Evolutionary Dynamics
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Lecture 14

Thank you. Hi, everybody. Welcome back to the next lecture of the course. As we have seen so far in the course, the mechanisms of generating diversity differ when we compare prokaryotic populations to eukaryotic sexually reproducing populations. In prokaryotic populations, the mechanisms of generating diversity primarily involve novel genetic variation, which occurs via the acquisition of new mutations.

Whereas in eukaryotic, sexually reproducing complex organisms such as us, diversity is primarily generated via recombination. One of the major reasons for this is that the number of mutations occurring in a population is proportional to the population size. As we saw, for example, in a bacterial population, even in a 100-milliliter culture flask of nutrient media, the population size can be as large as 10^10 or even 10^11. In contrast, the human population is of the order of 10 billion worldwide. Since the number of mutations in a population is proportional to its size, these mechanisms vary.

Another important factor is that in prokaryotic populations, the generation times are very short. A commonly cited statistic is that an E. coli bacterium, under nutrient-rich conditions with appropriate temperature and oxygen supply, can divide in as little as 20 minutes. In contrast, for sexually reproducing organisms like humans, a child is born, reaches sexual maturity in about 15 years, and procreates by the age of 25 or so. Thus, the generation time—transitioning from one generation to the next—is about 20 to 25 years for humans, compared to just 20 minutes for E. coli. This has important implications, especially if the environment changes rapidly.

Then for a E. coli bacterium, the next generation comes and because population sizes are large, that means the next generation has a huge number of individuals and it is very likely that one of those individuals is going to acquire a mutation which allows that individual to flourish in the environmental change that has happened. So there'll be someone in that changing environment which is well adapted or better adapted to survive in those conditions. Whereas so even for rapidly changing environments, which is changing every hour, every second hour, if growth conditions are

right and conditions Divisions are taking sufficiently frequently. You are generating those mutations as you transition.

You are generating those mutations as you transition from one generation to the other. But that's not possible for complex sexually reproducing organisms. And hence different mechanisms are required to generate those diversities. But but at this point in the course, we sort of take a call as to which of these two set of mechanisms of generation of diversity we are going to study for the rest of the course. If we go down the route of sexually reproducing organisms, then that field of study of evolutionary changes referred to as population genetics, which was born approximately 100 years ago, primarily due to work of people like Seawall Wright, Ronald Fisher, J.B.S.

Holden. What we'll be focusing on this course is sticking to prokaryotic systems and evolutionary change happening primarily via acquisition of new mutations. This will be our focus. And the primary advantage of doing this is that one, working with microbes, As we will see shortly that we can process six to seven to 10 order of magnitude, 10 generations every day by doing experimental evolution in lab.

So if we choose to work with an organism such as E. coli, yeast, or Bacillus subtilis, then we can process 10 generations every day, which means if we do this evolution experiment for one year, we have done more than three and a half thousand generations. And that is a sufficiently long number of generations that we can study a population's response to this changing environment in a lab setting. This is obviously really hard to do when the organisms are more complex and generation times are longer. If we move to even something as simple as Drosophila, which is another popular model organism of choice for lab studies, the generation time is roughly two weeks, which means we can do two generations every month and, consequently, about 25 generations every year compared to three and a half thousand for bacteria. That's one major advantage.

The second major advantage is that we'll sequence these bacterial genomes and compare them to what we started with. And this comparison allows us to identify what nucleotide-level changes happened in the genome of the organism over these three and a half thousand generations, which facilitated the adaptation of this organism to the environmental change that occurred. The third advantage that microorganisms offer, which complex species do not, is that, suppose during the course of this year, I'm doing this experiment and I started with generation zero. And at the end of one year, I am at generation three thousand five hundred.

Then, periodically—at generation one thousand, two thousand, three thousand— I can take the population at these times and freeze them in a minus 80-degree Celsius freezer. And the bacteria remain viable and metabolically active there for practically an infinite time. What that means is that

even after processing the generations for three and a half thousand, I can go back to the population that was there at generation 1000, take it out of the freezer, revive it, and compare it with what is happening now.

These are referred to as frozen fossils and they allow direct comparison of what happened at generation 1000 and what happened at generation 3500 after one year of evolution. So this is of course not possible with complex species. We can't preserve fruit flies for any number of generations. So these are some of the advantages that working with microorganisms offer. And that's why from now on onwards, we'll be exclusively talking of only microorganisms.

So it's again important to realize that these advantages, so advantages of working with microorganisms. microbial populations that small generation time this can be as little as 20 minutes and for human beings this could be Human beings are most complex species could be years, months, weeks, depending on which model system. So what we can do is process more generations in lesser number of time. And when we are doing an evolutionary experiment, the relevant timescale is not the quantum of time that the experiment was done for, but how many generations did we process.

For example, the experiment we just discussed, if you're working with bacteria, we can process 3000 generations in a year. But if you're working with Drosophila for the same amount of time, we're probably only processed 25 to 30 generations. So what is relevant is not the quantum of time one year, but how many generations did we process in that particular time. Genomes are small, easily sequenced. Genomes are small.

And this allows us to understand the molecular mechanisms for adaptation. The third advantage is that we can make deep freezer stocks. And what that means is that if I start an experiment today, a year down the line, I will be at generation 3650. If I'm here and this is generation zero, then this is the number of generations.

Then periodically, as the experiment progresses, however frequently I like—maybe every 500 generations—this is 500 generations, I can take the culture growing here and make a freezer stock in a minus 80 degrees Celsius freezer. And the bacteria remain viable, which means they can be revived at any point from the freezer stock, and then they will start growing. But they are also metabolically inactive in the state they have been stored in.

So they are stored as they are, in the same condition they were at generation 500, and as a result, this is referred to as frozen fossils. All right. So this allows me to make direct comparisons between what is happening now and what happened at a previous generation during the evolution

experiment. This, of course, is not possible for any species other than microbial species. And this offers a great advantage as far as the evolution experiment is concerned.

So these are some of the advantages that microbial systems offer. So now we'll discuss how a particular growth experiment of a microbial species is done in a lab setting. And there are typically two ways of doing this growth experiment. Let's discuss these two ways. The first way is called a batch culture.

In this, what we might do is take a flask. In this flask, we'll add nutrients. These nutrients will be sources of carbon, nitrogen, oxygen, sulfur, phosphorus, there'll be metals, and so on. And then we will add these nutrients. We'll seed this culture with a bacterial species of our choice.

And let's say we are studying E. coli. So what we'll do is add a bacterium to this culture, which has these nutrients. And then we will let growth take place. Grow at 37 degrees Celsius, which is the temperature where E. coli's growth is most preferred. And we will typically shake the flask so that oxygen is available.

Some species can grow in the absence of oxygen to equal I can, but it's just that both are more robust and faster if oxygen is available to the species. And that's why in a typical lab experiment, these would be the growth conditions. So in these conditions, let's say that the carbon we provided was in the form of lactose. So the carbon source that is available for the bacterial population here is lactose. And then as time moves forward, what is obviously going to happen in this culture is that the number of bacteria is going to increase.

But in a batch culture, what is important to realize is that since the availability of nutrients at t equal to zero was finite and these nutrients are being increasingly used up by the bacterial population, the supply of nutrients is going down with time. And in conjunction with that, the number of bacteria is going up, and each bacterium is growing. So the demand is going up. So these two processes are going on simultaneously: growth is being increased. Carried on forward by the growing population of bacteria because each one is dividing, but it is being constrained by limiting resources in this flask.

So if we track, if we do a time course measurement of how these trajectories look like, let's say this is time, And this is the number of bacteria. Then typically this growth curve is going to look something like this. And before we discuss the characteristics of this curve, let us first discuss how we get data for this. So typically, what you would do is not have a continuous curve because a continuous curve means an infinite number of measurements.

But what we might have is a finite number of points at which we take these measurements. So at this time, the number of bacteria might be this. At this time, it might be this, and so on and so forth. I will have these data points because of the measurements I take at different time points during the course of the experiment. And then I fit a curve through this to get this characteristic growth curve.

We'll discuss the mathematical form of this growth curve in just a little bit. So we get this growth curve. Now let's just spend a minute or two to discuss how each measurement comes in. So what is typically done is that, let's say this measurement was at two hours of growth. So two hours have passed since we seeded the culture.

And at the end of two hours, what I do is go to this flask and take a small volume Take a small volume out. Obviously, the density of bacteria in this small volume will be the same as this. And through this, I pass light. And this is the principle by which all bacterial counts are estimated in a lab setting.

When we do not have growth in the culture, most of the light is going to pass through the liquid media and go uninterrupted. So what I see at the output is roughly going to be proportional to what I see at the input. However, as growth more and more growth takes place in this flask, what is going to happen is that light is going to collide with these bacterial particles and then diffract. And as a result, the output light that I see is not going to be is going to be much lesser as compared to what went into the flask. As a result, this is a proxy for how much growth took place.

All right, so what's happening here is that at every given point in time, whenever I want to measure the density of the culture, I take this out, shine light through it. And depending on how much light makes it out of the tube, this is a proxy for growth. The lesser the amount of light that makes out, the more the growth. And this is one way to measure the number of bacteria in the tube with time. and this data would look something like this.

Depending on how frequently we measure and this is the data that you would get. This is one way to measure it and this is the most popularly used method in labs. Another way which is more accurate but much more cumbersome is that if we have growth taking place in a flask, so let's go back to our drawing of flask. And at any given point in time, what I will do is I will take a small volume V and I will plate this small volume on a solid agar plate. And when I plate this, this V is a very small volume.

So this contains, let's say, a very small number of cells in it. Let's say it contains 100 cells in it. When I plate this on this solid agar, I don't know where those 100 cells are. So after spreading this volume and spreading it here, I let it grow overnight. At 37 degrees Celsius.

And what's going to happen? Is that. I don't know the location of these 100 cells. But they are somewhere there. Because one bacterium.

Is too small to be seen. So I don't know where these 100 cells are. But they are there somewhere. And when I come in the next morning. What I am going to see.

Is. Because I let growth take place. At 37 for overnight. And this plate contained nutrients. These bacteria would have divided overnight.

And during the process of division, where there was one, there would be two bacteria and then two would have divided to four and eight and so on and so forth. So wherever there was a bacteria, I would see a small mound of cells like this. So while I did not see where I spread these 100 bacteria because too small to be seen, after overnight growth, they divided enough number of times so I can see this mass of cells and I know that each one of them came from one cell that I plated the previous evening. Each one of these mounts is called a colony. It's a word that we'll keep repeating throughout this course.

And this is what it means that we spread these bacteria on a solid media and without knowing where we are spreading them. But if we allow them to grow for 12 hours, 24 hours, depending on the environmental conditions and depending on which species we are working with, there will be sufficient growth such that these cells will form a mound. And this mound is referred to as a colony. But from the context of our experiment, what this tells us that let's say the number of colonies here were n. What this tells us that in the volume small v, there were n number of cells and hence in the flask where the total volume is v, I get an estimate of how many cells are there in the larger flask. So this is a more direct, but a much more laborious way of measuring.

I have to wait overnight for growth to take place, then count, and then back-calculate, and all those things need to happen. This here is a much more direct, faster, but indirect readout of what the density of cells in a flask is. What I'm counting here has another name that we'll keep coming back to in the course: CFU, which is colony count. Forming units. And the question we are asking here is: how many CFUs were there in this small volume V of the liquid that was growing in this particular flask at a particular given time?

So again, if we do this at different times, I will get a graph like this where it will be time versus CFU, and if I do this experiment enough times, I will get data such as this. So this is what growth data would look like in actual samples. Compared with this, if I track, because this is a batch culture, if I track the following, let's try this graph where this is time, On the x-axis, we are representing the number of bacteria.

And on the y-axis, we are representing the amount of lactose which is present in the flask that I am looking at. As you can imagine, as growth is taking place, the number of bacteria should increase. And because they are increasing, the only source of carbon available to them is this lactose. So the amount of lactose that's present in the media should decrease with time. And typically, in growth experiments, when we do this, we see the following trend.

The number of bacteria rises and then saturates, and the amount of lactose will be somewhere here at t equal to zero, and then it goes down. Let me draw this. So lactose is all used up, and this facilitated growth. Again, the numbers—the readings associated with these—will be readings taken at different times. So the actual data would look something like this.

And we'll fit a trend line, which represents the process of carbon being used up by the number of bacteria present in the culture. The same applies to the number of bacteria, where we'll be taking periodic measurements and not have a continuous measurement, as indicated by the curve. So this is the first way in which we will actually do growth curves. The second way to do growth is the following, which is called a chemostat—a continuous culture. This is also referred to as a chemostat.

In a chemostat, we have a continuously stirred tank reactor—a continuously stirred tank. So this is a stirrer; there is continuous shaking taking place. The volume of this tank is, let's say, capital V, and this tank has an inlet and an outlet stream. This is unlike the batch culture we saw, where the nutrients would get used up completely, leaving nothing for the bacteria to grow on. A chemostat is not like that.

We seed this with bacteria. So in these bacteria, because this capital V volume of liquid that's present contains nutrient resources, they would grow and divide. The inlet stream has a particular rate. Let us say the inlet is coming at V0 volume per time. The outlet stream has to match the inlet stream.

So there is something being supplied at the rate V0, and there is some volume being taken away at the rate V0. These two are exactly the same, which means that the volume being added to the chemostat is exactly the same as the volume being removed from the chemostat. This V0 that goes in is pure media only. It is just growth media, and it is sterile. By sterile, we mean that there are no bacteria in this.

So this is just nutrients. So, in some sense, I am supplying nutrients to this chemostat at a rate V0 volume per time. On the other hand, what is going out is called the spent media, which will contain

the metabolic waste of the bacteria, which in the process of growth they have been secreting into the media. This also contains unused resources. And, of course, they will also have bacteria.

Because if a bacterium happens to be right here, it's just going to get washed away by the outlet stream. So what happens is that these bacteria are being removed at a certain rate. They are being removed at a certain rate. And this rate is obviously dependent on the volume that I'm taking out per unit time. The more volume I take out, the faster I'm removing the bacteria from the chemostat.

This removal rate has to be exactly balanced with the rate at which growth is taking place. That is when a chemostat reaches equilibrium: the rate of removal of bacteria is exactly equal to the rate at which they are being generated. For instance, if I'm removing four bacteria per second, but if the growth is only three bacteria per second, then very soon what's going to happen is that I'm removing more but adding less.

As a result of this, the density present inside the chemostat is going to decrease with time, and the population would go extinct. So in equilibrium, these two rates have to match each other. And when I'm removing four bacteria per second, that is exactly balanced by the rate at which growth is taking place, which is also going to be four bacteria per second. So there is this balance that takes place in a chemostat. The advantage of working with a chemostat is that the environmental conditions do not change over time.

They stay constant as growth takes place. Whereas, if we go back and look at our batch culture, what you should note here is that the environment is changing continuously as growth takes place. When we started out, there were lots of resources, and by the end of the growth curve, almost all the resources were completely gone. So the environment that the population is experiencing in a flask like this is constantly changing, and it is responding to this constantly changing population. Whereas in a chemostat, because there is a constant supply and a constant removal process,

The system soon reaches an equilibrium where the environmental conditions do not change with time. So the bacteria are constantly responding to the constant concentration of every resource and every waste, and their own density and environment are thus invariant with time. These are the two popular ways of performing growth experiments on bacteria in the lab. And as we will see, for reasons that will become clear as we move forward, the chemostat, despite this advantage, is much less used compared to batch culture growth. Batch culture growth, despite its disadvantage that it has populations responding to changing environmental conditions, is something that is used as an experimental setup in comfortably more than 95 percent of all growth experiments.

And we'll continue this discussion in the next video. Thank you.