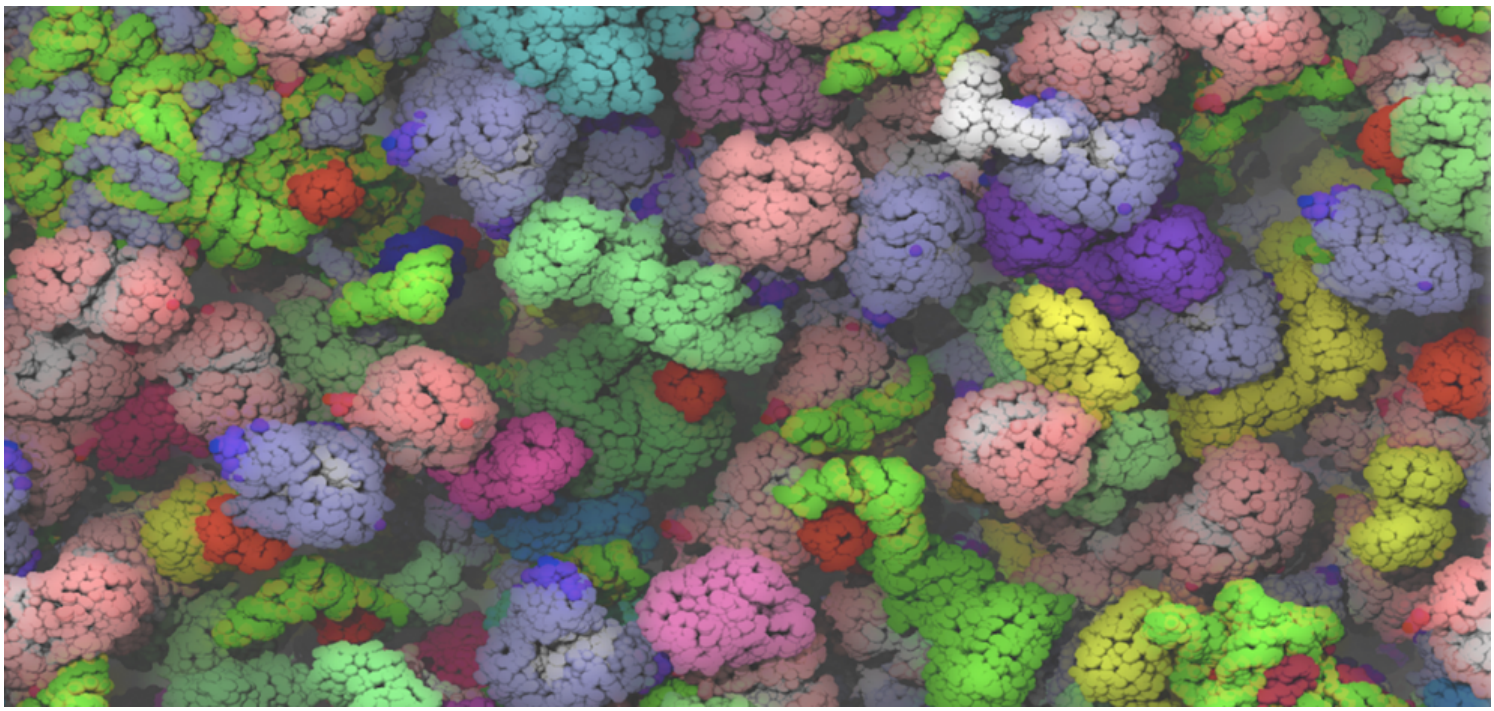

Molecules inside Escherichia coli. Adrian Elcock, PLOS Computation Biology

THE LOOM: 21 hours ago

Evolution Hidden in Plain Sight

by Carl Zimmer

It's hard to believe that *Escherichia coli* could have any secrets left.

For over a century, scientists have picked the microbe apart–sequencing its genes, cracking its genetic code, running experiments on its metabolism, earning Nobel Prizes off of it, and turning it into, arguably, the most-studied organism in history.

But as deep as scientists dive, they have yet to touch bottom. That's in part because *Escherichia coli* is not fixed. It continues to evolve, and even in the most carefully controlled experiments, evolution leaves behind a complicated history.

Twenty-five years ago, Richard Lenski used a single microbe to seed twelve lines of bacteria. He fed each line a meager diet of glucose, and the bacteria have been adapting to

this existence in his lab at Michigan State University ever since. (Here I've gathered together a few pieces I've written over the years about the 58,000-generations-and-counting Long-Term E. coli Evolution Experiment.)

In 2003, Lenski's team realized that something utterly unexpected happened. One of the hallmarks of *Escherichia coli* as a species is that when there's oxygen around, it can't feed on a compound called citrate. But one day a flask turned cloudy with an explosion of *E. coli* that were doing just that. The change was so profound that it may mean these bacteria had evolved into a new species.

For the past 11 years, the scientists have been trying to figure out how the bacteria gained this ability to feed on citrate. Thankfully, Lenski decided at the outset of the experiment to freeze some of the evolving bacteria every 500 generations. As a result, he and his colleagues can resurrect ancestral microbes, sequence their genomes, and probe their biology for clues.

After sifting through the frozen history of citrate feeding for a couple years, the scientists discovered an important step in this evolution. It involves a gene called citT.

The citT gene encodes a protein that lets *E. coli* feed on citrate when oxygen levels get low. The protein sits in the microbe's membrane and helps pull in citrate molecules from the environment. As it draws citrate in, however, it pumps another molecule–succinate–out. The pushing and pulling of these two molecules helps keep the chemistry of the cell in balance.

A small segment of DNA next to citT serves as a switch. If the microbe detects oxygen, a protein grabs onto the segment and shuts citT down. The microbe no longer feeds on citrate, instead feeding on better sources of energy, such as glucose.

The scientists found that around generation 31,500, a microbe that was copying its DNA in order to divide made a big mistake. It accidentally made an extra copy of a segment of DNA. That segment, it just so happened, contained citT. The microbe inserted the copy next to the original one, so that one of its daughter cells now had two copies of citT.

This sort of gene duplication happens from time to time in all living things. Human DNA regularly gets copied, too. And it can lead to important changes, because the two copies can start to do two different things. And that's what happened to the *E. coli*. In Lenski's

experiment, the new copy of citT ended up near a new bit of DNA that controlled genes in a different way. Instead of shutting down genes in the presence of oxygen, it keeps them always switched on. Thanks to this mutation to citT, the bacteria could start feeding on citrate in Lenski's oxygen-rich lab.

But the scientists found that this mutation was only one part of the story. The citT mutation allowed the bacteria to grow on citrate, but only slowly. Only after 1500 more generations had passed did the citrate-feeding bacteria begin growing quickly enough to dominate their flask.

During those 1500 generations, the scientists found, the bacteria made more copying mistakes, turning the new citT gene into four duplicates. Those extra copies enabled the bacteria to make more citrate-pulling proteins. But other mutations arose from generation 31,500 to 33,000, and the scientists had no way of knowing if they were important as well.

The story also turned out to have an earlier chapter. The scientists went back through the frozen archive to the very beginning and thawed out some microbial ancestors. They inserted the evolved citT genes into the ancestors, and found that the microbes could not feed on citrate. So the evolved citT gene alone was not enough to turn a microbe into a citrate-feeder.

The scientists did the same thing to bacteria from generation 20,000 and got a different result. When those more evolved bacteria got the citT gene, they could feed on citrate. Results like these suggested that early in the evolution of the bacteria, they picked up mutations that would later make it possible for the citT mutation to turn them into citrate feeders.

So, to recap: the scientists now had a story in three parts. Up to 31,500 generations, it was a story of groundwork mutations. Then came the big citT duplication. And after that came refining mutations, leading to world domination by generation 33,000. (The world, in this case, being a shot-glass-sized flask.)

In order to read this story in its full details, the scientists would need to understand the order by which every mutation arose, step by step. And they'd have to understand how each mutation helped produce a new kind of organism.

Despite the carefully controlled conditions of the experiment, this was a fiendishly hard problem. By the time the bacteria had evolved into full-strength citrate feeders at generation 33,000, they had acquired 79 mutations not found in their ancestor. Many of those mutations probably had nothing to do with citrate feeding. They may have helped the early bacteria grow better on glucose. Some might have had no effect on the bacteria one way or the other.

One of the scientists studying the citrate eaters was post-doctoral researcher Jeffrey Barrick. In 2011, he moved to the University of Texas to set up his own lab, and there he continued to study the citrate eaters, developing new methods to tease apart the evolutionary history of the citrate feeders.

He and his colleagues developed a new method of engineering bacteria in order to identify the mutations that were absolutely essential for full-blown citrate feeding. They combined portions of the citrate-feeding genome with that of the ancestral genome and then dropped these hybrids into dishes with only citrate to feed on.

Most starved to death. But a few grew. The scientists then plucked out the surviving hybrids and put parts of their DNA into other ancestral bacteria. Round after round of experimenting let them zero in on the essential segments for growing on citrate. Eventually, they could pinpoint the specific mutations.

Their results were weirdly few.

One result was no big surprise. Barrick and his colleagues found that in order to feed on citrate with maximal gusto, bacteria needed extra copies of the rewired citT genes.

But, as Barrick and his colleagues reported in a recent paper, they found just one other essential mutation.

This mutation affects a gene called dctA. When the scientists inserted the evolved versions of citT and dctA into an ancestral microbe, it became a full-blown citrate feeder. Neither gene on its own could achieve the same result. And no other genes were required for the metamorphosis.

This discovery prompted the scientists to look closely at the dctA gene. It encodes another membrane protein that's responsible for pumping molecules in and out of the

microbe. While citT pumps succinate *out* of the microbe, dctA pumps it *in*.

Barrick and his colleagues suspect that the evolution of a new kind of dctA gene allowed the bacteria to keep up a supply of succinate, which they needed on hand in order to feed on citrate. Together, the mutations to citT and dctA turned the mutant microbes into winners.

Which leaves the role of all the other mutations shrouded mystery. In the new study, *none* of the mutations that came before generation 31,500 proved to be vital for being a full-blown citrate feeder. They didn't lay the groundwork in any essential way. And yet the previous research clearly indicated that things were afoot before generation 31,500.

Given the new results, Barrick and his colleagues have a few ideas for what was going on before then. It's possible that some of the early, mysterious mutations were favored by natural selection because they helped the bacteria grow on their regular diet of glucose. And as a side effect, they helped build up a small supply of succinate. That succinate turned out to be a big benefit later on, when citT mutated. Now the bacteria had enough succinate (or some related molecule) to push out as it pulled citrate in. If the citT mutation had arisen before those mutations, the bacteria might not have been able to feed on citrate. And then later on, the dctA mutation arrived, kicking the citrate feeding into overdrive.

I contacted Lenski, who was not a co-author on Barrick's new study, to see what he thought of the results. "I love the fact that this paper shows just how complex evolution can be," he replied, "even for one little species in a tiny flask world for just a couple of decades."

(For more on E. coli's strange scientific history, see my book Microcosm*.)*

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Mike Lewinski
January 6, 2014

I like the point you make at the beginning here, and imagine that we'll probably never know everything there is to know about E. coli because E. coli is a moving target.

As Stuart Kauffman might say, the "adjacent possible" is always expanding and creating its own future

possibilities of becoming.

http://www.edge.org/conversation/the-adjacent-possible

Also thanks for the link to the blog by Zachary Blount on Ernst Mayr's Biological Species Concept. That saved me from asking a question I know I've asked of you before concerning the definition of speciation in asexual species.

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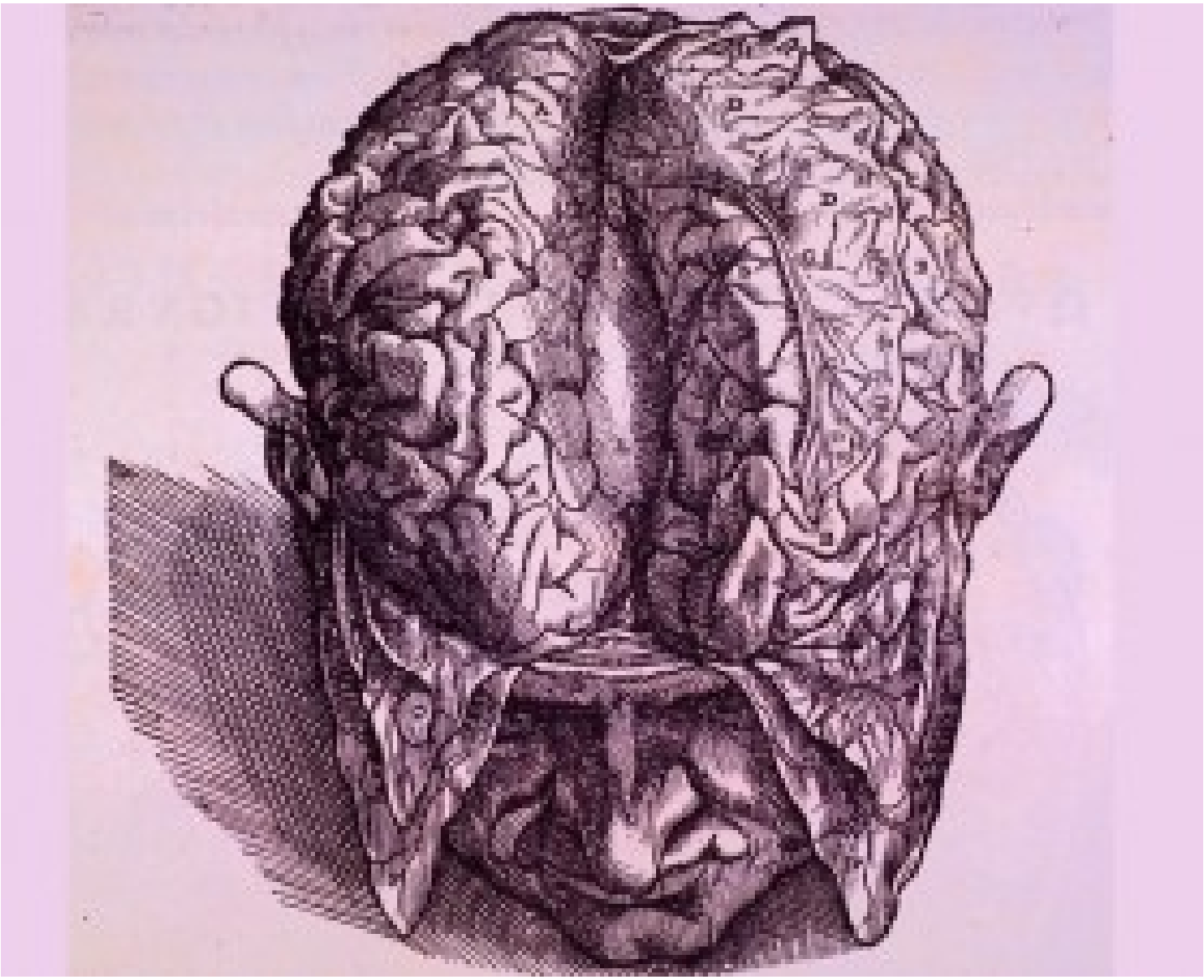

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Carl Zimmer is an award-winning science writer whose work appears frequently in the *New York Times, National Geographic*, and other publications. He is the author of 13 books, including *Parasite Rex* and *The Tangled Bank: An Introduction to Evolution*. You can find him on Twitter, Facebook, Pinterest, and Google+.

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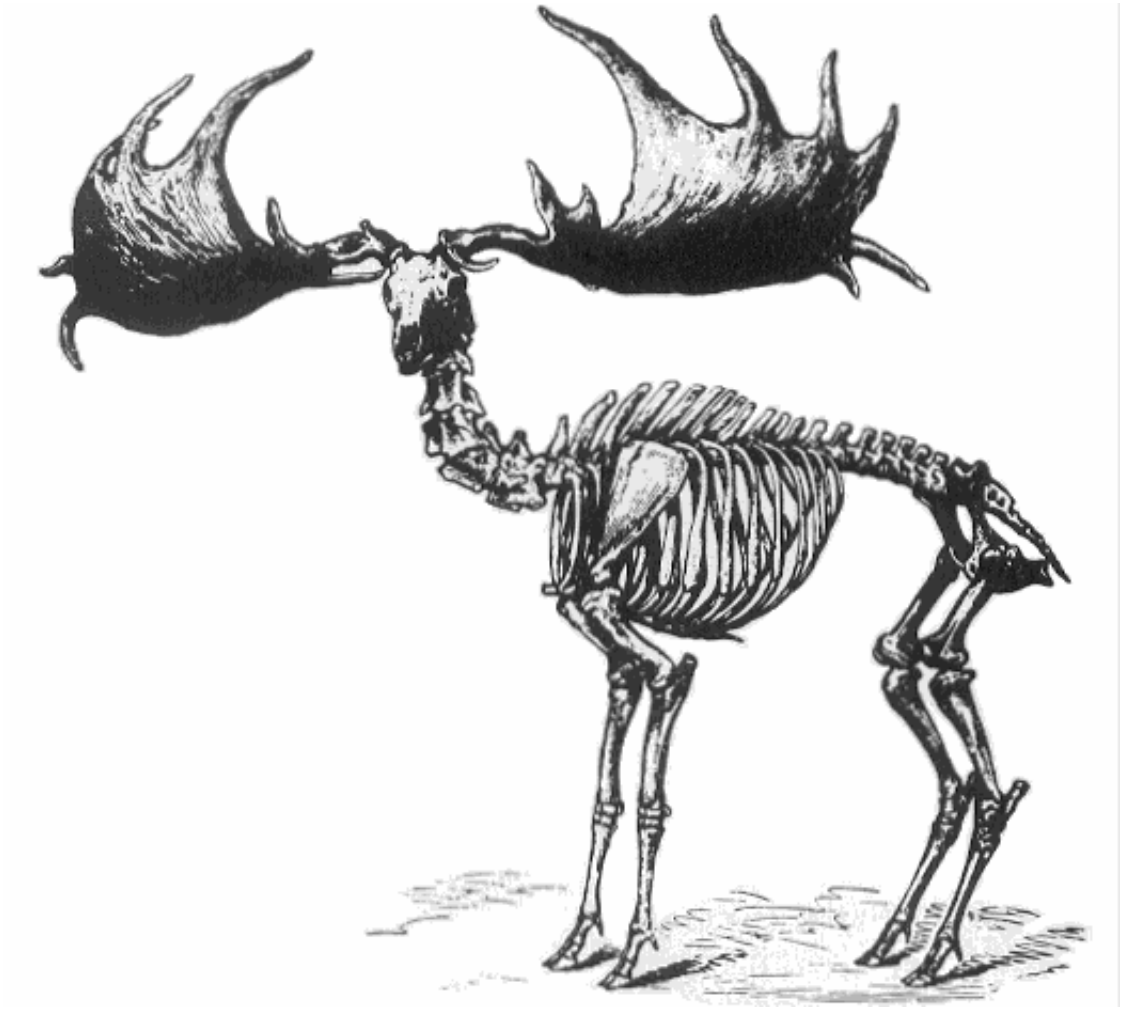

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