Darwin Under the Microscope: Witnessing Evolution in Microbes

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Charles Darwin spent his life elbow-deep in the flesh and sinew of nature. As a boy, he hunted for beetles and wandered among tide pools. At age 21, he boarded HMS *Beagle* and spent the next five years traveling around the world and closely observing the animals and plants along the way. He contemplated the crowded wealth of species in the jungles of Brazil. In Argentina, he dug up fossils of giant rodents. On the Galápagos Islands, he collected birds and tortoises. In the Indian Ocean, he mapped coral reefs. He brought home box upon box of specimens, which took him years to unpack and to describe.

Not long after his return to England, Darwin began sketching out his theory of evolution by means of natural selection. It was a bold new account of how nature's diversity came to be. But Darwin recognized that he needed to find new life to study in order to develop his ideas into a mature theory. At the core of that theory was natural selection. Darwin believed that natural selection occurred because variations emerged in every generation. Some variants had more offspring than others, and they passed down their traits to subsequent generations. At the time, most naturalists thought that every member of a species was pretty much identical to one another. So Darwin had to gather evidence that this was not so. He laid rabbit bones out on his billiard table and compared their lengths, documenting the range of sizes they came in. Darwin was particularly struck by the variations in barnacles, crustaceans that anchor themselves to rocks, boat hulls, and dock pilings. He borrowed collections of barnacles from other naturalists and spent years peering at them under a microscope. He published a two-volume monograph on the creatures, in which he showed how barnacles varied so much in their size and shape that it was often impossible to draw a clear line between barnacle species.

Darwin knew that skeptics would wonder just how powerful natural selection could be in shaping life. But he knew that humans had carried out a similar kind of selection when they picked out individual animals and plants to breed. Such artificial selection had brought about stunning changes in just a matter of centuries. Darwin drank gin with pigeon breeders and listened to them as they described how they went about selecting individual birds to breed. Artificial

ROBERTS & COMPANY PUBLISHERS

This essay is from ***In the Light of Evolution: Essays from the Laboratory and Field*** edited by Jonathan Losos (Roberts and Company Publishers). The book will publish in December, 2010. ISBN # 9780981519494. Other contributors include: James Curtsinger, Ted Daeschler, Douglas Emlen, Harry Greene, Luke Harmon, Daniel Lieberman, Jonathan Losos, Axel Meyer, Teri J. Orr, Naomi Pierce, Andrew Berry, David C. Queller, Neil Shubin, David Reznick, Michael Ryan, Marlene Zuk, and Carl Zimmer. Includes a foreword by David Quammen.
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selection, Darwin observed, could turn a plain rock pigeon into extravagant forms over the course of generations.

If life had indeed evolved, as Darwin believed it did, the history of that evolution ought to be recorded in the traits that were shared by different species—traits that had been inherited from their common ancestors. Darwin became familiar with the research of embryologists, who were finding striking similarities in the embryos of animals that looked very different as adults—animals as varied as fish, birds, and people. People carried many of the clearest marks of evolution, Darwin observed, as shocking as that might be. Darwin was especially struck by how many similarities he could find in the behavior of humans and apes. He was so struck, in fact, that he climbed into a cage at the London Zoo so that he could observe more closely the grins and pouts of an orangutan named Jenny.

In 1859 Darwin, published *On the Origin of Species*, in which he presented his theory of evolution. It is packed tight with details about animals and plants, but Darwin did not have room for more than a small fraction of his observations. Darwin lived for another 24 years, during which time he continued to discover new species to study. He raised orchids to learn how they had evolved their beautiful, elaborate flowers. Far from having been created to please the eye of man, Darwin discovered, orchids had evolved ingenious ways of sticking pollen onto visiting insects so that the animals could fertilize other orchids. He also kept carnivorous plants so that he could learn how they could devour animals, trapping them in sticky tentacles. In the spring of 1883, a paper was read to the Linnean Society in London describing Darwin's latest project: he and his son Francis were drawing pictures of cells in carnivorous sundew plants, trying to identify the molecular changes that took place as the plants trapped their prey. A few weeks later, Darwin was dead.

Darwin had seen evidence for evolution in all of the animals and plants he studied, but he never believed that anyone could see natural selection take place in his own lifetime. He summed up his view in *On the Origin of Species*: "We see nothing of these slow changes in progress, until the hand of time has marked the long lapses of ages, and then so imperfect is our view into the long past geological ages that we only see that the forms of life are now different from what they were."

Biologists now know that this is not always true. Natural selection can happen quickly enough, in some cases, for it to be documented over a matter of years. In fact, biologists can now carry out experiments in evolution, testing out different hypotheses about how natural selection works, over the course of a few months. And some of the most compelling results come from research on a kind of life that Darwin did not study: microbes.

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Ironically, the first experiment in microbe evolution took place during Darwin's own lifetime. In fact, Darwin even knew about it. In 1878, he received the details of the experiment in a letter from a Liverpool minister and amateur scientist named William Dallinger.

Dallinger had realized something simple and yet profound: while animals and plants might be poorly suited to evolution experiments, microbes might make such an experiment possible. For one thing, microbes are tiny. A single glass beaker can hold billions of them. In such a big population, there's a huge amount of variation upon which natural selection can work. Another advantage of microbes is that they can reproduce much faster than animals and plants. A thousand generations of humans may span 20,000 years or more, but a thousand generations of bacteria may span only a few weeks.

In his letter, Dallinger described to Darwin how he had designed a special copper vessel for his experiment. He filled it with water and added water-dwelling microbes called flagellates. Over the course of months, Dallinger slowly raised the temperature of the water. He was curious to know if the flagellates might be able to adapt to the warming water through natural selection, as heat-resistant microbes reproduced more than heat-sensitive ones. Over the course of months, he raised the water to 150 degrees Fahrenheit. That much heat was lethal to ordinary flagellates, but Dallinger found that the flagellates in his vessel continued to reproduce.

Dallinger concluded that the flagellates had indeed evolved resistance to heat. As they did, he wrote to Darwin, they also changed in other ways. In the process of adapting to their warmer environment, they lost some of their adaptations for surviving at cooler temperatures. Dallinger put some of the evolved flagellates into lukewarm water, whereupon they died.

Darwin was thrilled to learn of Dallinger's experiment. "Your results, I have no doubt, will be extremely curious and valuable," he wrote to Dallinger.

Yet experimental evolution did not immediately bloom into a new kind of science. Judging from his writing, Darwin didn't fully appreciate how important Dallinger's experiment was to Darwin's own theory. Other scientists also praised Dallinger's experiment, but none of them bothered to run an evolutionary experiment of their own. Dallinger tried to keep his experiment running, but, in 1886, his vessel was destroyed in an accident. Perhaps dispirited by the cool reception to his work, Dallinger never rebuilt it. For decades, no one followed up on his work. In retrospect, it's clear that Dallinger was just too far ahead of his time.

In Dallinger's day, for example, no one knew about DNA. It would be more than 20 years before the word gene would be coined. Without a clear understanding of how genes make heredity possible, Dallinger had no way to know for sure that natural selection was driving the adaptation of his microbes. It was

possible that the microbes were just responding to their experience, the way a bodybuilder develops bigger muscles and stronger bones by lifting weights.

Today, Dallinger is far from a household name, but he is revered in certain laboratories scattered around the globe. In those labs, scientists are finally making Dallinger's dream a reality. For the past twenty years or so, they have been running experiments on microbes to test hypotheses about the workings of evolution. Those experiments now shed light on the molecular changes that take place as organisms adapt to new challenges. They reveal how natural selection can alter behavior and even the social relationships among microbes. Those changes happen, as Dallinger had hoped, over a matter of weeks or months. And in some cases, it now appears, scientists can even observe the origin of a new species in their own laboratories.

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On February 15, 1988, Richard Lenski, a biologist now at Michigan State University, set up one of the earliest of these experiments. It's still running today. Lenski started his experiment with a single microbe. It belonged to the species *Escherichia coli*, which lives harmlessly in our guts. Lenski chose to study *E. coli* because it had emerged as the best-understood microbe known to science. It also had the advantage of growing quickly in laboratories on a diet of sugar. A single *E. coli* can produce billions of descendants in a single day.

Instead of challenging bacteria with heat, Lenski decided to challenge them with cycles of feasts and famines. He put the bacteria in a flask, which he kept in an incubator at body temperature. The bacteria floated in a standard laboratory broth, to which Lenski added some glucose for them to eat. The bacteria devoured the glucose in a few hours, but then had to survive without anything to eat until the following morning. Lenski and his colleagues would draw a little of the liquid from each flask and squirt it into a fresh batch of broth, where the bacteria could feast again.

Lenski wanted to see if the bacteria would be altered by natural selection. In each generation, some of the bacteria would mutate. A few of those mutations might make them grow and reproduce faster in the flask, and they would then outcompete the other bacteria. Over time, natural selection might transform the bacteria in measurable ways. A single run of the experiment would not tell Lenski much. The results might be a fluke of random mutations. So he separated the bacteria into 12 identical lines, each of which lived in a flask of its own. If evolution were at all repeatable, he hoped to get similar results in many of the flasks.

Lenski also realized that he could preserve the history of this evolution. That's because microbes can be frozen without killing them. They can sit in a freezer for years—even decades—until a biologist decides to take another look

at them. Once the bacteria thaw out, they come back to life, feeding again, growing, and reproducing. So Lenski and his students began freezing some of the *E. coli* from all 12 lines in his experiment every 500 generations. The frozen bacteria sit in coffin-sized freezers labeled with the names of mythical resting places of great heroes, such as Valhalla and Avalon, along with a motto: WHEN NEEDED THEY SHALL REVIVE.

These bacteria are, as Lenski puts it, a frozen fossil record. This frozen fossil record lets Lenski measure evolutionary change far more precisely than was possible in earlier studies. He and his students can, for example, thaw out the bacteria from early in the history of a line and then put them in a Petri dish with their descendants. The scientists can then observe how fast the two populations of bacteria grow under identical conditions. They can also sequence the DNA of bacteria at each stage of the experiment to pinpoint the mutations that were favored by natural selection.

By the early 1990s, Lenski had clear evidence that the bacteria had evolved. They were growing faster than their ancestors—and it wasn't just one line that was growing faster, but all 12. The longer Lenski let the experiment run, the more they evolved, and the more questions occurred to him that he could answer with the bacteria. So he kept moving the bacteria to new flasks every day, kept building up his frozen fossil record. Today, after 50,000 generations, the bacteria now grow more than 75% faster than they did at the beginning of the experiment. The rate at which they improve has slowed down, but they are still getting better. The bacteria in all 12 lines have also become roughly twice as big as their ancestors, probably as a side effect of mutations that made them better able to survive their daily famines. Lenski discovered more parallel evolution when he began to zero in on some of the mutations that had arisen in the bacteria. He and his colleagues found a few key genes that had mutated in just about all of the lines.

But the similarity was not perfect. Each line had different mutations to the same genes, for example. Another difference came to light when Lenski and his colleagues gave the evolved *E. coli* a new challenge. They switched the bacteria's diet from glucose to a different sugar, known as maltose. If the bacteria had evolved down an identical path, all of them should have fared about as well on their new diet. But the experiment turned out very differently. Some of the lines of *E. coli* could not feed on maltose, and they starved. Meanwhile, other lines thrived on their new food.

Lenski's research has inspired many other researchers to run similar experiments. As time has passed, they've developed more powerful methods for exploring how the microbes evolve, and they can get new answers to the questions Lenski raised. For example, why is it that bacteria evolve such a puzzling mix of similarities and differences? Bernhard Palsson and his colleagues at the

University of California at San Diego fed *E. coli* glycerol, a sweet-tasting alcohol that is used in soap and face creams. Normal *E. coli* do a bad job of growing on glycerol, but, after 44 days of Palsson's experiment, the bacteria were growing twice as fast as their ancestors. Palsson sequenced the entire genomes of some of the bacteria, starting with the original ancestor, and ending with the final generation of the experiment. He and his colleagues were able to pinpoint all the genes that had mutated in each line.

To determine the effect of each mutation, Palsson and his colleagues inserted copies of the mutated versions of the genes, one at a time, into the ancestral bacteria. In some cases, these evolved genes immediately allowed the bacteria to start growing faster on glycerol. But the order in which Palsson inserted the genes made a big difference on the effect that each gene had. Some of the genes, for example, could speed up the growth of *E. coli* only if Palsson had already inserted some of the other evolved genes. In fact, on their own, some of those genes were actually harmful, slowing the bacteria down.

Experiments such as these illuminate a crucial element of evolution, one that Darwin could not appreciate. Genes work together in an organism, and so the effect of a mutation on any one gene depends on the makeup of the other genes. This principle, called epistasis, can help explain why some of Lenski's bacteria could thrive on maltose and some failed. They all faced the same challenge to their survival, but the pattern of the random mutations that arose in each line was different. Once a particular beneficial mutation arose and spread throughout one of Lenski's lines of bacteria, it changed the effects that future mutations would have on the bacteria. As a result, each line accumulated some different mutations, even as they all adapted to a diet of glucose. In some lines, the unique combination of mutations they accumulated allowed them to thrive on maltose. In other lines, however, their glucose-adapted genes left them unable to feed on a different sugar.

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The survival of any organism depends on more than its ability to find food or to withstand heat. Any organism must overcome other threats as well. One of the most important is the onslaught of parasites. We humans are regularly attacked by viruses, bacteria, protozoans, fungi, and even parasitic worms. Microbes suffer infections of their own. Our guts are not just teeming with microbes such as *E. coli* (we each carry an estimated 10 trillion microbes in our bodies), but also with the viruses that infect our resident microbes. Viruses are often lethal to microbes, hijacking their biochemistry to build new viruses, which then burst out of the cell, leaving it to die.

Mathematical models of evolution suggest that parasites and their hosts should speed up each other's evolution. Parasites that do a better job of infecting hosts should make more copies of themselves. Hosts that acquire mutations

that defend them against parasites should be more likely to survive and to pass on those mutations. A number of scientists have tested this hypothesis by infecting bacteria and observing their evolution. As predicted, resistant bacteria evolve rapidly, and the viruses then evolve new strategies for overcoming those defenses.

But these experiments do not just end up producing a single strain of super-resistant bacteria. They actually give rise to many different strains that can coexist with each other for a long time. There is more than one way to defend against viruses, and if the viruses in an experiment evolve to get around one kind of defense, the strains with other kinds of defenses will flourish. This result sheds light on one of the questions that consumed Darwin throughout his life: How does evolution produce the diversity of life?

Other experiments on bacteria reveal that parasites are just one of many forces that spur the evolution of diversity. In the early 1990s, Julian Adams, a microbiologist at the University of Michigan, used a single microbe to found a colony. Adams and his colleagues supplied the bacteria with a low supply of glucose. Unlike Lenski, he replenished their sugar so that they never faced outright starvation. The bacteria began to evolve, adapting to the new conditions. But, to Adams's surprise, natural selection did not favor a single strategy. When he put the bacteria in Petri dishes, they grew into two types of colonies. Some formed big splotches, and others formed small ones.

Adams thought he might have contaminated his original colony with another strain, and so he shut down the experiments and started all over again. After the new colony had adapted to the low-glucose diet, Adams spread the microbes on more Petri dishes again. Once again, he discovered that some of the bacteria made big splotches and others made small splotches. Adams ran the experiment a few more times, and he found that it took about 200 generations for the two types of microbes to emerge. He realized that a single clone was evolving time and again into two distinct types of *E. coli*.

Those two types turn out to be ecological partners. The large colonies are inhabited by microbes that do a better job than their ancestors of feeding on glucose. One of the waste products they give off is acetate. *E. coli* can survive on acetate, although it grows more slowly on acetate than it does on glucose. Adams discovered that some of his *E. coli* evolved into acetate-feeders. They become more efficient at feeding on acetate than their ancestors. Instead of competing for the glucose, they turned the waste of glucose-feeders into their own food. The acetate-feeders grew slowly, but they weren't driven to extinction because they were taking advantage of a food that the faster-growing bacteria weren't eating. A food chain had emerged spontaneously in Adams's lab, as organisms began to depend on each other for survival.

Other scientists have confirmed Adams's results with experiments of their own, and they've created new kinds of ecological diversity from a single *E. coli* ancestor. Instead of a glucose-only diet, Michael Doebeli and his colleagues at the University of British Columbia supplied *E. coli* both with glucose and with acetate. After 1,000 generations, Doebeli found that the bacteria had evolved into big and small colonies, but the colonies were different from the big and small colonies that Adams had produced. Both types of colonies in Doebeli's experiment fed on glucose and acetate. The difference between them was a matter of timing. The big colonies fed on glucose until it ran out, whereupon they started to feed on acetate. The small colonies, on the other hand, switched over to acetate sooner, before the glucose ran out.

Doebeli and his colleagues then looked closely at how the genes in each colony had evolved. Typically, when *E. coli* is feeding on glucose, it keeps the genes for digesting acetate tightly repressed. If it made both sets of enzymes at the same time, they would get snared in a metabolic traffic jam. When the time comes to switch to acetate, the bacteria must first destroy the enzymes for glucose.

In the small colonies, Doebeli found, the bacteria had evolved so that they no longer repressed their acetate genes. Now they made enzymes for both molecules. Because their enzymes interfered with each other, the bacteria grew slowly on glucose, and thus produced small colonies. The big colonies contained bacteria that continued to feed only on glucose at first, and then slowly switched over to acetate after the glucose ran out. The small colonies were nimbler, able to feed on the acetate while the big colonies slowly retooled their metabolism.

The environment itself can also drive the evolution of diversity. Paul Rainey, a biologist now at the New Zealand Institute for Advanced Study at Massey University, discovered this complexity when he started to experiment on a species of bacteria that live on plants, called *Pseudomonas fluorescens*. Rainey put a single *P. fluorescens* in a flask of nutrient-rich broth, He put the flask in a device that constantly shook it in order to mix oxygen continually throughout the liquid. After a few days, Rainey found that all of the bacteria in the flask were identical. But then he put a single *P. fluorescens* in a flask and didn't shake it. The bacteria multiplied and quickly consumed the oxygen in most of the liquid. But the top layer of the liquid still had high levels of oxygen, because it was in contact with the air. Under these conditions, the bacteria diversified.

One strain of *P. fluorescens* specialized in living on the top of the liquid. It evolved the ability to make cellulose, which clumped together to form a floating raft. The bacteria that lived on these rafts could grow quickly with the oxygen from the air and the food in the liquid. Meanwhile, other forms of *P. fluorescens* evolved a lower depths. They established themselves in narrow layers of the liquid, including one strain that formed fuzzy carpets of cells at the bottom.

This diversity evolves every time Rainey seeds a new flask with the bacteria, but it's a dynamic diversity that changes over the course of the experiment. As the raft-builders grow on top of the liquid, they seal off the rest of the liquid, so that the bacteria underneath get even less oxygen and grow even more slowly than before. But the raft-builders then destroy themselves, thanks to a remarkable kind of evolution: some of the bacteria evolve into cheaters.

It takes energy for a *P. fluorescens* to make cellulose, energy that it can't use to grow. If a *P. fluorescens* mutates so that it stops making cellulose, it can use that extra energy to grow, while enjoying the raft built by its fellow bacteria. A cheater has a big evolutionary advantage over the raft-builders, and, over time, they make up a bigger and bigger fraction of the population. Eventually, there are so many cheaters and so few cellulose-producers that the whole raft collapses and sinks down into the flask. Without a seal covering the top of the liquid, oxygen can mix down into the lower depths of the flask, spurring the growth of the other strains.

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Darwin himself had given thought to the evolution of cheaters. He observed that, among humans and some animal species, individuals were often willing to cooperate and even to sacrifice their own self-interest for the sake of others. Darwin speculated that altruistic behaviors could evolve if the actions of an altruistic individual benefited family members. In the 1960s, the British biologist William Hamilton recast Darwin's argument in terms of genes: relatives are genetically similar, and so the cost of altruism may be smaller than the benefit that comes to relatives who carry the same genes.

It would probably have surprised Darwin greatly to find that scientists are now probing the evolution of social behavior with, of all things, microbes. It turns out that microbes are intensely social creatures, communicating with each other, making collective decisions, and cooperating for the greater good. Of course, what's good for one tribe of humans may not be good for another tribe they conquer; and what's good for a group of bacteria may not be so good for their victims.

In many cases, the victims are us. For example, another species of *Pseudomonas*, known as *Pseudomonas aeruginosa*, infects our lungs. In order to grow, the bacteria need iron, which is hard to find in a usable form in our bodies. To overcome the shortage, *P. aeruginosa* releases special molecules called siderophores that can snatch up iron compounds and make them palatable to the microbe. It takes a lot of energy for the bacteria to make siderophores, and they aren't guaranteed a return on their investment. Once a siderophore harvests some iron, any *P. aeruginosa* that happens to be near it can gulp it down.

Siderophores offer a potent opportunity for the evolution of cheaters. A *P. aeruginosa* that doesn't make siderophores can still get the iron it needs to grow, thanks to its generous fellow bacteria; and it can use the extra energy it saves by not making siderophores to grow and reproduce. These cheaters are not just theoretical possibilities: doctors find them all the time when they take samples from the lungs of people infected with *P. aeruginosa*. And yet, despite their advantages, the cheaters never come to dominate populations of *P. aeruginosa*.

Stuart West and his colleagues at the University of Edinburgh speculated that the bacteria continue to cooperate because closely related microbes share the same genes. If a relative scoops up the iron and can reproduce, that's all the same for your genes.

To test this hypothesis, the Edinburgh team ran an experiment. They filled 12 beakers with bacteria they produced from a single clone. While the bacteria were all closely related, half were cheaters and half were do-gooders. The team let the bacteria feed, multiply, and compete with one another. Then they combined all 12 beakers so that all of the bacteria mixed together. From that mix, the scientists took up a few drops and transferred them to 12 fresh beakers. Over time, the cheaters became rarer and rarer, while the do-gooders became more and more common. Eventually, the siderophore-producing bacteria made up nearly 100% of the bacteria.

West and his colleagues then ran the same experiment with a twist: instead of using bacteria that descended from one *P. aeruginosa*, they mixed bacteria descended from two different *P. aeruginosa* into each beaker. Now the do-gooders of one strain were not just helping out their close relatives in the beaker, but also strangers. The benefits of making siderophores were smaller in this experiment, and, as a result, the do-gooders did not take over the population. Instead, the cheaters became more common.

The altruism of bacteria goes far beyond helping each other find food. In the case of *Myxococcus xanthus*, a soil-dwelling species, microbes will sacrifice their own lives for their fellow microbe. *M. xanthus* are predators, hunting in packs for smaller bacteria. If they search for too long without finding any prey, they take a dramatic step so that they don't all die of starvation. They make the collective decision to come together to form a mound. A small number of the bacteria in the mound undergo a life-saving transformation. They become spores, covering themselves in a tough coat and shutting down their metabolism so that they go into a kind of suspended animation. The *M. xanthus* spores can be carried away by wind or water, and they can survive long enough to find a better home elsewhere. The bacteria left behind in the mound face an almost certain death from lack of food.

Biologists don't yet fully understand why certain *M. xanthus* in a mound become destined to form spores. It appears to be the random luck of the draw.

Biologists also don't know much about how a free-living *M. xanthus* turns itself into a spore, although it's clear that they can do so only inside a mound. In other words, the only way for some of the bacteria to survive as spores is for most of them to form a mound and then die.

Gregory Velicer, a former student of Lenski's who now teaches at Indiana University, wondered if the altruism of *M. xanthus* might change. He placed the bacteria in a rich broth, where they would never run out of food and thus never need to form mounds and spores. Mutations that might make them less altruistic might no longer harm their long-term survival. Velicer let 12 lines of bacteria live for 1,000 generations in their flasks and then took them out of their comfortable environment. He found that most of the lines of bacteria lost the ability to swarm or to form spores, or both.

Surprisngly, some of the newly evolved bacteria were not just asocial—they were positively antisocial. Velicer found that, if he starved a population made up only of cheaters, they could not form mounds. However, if he mixed some cheaters in with ordinary *M. xanthus*, the cheaters could join mounds. When Velicer looked at the spores produced by these mounds, he was surprised to find that the cheaters were far more common than you would expect if the spores were randomly selected from the bacteria in the mound. Somehow, the cheaters had found a way to exploit the spore-selection process so that they were 10 times more likely to form a spore as a normal *M. xanthus*. It was as if the crew of a sinking ship were drawing straws for spaces on a lifeboat, and a few of them figured out how to make sure they didn't draw a short straw.

Velicer wondered what would happen to a mixed population of cooperators and cheaters if they passed through several rounds of mound forming. Since the cheaters would be overrepresented among the spores, they might gradually become more common, while the cooperators might become rarer. Velicer set up a new experiment in which *M. xanthus* alternated between a rich broth and a dish with no food. As he had predicted, the cheaters became more common. In fact, if they became too common, an entire population could get wiped out, because there were no longer enough cooperating *M. xanthus* left to make the mounds during famines.

Like many researchers who carry out evolution experiments, Velicer has also had his share of surprises. As he and his colleagues were studying the evolution of cheating in *M. xanthus*, they discovered that a strain of cheaters had given rise to a cooperator that could form mounds on its own again. Velicer and his colleagues sequenced the genome of the new cooperator and discovered a single mutation. The new mutation did not simply reverse the mutation that had originally turned the microbe's ancestors into cheaters. Instead, the mutation struck a new gene. Velicer and his colleagues discovered that normally this gene, called Pxr, prevents *M. xanthus* from forming a mound when lots of

food is present. No one knew what Pxr did before. It took evolution to reveal its importance to scientists.

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Experiments such as these are transforming bacteria in some extraordinary ways. In at least one case, Lenski and his colleagues may have actually observed the evolution of a new species.

It came entirely by accident. One day in 2003 Lenski's lab manager, Neerja Hajela was performing the morning ritual of drawing a few drops of *E. coli*-laced liquid from each flask and adding them to a new flask. She noticed something odd: one flask was cloudy with a dense bloom of bacteria. At first, she assumed that the flask had been contaminated by a different species of bacteria that could grow faster in the liquid than *E. coli*. Therefore, she threw out the cloudy liquid and thawed out some of the most recently frozen bacteria from that line.Within a couple weeks, the same line had turned cloudy again. That couldn't be a coincidence, Hajela decided.

Lenski also thought it was a false alarm at first, but when the clouds returned to the flask, he enlisted his postdoctoral researcher Christina Borland and, later, his graduate student Zachary Blount to figure out what was going on. Borland quickly determined that there was no contamination. The bacteria exploding in the flask were the descendants of the original strain of *E. coli*—and yet they were also a new kind of *E. coli*, one that was doing something *E. coli* is not supposed to do.

The broth Lenski uses to rear the bacteria is a standard recipe that microbiologists developed decades ago to let bacteria thrive in labs. *E. coli* needs trace amounts of iron to survive, for example, but it can't draw in free iron atoms. The broth contains a molecule called citrate (the compound that makes lemons tart), which can bind iron, and, in that form, *E. coli* can absorb it. The citrate doesn't enter the microbe, however.

Blount discovered that the bacteria were now taking in the citrate and eating it. They were drawing energy from the bonds between its atoms and using some of the atoms to build new molecules. One of the hallmarks of *E. coli* as a species is being unable to eat citrate when oxygen is present. The "citrate-eaters" no longer had to starve when their supply of glucose ran out. Now they had a big dessert tray.

Blount returned to the frozen fossil record to figure out when the citrate-eaters first emerged. The first bacteria with any ability to eat citrate appeared after 31,000 generations but before 31,500 generations. Over the next 2,000 generations, they acquired new mutations that vastly improved their ability to exploit citrate, leading to their population boom.

The citrate-eaters offer some clues to how new species evolve. A new species needs a new ecological niche to occupy so that it won't be outcompeted into extinction. In the case of *E. coli*, the citrate in the broth was a niche just waiting to be taken over. At first, the bacteria did a poor job at feeding on citrate, but they survived because they had no competition. Then, as they acquired more mutations, they became better at their new way of life.

Lenski and Blount predict that the mutations that are making them better at eating citrate won't benefit the other lines of *E. coli* that are still depending on glucose. It's a prediction they can actually test. Blount plans to insert the citrate feeding mutations into the glucose-feeding bacteria to see if they grow more slowly as a result. If that proves to be true, Lenski may be finally ready to treat the citrate-eaters as a new species. He might call them *Escherichia blountii*, or maybe *Escherichia gouldii*, after the late paleontologist Stephen Jay Gould.

Gould wrote often about the contingency of evolution, and how random events steered its history in unexpected directions. The evolution of the citrate-eaters does seem to feature some major flukes. Blount tried to replicate the evolution of citrate-eaters, using their own ancestors from different points in the frozen fossil record, as well as other lines of *E. coli*. It was a massive project, demanding that Blount smear trillions of *E. coli* across thousands of Petri dishes.He found that, after about generation 20,000, some samples of ancestors of the citrate-eaters could evolve the ability to digest citrate. But none of the other 11 lines could. In that one lineage, it seems, some mysterious mutation opened the door to the evolution of a new way of life.

Experimental evolution promises to reveal deeper secrets in years to come, thanks in large part to the fact that it's getting so cheap to sequence DNA. When Lenski started his experiment, sequencing even a single gene would have taken years. Now it costs a few hundred dollars to sequence all 4 million letters in the genome of a single *E. coli*, and that cost is going to continue to crash.

Uncovering the molecular basis of new adaptations may lead to some important practical benefits. Scientists can engineer microbes to produce drugs and other valuable molecules. People with diabetes, for instance, cannot make their own insulin, and so they have to inject insulin into their blood. Before 1980, the insulin diabetics used came from the harvesting of pig pancreases. But then scientists figured out how to insert the human insulin gene into E. coli, which then began to churn out the molecule in huge supply. Today, most diabetics get their insulin from*E. coli*.

Genetic engineers are now tinkering with microbes to find ways to produce new compounds, ranging from jet fuel to drugs to treat malaria. But it still takes a long time to make these new discoveries, because scientists still don't know very much about the function of each gene in a microbe. Observing microbes evolving can reveal some of the important functions of genes.When Palsson

allowed *E. coli* to adapt to feed on glycerol, the genes that evolved had never been known before to be involved in the task. Genetic engineers can thus make evolution their guide. As Darwin predicted, Dallinger's experiments are indeed proving extremely curious and valuable.

SUGGESTED READINGS

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