine a whole range of ideas that there hasn't been an opportunity to look at before," says Graham Bell, an evolutionary biologist at McGill University in Montreal, Canada.

By thawing out frozen vials from generations past, Lenski can go back in time to look at intermediate stages of evolutionary change. "It's like the perfect fossil record," says Douglas Emlen, an evolutionary biologist at the University of Montana in Missoula. "They can pinpoint exactly when [a trait] arose." And Lenski can restart the experiment with those ancestral bacteria to see if history repeats itself. "A graduate student who is 22 today can study populations that come from before they were born and use techniques that haven't existed before," says Christopher Marx, a microbiologist at Harvard University and a former Lenski postdoc.

For the first decade, things hummed along. The investigators monitored the bacteria's fitness by measuring how fast they could multiply relative to their ancestors. At first, their fitness rapidly increased, but then the improvement slowed. Over the whole experiment to date, fitness has improved by an average of 70%—meaning the most recent descendants undergo 1.7 doublings in the time it took the original microbes to double once.

All 12 lines improved by about the same amount, showing that, broadly speaking, evolution is reproducible (*Science*, 25 June 1999, p. 2108). But some lines improved faster than others, and genetic analyses of the strains showed that they had taken different evolutionary paths. For example, six lines developed defects in DNA repair, and instead of dying out, began sustaining higher mutation rates than their counterparts. "Historically, which mutations arise early changes the subsequent evolution," explains Janette Boughman, an evolutionary biologist at Michigan State University. "But you really get to the same endpoint": better fitness in sugar solutions.

Other striking differences emerged among the flasks. At generation 6500, about 3 years into the experiment, two types of *E. coli* evolved in one of the flasks: one that made small colonies consisting of relatively small cells and one that made large colonies, with large cells. Lenski expected that eventually one type would take over and the other would disappear, or both would be ousted by a bacterium with an even more beneficial mutation. But to his surprise, both types have persisted, creating an ecosystem in which competition and other interactions between the colony types allowed both to be viable. "He created his own Galápagos Islands," Marx says.

Going digital

Having done so, Lenski almost gave up on the flask experiment. He had caught a glimpse of an experimental evolution system that promised to be even simpler and faster. "He's a shiny object researcher," says former student Paul Turner, now an evolutionary biologist at Yale University. "If there's something interesting to pursue, he will try to pursue it." During a squash game, a physics colleague invited Lenski to a seminar by California Institute of Technology physicist Christoph Adami. With his graduate student Charles Ofria, Adami had developed software that allowed self-replicating computer programs, aka digital organisms, to compete with one another for processing power and evolve new functions. Lenski was entranced. "One of the first data slides [Adami] put up looked like data from my long-term experiment," he recalls.

In 1998, Adami sent Lenski an advance copy of his book *Introduction to Artificial Life* (*Science*, 8 May 1998, p. 849). Lenski stayed up for 2 nights straight reading about the program and trying out simulations he had designed himself. The digital organisms replicated thousands of times faster than his microbes, and their "mutations" could be tracked in more detail. For the next 6 years, he focused as much on digital evolution as on bacteria.

In 2003, he teamed up with Ofria and Adami, both of whom are now at Michigan State, and with Robert T. Pennock, a philosopher there, to follow mutation by mutation how computer programs that initially could do no more than replicate evolved the ability to perform complex operations, such as checking whether one numerical string equals another. They found that many earlier mutations—some of them deleterious in the short term—had to accumulate before a final "enabling" mutation conferred the new trait. The work demonstrated that complex traits, such as the vertebrate eye, likely come about through a series of intermediate steps that open the way for future adaptation.

Lenski's fascination with digital life hasn't faded. In 2010, he, Ofria, and others got funding for BEACON, an NSF Center for the Study of Evolution in Action, which includes 400 investigators and students from five U.S. universities. They collaborate on studies of evolution as it occurs in both real and digital organisms.

Fortunately, he did not abandon his first love. The weekend that Lenski discovered digital organisms, he had told his wife he might shut down the long-term experiment. Only her nudging convinced him to keep it going—and in January 2003, the value of doing so became clear, when the bacteria served up yet another surprise. "Digital organisms have the advantage that you can get complete information about pretty much all aspects of an experiment," Lenski explains. "The bacteria, though, are a lot more complex and so they have a lot more tricks up their sleeves, a lot more potential to evolve in ways that one cannot anticipate."

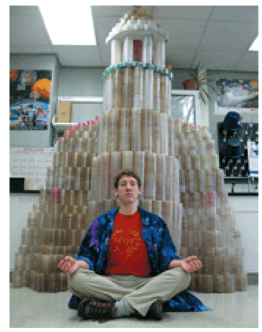
One morning, Lenski and his colleagues noticed that the medium in one flask had grown turbid, a sign that it was unusually thick with bacteria. They suspected contamination but could not confirm it, so they dug out the most recent frozen sample of Ara-3, as that population was called, and restarted it. Three weeks later, the turbidity reappeared. This time, they thoroughly tested the culture and ruled out contamination. By growing the Ara-3 bacteria on different types of media, they discovered that the bacteria in that flask had evolved a new way to nourish themselves. Instead of relying on scarce glucose, they drew on a different energy source in their medium, citrate, which enabled them to reach much higher densities than in other flasks. "This was the biggest event in the entire *E. coli* experiment," Adami says. "To have a complex new function develop seemingly from scratch is a big deal and quite remarkable."

It fell to graduate student Zachary Blount to figure out what happened. Was a single mutation responsible? And if so, why hadn't this ability appeared earlier, since the bacteria had already had plenty of time to experience mutations in every possible gene? Or were multiple mutations required, and if so, when and in what order did they arise? To find answers, Blount rewound the tape of life, unfreezing earlier generations and letting them evolve again in the same medium. Citrate use emerged in four of 72 cultures, always in ones derived from more recently frozen samples. That suggested that multiple mutations underlie the trait. Populations from those later generations had already acquired the predisposing mutations and so could more readily take the next step.

Blount and Lenski's results, published in 2008, drew lots of attention, and not just from the media and scientific community. On his website, Conservapedia, conservative Christian Andrew Schlafly questioned the conclusion that the bacteria had evolved this striking new ability and requested Lenski's original data. Lenski referred him to the original paper, but Schlafly persisted. Lenski responded with a sharply worded response that went viral. "He immediately became a rock star of science," Adami says.

Meanwhile, the citrate work continued. The cost of sequencing microbial genomes dropped dramatically, enabling Blount to look at the specific genetic changes involved. He found that citrate users had undergone a duplication of a 2933-base piece of DNA that activated an otherwise silent gene coding for a citrate transporter. Subsequent genetic changes then tuned up the efficiency of that transporter, he and Lenski reported in 2012.

These citrate users are enabling Blount, now a postdoc in the lab, and Lenski to look at another aspect of evolution: the formation of new species. The usual test of separate species is that they are unable to interbreed successfully, a criterion that can't be applied to bacteria because they don't mate. But because one of *E. coli*'s defining characteristics is the inability to use citrate for energy in the presence of oxygen, the citrate-consuming bacteria could be seen as a new species. And they may even meet the traditional definition. Researchers can't interbreed bacteria, but they can mix the genomes of separate strains. Bacteria that thrive on citrate do poorly on glucose, and melding the citrate users with the parent strain produces a less fit hybrid, Lenski reported at the June evolution meeting.



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Pileup. Zachary Blount used all these petri dishes to study how one flask of bacteria evolved the ability to consume citrate.

CREDIT: BRIAN BAER/MSU

Evolving endlessly

Lenski no longer thinks about ending his experiment. "It's become more and more apparent that it's not good to just do short-term experiments," he says. "Any microbiology lab with enough people should be thinking about doing a 20-year experiment." Funders agree. The project, once rejected by the National Institutes of Health, now has ongoing NSF support: a 10-year Long Term Research in Environmental Biology grant.

And it is continuing to pay off, Lenski, Wiser, Ribeck, and their colleagues report online this week in *Science* (http://scim.ag/MWiser). Researchers have long assumed that when organisms encounter a new environment, they will adapt very quickly at first, then, as long as conditions are stable, ultimately reach an adapted state. At that point—a "fitness peak"—adaptive evolution should virtually stop. Wiser tested the fitness of all 12 populations at 41 time points during their evolution, traveling back in time with the frozen samples. At 10,000 generations—about 5 years—it seemed the bacteria were reaching that fitness peak. But now, after 50,000 generations, the improvement has slowed down but not leveled off as expected, Lenski's team reports. "The notion of a fitness peak is more elusive than I anticipated," Lenski says. "I think fitness may well continue to increase for a million years." Evolution is endless, it seems, even in a stable environment. "That's a profound insight," Boughman says.

It's also an encouraging portent for the future of Lenski's cultures, which are now passing 58,500 generations. "If you asked me 20 years ago, I thought [the researchers] were running out of new things to learn," Levin says. Now, "I think they should go on indefinitely." Lenski agrees. During his presidential address at the recent evolution meeting, he made a plea: "If you know anyone who would like to endow a million-year experiment, have them get in touch with me."

↵* Evolution 2013, Snowbird, Utah, 21 to 25 June.

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