Evolutionary Dynamics

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Lecture 59

Hi everyone, welcome to the next lecture in the course Evolutionary Dynamics. So in the last video, we were discussing a case where, working with bacteria, we talked about the scenario of adaptive diversification. That is, how do we have this process of diversification where we start with a single genotype but diversify? The eventual outcome of the evolutionary process is that the population diversifies into various specialist groups. And we discussed this case of Pseudomonas bacteria, which, when evolved in a static tube, developed three specialists: one of which was a specialist at the air-water interface.

The second one was a specialist in the bulk liquid. And the third one was a specialist at the glass surface. And this diversification happened in a reasonably short amount of time. But the only caveat here was that this culture was kept static, so there was no shaking. So the environment in which this population is evolving is itself providing three different niches.

So we call this system one where space is a variable. And what I mean by that is that in the environment in which evolution is taking place in this tube, if this tube is shaking, then all its constituents are well mixed. There are no concentration gradients of oxygen, metabolic waste products secreted, or resources available in the culture. There are no gradients. Everything is well mixed, like the chemostat scenario that we've been discussing in the course.

However, if I keep this static, then what is going to happen is that maybe there is a gradient of oxygen in this direction. This is the oxygen gradient. Maybe the cells are going to settle down more at the bottom. So, the cellular gradient is going to be like this. And as a result of this, space becomes a variable.

My coordinates—what is the location—if I am a cell in this tube, the environment that I am facing is a function of my coordinates inside this tube. That will not be the case if this test tube was well mixed. So, in this scenario, we see adaptive diversification take place. When space is a variable. And this is a classic paper from 1998 by Travisano, which demonstrated that Pseudomonas, the bacterium, could diversify in a really short amount of time.

And then we move down to the case where our group has been discussing—our group has been trying to develop systems where we might see diversification as an evolutionary outcome. But space is not a variable. That means in well-mixed cultures, and the strategy that we've been using is one of public goods. So, in public goods systems, we have we have yeast—that's the model system that we use in our lab—and let's say there is a sugar which is a disaccharide where glucose and galactose are linked together with a bond, and what yeast does is that it secretes a certain enzyme in the environment, so this is an enzyme

Enzyme remember is just a catalyst and the goal of this enzyme is to break this glucose galactose disaccharide into glucose and galactose. And if this happens, then what has happened is that now we have created an environment which prior to hydrolysis, before hydrolysis took place, there was a single resource available which was this disaccharide, which is called melibiose. But post this hydrolysis, we have created an environment where there are actually not one, but two resources now. And what this, the availability of these two resources creates an opportunity

for diversification to take place. So when this is work that our group has published in the last couple of years is that when we evolve a population, an isogenic population is evolved in this environment. What happens in this case is that the end of evolution experiment and this process what we have tested is for a few hundred generations. So, of the order of 500 generations. After few hundred generations, if this was the DNA of the yeast that we started with, we get two different types of populations coexisting in the tube where one is carrying one set of mutations and the other one is carrying a different set of mutations.

And the difference between these two genotypes is that as evolution proceeds in this environment, This one group acquires mutations to become a specialist at using glucose. And the other genotype becomes a specialist at using galactose. So this diversification takes place In the period of a few hundred generations, when everyone, the starting

population was isogenic, it was identical as far as the genotype of every individual was concerned.

Interestingly, if we do the same evolution experiment in a mixture of glucose and galactose—so now I am starting with the same isogenic population, but the environment in which I am evolving it is glucose plus galactose. And the public good is no longer required, then what happens is that the entire population first uses up glucose in the flask. When all the glucose is gone, then the entire population switches to galactose, and we see this transition take place. So, at any given point in time—if we draw what happens in this particular case, let us say— This is time.

And in our flask, I am looking at OD. So, optical density in this particular scenario increases like this. This is the phase where growth on glucose is taking place. And then, at this point, all the glucose is gone. So, in the next phase of growth, growth on galactose takes place.

And these are sequentially used. What that means from our context is that if I track the sugars—the two sugars that we are using—if I am tracking glucose in this flask, glucose is going like this. All the glucose is gone. By the time growth on glucose is done. And if I am tracking galactose, the galactose profile looks something like this.

And this is how galactose is going to get used. The point being that at any given point in time, at any given point in time is growth is happening on a mix of glucose and galactose, then up until this point, up until this point, everybody in the population is growing on glucose. And after a certain point, after this point, everybody in the population is growing on galactose. So the entire population has shifted from glucose to galactose.

If I take a snapshot of the population at one instant of time and if that instant of time happens to be here, then I will see in that snapshot that every member of the population is growing on glucose. If that snapshot happened to be here, then I will see that every member of the population is growing on galactose. So, at a given instant of time, there is no diversification of strategies that is being employed by members of the population. That is not true for the public good system because at any given point in time, there is only a small amount of glucose and galactose that is available. Most of the carbon resources is packed away as this disaccharide glucose and galactose and this cannot be used by yeast unless it is first hydrolyzed into glucose and galactose.

So at any given point in time, there is a small amount of glucose and a small amount of galactose that is present. Most of the carbon is actually just present as melibiose. And hence that scarcity of resource. places of selective pressure on the population to not compete over the two resources that are available in very small amounts, but instead devise a strategy where one group specializes in using small amount of that sugar and the other group acquires mutations to become a specialist at using the other sugars. Hence, this diversification that we see

Diversification is contingent on public goods, public good which is melibiose's hydrolysis. And this is something that our group published in the journal called Evolution in 2022. Interestingly, this is not an isolated system that is present in yeast. There are several other systems in yeast which exhibit this property. For instance, there is this disaccharide called sucrose.

which is comprised of not glucose and galactose, but this time glucose and fructose. And in this occasion, the enzyme is not secreted out into the media. The enzyme is just secreted in the periplasm. So, enzyme in periplasm. And the sugar comes to the periplasm and there it is hydrolyzed by this enzyme resulting in release of glucose and fructose.

It is not square but triangle. So this is broken apart here and this is fructose in this case. and this is glucose. So, these two come to the periplasm are hydrolyzed there and then the resulting monomers which are glucose and fructose will then be utilized by the cell. Interestingly, if we look at the melibiose example, both glucose and galactose that are produced after hydrolysis of melibiose are present in the extracellular media to be used by any individual that is present in the population.

That is not the case in this particular example of fructose. What happens here is that because I am the individual cell in whose periplasm this hydrolysis is taking place, of all the monosaccharides that are produced, people have quantified that about 98% of all the monosaccharides are released into the environment. But about 2% are for my private use. So this is an example where this enzyme is producing a public good because most of the glucose and fructose is available to be used by anybody in the population. However, I retain a very small fraction of these two sugars for my own particular use.

And this is sort of called the phenomenon of partial privatization of a public good. And this has interesting ramifications that our group studies. Lastly, there is another interesting system in yeast, which is now a mixture of these two systems that we looked at. And the sugar in this case is called raffinose. So, it is galactose, glucose, and fructose.

This is galactose, glucose, and fructose. And now this can be hydrolyzed in a variety of ways. One of the ways could be that the first enzyme that we talked about is released into the environment. This enzyme hydrolyzes this particular bond. So what you get—remember, this enzyme breaks down the bond between galactose and glucose.

So what we get is galactose. Plus glucose fructose which are tied together which is sucrose. This trisaccharide of three sugars is called raffinose. Galactose can be used by the cell. The sucrose has to go to the periplasm where this other enzyme is also released.

And now the resulting sucrose that we have is going to be hydrolyzed into glucose and fructose. So that is one way. Alternatively, we could have the trisaccharide come to the periplasm and this enzyme in the periplasm is going to hydrolyze the bond between glucose and fructose. So, we are going to get fructose, but also the disaccharide of galactose and glucose, which is just mellebios, which is going to be used up which is going to be hydrolyzed by this enzyme release in the extracellular environment and these disaccharides molecule will be hydrolyzed into galactose and glucose.

So, all these are public good driven systems and they offer these opportunities of studying diversification in an environment where space is not a variable. For example, this particular study from our group, this diversification happens when growth is taking place in a flask, but this flask is being well shaken so that the environment inside the flask is the same everywhere. It's homogenized and it's like a CSTR working where there are no spatial gradients inside the culture volume. So these are some examples of diversification that we can see happen from the context of whether in static environment or in cultures which are shaking.

But then we need to move to these special systems where public good-driven dynamics dictate what is going to happen in a population. Let me quickly cover one aspect of evolution that we didn't cover. So, insofar, what we have assumed is that synonymous mutations—remember, synonymous mutations are those mutations where if this is a codon and a nucleotide change takes place, let us say here, but both these codons encode for the same amino acid. The identity of these two amino acids is the same. And in this case, we say that this particular mutation happened.

It did not lead to a change in amino acid. And because it does not lead to a change in amino acid, what is going to happen is that the protein sequence associated with these two cases— So, imagine that this is DNA sequence 1, this is DNA sequence 2, and this one has a mutation here, which is a synonymous mutation. So, what is going to happen is

that after transcription, we will get mRNA. And after translation, we will get a peptide chain.

Amino acids link together. After translation, we will get— And while this DNA sequence and this DNA sequence are different because of this mutation, this mRNA sequence and this mRNA sequence are different because of this mutation. At the amino acid level, because this mutation was a synonymous mutation, there is no difference between the identity of the amino acid chain in this case and the identity of the amino acid chain in this particular case. So, as a result, because—

the resulting amino acid chain is the same, we made this assumption that synonymous mutations are going to be largely neutral because they have no effect in terms of the protein amino acid that amino acid chain that is going to get synthesized and hence it may not have fitness effects. However, in the last 10-15 years or so, There has been lot of evidence which has suggested that synonymous mutations are actually not always synonymous. So let me just tell you briefly what are the different cases that we know of where synonymous mutations are actually not neutral. They have a fitness effect.

It could be beneficial. It could be deleterious. So let's imagine that we have a DNA sequence. DNA and then transcription is going to take place and the resulting will be an mRNA. This mRNA, mRNAs are usually they have a very short half-life compared to DNA.

It is an unstable molecule mRNA. This mRNA degrades. And there is certain half-life associated with it. But it is also going to get translated, translation, which will lead us to amino acid chain. And this is going to fold into protein.

That is the scenario that we have in front of us for any piece of DNA. Alternatively, let us imagine this scenario that we have this same DNA sequence except for the fact that there is a synonymous mutation here. Everything else is identical. So, we have transcription taking place leading to mRNA. The mRNA of course is going to be different now because of this synonymous mutation.

This mRNA also is going to degrade and this mRNA will also be translated leading to an amino acid chain whose identity is exactly the same as in the previous case leading to this protein. So, so far we have been saying that these two cases are near identical and there is no difference between the two. However, what evidence in the last decade or so has shown is that these two are not identical and very often synonymous mutations can

actually have fitness effects. So, let us see what are the different ways in which synonymous mutations will make sure that these two are not identical. So, the first way

it can happen is alter mRNA stability. A synonymous mutation, this mRNA molecule will have a structure which minimizes free energy and so will this one. And it's possible that acquisition of this particular mutation changes the structure of the mRNA and makes it less stable or more stable. Less stable mRNA means less mRNA molecules at any given point in time. As a result of this, less translation happens, as a result of which less amount of protein is made and the amount of protein may dictate what is your fitness level.

So, synonymous mutations can alter mRNA's stability. So, again, if this mRNA is less stable, that means at steady state, maybe in this particular version, there were 20 mRNA molecules at steady state. In this case, there will only be 10. And let us say this means that the number of protein molecules will be 100 in this case. In this case, it might be 50.

And 100 will do the job much more effectively, and hence there will be a fitness cost associated with having this synonymous mutation in the mRNA. So, this is the first scenario. The second scenario is that because the mRNA that has a synonymous mutation is going to fold differently compared to the original mRNA sequence, it might hinder the ribosome's access. Remember that translation starts by the ribosome coming and binding here and then moving across the mRNA. If the mutation is in a location such that the mRNA structure folds into a very tight hairpin or it makes a structure such that access to the mRNA is not very easy.

In that case, the translation rate is altered. Another mechanism in which this can happen is that we saw that for most amino acids, organisms have a choice of encoding that amino acid via different codons. Each species tends to use some codons, which are called frequently used codons, and each species has certain codons which are used very infrequently, called rare codons. And experimental evidence has shown that rare codons are translated more slowly. As a result, the rate of translation slows down.

And again, that has the same effect as blocking ribosome access to the Shine-Dalgarno sequence where it can come and bind the mRNA. So, synonymous mutation, the point being that synonymous mutation can also alter the rate at which translation is happening. which means that now mRNA numbers are the same this is 20 but because this is being translated better it has 100 copies this is not being translated better so this might only have 70 copies and because you have different amounts of proteins this will have a

fitness effect the third case the third final case is a very interesting case that we know of from the last few years And in this case what happens is that we have DNA and we get mRNA. Now this is the normal mRNA and ribosome comes and binds here.

And as it moves across the mRNA, the peptide chain is coming. This is the peptide chain. Let's say this translation process was really fast. So you have this peptide chain synthesized quickly. And this peptide chain is released and this folds to its free energy minimum.

And this gives me the protein structure. What we now know is that the following can happen. Now imagine that a synonymous mutation happened here. Transcription took place, so you have mRNA having a rare codon here.

What that means is, as this was being translated by the ribosome, remember rare codons are translated slower. As a result, when the ribosome comes to this rare codon, you have this amino acid chain, which has already been translated, coming from this part of the mRNA. And the ribosome, when it comes, pauses there because it has to wait for the appropriate tRNA carrying the amino acid encoded by this rare codon here. As a result of this, there is a pause here. There is a halt—a transient halt—in the process of translation.

In that process, this chain is not going to remain as it is. This folds into its free energy minimum. So what will happen is that I will get a structure like this. This has folded now. And then, when it resumes translation, I will get a structure like this.

Which comprises the folded part of the first half when the ribosome paused and the rest of the unfolded one, which is now going to fold. And I will get this particular shape of the protein instead of this. And remember, all of this has been obtained only because of a synonymous mutation, which slowed down translation. The amino acid identity didn't change. It just slowed down translation.

So a synonymous mutation, in fact, can also lead to a change in the shape of a protein. Change in protein shape. And this has to do with the fact that translational co-folding, or this is called co-translational folding, happens. The rate of translation was altered because of the synonymous mutation, and that leads to this change in protein conformation. Now, these could be beneficial or these could be deleterious, but the point is that a synonymous mutation can have different effects on the protein amount or protein structure via these three different ways.

We will continue this discussion in the next video. .