**Evolutionary Dynamics**

**Supreet Saini**

**Chemical Engineering**

**Indian Institute of Technology Bombay**

**Week 10**

**Lecture 50**

Hi everybody, welcome back to the next video. So, before we move to the next experiment that we want to discuss, I just want to highlight a couple of aspects associated with the previous experiment. We ended the last video with the discussion of this experiment that the authors did, where the concentration of the antibiotic on the plate transitioned from 0 to 1000 times MIC in one step. And the results that they report are that obviously bacteria quickly expanded to fill up this space, but thereafter there

wasn't a mutant which occupied the next region of the plate, which contained antibiotic at the concentration of 1000-fold MIC. Why did this not happen? One of the main reasons for that is that going from 0 to 1000 needed many mutations. This transition of the bacteria being able to resist the concentration at MIC to increase that thousand-fold probably needed many mutations. So, many mutations were needed.

Whereas, as we discussed very early in the course, when we had two sequences, when we had a sequence space, I want you to remember and go back to the discussion where we said that if we have a sequence of length L, then 4 to the power L sequences are possible. And when we made this sequence space where each of these nodes represented one sequence, represented one sequence, then we joined sequence i and j if they were connected by if the Hamming distance between i and j was equal to exactly one. We never connected Hamming distance; we never connected nodes which represented sequences which were separated by a Hamming distance of more than one. And that is important in this context because why we said we only connected Hamming distance 1 was that if we have a population here, then in a division event, what might happen is that one of these individuals might acquire a mutation and the progeny belongs to this node. However, if we have another node k such that the Hamming distance between j and k is 1 and

It's not very realistic to expect that an individual which was at node I picked up a mutation and landed up and the progeny landed up at a node which was at a Hamming distance of 2 from it. And that is what we see here that. Because an individual needed multiple mutations to increase its resistance from MIC to 1,000 times MIC, those mutations could not have happened in one individual as a result of which the population could not break through this frontier of 1,000 times MIC. So that's one. The second thing is that this was an environmental stress.

If you think of what we asked the bacteria to do here, this was a challenge that was too steep. In the original video that we saw, the challenge was broken down into smaller steps. Where we went from 0 to 1 to 10 to 100 and eventually to 1000. So this was broken down into four steps. But when we.

Remove those four intermediate steps and ask the population to come up with a solution to this environmental challenge that is posed in just one go. The population can't cope up with it. And if this was the environmental change that happened of 0 to 1000, it would just go extinct. And this is interesting in the context that. In the context of what we are seeing today in an ecological context, the earth has been warmer in its history than it is today.

The oceans have been more acidic than they are today. But the important parameter here is the rate at which the temperatures are changing and the rate at which the ocean's pH is changing. Species cannot cope with environmental change if it is too rapid. If the environmental change is slower, then species can adapt. They can acquire beneficial mutations and adapt to the stresses that are being posed.

However, if the stress that is being posed is too harsh, then it is not possible for populations to come up with the genetic solutions necessary to adapt. As a result, Because environmental changes are happening at a speed that has not been seen previously, what is happening now is that the extinction rates of species today on our planet are 1000 times higher than the background natural extinction rates present in ecology. So that is an important illustration, I feel, that comes out of this experiment. The last point about this experiment is that when we talk about antibiotic resistance, the popular mode of resistance is something called horizontal gene transfer.

So we say that we have bacteria, and this bacteria is susceptible to antibiotics. What that means is it has a very low MIC. And now, if it were to acquire resistance, the more popular path that is thought to lead to resistance is via the acquisition of genes from the

environment. That if this is the genetic content of the then maybe it will acquire foreign pieces of DNA, and this piece of DNA will confer resistance.

This has a certain gene on it, and that gene's job is to counteract the action of the antibiotic that's present in the environment and confer resistance. And these are mobile genetic elements that can move from one individual to another. And typically, these can move across via different processes. They can be taken up from the environment. Phages, which are viruses that infect bacteria, can move pieces of DNA from one bacterial species to another.

Or you can have actual exchange of genetic elements between bacterial species that are close to each other and have the right mechanisms to trade DNA strands with each other. So resistance is commonly thought to be easily spread. Through these types of mechanisms, where I just acquire a foreign piece of DNA that I did not have previously, and this foreign piece of DNA that I have acquired confers resistance in me, and I am able to survive the antibiotic. However, what we saw in the paper in the last video was that mutations can take place in the existing DNA of the organism. I did not acquire any foreign piece of DNA.

Mutations just happened in the genome that I'm carrying already, and these mutations facilitated an increase of MIC up to a thousand-fold. What that means is that we don't even need to invoke horizontal gene transfer to explain an increase in resistance. The genetic material present inside a cell is sufficient to acquire mutations and itself become resistant without the acquisition of foreign pieces of DNA, which will, of course, aid this process, but this in itself can acquire mutations and become resistant. All right.

So that was the first study. Next, we are going to discuss perhaps what is the most famous evolution experiment in microbiology, which is called long term. The experiment has a name which is called long term evolution experiment. Or in short, it's referred to as LTEE. This experiment is so famous that in fact it has a Wikipedia page dedicated to it.

So if we if we just read through the Wikipedia page, this is long term evolution experiment. LTEE is an ongoing study begun by Richard Lenski at California, Irvine. And then the experiment was moved to Michigan State University when Lenski moved from Irvine to Michigan State University. And in the last couple of years since Lenski has retired, this has been taken over by Jeffrey Barrick at University of Texas, Austin. And this was a study that was started more than 35 years ago in February 1988.

And this is a little bit dated. This is 73,000. But as of now, the experiment has crossed more than 80,000 generations have been processed in this experiment. So, in terms of volume and wealth of knowledge of what evolutionary processes can do from a lab experiment that we can gather, there is no other experiment which has told us more than this particular experiment. So, this has been going on since 1988 and to date it has processed more than 80,000 generations.

Literally, a hundred or so papers have resulted from these studies. Some of them are just classical papers that we will discuss in this course. This was started by a person called Richard Lenski. And much of the experiment actually took place at Michigan State University. Currently, the experiment is being led in Jeffrey Barrick's lab at Austin.

OK, so while this is a... While this is the experiment that was started roughly 35 years ago, the first microbial evolution experiment was done almost 100 years before Lenski started this particular experiment. In that experiment—this is the first microbial evolution experiment— This was started by somebody called William Dallinger. And what Dallinger was trying to do—the question he was interested in—was: suppose you have bacteria that can grow up to certain temperatures but not beyond that.

Can you evolve these bacteria so that they acquire the ability to grow at higher temperatures? And over the course of seven to eight years, as this experiment lasted, Dallinger began this experiment by growing the bacteria at very low temperatures, of the order of 20-25 degrees Celsius. And over 7-8 years, he gradually raised the temperature of the medium in which these bacteria were growing and was able to reach temperatures of the order of 70 degrees Celsius. Now, some people conjecture that we are not sure if there was contamination.

So the original species had died down, and some external contaminant had come out and was growing. And this is obviously the 19th century. So techniques weren't sophisticated back then. But Dallinger, assuming that there was no contamination and that this adaptive process occurred—that bacteria which were not able to grow at 70 degrees were now growing at 70 degrees as a result of this experiment that lasted several years. Dallinger was also able to show a phenomenon called trade-off in bacteria.

What he saw was that the ancestor he started with was able to grow at 25 but exhibited zero growth at 70 degrees Celsius. The evolved strain, which was growing fine at 70 degrees Celsius, when he compared its growth at 25, the evolved strain was able to grow at 25 but not as well as the ancestor strain at 25 degrees Celsius. So what Dallinger noted

was that the ability to grow at 70 degrees Celsius came at a cost, which reduced the fitness of these bacteria at 25 degrees Celsius. So it told him this idea of trade-offs—that you can't be very good at growing at both very low and very high temperatures.

As you become better at growing at higher temperatures, your ability to grow at lower temperatures is compromised. And this was the first demonstration of the phenomenon called trade-offs. The ancestor—so let us say this is 30 degrees Celsius, this is 70 degrees Celsius. So the ancestor exhibited high growth rates at 30 and zero growth at 70. It was not able to grow at this elevated temperature.

On the other hand, the evolved strain exhibited growth at 70 degrees Celsius, but the growth at 30 degrees Celsius was intermediate, was not as high as the ancestor. So, what this tells us is that in order to become better at 70, so in 70 the performance improved after this evaluation experiment, but that came at a cost of decreased performance at 30. Now, are these tradeoffs necessary or is this just the fact that you weren't going at 30 degree Celsius? You had not seen temperature of 30 degree Celsius for several years and hence there was no need to maintain the ability to grow fast at 30 degree Celsius. Are the kind of questions that we are interested in as a field and studying these tradeoffs which are encoded by cellular logic.

The experiment, I think, ended in a bit of a disaster because I think the equipment just burst. So almost 100 years on from Dallinger's experiment, Lenski started the following experiment. So LTEE has 12 identical lines. With some minor differences, there are groups of six each, but we will not consider that difference here. It's not relevant for the discussions that we'll be having.

And starting with an ancestor E. coli. So this is ancestor E. coli. It has its genome. This ancestor was placed in a flask. which contained low amounts of glucose as the carbon source.

And this ancestor was placed and growth was allowed to happen in this flask for a period of 24 hours. After which the flask would have lots of individuals present in them. And then 1 is to 100 dilution would take place to a fresh flask which contained the same media as the one that was started with. And that's pretty much the entire experiment. And this process has been continued on since Feb of 1988.

except for the fact that when this experiment was started, Lenski didn't start only one line, but he started this experiment with 12 identical lines. So, some of the ancestral cells were

added to another flask, which had an identical composition as the as the first flask and an identical treatment was meted out to this as well. So, this we will call this. So, these independent repeats, identical independent repeats are called lines.

So, this is line number 1, line number 2 and there are as we listed here that there are 12 identical lines which have been going on since. Each one of these lines has been evolved for 80,000 generations. And the idea is to understand what sort of phenotypic and genetic changes. So let's do it on the next slide. So what are some of the questions of interest here?

What are the sort of phenotypic and genetic changes that take place in these populations as they evolve for such a long period of time. Between the 12 lines, is the response parallel? Well, is the response identical? Distinct—what that means is, is it that line number one, for instance, exhibited many beneficial mutations very quickly, whereas line number two didn't do anything, and so on and so forth? Are the dynamics of the adaptive process the same or very different?

Are the kinds of mutations that these lines are acquiring the same or different in these populations? Are some of the kinds of questions that this work was started with? The last point to note here is that when this experiment was started, the carbon source in this flask was glucose, right? However, this environment—this liquid media that the experiment was performed in—also contained quantities of citrate. Citrate, as a carbon source in the presence of oxygen, cannot be used by E. coli for growth.

So, E. coli in oxic conditions These flasks were shaken well, so there was plenty of oxygen around. In oxic conditions, citrate cannot be used as a carbon source. So, what that means here is that you have this resource available, which is being used and competed for in the population. However, you have another resource present in the population, which cannot be used because of an environmental condition.

If the environmental conditions were different and suppose oxygen was not present, then then E. coli could use citrate as a carbon source. However, in the context in which this experiment is being conducted, citrate is not available as a carbon source. So, you have this environment where there is an additional resource present, but just out of reach of the population. And what would happen in that case?

So obviously, this experiment is a long, long story of 80,000 generations. My guess is that about 100 papers have come from these types of studies. We'll start with—it's

impossible to cover everything—but we'll start with a few key results that we'll discuss as part of this course. About 10 years into the experiment, in the late '90s, I think, Lenski himself said that there was nothing really exciting going on in the experiment, and he had thoughts about shutting it down. But close to the turn of the century, something interesting happened, something remarkable, which led to a very exciting discovery of how evolutionary change can take place and how we can study it through lab experiments.

Since then, this experiment has now become an institution, and there is no experiment that has gone on longer or told us more about the molecular basis of adaptive evolution. responses of populations in the context of bacteria, in this case. And we'll start our discussion of some of the key results from this LTE study in the next video. Thank you.