## Disclosure

of things evolutionists don't want you to know

Volume 12 Issue 11

www.ScienceAgainstEvolution.org

August 2008

# THE LONG TERM EVOLUTION EXPERIMENT

The Long Term Evolution Experiment (LTEE) has been going on since 1988. It finally proved Gould right about one aspect of microevolution. But it also proved him wrong about something much more important.

Evolutionists argue a lot about how evolution works. In an effort to explain how so many beneficial mutations can happen at once to produce a new feature, Stephen Jay Gould suggested that sometimes mutations happen that have no immediate benefit; but those mutations remain in the gene pool because they are not harmful. Those mutations are just floating in the gene pool waiting to be used. Then a subsequent mutation that depends upon those previous mutations can occur that causes some benefit to the organism. In other words, a new mutation might depend upon other mutations that happened in the past. Scientists call this, "historical contingency." "Historical contingency" simply means, "it depends upon something that happened in the past."

The role of historical contingency in evolution has been much debated, but rarely tested. <sup>1</sup>

Stephen Jay Gould maintained that these historical contingencies make evolution largely unpredictable. Although each change on an evolutionary path has some causal relation to the circumstances in which it arose, outcomes must eventually depend on the details of long chains of antecedent states, small changes in enormous long-term which may have repercussions. Thus, Gould argued that contingency renders evolution fundamentally On the other side, Gould's opponents say that the environment drives evolution to a particular solution, so it doesn't depend on past accidents. Furthermore, a mutation that has no immediate benefit will likely disappear from the gene pool before it is eventually needed.

Simon Conway Morris countered that natural selection constrains organisms to a relatively few highly adaptive options, so that "the evolutionary routes are many, but the destinations are limited". He and others point to numerous examples of convergent evolution as evidence that selection finds the same adaptations despite the vagaries of history. Evolution may thus be broadly repeatable, and multiple replays would reveal striking similarities in important features, with contingency mostly confined to minor details. <sup>3</sup>

This has been widely debated because it is hard to test. But, the Long Term Evolution Experiment (LTEE) did test it. It was a real, honest-to-goodness scientific experiment which produced a valid result.

It comes as no surprise to us that the results were misreported by New Scientist as a "major

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quirky and unpredictable, and he famously suggested that replaying the "tape of life" from some point in the distant past would yield a living world far different from the one we see today. <sup>2</sup>

<sup>&</sup>lt;sup>1</sup> Blount, et al., Proceedings of the National Academy of Science, June 10, 2008, "Historical contingency and the evolution of a key innovation in an experimental population of Escherichia coli"

<sup>&</sup>lt;sup>2</sup> ibid.

 $<sup>^3</sup>$  ibid.

evolutionary shift in the laboratory." <sup>4</sup> What the experiment actually proved was that Gould's theory is possible, but so unlikely that it can't be a plausible explanation for macroevolution. But let's not get ahead of ourselves. Let's look at what they actually did; what they actually found; and then we can discuss what it actually means.

#### THE EXPERIMENT

Here is how they described their experiment, followed by our translation into plain English.

To address the repeatability of evolutionary trajectories and outcomes, the long-term evolution experiment (LTEE) with Escherichia coli was started in 1988 with the founding of 12 populations from the same clone. These populations were initially identical except for a neutral marker that distinguished six lines from six others. They have since been propagated by daily 1:100 serial transfer in DM25, a minimal medium containing 25 mg/liter glucose as the limiting resource. Environmental conditions have been controlled, constant, and identical for all 12 lines. To date, each population has evolved for >44,000 generations, and samples have been frozen every 500 generations, providing a rich "fossil record". Moreover, these samples remain viable, allowing us to perform simultaneous measurements and experiments with bacteria from different generations. The founding strain is strictly asexual, and thus populations have evolved by natural selection and genetic drift acting on variation generated solely by spontaneous mutations that occurred during the experiment. Thus, the LTEE allows us to examine the effects of contingency that are inherent to the core evolutionary processes of mutation, selection, and drift. 5

They started out with 12 individual, identical *E. coli* bacteria and put each one in its own little Petri dish; and they grew into 12 colonies.

*E. coli* bacteria reproduce asexually. In simple terms, the bacteria grow bigger and bigger until they get large enough to split in half, so the parent cell turns into two identical child cells. At the microscopic level, the division process isn't nearly so simple as it appears. Although the division process nearly always produces two identical children, sometimes the process partially fails, producing mutant offspring. It takes nearly 2

hours for *E. coli* bacteria to reproduce, so there are about 6.5 generations born every day. This is convenient for scientists because it allows them to study lots of generations in a reasonable amount of time.

So, if you start out with 1 bacterium, you will have 2 in about 2 hours, then 4 two hours later, then 8 two hours after that, then 16, then 32, then 64, and finally 128 in just over a day. It is very convenient that the colony grows by a factor of roughly 100 every day.

The little Petri dish the bacteria live in can only hold a certain amount of bacteria. They would crowd themselves (and starve themselves) to death if you didn't do something. So, every day, 7 days a week, 52 weeks a year, for the past 20 years, someone (probably the lowest graduate student on the totem pole ③) has had to go into the lab and take 1% of the bacteria in each of the 12 Petri dishes and transfer them to 12 new Petri dishes. Then they sterilized and recycled the 12 old Petri dishes, killing 99% of the bacteria. But once a month (every 500<sup>th</sup> generation), they didn't recycle the old Petri dishes. Instead, they labeled and froze them for future use.

Everybody has to eat, and bacteria are no exception. Near the end of the report, they tell exactly what the bacteria were fed.

The twelve populations have been propagated for almost 20 years by daily serial dilution in DM25, a minimal salts medium that has 139  $\mu M$  glucose and 1,700  $\mu M$  citrate. Given 1:100 dilution and regrowth, the populations achieve \$\infty\$6.64 generations per day, and they have evolved for over 40,000 generations in this experiment to date. Every 500 generations, population samples are frozen at  $-80^{\circ} C$  with glycerol added as a cryoprotectant. These samples contain all of the diversity present in a population at that generation.  $^6$ 

In other words, they fed them glucose and citrate, and gave them 12.2 times more citrate than glucose. This was critical because *E. coli* can't digest citrate. In human terms, it would be like being fed sugar and artificial sweetener. Artificial sweetener has 0 calories, so we would die on a steady diet of nothing but artificial sweetener. *E. coli* would die on a steady diet of citrate.

The goal of the experiment was to try to force the *E. coli* to evolve in such a way that they could digest citrate, like some other bacteria can. They did this by giving the bacteria just enough sugar to keep them alive, and an abundance of artificial

<sup>&</sup>lt;sup>4</sup> http://www.newscientist.com/channel/life/dn14094-bacteria-make-major-evolutionary-shift-in-the-lab.html <sup>5</sup> Blount, *et al.*, *Proceedings of the National Academy of Science*, June 10, 2008, "Historical contingency and

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sweetener. The expectation was that if the E. coli did evolve into a form that could digest citrate, that new variety would flourish in the citrate-rich environment and drive the old variation to extinction.

If you read the fine print, you will discover that E. coli can ALMOST digest citrate. In their words,

Throughout the duration of the LTEE, there has existed an ecological opportunity in the form of an abundant, but unused, resource. DM25 medium contains not only glucose, but also citrate at a high concentration. The inability to use citrate as an energy source under oxic has long been a defining conditions characteristic of E. coli as a species. Nevertheless, *E. coli* is not wholly indifferent to citrate. It uses a ferric dicitrate transport system for iron acquisition, although citrate does not enter the cell in this process. It also has a complete tricarboxylic acid cycle, and can thus metabolize citrate internally during aerobic growth on other substrates. E. coli is able to ferment citrate under anoxic conditions if a cosubstrate is available for reducing power. The only known barrier to aerobic growth on citrate is its inability to transport citrate under oxic conditions. Indeed, atypical E. coli that grow aerobically on citrate (Cit<sup>+</sup>) have been isolated from agricultural and clinical settings, and were found to harbor plasmids, presumably acquired from other species, that encode citrate transporters.  $^{7}$ 

So, the ability to digest citrate is one small step for a bacterium, not a giant leap for bacteria-kind. They were just on the edge of having this digestive ability. The potential was always there.

Despite this potential, none of the 12 LTEE populations evolved the capacity to use the citrate that was present in their environment for over 30,000 generations. During that time, each population experienced billions of mutations, far more than the number of possible point mutations in the \$\infty4.6-million-bp genome. This ratio implies, to a first approximation, that each population tried every typical one-step mutation many times. It must be difficult, therefore, to evolve the Cit<sup>+</sup> phenotype, despite the ecological opportunity. Here we report that a Cit<sup>+</sup> variant finally evolved in one population by 31,500 generations, and its descendants later rose to numerical dominance. 8

So, it took more than 30,000 generations to fully evolve a capability that was already almost there, and it only happened in 1 out of 12

<sup>8</sup> ibid.

populations. Thirty-thousand generations isn't a long time for a bacterium, but 30,000 generations for people is about 600,000 years. If it would take people more than a half million years to evolve just a minor improvement in digestion in an environment where 99% of the population starves to death every 130 years, just think how long it would take any animal to evolve any significant new feature in an environment with much less evolutionary pressure. Just try to imagine how many generations of E. coli bacteria existed in the wild without evolving the ability to digest citrate. They really had to try hard, using all the evolutionary pressure they could muster, to produce this minimal change.

#### IT'S HARD TO SWALLOW

In a previous quote, you might not have understood what they meant when they said, "It uses a ferric dicitrate transport system for iron acquisition, although citrate does not enter the cell in this process. It also has a complete tricarboxylic acid cycle, and can thus metabolize citrate internally during aerobic growth on other substrates. E. coli is able to ferment citrate under anoxic conditions if a cosubstrate is available for reducing power." Even if you didn't understand half the words in those three sentences, you certainly can understand that digestion is a complex process. Those steps all had to happen by accident if evolution is true.

There are a lot more digestive improvements that had to happen by accident to get to our digestive system, if evolution is true. We had to evolve teeth, saliva, a stomach, stomach acid, intestines, a liver, a pancreas, and insulin by historical contingency. Think about it. Not only did insulin have to happen by chance; it must also have been a lucky accident that an organ (the pancreas) that produces insulin happened by chance, and that organ just happened to be able to figure out how much sugar is in the blood stream, and produce the right amount of insulin in response. Ask a diabetic friend how important it is to have the right balance of insulin and sugar in the blood.

No doubt there are biomedical engineers trying to invent an artificial pancreas. I truly doubt that they are trying to do it by trial and error, hoping to find the right design completely by accident.

#### **CHANCES ARE SLIM AND NONE**

Even among the potentiated clones, the rate of mutation to Cit<sup>+</sup> [the ability to digest citrate] is extremely low. Cit<sup>+</sup> mutants arose in 2 of the 280 new cultures, giving an estimate of 6.6 x 10<sup>-13</sup> for the mutation rate, with the 95% CI extending from 7.9 x  $10^{-14}$  to 2.4 x  $10^{-12}$  . . . . A typical mutation rate in E. coli is ≥5 x 10<sup>-10</sup> per

<sup>&</sup>lt;sup>7</sup> ibid.

base pair per generation. Such a low rate suggests that the final mutation to Cit+ is not a point mutation but instead involves some rarer class of mutation or perhaps multiple mutations.

The potentiated clones are the bacteria that had an increased potential (that is, an increased ability) to evolve this improved digestion.

The mutation never happened in the unpotentiated clones.

For more than 30,000 generations, none of them evolved the capacity to use the citrate, although billions of mutations occurred in each population, such that any typical base pair mutation would have been tested many times in each one. It is clearly very difficult for E. coli to evolve this function. In fact, the mutation rate of the ancestral strain from Cit- to Cit+ is immeasurably low; 10

Let's review a little bit. For normal E. coli to evolve the ability to digest citrate, the mutate rate is "immeasurably low." In other words, they could not measure the rate because it never happened. In the "potentiated" E. coli, Blout said the rate was "extremely low." So, the question is, "What potentiated [enabled] some clones enough that their mutation rate changed from immeasurably low to extremely low?" Here is where the historical contingency comes in.

#### **TRYING TO PROVE THEIR POINT**

Remember that historical contingency was Stephen Jay Gould's unproven hypothesis. Blout and his colleagues were attempting to prove Gould correct. In his honor,

We started the first replay experiment on the 3rd anniversary of Stephen Jay Gould's death; we ended it on the 66th anniversary of his birth.

Let us emphasize that there is nothing wrong with this. Scientist always try to prove what they already believe to be true is actually true. They have a bias going into the experiment. It is what gives them the motivation to do the work. **Evolutionists** sometimes reject creationist research out-of-hand simply because creationists are biased: but evolutionists are biased, too.

Having a bias does not invalidate the results. so long as the work is done carefully and Blout did the work carefully and honestly. honestly. His results and conclusions are valid.

We demonstrated that the evolution of this new function was contingent on the history of the population in which it arose. In particular, we showed that one or more earlier mutations potentiated the evolution of this function by increasing the mutation rate to Cit<sup>+</sup>, although even the elevated rate is much lower than a typical mutation rate. 12

#### FORCING EVOLUTION

If you thought the quotes about the experiment we have shown you so far were hard to understand, you aren't going to have a chance to comprehend the rather long and obtuse explanation of what they did. So let's skip the quotes and just explain in plain English what they

They had 12 colonies of *E. coli* bacteria, each living in a different Petri dish. After about 31,500 generations, one of the colonies evolved the ability to digest citrate, and the other 11 didn't. They wanted to know if a mutation in a previous generation of that one particular colony made it more likely for that colony to evolve the ability to digest citrate. So, they thawed out the frozen samples from the 500<sup>th</sup>, 1000<sup>th</sup>, 1500<sup>th</sup>, etc. generations and started new colonies from them. They discovered that something must have happened just before the 20,000<sup>th</sup> generation because none of the colonies from the previously frozen bacteria evolved the ability to digest citrate, but a few of the bacteria regenerated from the 20,000<sup>th</sup> generation did. This confirms what Gould thought was possible.

We need to stress, however, that they discovered that although it is possible that a mutation with no apparent immediate usefulness might hang around in the gene pool long enough for it to become useful in conjunction with another mutation that occurs in a later generation, it is extremely unlikely. Such mutations are so rare, and produce such minor changes, that it is inconceivable that all the novel features that supposedly evolved over time (eyes, ears, mammary glands, blood, etc.) could have originated that way.

#### MISSING MISSING LINKS

There was also an unexpected result from the experiment.

The origin of the Cit<sup>+</sup> function also had profound consequences for the ecology and subsequent evolution of that population. This new capacity was refined over the next 2,000 generations, leading to a massive population expansion as the Cit<sup>+</sup> cells evolved to exploit

 $^{10}ibid.$ 

<sup>9</sup> ibid.

<sup>&</sup>lt;sup>11</sup> ibid.

more efficiently the abundant citrate in their environment. Although the Cit<sup>+</sup> cells continued to use glucose, they did not drive the Cit<sup>-</sup> subpopulation extinct because the Cit<sup>-</sup> cells were superior competitors for glucose. Thus, the overall diversity increased as one population gave rise evolutionarily to an ecological community with two members, one a resource specialist and the other a generalist. <sup>13</sup>

One of the fundamental ideas behind the theory of evolution is extinction. Natural selection allows the group that is more fit for survival to drive the less fit ancestors to extinction. That's why the missing links are missing. The alleged common ancestor of chimps and humans is missing because we, and the chimps, drove it to extinction. All the transitional forms are missing because they were driven to extinction. That's how evolution is supposed to work.

But in this experiment, the new variety did not drive the old variety to extinction. Presumably that is because there was enough food for both varieties. Hasn't there ever been enough food for just one transitional form to survive, at least long enough to leave some fossils?

#### TOO UNLIKELY TO HAPPEN

If you toss a coin in the air, it will land heads or tails; but with a steady hand, and a certain amount of patience, one can eventually get a coin to stand on edge. That proves it is possible for a coin to assume that position. But after trying and failing to stand a coin on edge a number of times, one begins to appreciate how difficult it is to do, and how unlikely it is that it might happen by chance.

If you see a table with dozens of coins standing on edge on it, it certainly is possible that someone tossed a handful of coins into the air, and they all landed (and stayed) on edge. But knowing how difficult it is to do, you know that it probably didn't happen that way. Therefore, the primary thing we learn from this experiment is how difficult it is to cause even the smallest amount of evolution.

Let's not gloss over this point. Being able to do something intentionally does not mean that it happens by accident. Trying to stand a coin on edge does not teach you that it happens by chance. Instead, it teaches you that it is so hard to do that it won't happen by chance.

The LTEE showed that after 20 years of extreme, relentless pressure, it is possible for a minimal improvement in digestion to occur (after just 30,000 generations ©). Imagine how many generations it would take to develop a whole

digestive tract from teeth to anus. That's just the superficial parts we can see. When you think about it on the cellular level (don't forget about the ferric dicitrate transport system for iron acquisition, and complete tricarboxylic acid cycle ③) it gets even more mind-boggling.

The experiment just goes to show that even with historical contingency and extreme selection pressure, the probability of random mutations causing even a tiny evolutionary improvement in digestion is, in the words of the researchers who did the experiment, "extremely low." Therefore, it can't be the explanation for the origin and variety of all the forms of life on Earth.

Creationists have calculated that the probability is too low for it to happen. Now evolutionists have experimentally proved the creationist calculations right.

**Email** 

### EVOLUTION ISN'T JUST CHANGE

James proves our point.

It is clear that many of the people who send us email don't even bother to read what we have written, but disagree with it anyway. We received this email five days after the July newsletter was posted on-line.

From: James
Date: 7/20/2008
Subj: An Error
Hello,

I, like you, am retired. I am a geologist and I've been corresponding with one of your disciples. I noticed on your home page that you say:

the theory of evolution is not consistent with physical evidence and is no longer a respectable theory <u>describing the origin</u> and diversity of life.

Sir, what does EVOLVE mean? What is the definition?

It's CHANGE isn't it"
and that's all.

Evolution has nothing to say about the origin of life. That, like the definition of "theory," is one of the most abused terms in all of creationland.

You, no doubt, read that newsletter, and know that approximately 2/3 of it was devoted to the definition of "evolution." You also know that we claimed evolutionists try to change the definition of "evolution" in order to avoid addressing the real issue. We want to thank James for proving us right.

You are permitted (even encouraged) to copy and distribute this newsletter.

<sup>13</sup> *ibid*.

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by Lothar Janetzko

#### **ANSWERS IN GENESIS**

#### http://www.answersingenesis.org/creation/v22/i1/creation.asp

"Believing it. Defending it. Proclaiming it."

This month's web site review looks at an article from the Creation Archive of the *answersingenesis.org* website. The article is from Volume 22 Issue 1 and was first published in December 1999. The title of the article is "Creation: 'Where's the proof?' When the person you talk to on creation insists that you 'leave the Bible out of it', they are really saying the deck should be stacked one way."

The author of the article, Ken Ham, points out that "Creationists and evolutionists, Christians and non-Christians all have the same evidence—the same facts." The difference is in the way we interpret the facts. He points out that this is due to starting out with different presuppositions.

In the section of the article with the title "Past and present," he describes what he believes are the presuppositions of Christians and non-Christians. He comes to the conclusion that a person will not interpret evidence differently until he or she realizes what the presuppositions are and be willing to change them.

In the article, Ham also points out that he is a teacher and has learned "to teach the students how to think rather than just what to think."

On the home page of the article you will find a link to *Answers Magazine*. This magazine is issued quarterly and features "fascinating content and stunning photographs that present creation and worldview articles along with relevant cultural topics from different authors".

You can use this article as a launching point for exploring the whole answersingenesis.org website.

#### Disclosure

The official newsletter of

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