Watching Evolution Unfold

" In looking for the gradations by which an organ in any species has been perfected, we ought to look exclusively to its lineal ancestors; but this is scarcely ever possible, and we are forced in each case to look to species of the same group, that is to the collateral descendants from the same original parent-form."
—Charles Darwin, *On the Origin of Species*, 1859

Richard Lenski has a front-row seat in the arena of evolution.

Back in 1988, he put 12 genetically identical strains of the bacteria *E. coli* in 12 flasks. He and his students then kept the bacteria on a glucose diet while the separate populations reproduced at a rate of more than six generations per day. Every 500 generations, they captured samples from each population and froze them for later comparison and experimentation.

Now, more than 54,000 generations later, Lenski's experiment encompasses the most generations ever examined in experimental detail. Armed with modern sequencing technologies and the vast stores of data contained in 23 years of frozen samples, he and his collaborators are learning a great deal about long-term evolutionary processes that in other species would take millennia to unfold.

Lenski, a member of SFI's Science Board, is the Hannah Distinguished Professor of Microbial Ecology at Michigan State University.

"My work uses *E. coli*, but it's not primarily about

E. coli," he says. "It's using *E. coli* in a very simple, artificial world to ask general, abstract questions about evolution, and explore the roles of chance and contingency."

It's the sort of experiment Charles Darwin might never have imagined as he sketched plants and animals in his notebooks, inferring their evolutionary histories from their modern characteristics. But, by expanding the boundaries of human perception, much as the telescope or radiography have, this experiment could serve as empirical high beams for modern evolutionary theory.

punctuated by periods of rapid mutation. By generation 20,000 in one population, for example, the team had found 45 mutations. At generation 26,000, a mutation affecting the bacterium's DNA metabolism arose, upsetting a relatively constant rate of genetic change and sparking a flurry of new mutations. By generation 40,000, some 653 mutations had occurred.

The team also found that a population of a given generation is in many respects more similar to the other independent lineages than to its own ancestors. For example, the levels of gene expression

for 54,000 Generations— and Counting

BY JOHN GERMAN

Lenski's *E. coli* strains are now distinct, each possessing unique traits that have resulted from many iterations that introduced both mutations—one of the random processes in evolution—and adaptation to their environment—a result of natural selection. By sequencing the genomes of generations of the bacteria, the researchers have been able to quantify rates of change and genetic differences among the populations.

They have gained important insights. Early changes in the bacteria, for example, appeared to be largely adaptive as the strains improved their fitness, and those early adaptations tended to progress in step-like sweeps of beneficial mutations. But adaptation-driven changes tended to slow down as the populations approached peak fitness, and later evolutionary changes tended toward the random.

These adaptive slow-downs were sometimes

are strikingly similar for two strains that evolved separately—but in the same environment—for 20,000 generations. This suggests that overall, most of the genetic change in *E. coli* occurs as a result of selection and not by random drift.

In other words, if random drift were the dominant process, given enough time, the genomes and phenotypes from different lineages could be expected to diverge significantly. Instead, because different lineages evolve similarly, if not identically, there may be a common solution to the problems imposed by the glucose-limited environments in which all the populations have been living and evolving.

Still, adaptive and random genomic changes don't necessarily follow the same patterns. Even in a consistent environment, the interplay between adaptive and random is complex and can be counterintuitive, Lenski says. The researchers discovered, for example, that although most of the *E. coli* lineages continually adapted to the glucose diet, one population eventually figured out that the flasks contained citrate too, and evolved to

Left: Because they reproduce so rapidly, *Escherichia coli* bacteria, often found in animal intestines, offer a unique opportunity to study evolutionary processes.

take advantage of the citrate as well as glucose.

Fortunately, with frozen samples, the researchers can replay the tape of the *E. coli's* evolutionary history. "We can go back and see how evolution might play out differently if given another chance," Lenski says. When the researchers took a second look at the evolution of the citrate-using bacteria, they found nearly two dozen more cases in which the bacteria evolved to use citrate. They also found that this change did not come about in any one mutational step, but instead required a series of mutations. Earlier mutations having nothing to do with citrate were required to set the stage for the eventual evolution of the new function, he says.

In another set of experiments spun off from the main experiment, Lenski's team pitted strain against strain in a battle for flask dominance, with some surprising results. Over time, one strain had dominated all others in one of the

populations, as determined by accumulated mutations; the team dubbed this strain the "eventual winner." But they wanted to understand how it had achieved its victory, so they collected samples from the 500-generation freezer sample, and had the presumed winners and presumed losers compete against each other.

To their surprise, at the new 500-generation mark, the presumed losers had grown faster than the presumed winners, at a rate that would have driven the winners to extinction in 350 more generations. The presumed losers appeared, in other words, to be headed for victory. So what happened to change the outcome?

In a paper published in *Science* in March 2011, the team showed that the presumed losers (which a press release called the "hare" bacteria) had pulled ahead early with mutations that had given them a short-term advantage. But because of these early adaptations, the hares were not able to take advantage of later, more beneficial mutations. The ultimate winners (which the press release called the "tortoises"), on the other hand, enjoyed a large benefit from later mutations, allowing them to prevail.

Overall, says Lenski, the research shows that "mutations and their effects can't always be understood in isolation. With both the citrate users and the eventual winners, we showed that what had happened in earlier generations had unexpected and nonlinear effects in later generations."

More generally, Lenski emphasizes that "evolution in action"—that is, the fact that evolution is ongoing in the world around us—has many important implications and potential applications. "Evolutionary methods and concepts are used all the time to track the source of emerging pathogens and to understand the rise of resistance to antibiotics," he says, "and as human activities are changing the natural world in so many ways, we also need to ask how microbes and other organisms that perform key ecosystem services will respond." ◄

The study has utilized thousands of petri dishes. Here, Zachary Blount, a researcher on the project, contemplates the vastness of *E. Coli* reproduction.

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