Evolutionary Dynamics

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Week 10

Lecture 49

Thank you. Hi everyone, welcome to the next video. We start our discussion of the first evolution experiment that we want to discuss. This is a study that was published in 2016 in the journal Science by the group of Roy Kishony at Harvard. So this study asks how easy it is for the evolution of antibiotic resistance in bacteria.

First, historically, we discussed this briefly in an earlier video that antibiotics were discovered in the first half of the 20th century. And when that happened, it was thought that we had found this great tool to control the growth of bacteria in places where we didn't want them growing. So suppose we had an infection; you take antibiotics, and the bacteria are taken care of. However, in the decades of the second half of the 20th century, we soon found out that bacteria do not just statically stay while antibiotics are being used. They can change their physiology via the accumulation of genetic changes so that they become resistant to antibiotics.

And an antibiotic that was working historically would no longer be functional in the case where antibiotic resistance has been acquired. So that's why antibiotic resistance is such a big health concern today—because of the overuse of antibiotics, there are so many pathogenic strains which are resistant to many, many antibiotics that we know. In some cases, we have isolated strains where hardly any antibiotic that we know works because the bacteria are resistant to everything that we know. So when we think of antibiotic resistance, we Before we discuss the results of this paper, it's a very nice two-minute video that the authors produced, which we will look at in just a few minutes.

But first is that if we have a bacteria, when we say this is resistant to an antibiotic, so what that means is that this can resist clinical level of antibiotic. So, let us say this bacteria is not resistant. So, we say that this is a bacteria which is susceptible to an

antibiotic. What that means that, so let us say the antibiotic we are talking is called trimethoprim. That's just one of the antibiotics that is commonly studied.

But it could be ampicillin, it could be tetracycline, any antibiotic you like. So this is susceptible to trimethoprim. What that means is that there is a concentration of antibiotic. So this is the bacteria that this bacteria will grow. Will grow.

in an environment if antibiotic concentration is less than C naught. If the environment has concentration which is greater than C naught, then this bacteria will not grow. This concentration below which growth happens and above which growth does not happen is called the minimum inhibitory concentration of an antibiotic. Minimum inhibitory concentration of an antibiotic against a particular strain. So, let us imagine that this is a scale which indicates antibiotic concentration.

This is zero; this is very high, and in between, we have intermediate ranges of intermediate values of concentration of the antibiotic. Then, because this strain is susceptible, its MIC would be towards the lower spectrum—it would be something like this. So, this strain has an MIC which is here. Let us call this C naught. So, it can exhibit growth if grown in an environment which contains antibiotic in this window.

But this cannot grow if it is grown in an environment which contains antibiotic in this window. So, very small MIC. However, what can happen is that this individual can evolve to acquire mutations. This is the genome, and it can acquire mutations, and these mutations can facilitate it becoming resistant to the antibiotic. And as a result of this, the MIC associated with this new genotype will increase and maybe go here.

This is the new MIC. And maybe, if we keep increasing the pressure on the bacteria by using more and more antibiotic, we are facilitating the selection of newer genotypes, which are even more resistant. And by that, what we mean is whose MIC is even higher than the strain that we just described. Maybe these sorts of changes are getting selected for. And as a result, this moves to even higher MICs.

This is the MIC of the third strain and so on and so forth. So this MIC values can change depending on what is the selection pressure that we are applying. So now let us think of an experiment where we are growing these three strains in an environment. Let us imagine an experiment where we are growing these three strains in an environment where the antibiotic concentration is given by this value. Let us call it C1.

If growth is taking place in C1 and all three of these genotypes are present, let us call them A, B and C, then what is going to happen is that because C1 is above the MIC of B, is also above the MIC value of A, A and B cannot grow in that environment. Only C can grow. So in this environment at C1 concentration, strain C is going to win. This is going to get selected. The other two cannot grow and hence they are going to go extinct.

However, what happens if we do this experiment at this concentration? Let us say C2. Since C2 is above the MIC of A concentration, A will go extinct, but B and C should both be able to grow in this environment. And that is indeed what will happen.

But what typically happens is that increasing MIC comes at a cost because you are investing cellular resources toward becoming resistant. The growth rate of these two will not be equal. And typically, what will happen is this individual will have a higher growth rate compared to C. So, in this environment, B and C will both grow. However, B will likely grow faster.

As a result of this, selection will favor B, and eventually, if enough time is given, B will be selected, C will go extinct, and A will never grow. So, this phenomenon is the cost of becoming resistant. You became highly resistant, and if the environment has a lot of antibiotics, that resistance will come in very handy because, in this case, only C will survive. However, if the antibiotic in the environment is lowered to C2, then A will go extinct.

But between the competition of B and C, B will win because C, in order to become highly resistant, paid a cost in terms of its growth rate. And by the same token, if the antibiotic concentration was here, perhaps A would survive, and so on and so forth. So, all these dynamics are present. There are trade-offs involved: the more resistant you are, the slower your growth rate is in the absence of antibiotics. So, again, fitness is a very environment-dependent phenomenon. In an environment where C1 is present, C is the fittest, but in an environment with no antibiotics, A will be the fittest.

So, the environment decides which genotype is the fittest in a context. So, suppose in this experiment that we are going to discuss, we want to study how we can evolve E. coli. How easy is it? The question we are asking is: How easy is it for bacteria to become resistant? And becoming resistant in this context means just increasing the MIC.

So, how would we design such an experiment? So, in this study, they used a very clever approach to answer this question. What the authors do is, they take a plate which is not

liquid, because if it were liquid, then everything would mix with each other. This is not solid, because if the plate is solid, then bacteria can't move in that medium.

This is semi-solid. This is typically used for studies where we are studying the ability of bacteria to swim. So, this is a plate which is solid. Very large in dimension. Its dimensions are of the order of several feet by several feet.

A typical petri plate is only about this much. This is, I think, 4 feet by 2 feet. They divide this plate into several sections. The media in this plate is prepared so that bacteria can swim. This is important and will become clear in just a second.

So, they divide this plate into several sections. At the start of the experiment, they introduce bacteria at the left edge of this plate, as I'm showing. I just want to give you some context before we watch this two-minute video, which will show us exactly what happened in the experiment. So, at this edge, they introduce bacteria. Now, what will happen is that these bacteria will grow, divide, and consume the resources available near this edge.

As they consume resources, all the resources near the edge will get used up. As a result, bacteria will sense this gradient: all the resources here are gone, but the resources a little farther away are still present. Bacteria have a sensory system called the chemotaxis system, through which they can sense gradients and move up a gradient if it's an attractant they are seeking. As a result, bacteria will move up the gradient and start consuming. Very soon, we will see that bacteria start spreading in this direction.

As they are eating resources, they are swimming and moving towards right. Now these dotted regions indicate split in different parts of the plate and the first region has no antibiotic. So in this region there is absolutely no problem. The bacteria keep eating and keep moving to the right until they hit this first barrier beyond which in this region of the plate there is antibiotic at concentration equal to the MIC. And this is the ancestral strain.

So It would be worthwhile if you just pause the back. If you just will just take a 10, 15 second break here. And I want you to think about what would happen as these bacteria are moving towards the right and they hit this frontier beyond which there is MIC concentration of an antibiotic. What would happen as you see this bacteria moving right, hitting this frontier?

What would happen thereafter? I'll just give you 15 seconds to think about this problem. so what you should note here is that once bacteria will reach here just fine but once they

are here at this front they can't grow beyond this because this region has mic concentration of antibiotic they can't grow as a result of that they will pause here and now growth will not happen until an individual hair acquires a mutation which allows for growth in MIC concentration of antibiotic. And this individual will now spread.

And this spread will continue till the bacteria reach this front, at which point growth will halt again because this region has concentration of antibiotic which is 10 times MIC. And the same phenomena is going to keep on repeating with every rightward section of this plate has 10 times the concentration which was present on the section to its left and so on and so forth. So the question that they are asking is that how far can bacteria go towards acquisition of this resistance? And when this paper came out in 2016, the authors of the study also made, when this experiment was going on, it was several days long. They made a...

video of the entire process and they added a dye to the plate background so that the visualization of bacteria moving on the plate is made easier. So, we can actually see this experiment. So, they condensed something like 10 days to 2 weeks of experiment into a 2 minute video that is present on YouTube which is what we are going to watch now and we can actually see what happened in this experiment as concentrations go up. So let's watch this two-minute video, and we'll continue our discussion after that. So what we ended up building was basically a petri dish, except that it's two feet by four feet.

And the way we set it up is that there are nine bands, and at the base of each of these bands, we put a normal petri dish, thick agar, with different amounts of antibiotic. On the outside, there's no antibiotic. Just in from that, there's barely more than the E. coli can survive. Inside of that, there's 10 times as much, 100 times, and then finally the middle band has 1,000 times as much antibiotic. And then across the top of it pours thin agar that bacteria can move around in.

The background is black because there's ink in it, and the bacteria appear as white. First, you see them spread in the area where there's no antibiotic up until the point they can no longer survive. Then a mutant appears on the right. It's resistant to the antibiotic. It spreads until it starts to compete with other mutants around it.

When these mutants hit the next boundary, they do have to pause and develop new mutations to survive in 10 times as much antibiotic. Then you see the different mutants repeat this at 100. And after about 11 days, they finally survive in 1,000 times as much antibiotic as the wild type can tolerate. And so we can see by this process of

accumulating successive mutations that bacteria, which are normally sensitive to an antibiotic, can evolve resistance to extremely high concentrations in a short time. Okay, so that was the—hopefully you got the gist of it.

So we'll walk through what happened here. This is the petri plate, which is four feet by two feet in size. And the challenge is maintaining this soft agar, allowing for growth, and keeping the whole condition sterile for a period of 10 to 11 days. So they made this lovely video out of this, and as they highlight in the video, this plate is sectioned into these concentrations. So 0 indicates no antibiotic, and then 1 is 1 times MIC, then 10 times MIC, and 1000 times MIC.

So what you are looking at in this video is the evolution of resistance to an antibiotic and But a thousand-fold—a bacteria that couldn't live at one times MIC is able to survive at a thousand times MIC. Now, is that a very hard thing to ask of a bacterium, or is that a very easy challenge to ask of a bacterium? And before this, we didn't really have a good sense of it. And then, of course, they introduce the left and the right halves, which are just mirror images of each other.

So they introduce these bacteria together. And they quickly occupy both the left and the right. They quickly occupy the first band where there is no antibiotic. And that's not a challenge. They're just using up resources, spreading, and filling the space available.

But then interesting things happen—now, once they arrive at the interface between zero antibiotic and one times MIC, there is a pause because this genotype that expanded in the zero region cannot occupy the one region because it can't grow and survive in that antibiotic concentration. So there is a waiting time associated here at this front. That one of these individuals should be able to pick up a beneficial mutation and then grow in the subsequent section of the plate. And then we begin to see this happen—here is where the mutation first happens. And it starts to spread radially.

These are the progeny of this mutant that arrived at this particular location on the plate. However, as you can see on the left section, something interesting is going on—both on the left and the right, actually—that mutation that has facilitated growth in this next section has not just happened at one place, but there is one, two, three, four, five, six, seven, at least seven places where this mutation has happened. And all seven are now spreading into this region. In fact, we also see more mutations emerging on the right-hand side where these mutants are now spreading. So, they quickly grow up, fill up the space,

and then pause again at the next front—not everyone that was able to grow at 1 times MIC can grow at 10 times MIC.

As you can see here, there is a mutation that quickly grows to expand in the 10 times MIC region. This mutation is showing an interesting trajectory of growing in 1 and then 10. And so on and so forth. This growth process continues. And every time it hits a section of the plate, there is a pause associated with the acquisition of new mutations.

And eventually, this plate is occupied by the evolved bacteria. They acquire mutations to be able to grow in a region of the plate which has 1000 times its M.I.C. And because this is a plate, I can sample bacteria from different parts of the plate and actually study where mutations were acquired, which mutations were acquired, and so on and so forth. And completely understand the evolutionary trajectory of these populations as they evolve from their ancestor state to a state where they grow up. They acquired 1000 times its MIC.

It's a fascinating experiment. And interestingly, in this section, they collect bacteria from different parts of the plate. And this is the study. This is the lead author. And what they find then is that from the final map we saw of the entire plate, we can isolate bacteria from different sections of the plate.

We know how they performed on the plate because we made the video. We know which one came early and which one spread faster. So we know their relative fitness. We also know which part of the plate. So we know which antibiotic conferred how much resistance.

And that is linked, and then we can isolate these bacteria from the plate, get their genomes sequenced, and identify which regions of the genome were targeted the most. So, for instance, in this one, the X-axis represents the nature of mutations that happened—which genes were targeted, which genes acquired mutations. And the Y-axis represents how many times that particular gene was targeted in the entire sampling I did from different places on the plate. So, as you can see, there is a gene called FOLA, which encodes for a protein responsible for folate biosynthesis and is also associated with resistance to trimethoprim. And there's a lot of study being done on this gene.

That was targeted almost 50 independent times on this plate. Then we have genes, something like marR, which stands for multiple antibiotic resistance. Then we have another gene called soxR, which is associated with handling superoxide stress, and so on

and so forth. And then the frequency quickly decreases a lot. Some of these occur only one or two times, and there are many mutations which occur only once on the entire plate, no matter where you sample it from.

But there are these signatures of conserved evolution—that if you evolve bacteria in a particular stress, certain regions acquire mutations, and so on and so forth. So we can look up the reference, but the idea is that we can do this sort of experiment and trace the phenotype with the underlying genetic changes taking place in the organism. Interestingly, one last thing before we wrap this experiment up is that the authors did the following experiment too. They divided the plate into two regions.

The left region had zero antibiotic. The right region had 1000 times MIC. Remember, in the original scheme they have, the regions are 0, 1, 10, 100, and then 1000. So you allow for a more graded response in the actual experiment. But they also did this experiment where you only have binary regions in the plate.

Either there is no antibiotic, or there is 1000 times the antibiotic. And when you do this experiment, obviously, the bacteria quickly spread through the left half and occupy this region. But then they come and halt here, just as they do in the previous one. And once they are here, now you are waiting for a mutation to take place at this front, such that because of that mutation, the MIC goes up 1000 times, and that mutation never happens. So what that means is that the challenge posed to the population here—going from 0 to 1000—was too steep a challenge, such that no single mutation could accomplish it.

This never happened. Whereas when you do it in a more graded response, you saw that antibiotic resistance could be increased by a factor of 1000 in a matter of just 10-11 days. So that was the first experiment we discussed. Next, we will move to a classical E. coli experiment, which has been going on for more than 30 years now. It's called the long-term evolution experiment, which we'll start with in the next video.

Thank you.