Evolutionary Dynamics

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Lecture 47

Hi, welcome back. Let us continue our discussion on the Serial Subculture Technique of Doing Evolution Experiments. So, what we saw is that we have a flask. We give a delta T amount of time for growth to take place. So, this is the time when growth takes place.

This was seeded by a few bacteria, and then after growth, this is full of bacteria and going from one flask to another. I take what is simply referred to as a 1:1000. So, this is the small volume V, which is capital V upon 1000. In literature, you will see this referred to as a 1:1000 dilution, which goes into a fresh flask. So, this goes into a fresh flask. This volume will contain some bacteria, and again, I will give delta t amount of time for growth, and this process just keeps repeating itself. So, if I look at the number of bacteria, this trajectory goes like this.

At t equal to 0—so this is time—at t equal to 0, I am starting with n naught number of bacteria. I give it delta t amount of time, so 0 to delta t, and then this growth takes place in delta t amount of time. After delta t time, I take one-thousandth of this number and put it in a fresh flask. So, there is a sharp catastrophe where the population size goes from a large number to n naught again. Let us call this as

And then growth in the second flask takes place, which allows for increase of numbers from N0 to N1 again. And this is now 2 delta T time. So you can see that there is this bust and boom type of phenomena associated with numbers of bacteria in the flask. And this will keep on happening with every flash. So simply we can sketch this as growth patterns like this.

That's the first thing that population sizes their numbers. They keep expanding gradually over time delta t. And suddenly then we apply a shock and the number just collapses by a ratio of 1000. The second thing we want to discuss here is that what does this number 1 is to 1000 mean? How many generations is it? So in a flask, I first add N naught number and this number increases 1000 fold.

So, this becomes 1000 fold. So, this reaches 1000 n naught and then I transfer 1000th of it that is only n naught to the next flask and then allow growth to take place. So, in a single flask the number increases from n naught to 1000 n naught. This is equivalent to we know the number of the formula for number of generations is k is equal to ln of base 2 of 1000 n naught divided by n naught. This just cancels.

This is of ln base 2 of 1000 and this is approximately we can check this. This is approximately 10. So, a 1000 fold expansion corresponds to 10 generations. And this is typically done. This is this number of thousand fold expansion and then contraction is a very typical number associated with how bacterial evolution experiments are done in a lab setting.

Some people do it 1 is 2000, 1 is 200, which corresponds to ln of base 2 of 100 n0 upon n0. And if you work out the math, this corresponds to 6.6 generations every day. An expansion of 1000 corresponds to 10 generations every day. So that is another aspect of it. Secondly, the time delta t is important.

So, if I have n naught cells here and I give it delta t and this number goes up to let us say n 1 and out of this I take the idea is that I will take 1 is to 1000. and I will get back n naught. But suppose the following happens. That when I start the experiment, I start with this is number of bacteria in flask, this is time. Suppose the experiment starts with N naught number of individual and then expansion happens and this becomes 1000 times.

And then I dilute this 1000 times and put it in the next flask here. And in the next flask, for some reason, maybe I forgot, for some reason or I added less nutrients here. For some reason, I didn't let it reach N1. I stopped it before it reached N1 and did a 1 is to 1000 dilution at this point. So remember this N0 was 1000th of this N1.

But now if I did a 1000 dilution at this number N2, the next flask is going to get far fewer number of bacteria, which is going to be N2 by 1000. N naught is simply N 1 by 1000 and since N 1 is bigger N 1 by 1000 is going to be bigger than N 2 by 1000 because N 1 is bigger than N 2. Now, maybe I was doing this after 24 hours, but now I am doing it after only 15 hours and in 15 hours the population cannot expand 1000 times. So in the next one what's going to happen is that since I'm only going to give it 15 hours, this will not expand 1,000 times because if this expanded 1,000 times, I will get back to N2 but this now is only able to go up to N3. And I will do N3 by 1,000 and so on and so forth.

So what you will see is that the population starts to collapse And you will see this in your flasks that as the number of days progresses, as the number of days increase, the density of the flask approaches zero. And this is happening because this shock that you are providing to the system causes for a reduction in the population by a ratio 1 is to 1000. Whereas the delta t time you are giving is not able to expand the population 1000 fold. So maybe this expansion only took place 600 fold.

That means when you did another dilution of 1000 fold this came to 0.6 instead of 1. and so on and so forth. So, this number will keep on decreasing because you are only allowing for 600 time expansion whereas the bust cycle that you are operating causes for a 1000 fold reduction. So, numbers will keep on dropping. So, whenever you are designing an evolution experiment you have to make sure that there is sufficient time and opportunity

for an equivalent expansion to take place such that when you apply the dilution factor of one is 2000 or one is 200, that has been recovered in the expansion phase that you did over 24 hours or sometimes it's done over 12 hours and so on and so forth. Otherwise, the population cultures are just going to crash. Okay. Now, let's compare two scenarios In one scenario, we are doing this experiment in a test tube.

I let this grow for 24 hours and then I take 1 is to 1000 in a fresh tube of identical media conditions and I let growth take place for 24 hours and this cycle keeps repeating. So in this test tube, it's 10 generations every day. Because in every generation as the number expands from N naught to N1, 10 generations happen and we know this because this expansion is 1000 fold. So let's say I do this experiment for 50 days. That means I have processed about 500 generations.

On the other hand, I do the same experiment in a flask now, a much larger flask. So I allow for growth for 24 hours, and then after growth, I do a 1:1000 dilution, and the same story repeats. At the end of 15 days, this case also has 500 generations. So as far as the number of generations, the duration of growth, and the number of subcultures that took place, both these are identical to each other. However, the only difference between the two scenarios was that the number operating here was, let's say, N0. After growth for 24 hours, the number of bacteria in the tube was N0.

Whereas after growth in a flask for 24 hours, it was N1. And let us assume that N1 is much bigger than N0, maybe because this was done in a 50-milliliter culture, whereas this was done in a 5-milliliter culture. Again, these are typical values used in lab

experiments. So, does this make any difference? Now, if you think about it, let us say N0 is simply 10 to the power of 10, and this is 10 times that.

So, this is, let us say, 10 to the power of 11. What is happening in the test tube case is Let us look at one cycle. So, the number increases from 10 to the power of 8—I am sorry, this is a 1000-fold increase. So, the number increases from 10 to the power of 7, increases 1000-fold, goes up to 10 to the power of 10, and then I apply a 1:1000 dilution, and the same story repeats itself, and this will keep on going.

Now, in one test tube, the number of cell divisions that have taken place is equal to 10 to the power of 10 minus 10 to the power of 7, right? We saw this in an earlier video in the course that if we start with an N1 population and the population expands to N2, the number of divisions that have taken place is simply N2 minus N1. That is exactly what is happening here. So, this is the number of divisions that took place, but I also know that the mutation rate is equal to mu. So, the number of mutations that happened in the test tube is equal to 10 to the power of 10 minus 10 to the power of 7 times mu, which is approximately 10 to the power of minus 3.

So, this gives me the total number of mutations that happened in a test tube every single day. On the other hand, In the case of the flask, the starting point of the population was 10 to the power of 8. And this population expanded to 10 to the power of 11 in one day. And then I did a 1 to 2000 dilution, and the same story repeated itself.

So, in one day of growth in the flask, the total number of divisions that took place increased. It was 10 to the power of 11 minus 10 to the power of 8, and then the mutation rate is mu. So, the total number of mutations is equal to 10 to the power of 11 minus 10 to the power of 8 times mu, which again is 10 to the power of minus 3 because it is the same organism. Now, if we compare these two scenarios, this is the test tube. This is the flask.

Number of generations, number of transfers, the 1:1000 dilution factor, the organism of choice, Everything between these two scenarios—the media in which this is happening, everything or everyone among this list—is identical in the two contexts. The only difference is that in one round of growth in test tubes, the number of mutations taking place is 10^10, I think this was 10^10 minus 10^7 multiplied by μ, which is 10^10. I take 10^10 common, and this becomes 0.001 multiplied by μ. This is 0.001.

999 multiplied by 10^10 μ. Whereas in the flask, this number is 10^11 minus 10^8 multiplied by μ, which is going to be 10^11 (1 minus 0.001) multiplied by μ, which is

0.999 multiplied by 10^11 μ. So we see that the number of mutations happening in the flask is actually 10 times higher than the number of mutations taking place in a test tube. What do you think is the difference that this particular aspect of the evolution experiment changes? The fact that everything is the same—the number of generations is the same,

However, in one scenario, because the population size was much larger, there are tenfold more mutations taking place compared to this scenario. And we will discuss the implications of this, but population size as a variable is an important consideration in the design of evolution experiments. The last idea that we want to discuss with serial subculture is actually the disadvantage of doing it. The disadvantage is that in one cycle, the number of bacteria—so the population size—increases from N_0. So let's say this is the population size.

This increases from N naught to N1. On the other hand, the resources available, let us say glucose concentration, decrease from some starting concentration to almost nothing by the end of this. This is time. The glucose is almost gone. In the window of this 24-hour period, what is happening is that the environment that cells are witnessing, the environment that cells are witnessing,

continuously changes with time during these 24 hours. Here, the number of individuals was very few, and there were lots of resources. After some time, there is a greater number of individuals, resources have become fewer, but perhaps the situation is not that bad anyway. And at the end, towards the end of this 24-hour window, there are lots of individuals. So the culture is now extremely dense.

However, there are no resources left. And of course, we are looking at these three extreme points of this growth curve. But what's happening is that this is gradually transitioning from being an extremely rich, resource-rich environment at the start of this 24-hour window. And transitioning to becoming an extremely nutrient-starved environment by the end of this 24-hour window. And this transition takes place in the course of one culture of this batch growth that we do in serial subculture.

And we repeat this process every single day. So as a result of that, cells are not responding to a constant environment. to a constant environment, but in fact an oscillatory environment. Because right after this stage, we are going to go back. This is going to get a 1 is to 1000 dilution and come back here.

And this is going to get replenishment in the new flask and go back here. So this process is going to keep repeating itself. So the environment that cells are seeing is not a constant environment, but it's an oscillatory environment which follows this cycle of 24 hours. So, what I am seeing at the after 500 generations when I characterize the performance of the populations in test tube or flask and I genome sequence it, I am not seeing the response of the population to a constant selection pressure. However, I am seeing the response of the population to this oscillatory selection pressure that I have placed on the population.

That is the big drawback of using these batch type of cultures with serial subculture built in, that selection is not uniform. It changes every instant and it forms this cyclical pattern of every 24 hours. However, despite that, well over 90% of all lab evolution experiments use this technique. It is easy, it is cheap, there is robotics available now to do all the serial subculturing and the last benefit associated with it is that suppose I am doing this experiment that there is growth for delta T and then there is subculture in a fresh tube and then there is growth for delta T And so growth takes place, growth takes place and then there is subculture 1 is 2000 in a fresh tube and there is growth and so on and so forth.

So, this pattern continues for as long as we want to propagate the evolution experiment. What is also very easy in a context like this is not just to have one such line of evolution. So, suppose I'm studying the response of E. coli to glucose. This is one population which is evolving in an environment where glucose is the only carbon source. But in parallel to that, I can start this evolution experiment with another tube,

which carries the same bacterial species, everything is the same, and I am doing the identical experiment. So, these two are identical experiments. I had an E. coli genotype to start with. Let us say this is the E. coli, this is its genome.

I added N_0 individuals to this tube and N_0 individuals to this tube, and I started the experiment. So, the initial starting point of this experiment is identical because I'm adding the same bacteria with the exact same genetic sequence to the culture tubes. The recipe, the nutrient conditions that I'm providing in the two tubes are identical. Both these tubes contain the exact same amount of glucose and every other resource.

So this is identical with respect to glucose, other nutrients, temperature, rotations per minute for shaking. Every aspect of their growth is identical. The subculture ratio of 1 to 1000 is identical. The time for which I allow growth in either one is exactly the same. So, these two experiments are identical in every aspect that we can think of.

However, what will happen is that at the end of this experiment, I will get one bacterium from here and another one from here, and if I look at their DNA, this is this DNA, this is that DNA, and I identify where all mutations have taken place. This individual will have maybe these three mutations, whereas the other individual may have some other mutations. This is despite the fact that everything we did with the experiment was identical in nature. So where did this difference come from? And that has to do with the stochastic nature of evolution.

Because the first mutation will take place at some part of the genome in one of them and in another part of the genome in the other, and subsequent mutations also have this chance event of happening or not happening. So, each line will acquire its own unique set of mutations, and typically when we do this these days, we do not do it for one or two such lines; we do it for several hundred, several parallel lines. Or let's say of the order of 100 parallel lines. Each one is given the identical treatment. We process each one for G number of generations.

Let's say G is 1000. And then, at the end of 1000 generations, we sequence all of them. And we find out the nature of identical mutations, which is conserved evolution, and the nature of unique mutations associated with each one of these lines. So, this sort of wraps up our preliminary discussion of serial subculture, by far the most widely used experimental technique. And next time, we will start discussions on some evolution experiments.

We will continue this in the next video. Thank you.