Evolution in Action: a 50,000-Generation Salute to Charles Darwin

The evolutionary process can be directly observed by watching microbes as they change and adapt over time

Richard E. Lenski

Like cuneiform on clay tablets, the history of life itself is written in minerals and in code. The minerals are fossils of long-dead organisms, while the code is the language of DNA shared by all organisms, revealing the family tree of life.

What story could be more exciting than the history of life on Earth? As an evolutionary biologist, I'm lucky to live at a time when legendary predecessors and talented contemporaries have turned the pages of this grand story by prospecting fossils and decoding genomes. Thrilling as it has been so far, I want to keep turning the pages of time. I like to watch evolution as it happens.

The year 2009 was the 150th anniversary of Charles Darwin's world-changing book, *On the Origin of Species by Means of Natural Selection*. In the closing passage, he emphasized that evolution is an ongoing process: "There is grandeur in this view of life, with its several powers, having been originally breathed into a few forms or into one; and that, whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved."

But can anyone really observe evolution in action? Though Darwin realized that evolution was ongoing, he emphasized its gradual nature: "It may be said that natural selection is daily and hourly scrutinising, throughout the world, every variation, even the slightest; rejecting that which is bad, preserving and adding up all that is good . . . We see nothing of these slow changes in progress, until the hand of time has marked the long lapse of ages, and then so imperfect is our view into long past geological ages, that we only see that the forms of life are now different from what they formerly were." Darwin also recognized the power of humans to exert selection and cause evolution—he began *On the Origin of Species* by explaining how humans had domesticated animals and plants by breeding those with desirable features, in order to introduce his idea of natural selection—but even these changes he thought would be imperceptible ". . . unless actual measurements or careful drawings of the breeds in question had been made long ago, which might serve for comparison."

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The Dawn of Experimental Evolution

Despite Darwin's sense that evolution was too slow to be directly observed, one of his readers pressed ahead with the idea of watching evolution in action. The Rev. William Dallinger (1839–1909) was not only a Methodist minister, he was also skilled in the methods of microbiology. For several years, he grew protozoa in an incubator, gradually raising their temperature. The organisms he used to start the experiment struggled even at 73°F, while those at the end tolerated 158°F but were unable to grow at the initial temperature of 60°F. In 1878, Dallinger wrote to Darwin that his findings "palpably demonstrate your great doctrine," while Darwin replied that the experiments were "extremely curious" and "very remarkable."

Dallinger showed that it was possible to observe the process of evolution over a human timescale by studying fast-reproducing microbes. Others also saw the possibility of studying evolution in action. In 1892, Henri de Varigny published a book called *Experimental Evolution* in which he proposed long-term experiments that would outlast the lifetimes of the participating scientists. Beginning early in the 1900s, fruit flies in the genus *Drosophila* became widely used for genetics research, and experiments were performed that demonstrated the effects of natural selection and random genetic drift.

Bacteria had to wait in the wings for many years before they could star in evolution experiments. While the science of genetics took hold with the rediscovery of Gregor Mendel's experiments on pea plants, the experts were baffled by the question of heredity in bacteria. Microbiologists saw that bacteria could adapt to challenges, but they couldn't tell whether spontaneous mutants had been selected or, alternatively, whether the challenge had induced the cells to change themselves. In 1934, a microbiologist, I. M. Lewis, wrote that "The subject of bacterial variation and heredity has reached an almost hopeless state of confusion . . . There are many advocates of the Lamarckian mode of bacterial inheritance, while others hold to the view that it is essentially Darwinian." In 1942, Julian Huxley wrote a book entitled *Evolution: The Modern Synthesis* that excluded bacteria from that synthesis on the grounds that "They have no genes in the sense of accurately quantized portions of hereditary substance . . ."

This confusion cleared the next year with the publication of what is, to me, the single greatest experiment in the history of biology. Working as a team, a biologist, Salvador Luria, and a physicist-turned-biologist, Max Delbrück, employed subtle reasoning and an elegant design to demonstrate that certain mutations in *Escherichia coli* occurred *before* the selective challenge was imposed and hence could not have been induced *by* the challenge. In other words, mutations are random events that occur whether or not they prove to be useful, while selection provides the direction in evolution by retaining those mutations that are advantageous to their bearers and discarding others that are harmful.

Luria and Delbrück's paper launched a tidal wave of research that led to the discovery of DNA as the hereditary material and to cracking the genetic code, among other achievements. But it had little immediate impact on evolutionary research. The new molecular biologists pursued their reductionist methods, while evolutionary biologists, grounded in natural history, didn't want to study things they couldn't even see. These naturalists preferred beautiful butterflies and even homely fruitflies to *E. coli* that, after all, come from a rather uninviting habitat. It was also difficult to tell bacterial strains and species apart, and many evolutionary biologists were focused on using patterns of visible similarities and differences to unravel the relationships among organisms.

But the field of microbial evolution eventually awakened, and for several reasons. In the 1970s, Carl Woese used differences in DNA sequences to study the evolutionary relationships among bacteria and other microbes, revealing extraordinary diversity beneath their outwardly simple appearances. Meanwhile, pathogenic bacteria that had been successfully treated with antibiotics often evolved resistance to those drugs, while other microbes emerged as pathogens, sometimes by acquiring new capabilities. In time, a few visionaries realized that microorganisms could be used—just as the Rev. Dallinger had foreseen a century earlier—in experiments to test Darwin's ideas and, more generally, evolutionary theory as it had developed over that century.

A Personal Odyssey

I was drawn into this field as a postdoc in the early 1980s. I had done my doctoral research in zoology, studying insects in the mountains of North Carolina. Despite the pleasures of working outdoors, data collection was slow, heavy rains drowned my beetles in their pitfall traps, and it was difficult to imagine feasible experiments that would really test the scientific ideas that most excited me. As I pondered future directions, I remembered the beautiful experiment by Luria and Delbrück that I had encountered as an undergraduate. I recalled not only its elegance, but also the insight it provided into the tension between randomness and direction in evolution. Evolution is like a game that combines luck and skill, and perhaps bacteria could teach me some interesting new games.

Games that involve both luck and skill rarely play out the same way twice, and that uncertainty is part of their fascination. Using bacteria, I could watch replicate populations—all starting with the same ancestral strain, and all living in identical environments—to see just how similarly or differently they would evolve. So in 1988, I started an experiment with 12 populations of *E. coli* that I intended to keep going for at least 2,000 generations, maybe longer.

Today, these bacteria have been evolving in and adapting to their separate little worlds for over 50,000 generations. Each population lives in a flask containing 10 ml of a solution with the sugar glucose as the limiting resource. Each day, including weekends and holidays, someone in my group withdraws 0.1 ml from a culture and transfers that into 9.9 ml of fresh medium. The bacteria grow until the glucose is depleted, and then sit there until the same process is repeated the next day. Bacteria grow by binary fission—that is, a cell grows in size before dividing into two daughter cells—so that the 100-fold dilution and regrowth allows almost 7 doublings, or generations, per day. Although this might sound like an easy existence for the bacteria, there is fierce competition to get the glucose and grow faster than anyone else. And every day, 99% of each population is consigned to oblivion by the random draw of a drop into a pipette that determines which lucky cells will continue this small, but great, struggle for existence.

However, survival and oblivion aren't the only two fates for these bacteria. A third possibility—suspended animation—is one of the most important features of this experiment. Every so often, instead of discarding the leftover bacteria from the previous day, we add a cryoprotectant and store the cells in a freezer. The result is an extraordinary "fossil record" where we can revive the bacteria and compare living cells from different generations. We can even compete bacteria against their own ancestors from thousands of generations earlier. These competitions allow us to measure the improved adaptation of the bacteria to their flask-world that has resulted from natural selection—the process that Darwin realized would give rise to organisms that were well fit to their environment. Imagine Neanderthals brought back to live among us. How would they fare at chess or football? How far have we come in the nature of our genes and the nurture of our culture? When I switched my research to bacteria, their speedy reproduction was an obvious attraction for watching evolution in action. But I've come to realize that the ability to freeze and revivify the bacteria—to study true living fossils—is just as important to my research.

When I began this experiment, I thought big differences among the 12 lines would soon be apparent. The random occurrence of mutations meant that some populations would get lucky by generating a beneficial mutation (and one that survived the daily dilutions) sooner than others. And just as in a game, different early moves—mutations—might open some doors while closing others. Some populations might get stuck with beneficial mutations that ultimately led nowhere, while others would follow paths that had long-term potential.

To my surprise, evolution was pretty repeatable. All 12 populations improved quickly early on, then more slowly as the generations ticked by. Despite substantial fitness gains compared to the common ancestor, the performance of the evolved lines relative to each other hardly diverged. As we looked for other changes—and the "we" grew as outstanding students and collaborators put their brains and hands to work on this experiment—the generations flew by. We observed changes in the size and shape of the bacterial cells, in their food preferences, and in their genes. Although the lineages certainly diverged in many details, I was struck by the parallel trajectories of their evolution, with similar changes in so many phenotypic traits and even gene sequences that we examined.

At the same time, we pursued other evolution experiments with *E. coli*. We varied their diets and the temperatures of their flasks. We allowed some to have sex by introducing special genes that let cells conjugate and recombine their genomes. We branched out and studied another bacterial species, *Myxococcus xanthus*, that has fascinating behaviors in which cells cooperate to form multicellular fruiting bodies as well as to hunt down and consume our beloved *E. coli*. Social norms evolved, including the appearance of cheaters that benefit from belonging to a group but harm the group's overall performance. We saw the evolution of predators that swarmed outward and found patches of their prey more quickly than their ancestors.

But back to the main story: the generations marched by, thousands upon thousands. The rate of improvement progressively slowed as the *E. coli* became better adapted to their new world. My colleagues began to track down the mutations responsible for that adaptation. Those analyses required skills I lacked, as new technologies emerged that could be applied to our experiment. As for myself, I branched into a new line of research.

From Bacteria to Computers, and Back Again

Perhaps it was a midlife crisis: my bacteria were slowing down, and I was looking for some new action. So I had an affair— one that continues today, though with slightly less feverish intensity—with some artificial creatures. Avidians are computer programs that copy their own genomes, and they live in a virtual world that exists inside a computer. But their replication is imperfect, so Avidians sometimes mutate. While most mutations are deleterious, some provide an advantage that allows a mutant to obtain resources and replicate faster than its competitors in that virtual world. Because they start in a primitive state—the ancestral Avidian was written by a computer scientist who endowed it with the capacity to replicate, but gave it no other function—we could watch not just subtle improvements but the emergence of brand-new capabilities as they evolved a rich computational metabolism. However, it seems the *E. coli* became jealous of the attention I lavished on the Avidians, because one lineage decided to show me that it could do something new, too.

The long-term experiment was designed to be a simple one in which time and the bacteria would do the work of evolution. In that spirit, I had chosen to propagate the lines in an environment with just one sugar, a constant temperature, no predators—in essence, just about as simple as I could make it. So while there were many opportunities for improvement, it couldn't be expected that the bacteria would learn some entirely new trick. What would be the point? The bacteria were becoming specialists—*Escherichia erlenmeyeri*, I sometimes call them— exquisitely adapted to a simple life in an Erlenmeyer flask, as opposed to the complicated world of the colon they had left behind.

However, I had left them an opening. Although glucose is the only sugar in their environment, another source of energy, a compound called citrate, was also there all along as part of an old microbiological recipe. One of the defining features of *E. coli* as a species is that it can't grow on citrate because it's unable to transport citrate into the cell. For 15 years, billions of mutations were tested in every population, but none produced a cell that could exploit this opening. It was as though the bacteria ate dinner and went straight to bed, without realizing a dessert was there waiting for them.

But in 2003, a mutant tasted the forbidden fruit. And it was good, very good. The descendants of that mutant rose to dominance owing to their access to that second course. At first, I thought this flask had been contaminated by some other species that consumed citrate. However, DNA tests showed the citrate-eating cells were descendants of the *E. coli* ancestor used to start the experiment.

The citrate-eaters still eat glucose, but they aren't quite as successful at competing for that sugar as they were before. As a consequence of that tradeoff, their cousins persist as glucose specialists. So the bacteria in this simple flask-world have split into two lineages that coexist by exploiting their common environment in different ways. And one of the lineages makes its living by doing something brand-new, something that its ancestor could not do.

That sounds a lot like the origin of species to me. What do you think?

Happy anniversary, Mr. Darwin! One hundred and fifty years after you revealed your ideas to the world, evolution continues to fascinate. And remarkably, we can now observe "these slow changes in progress" even before "the hand of time has marked the long lapse of ages."

SUGGESTED READING

Electronic Version of Darwin's *On the Origin of Species* (First Edition). http://www.gutenberg.org/ebooks/1228
Project Site for Lenski's Long-Term Evolution Experiment with *E. coli.* http://myxo.css.msu.edu/ecoli/
Project Site for the Avida System for Computational Evolution. http://devolab.msu.edu/