Evolutionary Dynamics

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Lecture 58

Let us continue our discussion on evolution of multicellularity. So, as we were discussing last time that we have a tube and The first phase of this experiment is only selecting for fast growth. After this fast growth, when this culture is occupied by yeast, we let the cells settle for some delta T amount of time. And in this time,

cells will have different differential propensity to sink in the tube some cells will settle faster some will float and at this time i sample from the bottom of the tube and only transfer that one is to 100 fraction to the next tube and this selection pressure is important to understand because what you are doing in this scenario is placing a hard constraint At this point, what is being done is that if you settle first, then you make it to the next round and you make it to the next round and you stay in the experiment. If you settle first, then you stay in the experiment. If, however, you are not settling fast, then you go extinct. And we never see these cells again in the evolution experiment.

So the selection pressure is a combination of fast growth and settling fast. What transpired in this experiment was that each cells have a transcription factor called ACE2. And a mutation was acquired in ACE2 such that this transcription factor, remember transcription factor controls transcription of other genes. So this transcription factor controls expression of proteins of these genes whose resulting proteins are responsible for, so how cerviciae divides is that it forms a bud, the bud then grows and then finally some pinching off happens here and you get two individual progeny cells.

But this final event of pinching off is dictated by several proteins, and the expression of these proteins is controlled by ACE2. What happened in this experiment was that the gene responsible for ACE2 picked up a mutation. So, a mutation happened here, as a result of which ACE2 was not functional or was not formed. Because there was no ACE2, the expression of these genes did not take place.

These proteins were not formed, and as a result, this pinching did not take place. And as a result, because these cells could bud, but these buds could not separate to become individual unicellular cells, what we end up getting are these multicellular clusters. And there are beautiful images of this if you just search for snowflake yeast. Because they look like snowflake clusters. This was first discovered around 14 to 15 years ago.

Since then, at Georgia Tech, there is a lab run by William Ratcliffe, which has started an evolution experiment that begins with multicellular yeast and evolves this multicellular yeast. It tries to study what happens to this snowflake yeast cluster that I get if I evolve it for a really long time. Some recent results from that experiment show that as these clusters—so let me just draw this cluster. So, let's say this is a cluster; it's much bigger than this.

I urge you to sort of just do a Google search of Snowflake yeast and the images are quite stunning. So as now this experiment has going on for a long time and this experiment is around 10,000 generations have already been processed, starting with a multicellular cluster. Now what is beginning to appear is that the cell at the center is sort of tasked with holding the entire cluster together. So then its mechanical properties... are quite different as compared to a cell at the periphery, which doesn't quite have the same mechanical stress that this central node of the cluster has to face.

On the other hand, the central node has very little access to resources because it's just embedded at the center of this cluster with cells all around. So this has limited access to resources. The cells at the periphery, however, have great access to resources. So the access to resource is differentially spread over the cluster. And the mechanical loads, the mechanical demands that are being made up of different made of different cells in the cluster are also differentially spread.

The demand is much higher at the center of the node as compared to periphery and access to resource is much higher at the periphery as compared to center of the node. So what is beginning to be seen is patterns of differential gene expression where the gene expression that this central cell is exhibiting has to do with greater mechanical strength and fewer protein expression which are responsible for bringing nutrients in. On the other hand, cells at the periphery, their gene expression is more to do with import of resources, which will then be shared with other cells in the cluster, and has less to do with mechanical strength because its location in the cluster doesn't demand that. So these are first

signatures of diversification happening in a cluster where all cells are genetically identical. And this is important because this is what our cells are also doing.

All of our cells are genetically identical. However, their manifestations are very, very different. A nerve cell looks very different from a skin cell or a liver cell, and so on and so forth. This is despite them having the same genetic content. And that is because the genes being expressed in one cell line are very different from those expressed in another cell line.

Hence, that differential gene expression gives the cells different properties. Hence, they are able to complete the respective tasks they perform in the body. And the signatures of the same phenomenon are beginning to appear in this evolution experiment in Ratcliffe's lab. But I also think that, going forward in the years and decades to come, this experiment will reveal some unexpected findings, which will be of great value in understanding how evolution works. All right.

The next experiment we'll be discussing has to do with how diversity is brought about. So if I'm starting with an isogenic population—suppose I have to design an experiment where, starting from an isogenic population— what should I do such that this population splits into two or more groups, where one group of cells has picked up one type of mutation and the other group has picked up another mutation? Starting from an isogenic population and leading to this diversification process increases the genetic diversity in the population. This process is also called adaptive radiation.

I started with one type, and it just diversified into many, many types and occupied all the different niches that were available in the environment that I provided the cells. This is obviously important from the context of understanding how niches are occupied. If I have, how many species will be supported in a particular niche in an ecological setting? It's important from the process of understanding, from the context of understanding what the processes and forces are that generate biodiversity. Because if you think of it starting from one and ending with five, that is the process of generating diversity in species.

So, it's an important question from a variety of contexts. And I want to discuss two papers that have demonstrated the generation of diversity in microbial populations. The first paper is by Rainey and Travisano. in 1998. This is the paper not with E. coli but with Pseudomonas, and what they do is grow Pseudomonas in a test tube with a defined growth media.

However, what they don't do is shake the tube. They just keep the tube static. So, no shaking. What this does is create different niches available in a microcosm as small as a test tube. So, if there is shaking taking place, then each cell is being mixed and driven to different parts of the tube.

Any gradient that might accumulate—so, let us say that this part of the tube, if this is kept static, will have more oxygen available compared to here. But if you shake it, So, let us go back. If this is static, then this part—the top part—will have more oxygen compared to the bottom part, and this establishes an oxygen gradient. So, what it does is create niches here in a single test tube: one niche with low oxygen availability and another with high oxygen availability.

Usually, we shake these culture tubes—like the flasks carrying the LTEE populations—which are being shaken. But if you do that, you destroy that gradient, and the whole environment inside the test tube is homogenized into one setting. So, In that context, there is only one niche. Space is not a variable.

In this context, when it is not shaking, space is a variable because, depending on my location—my coordinates in the tube—the environment I see is quite different. So, if you think about this tube, there are three different niches available here. The first one is this—the air-water interface. The second one is the glass surface. And the third one is the bulk liquid.

And starting with the population of isogenic Pseudomonas, if we propagate the population like this by just letting it sit without shaking, very soon what we find is that the bacteria occupying these three niches are genetically no longer distinct. So let's say these are three Pseudomonas populations: one sticking to the surface of the tube, a second one floating in the bulk liquid, and a third one at the air-water interface. Each one of these niches is occupied by

And what was shown is that the number of mutations—so we can think of this as this particular genotype being the specialist at surviving at the interface. This one is a specialist in surviving in the bulk, and this is the specialist that survives best at the glass surface. So this adaptive radiation—starting with just a single genotype and evolving into these three specialist genotypes fairly quickly—is an important study in understanding processes that lead to the generation of diversity. However, what this does is study diversity in an environment where there are three different niches available to the population. Can we create experiments where diversity is generated

despite there being no separate niches? Suppose this tube was shaking—would we see that diversity? And the answer is no. People have studied the adaptation of Pseudomonas in shaking flasks and shaking tubes, and there we don't see this diversity. So the evolution of this diversity is contingent on the presence of these three niches available to the population.

So the question that we ask for the next paper we discuss is: Can diversity be generated in homogeneous, spatially homogeneous conditions? So, the work that we just discussed has these three niches, as we discussed. The air-water interface, the glass surface, and the bulk liquid. But if this tube was shaking, then these three wouldn't exist because any individual— So the oxygen concentration would get homogenized because of the shaking.

Anybody sticking to the glass surface would be swept off by the convective currents, and so on and so forth. So the question is: Can diversity be generated in homogeneous environments? And this is sort of an area where our group has been working for a few years now. And the system that we work with is these disaccharides. So let us consider a molecule of lactose, which is comprised of glucose and galactose.

Now, interestingly, glucose and galactose can be joined to each other in another way. So another kind of bond occurs. It can link glucose and galactose together. So this square is now rotated 45 degrees and linked with glucose. This disaccharide is called melibiose.

E.coli bacteria can grow on both these sugars. And the way it grows on these sugars is that it first internalizes these sugars. So the lactose goes inside. The same happens with millibars. Then we know that lacZ acts on this.

And what we get as the next step is hydrolysis of lacZ inside the bacteria. And we get glucose and galactose released inside the bacteria for its consumption for growth and energy purposes. So the key idea here is that this hydrolysis step, the disaccharide, whether be it lactose or mellibers, is first internalized. and then hydrolyzed to monosaccharides. So if that is the case, then what is present in the environment is only lactose or mellibers because this molecule is just internalized and then hydrolyzed inside the cell.

However, yeast utilizes these disaccharides slightly differently. And we'll discuss three, I'll show you three different cases of how yeast processes these multiple monosaccharides which are linked differently. In the first case, the example is disaccharide which is called

sucrose, which is a combination of glucose, let me see here, And what happens in this case is that yeast does not internalize sucrose and then hydrolysis. In fact, yeast brings it to its periplasm and does hydrolysis here with the help of an enzyme called suc2.

The name is not important, but there is some enzyme which is encoded for in the yeast genome that it uses to hydrolyze sucrose without internalizing it. And the resulting monomers, glucose and this is a triangle, glucose and fructose, 99% of them are diffused away in the environment and So this is much over 95% and only a few percent of them are retained by the cell for its own use. The rest of the monosaccharides which are released into the environment are available for everyone in the population including itself. So this creates a different dynamics as compared to an E. coli growing on these monosaccharides because for E. coli, the disaccharide is first internalized and then hydrolyzed.

So whatever is hydrolyzed. The product of hydrolysis are mine only because I first internalized it and it was inside me that I hydrolyzed it. So the glucose and galactose resulting from that process are only to be used towards my growth. That is not the case in this context where sucrose is being hydrolyzed outside in the periplasm of the individual. And from there, the resulting monosaccharides, glucose and fructose, an overwhelming majority of it is released into the environment.

And I only keep a very small fraction for my own use. Now, that is not to say that this 95% is not available for my use, but it is to be equally shared between all members of the population that are surrounding me. The next case that is there in yeast is a sugar called melibiose. Remember Melibiose is glucose and galactose and these two are linked differently than lactose. So this is glucose, this is galactose.

In this scenario, We have yeast, which releases an enzyme. So, an enzyme which is encoded by a gene called MEL1. Again, the name is not important. This enzyme's job is to hydrolyze the melibiose present in the environment into glucose and galactose.

In the previous case, we saw that hydrolysis took place in the periplasm, and for its own use, it kept 2-3% of the monosaccharides. In this case, even that is not there. The enzyme is being released into the environment, and hydrolysis takes place in the extracellular environment. So, these monosaccharides that are released are available for anybody in the population. Right.

This is an example of what is called a public good. Where the resource does something which is for the benefit of the entire population. So imagine that there is a species present here which cannot grow on melibiose. So if yeast is not there, then this species cannot grow because it can't utilize melibiose.

However, if yeast is present, it hydrolyzes melibiose, leading to the release of glucose and galactose, which can then be used by the species that is present. So the question then is, why is yeast working towards such an inefficient process? Scheme of utilizing melibiose where the benefit—where the work that it is doing to hydrolyze melibiose into glucose and galactose—is actually being reaped by other species and individuals present in the environment. The third is a sugar called raffinose, which is a link between galactose, glucose, and fructose. And the way this is used is via a combination of the two strategies that we have already discussed.

So if we have this sugar in the environment, then MEL1 is secreted, and MEL1 hydrolyzes this particular bond, which releases galactose and fructose. The fructose is brought to the periplasm and is hydrolyzed here, leading to the release of glucose and melibiose. This is sucrose. The sucrose is then brought to the periplasm, leading to hydrolysis of sucrose and the release of glucose and fructose.

Alternatively, raffinose can be brought to the periplasm, and SUP2 acts on this, which leads to the release of melibiose plus fructose. This melibiose can then be acted upon by MEL1, which leads to hydrolysis of melibiose and the release of glucose and galactose. So the point is that there are these several public good-type systems in yeast, and our group has been using these systems to understand processes of diversification and how populations interact with each other in the context of these public good systems. We'll continue these discussions in the

Next video of the course.