Evolutionary Dynamics

Supreet Saini

Chemical Engineering

Indian Institute of Technology Bombay

Week 11

Lecture 55

Hi, everyone. Welcome back to the next video. We will continue our discussion of the evolution of this E. coli line in LTEE to utilize citrate as a carbon source for growth and energy. So, with the question before us, the conundrum in this evolution experiment is that, let us say we have line 1,

which has evolved for 80,000 generations, or if we talk of the time at which this happened, let us say this has evolved for 35,000 generations, and this has no citrate utilization capacity. On the other hand, I have 11 such lines. So, 11 such lines. On the other hand, I have this one line in which something seems to have happened such that the individuals in this population are able to utilize citrate. And the question is, why is it? This question is more pertinent now because we know that another 50,000 generations have passed and none of these 11 lines are still able to utilize citrate.

So the question is, what happened in this line in terms of the genome of the individual that allowed it to utilize citrate? And there were two hypotheses of why it could have happened. In the first hypothesis, it was an extremely rare mutation that happened in this particular line which allowed for the utilization of citrate. By rare mutation, what we mean is that, let's imagine that this is the DNA of the organism.

And maybe what needed to happen was that this part of the DNA needed to duplicate itself. Now, duplications happen all the time. But what made this rare was that this exact part of DNA needed to be duplicated. If it was slightly shorter, it wouldn't work. And if it was slightly longer, it wouldn't work also.

So the exact same region of DNA needed to duplicate itself. That is a very rare mutation. So maybe that's what happened and maybe that happened somewhere around here in this line and that's why we detected once that mutation happened you had one individual in

which it happened and that individual had competitive advantage over the others in the population and then it spread in the population and that's why we saw, that's why one morning at the time of subculture we saw that this flask was much more densely populated as compared to the other 11. That's one hypothesis.

The second hypothesis is that there were a series of mutations of mutations leading up to final mutation. None of these was rare. These are regular single nucleotide mutations. These set of mutations happen in a series in this particular line.

Leading up to a final mutation which conferred the ability to utilize citrate. So maybe these mutations had happened all along the way in this particular line. And finally, a mutation occurred. And when all five mutations were present together in a genome, the ability to utilize citrate evolved. Only this mutation would not confer the ability to utilize citrate.

It was only when this mutation, in conjunction with these four previous mutations, was present in the same individual that the ability to utilize citrate emerged. So, if these are the two competing hypotheses, then we need to think about what experiment one would do to test which of these two scenarios actually played out in the case of citrate utilization in LTEE. To test this possibility, What was done in this study was that, remember, for each of these lines, we have freezer stocks made at every 500 generations or so.

So we have access to these frozen records of this evolution experiment, allowing us to go back, revive from the freezer stock, grow them again, and so on. So, the strategy used was that for each one of these 12 lines, if we go back a few thousand generations—let’s say to 28,000— At 28,000 generations, I will revive this population in each of these 11 lines and check for the ability to grow in citrate. Check if it grows on citrate. And obviously, it was found that there was no growth on citrate.

That's not surprising because even at 35, there was no growth on citrate. But when we do the same exercise for the line which actually did evolve the ability to citrate, we check if grows on citrate. And we found that there was no growth on set rate. Now, what that means is that at 20000 generations, there was no ability to grow on set rate. But at 35000, for sure, I know that this line is growing on set rate.

These 11 lines are not. That means something happened between 28000 and 35000. Now, That is something that enabled utilization of citrate in this particular line could be either this hypothesis or this hypothesis. So, what would be the experiment here?

The experiment that was done to check which of these two hypotheses played out was that now if I go back to the freezer stock, from 28,000 and evolve again. All these 11 lines and this particular line. So, remember this is happening like this that there is an evolution experiment going on. This was 28,000 generations.

At this point, I made a sample in a while and I stored it in minus 80 degree Celsius freezer. While the experiment continued via this transfer every day, this continued and at I'm at 35,000 now. So I have the culture that was growing on this day stored away in minus 80 degrees Celsius that I can go back to revive and then start a parallel evolution experiment again from this point only. So let's do this on a fresh slide.

This is let's say 35,000 this is 28k, this is 11 lines and the line where the ability to utilize citrate did evolve, let us say is this, this is 35k, this is 28k and so on and so forth, this is one line. So, if hypothesis 1 is right, If hypothesis 1 is correct, then ability to utilize citrate came as a result of a very rare mutation, a very rare single mutation. If that is the case, then this rare mutation could have happened in any line.

could have happened in any of the 12 lines because it's so rare It only happened in this particular one because this was the fortuitous one which happened to acquire that mutation. But really it could have happened in any one of them. There was no particular reason that it needed to happen in this particular line. So the experiment to test this is that if I take the 28000 stock from these 11 lines and evolve them again and I take the 28000 stock which I know is not growing on citrate.

So by 28000, that rare fortuitous mutation had not happened. If I take this and evolve this again, then the question I ask is: which line should acquire the mutation? Which line should acquire the ability to utilize citrate? And if hypothesis 1 is correct, then the ability to utilize citrate is conferred because of this very fortuitous single rare mutation. And that single rare mutation could happen in any one of those 12 lines.

So, the ability to utilize citrate is The ability to utilize citrate could come up—or let's say should come up—in any one of the 12 lines. It doesn't need to be one in any of the 12 lines. Should hypothesis 1 be correct? Because that rare mutation can happen anywhere: in any line, in any individual, in any of these flasks. So, there is no reason that it should again arise in the line which had already acquired the ability to utilize citrate.

So, every line has the same probability of evolving to utilize citrate. So that is the experiment they did to check whether hypothesis 1 was true or not. And if hypothesis 1

was true, this is our null expectation. Suppose the outcome from this was that when we redid the evolution experiments, line 3, line 5, and line 9 evolved the ability to utilize citrate, whereas in the first case it was line 7. Then hypothesis one would bear out that this rare mutation is now happening at some other place.

Of course, it is rare. We are saying that this has to be a very rare and lucky mutation. So this evolution has to be carried out for a long time for us to statistically be able to confidently say that this is what is happening. It turns out that literally trillions of cells were checked for their ability to utilize citrate. And in these 11 lines, the number of cells that evolved the ability to utilize citrate was zero.

So not even once did these 11 lines acquire the ability to utilize citrate, even when started from 28,000 generations. On the other hand, when individuals from this particular line were started for this re-evolution experiment from 28,000 generations, 19 different times individuals were found which acquired the ability to utilize citrate as this repeat evolution experiment was done. So, this gives us a pretty clear indication that it is not hypothesis 1, but in fact hypothesis 2, which is responsible for the ability to utilize citrate in this one line. Because what is happening is that this line already has these mutations available, And all it needs now for it to utilize citrate and exhibit higher growth rates is this one more mutation to happen.

And sooner or later, that one more mutation will happen and that shows as increased density in the culture media that we are using. On the other hand, these mutations can happen here, but they will have no effect because these other prior mutations that are needed for this functionality have not happened in these 11 lines. So through this, it was found that the ability to utilize citrate was actually acquired through a series of mutations and not just one very lucky, rare mutation in this particular flask. And then it took a few years, but a few years later we found out the exact mechanism as to what had happened, which led to this flask exhibiting growth on citrate.

And it's actually—we won't go into much detail—but broadly, what had happened was the following: the gene which encodes for transport, which encodes for the protein responsible for the transport of citrate, is called citT. Now, citT—its gene expression, first transcription, then translation, then getting embedded in the membrane, and then bringing citrate in. This is controlled by the region upstream, which is called the promoter region of citT. And this promoter region does not allow transcription in oxic conditions. So, this promoter region only allows transcription in anaerobic conditions.

As a result, the cells of E. coli in LTEE don't make this protein, and they don't have the citT transporter embedded in the membrane, which brings in citrate. So, what happened in the line that eventually exhibited the ability to utilize citrate was that this gene was duplicated into two. So now, this evolved strain has two copies of citT, and via this duplication event, in one copy, its expression was driven by the citT promoter, which is the same promoter as here. But in the duplication event, only this part was duplicated, and upstream of this gene was another promoter. This was not citT's promoter, but another promoter that was present here on the DNA.

So, this is another promoter. And now, even in the culture conditions we are talking about, growth is happening on citrate because this promoter is not dependent on anaerobic conditions to facilitate transcription. This promoter is actively transcribing, and then the transcript is being translated, and we get citT protein, which leads to the metabolism of citrate as a carbon source and enables growth to take place. So that...

So it turns out that even this much, even this fortuitous mutation of duplication taking place, but if duplication had taken place starting from this point, then its own promoter would be copied again, and that would be driving transcription. That wouldn't work because this promoter doesn't allow transcription in auxic conditions. So this was the fortuitous event that needed to happen. But it turns out that even with this genome, the growth rate benefit that is conferred is only about 1 percent. And that is—that is barely anything. That's a very small mutation.

Remember, that tells me that this is S equal to point zero one. This mutation, when it happens, only has a 1% chance of surviving drift. As we have discussed in class, that probability of surviving drift is actually just equal to S. So this mutation per se doesn't confer a huge benefit to the individual. However, what happens is that more mutations take place after this, which lead to a further increase in fitness.

So this is the molecular detail of what happened in this case, which allowed one of these lines to exhibit growth on citrate as a carbon source. So now, these complex evolutionary processes are thought to happen via three different steps. The first step is called potentiation. Potentiation is—again, let us go back to the evolution experiment—and this is at 35,000 generations. Potentiation is the occurrence of these prior mutations, which did not confer the ability to utilize citrate, but it brought the cell to such a point that the acquisition of one more cell...

precise mutation and the cell would become cit plus, which is how we refer to citrate utilizing cells. Up until this mutation, the cell was cit minus. But what these potentiating mutations did was that it brought the cell to a point that one more and it would become cit plus. If these had not happened, then this mutation wouldn't be able to make the cell cit plus. So, these are called potentiation mutations.

The second step then is actualization. So these three collectively belong to this group. The second is called actualization, which is the mutation that actually changes the phenotype from cit minus to cit plus. But actualization mutations will only realize this transition if they happen after the potentiation mutations have taken place in the genome of the organism. And third one, third set is called refinement.

And in refinement, what we saw that even with that duplication event in citT, this was only growing 1% better as compared to the cit minus cells. So the advantage isn't huge. But something novel has happened. The novelty is still bad and it can be improved upon by subsequent mutations. So, these are the refinement mutations that happen after actualization has taken place which further improve the trait that has been evolved as a result of potentiation and actualization mutations.

So these are roughly the three steps in which these complex traits are thought to evolve. One thing that this experiment should make abundantly clear is that so far we have been saying that evolutionary fate is decided by Selection. What sort of environment this population grew on is extremely important, but also drift or chance events. That is what we've been discussing so far.

However, what this experiment also shows us is that there is an extremely important determinant of evolutionary processes, which is the history of the population, the evolutionary history of the populations. And this was abundantly clear in the citrate example, because we saw that the ability to utilize citrate was only possible because, in this particular line, these potentiating mutations had already happened in its evolutionary history. On the other hand, in these other lines, other mutations had happened, which were not potentiating with respect to citrate utilization. So, at this point, when I do this re-evolution experiment from the freezer stock, even though—so at this point, this is cit minus, at this—these are the 11 lines, this is the one line.

At, let us say, this is 35,000 generations, this is cit plus, and this is cit minus. However, if I go back in time to 28,000, then even this is cit minus, and this is also cit minus. But when I re-evolved them, one of them acquired the ability to transition from cit minus to

cit plus 19 times. Whereas the other 11 combined transitioned from cit minus to cit plus 0 times. And the reason is because of the evolutionary history of these populations—what has been their trajectory, which has brought them to this particular point in these 30,000 generations or so.

So, evolutionary history is extremely important in the context of what can and cannot be achieved via evolutionary change by populations. In fact, in the early days of the LTEE, there is a classic paper in Science from 1995 by someone named Travisano and Lenski. which talks about very simple, basic experiments that allow us to figure out how much of evolution is driven by selection, how much evolutionary change is brought about by chance, and how much evolutionary change is influenced by the population's evolutionary history. We'll discuss this particular paper in our next video. It's also a paper that is a great illustration of how, by asking very precise questions and conducting simple experiments, we can gain deep insights into evolutionary questions.

We'll continue with that discussion in the next video. Thank you.