Legendary bacterial evolution experiment enters new era

On 24 February 1988, evolutionary biologist Richard Lenski filled 12 flasks with sugary growth medium and seeded each with *Escherichia coli* bacteria. For the past 34 years, Lenski, at Michigan State University in East Lansing, and his colleagues have nurtured the bacterial cultures, refreshing growth media daily and freezing samples for future study every couple of months. The long-term evolution experiment (LTEE) has become a cornerstone in evolutionary biology that researchers continue to mine for insights. During their 75,000 generations of growth, the bacteria have made gains in their fitness and evolved some surprising traits. Last month, Lenski and his lab tended to the LTEE for the last time. The 12 *E. coli* lines are now frozen and will soon be revived to begin a new life in the laboratory of Jeffrey Barrick, an evolutionary biologist at the University of Texas at Austin. The two spoke to *Nature* about one of biology's longest-running experiments.


LAURA GERSON/UT COLLEGE OF NATURAL SCIENCES, SIPA US/ALAMY

Jeffrey Barrick (left) will take over the *E. coli* experiment from mentor Richard Lenski.

What inspired you to begin the experiment?
Richard Lenski: I like big, open-ended questions. I wanted a very simple, long experiment to look at how repeatable evolution was. The original goal was 2,000 generations. And I figured that deserves the moniker 'the long-term evolution experiment'. I had no idea that it would go as long as it has actually gone — and hopefully will go much longer.

Why did you keep it going?
Lenski: It's a very easy experiment to maintain. The amount of work for an individual on a typical day is maybe half an hour. It is 365 days a year, in principle, but the amount of work per day isn't huge.

And then, of course, the bacteria were doing very interesting things over time. New technologies emerged, such as the ability to sequence genomes cheaply. People like Jeff joined the lab and brought new ideas and questions. And the bacteria kept doing interesting things. I realized that it should just go on for as long as humanly possible.

How many generations had passed when Jeff started working on the experiment?
Lenski: Did you join the lab in about 2007? It was probably around 40,000 generations.

Jeffrey Barrick: Sounds about right. I knew less going in about the history of the experiment — maybe less than a lot of Rich's postdocs and graduate students. But I was at a time in my career where I had been studying evolution at the molecular level, looking at all kinds of bacterial genomes. I wanted to study evolution of whole organisms in the lab and be able to watch evolution.

Was it ever a challenge to keep the experiment going?
Barrick: Like Rich said, it's fairly simple. There are some major snowstorms and other things that go on, and other infrastructure anomalies that can make it sometimes challenging, but you can always go back to the freezer, which is one really nice thing about the experiment and makes it much more doable than other experiments. People have tried long-term experiments with mice and flies and other organisms where it's really difficult if something goes wrong.

Lenski: One of the advantages of the long-term experiment is everything is so simple. We work with a chemically defined medium, we can freeze strains and revive them. I think it should continue smoothly in the new home.

What has the experiment taught us about the repeatability of evolution?
Lenski: My bias going into the experiment was that all the strains would go off in very different directions. I was thinking that the roles of chance and contingency in evolution would have been larger than they were. And over the years, we've actually seen just striking amounts of reproducibility. So although a typical line has improved its relative fitness compared with the ancestor by maybe 70% or 80%, the variance in competitive fitness between most lines is more like just a few per cent. So they've all tremendously increased, but very similarly to one another.

But then, over the years, we've also seen quite striking divergences between the lines. Thirty thousand generations into the experiment, one of the 12 lines evolved the ability to consume citrate, instead of just glucose. And that garnered quite a lot of attention, and even some, shall we say, hostility from some people who are sceptical of the power of evolution. And after 75,000 generations , it's still the only one of the 12 lines that has evolved that ability.

Are there big questions about evolution that you hope to answer by carrying on for longer?
Barrick: For a lot of bacteria that ended up in simple, constant environments — especially simple endosymbionts that live inside the cells of insects — their genomes gradually shrink over time. And I'd say one of the more

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surprising things to me is that these *E. coli* have been in a very constant environment, yet their genomes have not shrunk much.

Lenski: I think part of the issue on the genome shrinkage is that that's a slow process. Thirty years and 75,000 generations — it's a drop in the evolutionary bucket. So I would guess that if we could come back — in one million years or whatever, the bacteria probably would have extremely reduced genomes. That's a reason to keep it going.

Why did you decide to pass the torch?
Lenski: I'm not going to be around forever. I think it's better to do those things now, to plan them carefully and thoughtfully. So it just made sense. I'm 65 years old, and although I don't plan to retire any time in the next few years at least, the lab is getting smaller. And one of the important things for keeping the long-term lines going is this daily rhythm. I think a lab that has half a dozen or more people is ideally suited to the weekend and holiday coverage that the experiment benefits from.

So I asked Jeff, maybe in 2018 or 2019. I have a National Science Foundation grant to run the experiment, and Jeff is now a co-principal investigator on that.

Why did Jeff take it on?
Barrick: I'm a big proponent of open science. This is a great resource that I want to support and share and continue. It's become kind of a common touchstone for a lot of stories about bacterial evolution. And something that people can take in so many directions. I'm excited about supporting the community.

Rich, what's your advice for Jeff?
Lenski: Keep calm and carry on. Frustrating things will happen. But the experiment is quite forgiving. As long as my brain is working, I'll be really excited to see what new spin-off experiments he and collaborators generate, what new analyses he and the broader community generate to make sense of what's going on with the long-term lines. Probably the most important thing Jeff will have to think about in 20 or 30 years is, who's next?

Interview by Ewen Callaway
This interview has been edited for length and clarity.

Q&A

ANCIENT DNA TRACES ORIGIN OF BLACK DEATH

Plague-causing bacteria from Kyrgyzstan are direct ancestors of those that sparked medieval pandemic.

By Ewen Callaway

A Silk Road stopover might have been the epicentre of one of humanity's most destructive pandemics.

People who died in what is now Kyrgyzstan in the fourteenth century were killed by strains of the plague-causing bacterium *Yersinia pestis* that gave rise to the pathogens responsible for the Black Death several years later, shows a study of ancient genomes.

"It is like finding the place where all the strains come together, like with coronavirus where we have Alpha, Delta, Omicron all coming from this strain in Wuhan," says Johannes Krause, a palaeogeneticist at the Max Planck Institute for Evolutionary Anthropology in Leipzig, Germany, who co-led the study, published on 15 June in *Nature* (M. A. Spyrou *et al. Nature* **606**, 718–724; 2022).

A pestilence's roots

Between 1346 and 1353, the Black Death laid waste to western Eurasia, killing up to 60% of the populace in some places. Historical records suggest that the bubonic plague emerged from the east: Caffa, on the Crimean peninsula, experienced one of the earliest-recorded outbreaks of plague during a 1346 siege by the army of the Mongol Empire. The Caucasus and other locales in Central Asia have been put forward as potential epicentres.

China hosts some of the world's greatest genetic diversity of modern *Y. pestis* strains, hinting at an East Asian origin for the Black Death. "There were all kinds of hypotheses in the literature. And it was not really known where it exactly came from," says Krause.

Signs of the plague

Several years ago, Philip Slavin, an economic and environmental historian at the University of Stirling, UK, and a co-lead author of the study, came across records from a pair of fourteenth-century cemeteries in Kyrgyzstan that, he thought, might hold clues to the origins of the Black Death. The cemeteries, known as Kara-Djigach and Burana, held an unusually high number of tombstones dated to 1338 and 1339, ten of which made explicit reference to a pestilence.

"When you have one or two years with excess mortality, it means something funny is going on there," Slavin said at a press briefing.

To determine whether the burials held any relevance to the later Black Death, Slavin worked with Krause to track down the remains from the Kyrgyz cemetery — which had been excavated in the 1880s and 1890s and moved to St Petersburg, Russia. The team, led by archaeogeneticist Maria Spyrou at the University of Tübingen, Germany, sequenced ancient DNA from seven people whose remains were recovered, discovering *Y. pestis* DNA in three burials from Kara-Djigach.

A pair of full *Y. pestis* genomes gleaned from the data showed that the bacteria were direct ancestors of strains linked to the Black Death, including a *Y. pestis* sample from a person who died in London that Krause's team sequenced in 2011. The Kara-Djigach strain was also an ancestor of the vast majority of *Y. pestis* lineages around today — a sign, Krause says, of an explosion in *Y. pestis* diversity shortly before the Black Death. "It was like a big bang of plague," he said at the press briefing.

Other evidence also puts the origins of the Black Death in this part of Central Asia. Among

An engraved tombstone of a person who died from the Black Death, from the Kara-Djigach cemetery in what is now Kyrgyzstan.

P.-G. BORBONE, M. A. SPYROU ET AL./NATURE

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