So, we have another kind of very interesting piece of the course right now. We're going to continue to talk about genetics, except now we're going to talk about the genetics of diploid organisms, which apart from bacteria, most of the organisms including us are diploid. They have more than one copy of each chromosome, and so we'll go through a segment on this, and also talk about mitosis and meiosis, the central processes of cell division and the segregation of genetic material that underlie life as we know it.

And then, were going to charge into a session of recombinant DNA, and some of these technologies, PCR and various things that you see in the newspapers all the time. And then, I'll finish up with the session on the immune system, which a few of you thought was surprising that bacteria recombines. I'll tell you in that system it will feel like science fiction relative to what I've told you up to now. It's an absolutely amazing system.

So, we are going to start today with genetics of diploid organisms.

So I'm going to go back to how this was first understood.

And most of you have probably heard of Gregor Mendel, who discovered this, and surely some fraction of you have run into an exposure to this topic before. But in keeping with what I'm trying to do in this course, could you guys watch out there?

I think I'm just going to unplug, yeah, it's OK. I think I'm just going to unplug it for just a minute here. You've probably heard of Mendel. Some of you have seen these different squares.

You might have memorized it from a textbook or something like that.

I'm going to try and see if we can go through this material up another level of sophistication because, again, and I'm saying, science didn't just come down from on high and end up with facts in a textbook. What's in a textbook is somebody's effort to take a current state of understanding which is based on experimentation and come up with models. And what you're seeing in the textbooks are the models such as they were as of the time the textbook was submitted for publication. Sometimes they change even before the textbooks get out. But anyway, it's a process.

Mendel was one of the starting people who started this process, really a key figure, and a guy with an amazing intellect.

But before we start in, I just want to show you a couple of pictures because these kind of knocked me over when I saw them.

I don't know what kind of image you have of Mendel.

You probably know he was a monk, and he did something with peas, and he figured out this stuff about genetics.

And most people probably carry around an image probably somewhat like this sort of romanticized drawing. He was a monk all right, but it was at an Augustine monastery in Bruno in Austria that was a very major intellectual center. They even published a scientific journal. They sent Mendel off to Vienna to go to university.

While he was there, he studied physics, math, as well as botany. So he had, in many ways, a background that is very similar to you guys, very heavy on the quantitative physical science, mathematical sort of background.

And then he went on to do some experiments in biology.

And I think you maybe can get a sense of this.

You can see a picture of what Mendel actually looked like.

Here's one picture of him. But the one that really blew me away, I have a picture of the monks. Just think of what ever image you had of the monks that Mendel was at.

Well there's a picture of them. To me, they look nothing so much like a group of university presidents or something sitting around for a portrait. And he traveled very widely.

Here he was on his way to London here. Here is a picture of him with a group of people on his way to, I think he was in Paris on his way to London. So this wasn't a little isolated monk in a garden who stumbled across stuff. He was a rather sophisticated guy going after some interesting problems.

And this was the garden in which he did his experiments.

Here's a picture of it. So this was a straightforward experimental setup for these really amazing things he did.

So, with that kind of background, Mendel was interested in a problem of inheritance. And people have been aware that traits were inherited. That was the whole principle of domesticating animals and domesticating crops, that if you took parents with certain characteristics and crossed them together, the offspring then had the traits that were associated with the parents. And so people have able to get better domesticated animals or better domesticated crops.

But up until then, this mixing was thought of sort of like blending liquids, stir together some green and red, and a little this and that. And it all stirred together.

And as you'll see, what one of Mendel's great insights was, was that it wasn't like mixing liquids. And to study this problem, then, he picked a system. It wasn't that he was just fiddling around with peas. He was a pretty sophisticated guy, and he picked peas as an experimental organisms for three reasons. And so, why peas?

Well one, they were easy to grow, and that's still a major consideration of any model system that you want to use and science today. It's really tricky to grow, it's very hard to work with. It was easy to pollinate in a controlled way.

Just the structure of the pea flower makes it very easy to make sure to either put the pollen right on the pistol of that same flower, which is a kind of self fertilization, or to make sure that the pollen goes from one flower to another, which is basically cross-pollination. And the third thing, and this was a really important thing, was the system had worked on before. And there were a number of what were called pure breeding lines. If he had just grabbed peas out of the wild it would be sort of like me starting to do genetics by crossing a couple of you guys. We'd get offspring, all right, and we'd cross those offspring, we'd keep getting things, people that look different and different, maybe something like the parents, but what had happened with peas is people had taken a pea, and then they continually inbred it until it finally sort of settled down. It always had white flowers.

It always had wrinkled seeds. It always had whatever the particular trait was. And so, I think I showed you the slide earlier. This shows two things.

You can see there are smooth seeds and then wrinkled seeds.

And it may be a little hard to tell in this light, but there is sort of two colors here. One's kind of greenish and one's sort of yellowish. So there, you see two of the traits right there. There were also flower colors, and height, and other things that had the characteristic.

They were pure breeding. Every time you took that line and if you self crossed it and put out its progeny, you'd see the same characteristic each time. So, this was the system that Mendel started to study this problem of inheritance. And how does blending come together when two organisms brought pollen?

Or, if it was other animals like us, it would be sperm and egg.

But somehow, you did something that ended up giving you an egg that got fertilized. And out of that came the progeny. So, what Mendel did, the term that's used, they say he carried out a cross. This is genetic-speak here now. So he took pollen from one plant.

And used it to fertilize another plant, collected the seeds that came out of this, and he examined the progeny. And I think an interesting way of thinking about this is a UROP project.

You'd come into Mendel's lab and wanted to do a UROP project, it was pretty easy. I could probably show you all the techniques you needed to know, and this is it. You'd show how you pollinate, collect seeds, and then we'd look at the characteristics. So, it's just some fairly simple manipulations and some observational stuff. So, let's suppose you're doing Mendel's thing as a UROP project, and let's just see where that will take us. So, what Mendel did to

begin with, he took one of these pure breeding lines that was smooth or all abbreviated as a capital S. And he pollinated it with something that was wrinkled. I'll do that as little S.

And then he collected the seeds from what's known as the first generation, starting with something like this.

And geneticists use the term F1 for this first generation in a cross like this, and what he found was everything, all the seeds, were smooth. So, the wrinkled trait had, if you will, disappeared.

That's your first UROP experiment.

[Time doesn't?] submit to nature, or science, or something, and read a little paper like Watson and Craig that turns the world on its end. What would you do next? No gels, no Whitehead Sequencing Facility. Anybody got any ideas? I've showed you all the techniques that he knew about. Pardon? Cross it again?

What's your thinking? Do you think the traits disappear, or do you think it's hiding? It might be hiding, right?

I don't know what he thought, but I think that's a reasonable to think about it is he's probably trying to figure out, did this wrinkled trait just disappear from the face of the Earth, or is it hiding in those first-generation seeds?

So, let's put that up here. So, that's exactly what he did.

So, he took these seeds, now, that were the smooth F1.

These are not the same smooth as the parental up here.

Just to make clear, you guys understand these are pure breeding.

These are the ones that people have been breeding for a long time, this one and this one. In this case, even though I'm trying it in circles, this is a smooth F1, smooth F1. And, this time when he did the experiment he looked at the second generation, or the F2 generation as a geneticist would call it. What he found, he got some smooth and he got some wrinkled.

So, the wrinkled trait reemerged in the F2. So it wasn't really gone.

It was hiding. OK, time to submit to nature's science cell. Got it? He didn't try and publish it at that point. He did something else. He had the same kind of background you guys have. Can he think what he might've done?

He could cross again. There's something else he did with this experiment, though. Well, you're thinking of new experiments. He's got a little data processing he can do here. What did you say? I'm not able to hear, sorry.

Statistics, OK. I'll simplify it even slightly before that. I've got some of each. Count them, right, exactly. So that's what he did, and I think the numbers were, if I remember, five, four, seven, four in 1850.

So, what should I do now? Ratio: absolutely. We could count another million of them, but that probably would not be terribly productive. And what he found out is that when he did that, he found that he got a ratio that was pretty close to 3:1. And, so that was sort of what he found out from this by doing this sort of thing over again was a pattern. A trait disappeared in the F1. The trait reemerged in the F2, and the two traits had this. The ratio of the two traits would be about 3:1. If you don't think this is sort of like a UROP project or something, that's a page out of some of Mendel's actual notes while he was doing his crosses. And what he did next, then, was to take some other traits. Yeah? Sorry? Excuse me?

Reemerged in F2. So, he took some other traits, white and purple flowers, tall, short, I found that there were at least certain other traits. It didn't work for everything he studied, but some of them he could see the same pattern.

One of the traits disappeared. It reemerged in the F2, and when he counted them, he'd find that the trait that reemerged was at one, and the other one there were three times as many.

So, he'd seen a pattern. And all he's done at this point is to cross flowers and count the progeny. So, at that point Mendel tried to explain his data. So, he had to, now, take the next part of the scientific process. And what's kind of nice about thinking about Mendel, in this sense, is we are not overwhelmed by complicated techniques.

You can see, I think, the scientific process.

And it's a very bare bones thing marching along.

So now he's got some data. He's quantitated his stuff.

He's founded reproducible. It's not only for seeds.

It seems to be some general feature. And the thing about this, I guess, I don't know what he thought but it seems to be likely that he could see that this didn't fit very well with the blending idea.

Like, you'd pour together two liquids, and you'd stir them all up. Instead, he really made this monumental leap in thinking that genetic information must come in some sort of particular form, come in particles, or units, or quanta if you want to think about if it if you're a physicist.

We know those units as genes right now. We grow up with it right now, but to go from the idea that genetic information was kind of like two liquids blending to the idea it was a little particle, so it was just about the same kind of leap as thinking that energy comes in particles instead of a continuous sort of thing.

So, that was the kind of insight that Mendel had.

And so genetic info comes in particles, units.

I was going to say, we now call these genes, and if that was what it was then he started to think about these traits as particles that had a different character associated with each of them. That would mean there would be one particle that was a big S, and that was smooth. There was some other particle that was specified.

The wrinkled character, that would be called a small s.

So, what was happening in these crosses, then, he was now mixing particles instead of liquids. Again, I don't know how he got to, how many particles there had to be of each per organism. It could have been anywhere from two upwards. He had to have two in order to explain the sort of stuff he was working with. There's no reason he couldn't have thought of 12 or something. But I assume you start with the very simplest thing, number that you can think of, and see if you can make this work. So, what he hypothesized, then, was that each organism had two copies of each of these particles.

So, two copies of each particle, so this would mean that there were two types of particles. So, there would be the S and the smooth. So, he can get three types of things. He could get one that was both big S or smooth. Or you could have the ones that were the two little S's. And these would be wrinkled.

Or, if you had the other combination, what he figured out, what fit with this model is these would have to be smooth.

That would have to mean that one of them is dominant over the other when you put them in combination. So, in this thing, the big S would be said to be dominant. And the little s Would be said to be recessive. There's another little term here that I'm going to introduce because it'll help us talk about this stuff over the next few days, terms geneticists use all the time. Because these both have two of the same, they're said to be homozygous, do the same.

And this one, with one of each, is said to be heterozygous.

OK, so there is, I think, sort of the setup for Mendel's model. He had to contend with one other issue, though, and that was if every organism has two, and two parents get together and each donate something, unless you did something, the offspring would have four.

When those offspring got together, the next one would have twice as many, and so on. So, from probably, I don't know whether it was just from first principle, but I would imagine he figured out that if organisms had sex with

pollen and whatever, or egg and sperm, that something had to be done to get around this problem of an ever increasing number of particles. So, he envisioned that when there were specialized cells for sex, and that they have the number so that the sex cells would have half the number of particles so that when each parent donated one, you'd be back up to two.

It's pretty simple, straightforward thinking once you've gotten the idea that these things are coming in a particular form.

So, with this, could he now explain his results?

Let's do it over here. So, what happened in the first cross? He had a smooth, pure breeding line crossed with, so the sex cells from this, each one would have been a big S, and the sex cells from each one of this would have been a little s.

And as you recall, what he got was all smooth, right? Remember?

So, if we try and figure out what's happening here, a way of representing this would be to think what happens if all of the combinations that you could get, so if we paired them in all possible ways, then every combination would be identical from that first cross, one from one parent, one from the other.

And, if one was dominant over the other, it's going to look like this.

This is really the word I introduced you to.

That's the genotype. That's what's going on down at the genetic level. What you're seeing up here is the observable characteristics of the organism.

That would be the phenotype. So, then what would happen then with this if he crossed the F1's? Well, as you recall, they were smooth, but he was now seeing them as being like this.

So that means that the sex cells that are generated from this, each one will generate one big S, and one little s.

And then, if you put them together to see how this would work out, well, this one's two big S's. This is a big S and a little s, a big S and a little s, and two little s's. So, what he's got over here is SS, SS, and a ratio of 1:2:1.

But when you look at the phenotype, what would we expect? Well, this would be smooth, big S and little s.

That's smooth. Big S, little s, smooth again, and two little s's, that's wrinkled. There's his ratio of 3:1.

Has he proved anything? It works. Beautiful. The model must be right?

What do you think? Are you ready to publish?

Why is that? Why did the model work?

Has the model predicted anything yet? No, it works because it describes the data. To some extent, it's kind of like hitting a curve.

You said it, but you don't really know yet. Of course it's going to work, because if you got different data it would've had a different model. So, you're putting your finger on a really important point, and that is that you can do an experiment. You can get data.

It doesn't have to involve DNA sequencing or fancy technique.

You're getting data out of a biological system.

You've come up with a hypothesis that explains it.

But of course it's going to explain it because you wouldn't publish a model that didn't explain your own data. But what he hasn't done is tested it. Will his model predict the outcome of something that he hasn't already done? So, the suggestion was that he should carry out another cross. And that's what Mendel then did again. This is what he had to work with.

He could cross, and he could count, and he could do some calculating and some thinking. But, those were the techniques.

I really like thinking about this, because you can sort of put yourself in his shoes. So, what would you guys like to cross?

We haven't got much, right? One, he did. One cross he did, the F1 with the homozygous dominant parent, the pure breeding lines.

So, he's got this smooth, that's a big S and a little s, and he's crossing it with something, two big S's. So, the sex cells that you'll get out of this, so if you set this one up and see what happens, there's the two. You will get to big S's, big S, little S, the big S, little S. This is sort of uninformative. If you want to look at your notes afterwards, and have this be consistent, let me just flip this slightly. I put the two big S's up here, and this is our F1 down here. That way I'll be following the same pattern as I did before. OK, so there we are. In any case, they're all smooth, but that's not particularly helpful.

He's seen that result before. It hasn't really proven out. Given the sort of unexpected result that's predicted by his

model.

But he tried another one that's very, very similar.

And in this case, he crossed the F1 with the homozygous recessive parent. This is a really important process in genetics. And the reason, because it's so important, it's given a special term that's called a test cross. Let's see what happens with this one, because this one's more interesting. So, we take the F1. So, this is the F1. He's now crossing it with this homozygous recessive, so a parent that's got two particles that are little s. And so, will the sex cells look like this, a big S little s as before, to little S's here? So, if we set up this, as we've done, there are the sex cells from this F1.

Here are the sex cells from the homozygous, recessive parent.

Up here, we get a big S and a little s for each of those.

But here, we get two little s's. So, if we look at the phenotype, if you're out on the field or out in the garden sitting in the kitchen, after you brought your seeds in or wherever he was working, what would you predict you'd see in a cross like this?

These would be both smooth, but these would both be wrinkled.

So, here at the genotypic level, we've got a ratio, now, of 1:1. And here as well, there's now a ratio of 1:1. So, there you have a result that you haven't seen before. And, if you do that cross and get that result, again, it doesn't prove your model.

Scientific proof is never a QED.

Somebody can always come up with an experiment tomorrow that disproves it. It tends to work more. You just keep shoveling on evidence, and finally someone says, enough, enough, I believe you.

So, this was at least a test of the model, and the model survived this task. Now, he did one other experiment. Of the things that we've got on our plate right now, is there anything else we might do you can think of? He did another cross.

Pardon? Two of the heterogeneous ones, we did the F1's against each other. That's where we got the 3:1. We've already crossed the F1's, but I like your idea. What if we took the F2's?

In this case, it's going to be pretty complicated because they've got this 2:1:1, but one of the things you can do with peas is you can self fertilize them as well as cross them with their neighbors because they've got the ability to

make the eggs.

It'll become the seeds, and they have the pollen, which would be equivalent to the sperm. So it got both.

So, as long as there's some plants that you can self fertilize and some you can't, one of the really nice things about peas, they have the property that you can self fertilize them.

So, another kind of experiment that Mendel did, then, was to self fertilize another test of the model, self fertilize the F2's. Well, the model predicts, if we look back over there, that what you'll have there is a mix of things in a ratio of 1:2:1.

So, if you were to take seeds from that F2, and then cross them with themselves, you could figure out all the different outcomes, and then sum them up. You'd have a prediction for what this model would suggest. All right, so let me just take you through the pieces. Let's think about a quarter of them, according to the model, a quarter are that. So, if we self cross those, what we should get is all wrinkled because the only thing we're dealing with is the wrinkled trait.

So, that would be a quarter of the F2 seeds would be expected to give that outcome. So, three quarters of them are smooth, but it's tricky because there's two types of them in there, right? So, of these, one third are this, and two thirds have that.

So, what would happen if we thought about each of those individually, and thought about the outcome? Well, if we take the SS type, and we've self crossed them, what we're going to get is all smooth because all we've got in the cross are the traits for the smooth characteristic. And, if we take these guys and we've self-crossed them, we've already done that.

We know what we will get is we will get smooth to wrinkled in a ratio of 3:1. So, again, you could now sit down with that and figure out in total what you would predict in terms of smooth and wrinkled if you self crossed the F2, and if the model is correct.

So, that was basically [your? first UROP project, or the end of the first term, or the first year, or something like that. And he did publish those results that were published in 1866, which was the same year as those, about the same time as those results that Pasteur was publishing that I told you about earlier on. So, it was a very impressive in retrospect, a truly major intellectual leap.

But, it had almost no impact at all on the world. And there's a theme here that it sort of tried to hit up several times.

And we saw it, to some extent, with the early work on DNA, that someone can get evidence for an idea. But if the scientific community is ready to accept it, it can take quite awhile before that idea becomes credible even if it's correct. The data was there. The DNA was genetic material quite a bit before the general scientists thought it was, and it just seemed like it was too simple a molecule, too boring a molecule to be possibly able to encode anything.

And the same sort of thing happened here. It was some geneticists picked up on this but not until about 1900.