Introduction to Developmental Biology

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Lecture-09

Genetic basis (Part 2 of 5)

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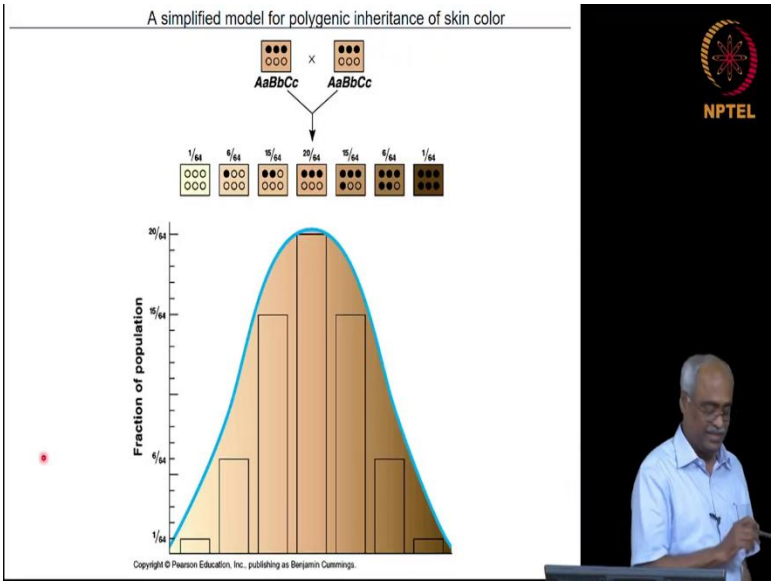

So, this an example of a polygenic trait. So, the nature of polygenic trait is they are not binary; it is not like a tall and short or terminal flower and an axial flower or purple and white. So, they have variation; there are shades, for example, the height of human beings; it is not like there are a set of dwarf people and a set of tall people. You have an entire range if you measure the height of a certain number of individuals like, for example, in this class. If you measure the height of all of us and plot the graph, it will look like the one seen in this slide. So, extremely short or extremely tall individuals will be fewer in the population, and the majority will be somewhere in between the

two. So, there will be an average, and its number will be maximum, so it gives you a bell-shaped curve. That is because of multiple genes having different alleles, and what combination come together could determine the phenotypic outcome.

So, here we are talking about skin color in an organism. Three different genes determine skin color here. So, let us say you have a parent who is heterozygous for all the genes, and then you cross it with a parent, which also heterozygous; you allow selfing. You will get these many combinations of genotypes; in one extreme, $1/64^{th}$ will be completely recessive and so on. And the other extreme again is 1/64 being completely dominant and then in between these many combinations are seen.

Due to the contribution of a given allele of a gene in combination with another allele of another gene, you get variations in the color. So that tells that variation in the phenotype is a good indicator of polygenic inheritance; human height is a good example, and in this particular organism, the color shade is a good example. So, this polygenic inheritance

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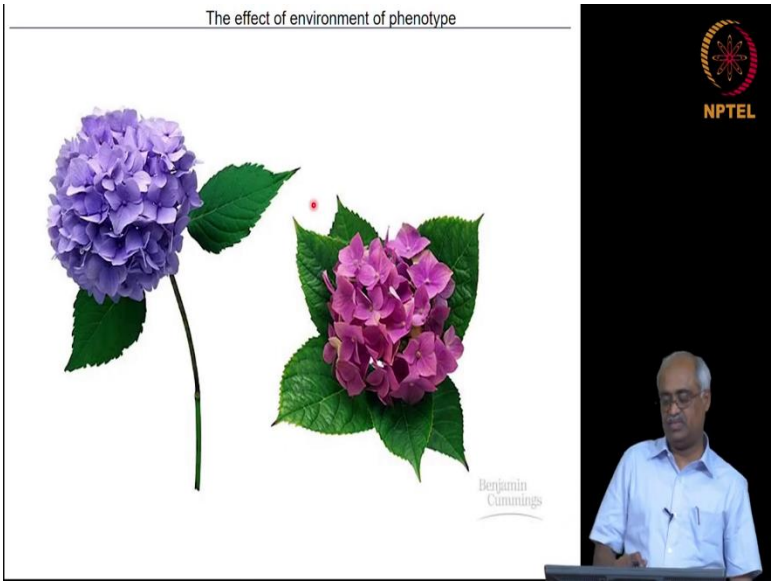

So, another example where environments do influence the gene function. A single gene might produce a certain pigment, and the pigment color could be sensitive to the pH. So, the plant grown in alkaline soil versus acidic soil can have different colors; this does not mean in this plant, flower color inheritance failed to follow Mendelian inheritance.

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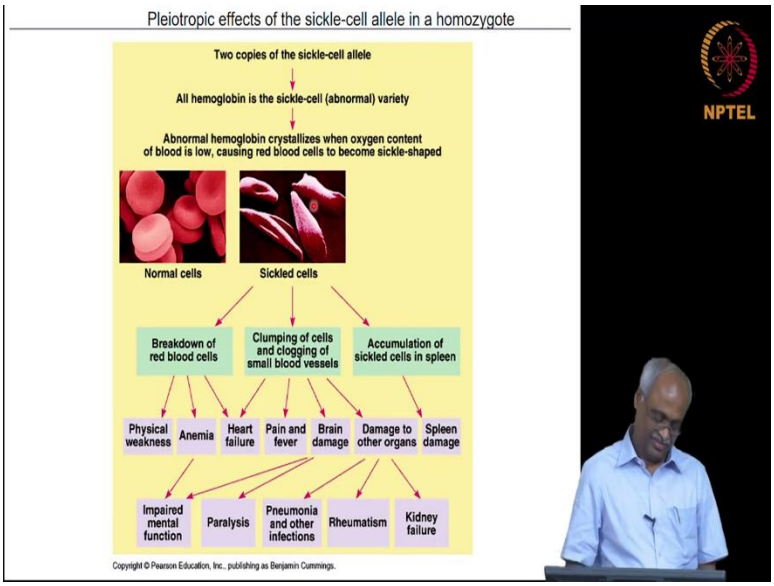

And there are opposite situations where you will get multiple phenotypes if you do one single gene mutation, and it depends on which gene is affected. A good example is sickle-cell anemia. Here you have one amino acid mutation, a glutamic acid to valine mutation caused due to change in one base. But then that affects the three-dimensional structure of the globin polypeptide such that the hemoglobin molecules can aggregate and form long rod-like aggregations and that affects the shape of the RBC and the RBC becomes sickle-shaped, and that is why this is called sickle-cell anemia.

So, the genotype is one single variation. Hence, two different alleles for a single gene, one has glutamic acid, and another has valine. The biochemical nature of that protein causes multiple phenotypes; due to the sickle-shaped, those RBC are going to be broken down, since they are not available for oxygen transportation you will have problems like physical weakness, anemia. Since they clog the arteries, the heart is also affected. Since blood vessels will get blocked, the brain will get affected too. And these cells accumulate in organs where these are degraded and cause damage to other organs as well. So, five different phenotypes are coming from a single mutation. So, here again, you have Mendelian inheritance being followed at the genotype level but not at the phenotype level. And you understand the phenotype when you understand the biochemistry and physiology of how that particular gene product functions.

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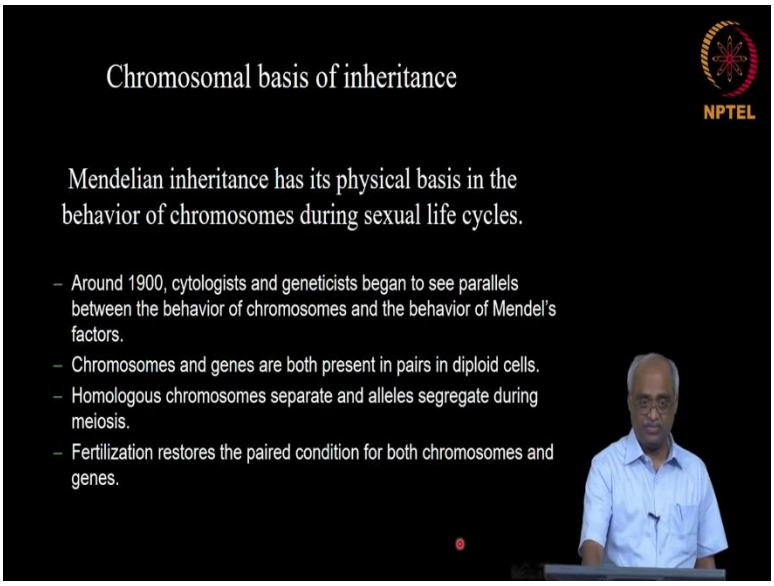

So, to understand all of these, you do not need to know the molecules or chromosome or DNA sequence. So, you can generate mutations and set up genetic crosses, follow the inheritance pattern faithfully adhering to Mendel's laws, then you will learn a lot about what those genes do without knowing even the protein sequence.

For example, we found a gene involved in garden pea flower color, but we did not know what protein it is, but we know there is a gene that is determining the flower color. So, in that same way, you can do complex genetic experiments and learn a lot about gene function without really knowing protein products.

So, then we are going back to history and trying to recollect the historical sequence of events very quickly.

So, people wondered that based on the phenotype, we understand what Mendel is talking about the gene, etc., but what is the physical basis of it? What is the nature of gene where are they present? So, then people who were observing cells using a microscope called cytologists found that chromosomes segregate very much like the way Mendel's genes. For example, chromosomes and genes are present in pairs; in our cells, 23 pairs of chromosomes are present. If you take chromosome 1, there is one more chromosome among the 46 that looked like chromosome 1. So, like that, you can sort that 46 into 23 pairs, so, therefore, they exist in pairs like Mendel's PP or pp. And homologous chromosomes separate, and alleles segregate during meiosis.

So, you get only one of the two allelic chromosomes in the gametes, and Mendel's assumption also was, only one of the two P's will be there in a gamete. And fertilization restores the two P's together, or a P with p and similarly the homologous chromosomes come together again. So, all of this told that probably genes are on chromosomes. So, you celebrate the solving of double helix right, but these were the bedrocks to that discovery.

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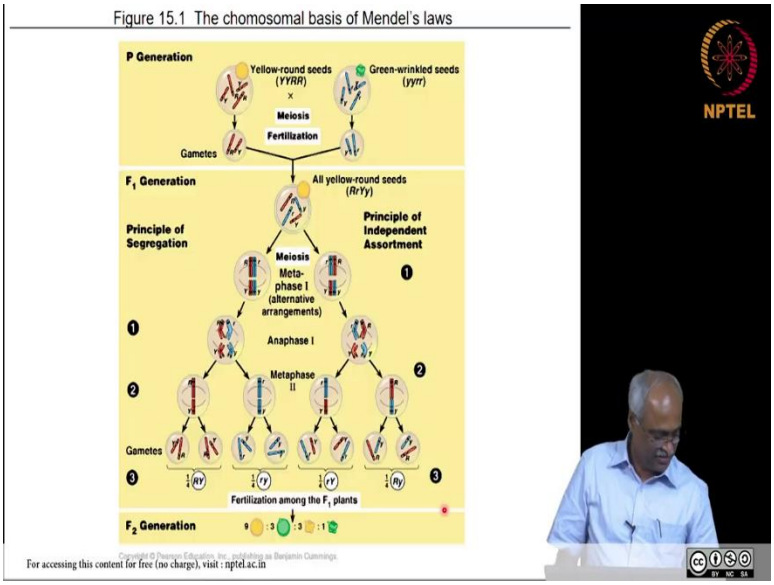

So, the chromosomal basis is illustrated in a cartoon here. So, you assume that flower color is on one chromosome and shape on the other chromosome, and then you find only one each in the gamete. We are assuming that they are in two different chromosomes, and therefore we are only taking two chromosomes here. And during fertilization, these two chromosomes come together, and then you have different combinations.

So, about this we will see in some detail in the next slide and then you have this homologous chromosome separation and then the sister chromatid separate, and then they come together to make the next generation. So, if you assume that the color is on one chromosome and shape is in another chromosome and follow through this meiotic process, then you get these gametes. Then when you bring it to the F2, you will find they follow the same ratio as Mendel's experiments.

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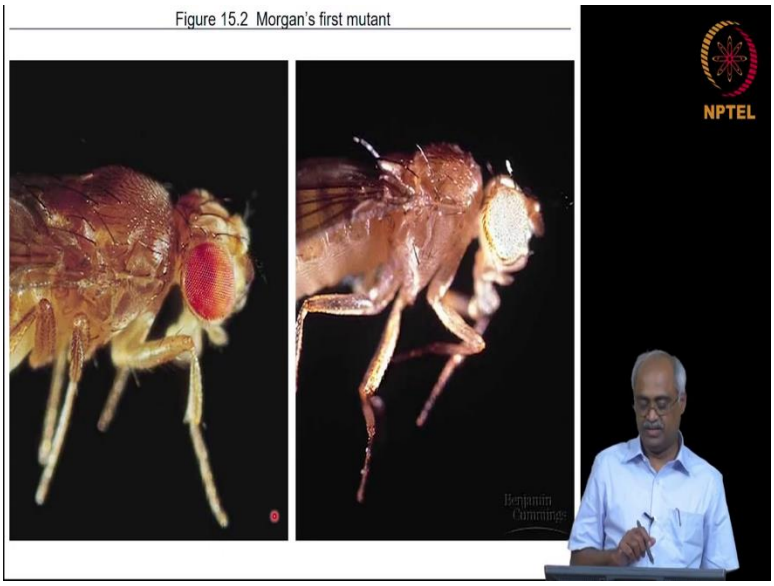

And so, there is another evidence that conclusively proved that genes are on chromosomes that came from Morgan's work. So, I think I mentioned this earlier while talking about preformation and epigenesis. So, Morgan identified that unlike the pea plants where there were a lot of characteristics or phenotypes in which variations were naturally available, in Morgan's experimental model Drosophila, there were no variations. So, he did not find any and after a lot of search he ended up finding just one male that had white eye and successfully setting up mating with that one male; he discovered eventually that genes are on chromosomes, we will see that.

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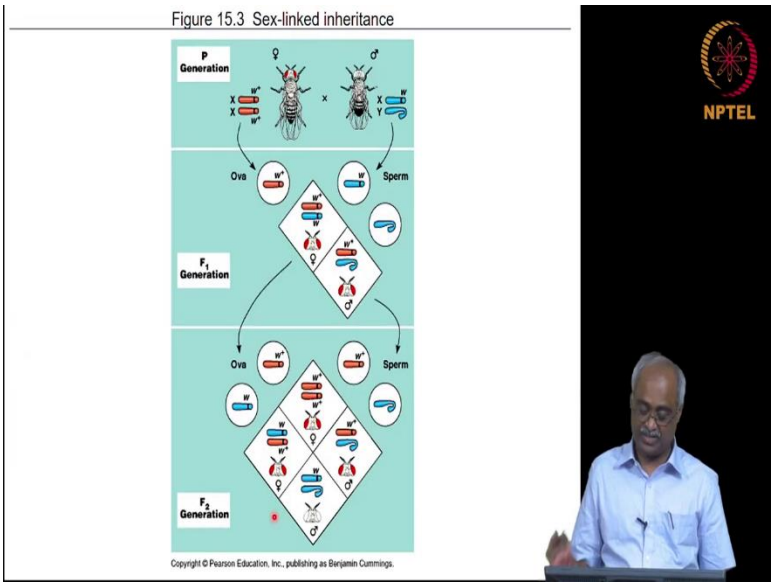

So, now let us assume the gene for eye color is on the X chromosome. Now we will call this **w+**, that is how the Drosophila geneticist name, they put a + for the wild-type or the one where the function is like dominant allele and - for the one that is mutant or where that gene function is not there. In this particular example, the + is absent here in this cartoon. So now the female will have two copies, males will have only one copy. So, Morgan reasoned that one copy was mutant in that male and the male did not produce the red pigment, and therefore it is white. During gametogenesis, the male will have 50% of the sperm with the Y chromosome and 50% of the sperm with the X chromosome. But the female will have only one type that is the X chromosome, and when they are brought together in fertilization, you have one wild type copy from the female; as a result, in the progeny, the female will have red eyes. And the male progeny will have one wild-type allele coming from the female parent, and only Y comes from the male parent; therefore, the F1 male will have red eyes. Now let us look at the segregation when you go to the next generation. Now this mutant X chromosome that came from the male is in this F1 female, and she will produce two kinds of gametes; one will have the mutant X chromosome; the other will have the wild-type. Now to make a male, you need the Y chromosome, and therefore, you will have two kinds of males, one getting the wild-type allele from the female another one getting the mutant allele. And now, you will end up finding males having a 1:1 ratio of white color and red color. So, this sex-linked inheritance immediately told the gene for eye color is not just on a chromosome; it is actually on the X chromosome.

So, this conclusively proved whatever the cytologists were observing and comparing with the Mendelian inheritance of genes is true and that genes are on chromosomes. This is something you should know even if this looks elementary education, but it is worthwhile knowing how we found the genes are on chromosomes.

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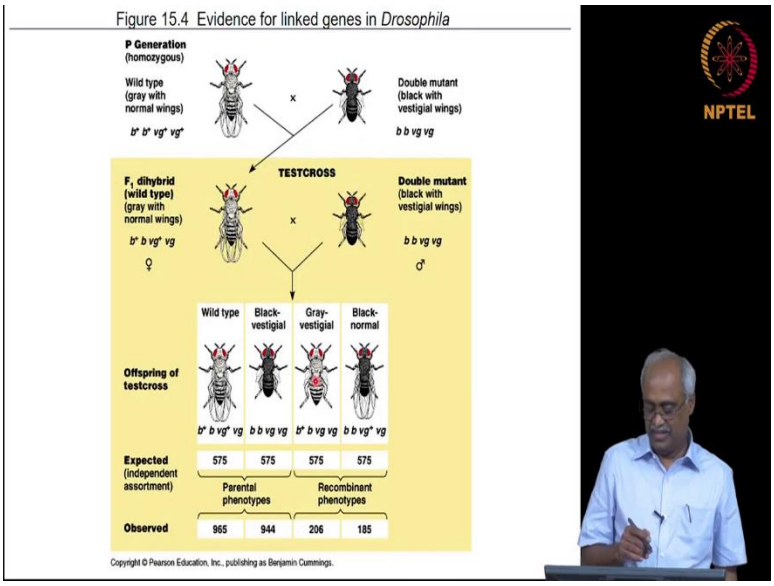

So, I will tell you a little bit of history here before we go into the next new idea. The new idea is not that genes are always on two different chromosomes, and one chromosome has multiple genes. If they have, then what kind of inheritance will be followed, so that is what is it in this slide. So, before going into this, you would be wondering if Morgan struggled hard to find one white male then now how you have the black body, grey body, and the normal wild-type wing, the vestigial wing, how did you get all of this?

So, Morgan and his students had no idea at that time how to mutate genes. Mendel used what is available in nature. So, Morgan searched hard and found one that was found in nature. So, now how do you create variations so they decided to mutate, and they did not know what is the nature of gene so as a result they did not know how to mutate that material. If you know the material, you will know how to handle it. So, they tortured the flies by putting them on low-pressure, high-pressure, heat, cold, pluck one leg or one wing and see what happens; they tried everything, and nothing happened. One of his students, Muller, learned that some physicists called Roentgen just discovered a thing called x-rays, and they have wavelengths in terms of the distances among atoms in molecules. Since gene is probably of that dimension, they thought to perturb at that level, and these x-rays are probably going to perturb at that level because of its wavelength being at inter-atomic distances in a molecule. So, they shined x-rays, and then they got all kinds of mutant flies, so x-rays were the first mutagen used.

So, now we can start with this experiment. So, Morgan experimented by crossing one fly strain that had wild-type wings, but grey abdomen with a fly with vestigial wing and black body. So, black is this body color, and when you have the wild-type gene, then you call it $\mathbf{b}^{+,}$ and when you have the normal wing, you call it as $\mathbf{vg}^{+}$. So, he took one where this is now pure breeding for these two phenotypes then they took another one where you have the black body and vestigial wing. Then they crossed and got the F1 hybrid where the dominant is shown. Here the dominant is the wild type copy, so you have one good copy that is enough to make normal wing and grey abdomen. So, now in the test cross, you should get 1:1 ratio; instead, Morgan got this ratio at the bottom; it was not 1:1 ratio, which would have indicated that each one is following the 3:1 ratio monohybrid wise. At the same time, this would have indicated an independent assortment. Still, if it was not independently assorting, then you should have gotten in this testcross exactly 1:1 ratio. So, he did not get that, which made him think they are probably on the same chromosome. If they are on the same chromosome, we will think that they should follow the rules of the monohybrid cross, like if they are together in one chromosome. If they are in two different chromosomes then they will follow a 9:3:3:1 ratio in a normal cross from F1 to F2 without test cross.

So, they did not get either, so he reasoned that they are probably on the same chromosome, but they have a way of sometimes separating probably the chromosomes break and join. So that is the idea that Morgan thought of to explain this result, evidence for linked genes. So, why did not Mendel see something like this when he has used so many different phenotypes? That is simply because, fortuitously for him, the chromosome size is extremely small. So, he got lucky, so every phenotype he chose were on different chromosomes because the chromosome size is small. So, maybe if he had looked like 500 different phenotypes, then you are likely to find two genes on the same chromosome. So, here Morgan saw this, and then he proposed multiple genes can be on the same chromosome, but they do not stay there forever; the chromosome seems to exchange parts. A part of chromosome 1 in Maternal (M) exchanges equal part with chromosome 1 in paternal (p).

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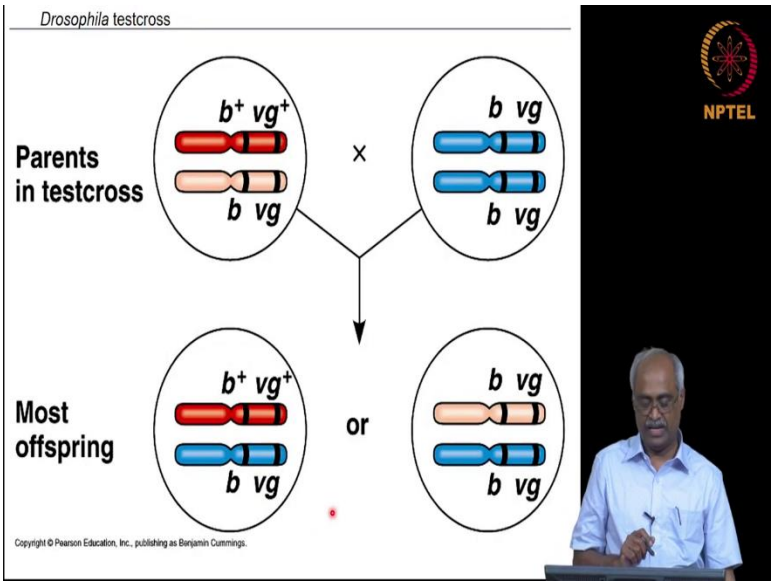

And that is explained here in this slide. So, normally these are the parents and most offspring's will be like this because they are not separating and they are together, this is what normally you would expect.

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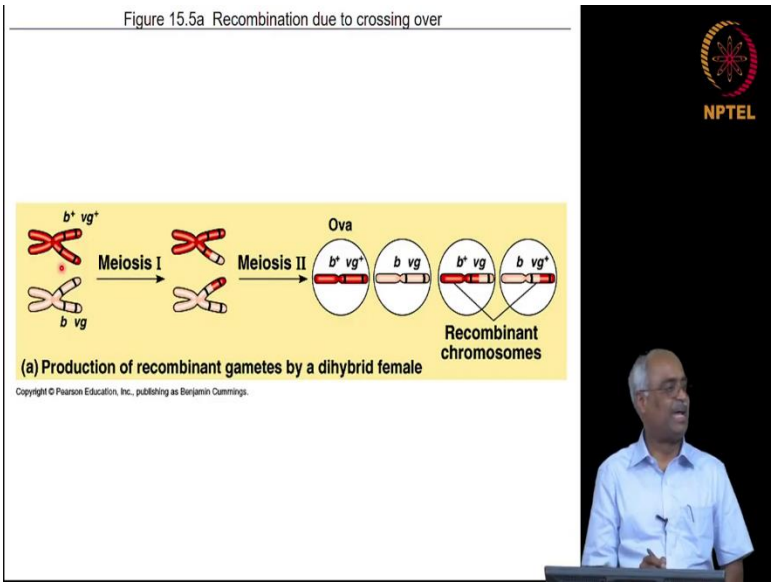

But if you imagine the exchange of parts taking place between these two chromosomes and the chromosomes get duplicated. Then you end up producing these recombinant chromosomes; we started with $\mathbf{b^+}$ and $\mathbf{vg^+}$; now, you have a situation where you have $\mathbf{b^+}$ and **vg**, and that is because of this swap. So, this was something the cytologists had observed during meiosis, and Mendel

proposed something like this can happen, and you will have these recombinants. So, when you get the black-bodied fly having the normal wing; and the fly with a grey abdomen and vestigial wing, unlike any of the parents, then they are called as recombinant progeny. So, this how you identify recombinants. The two phenotypes get mixed in the progeny and because that is essential to think about the next topic in this.

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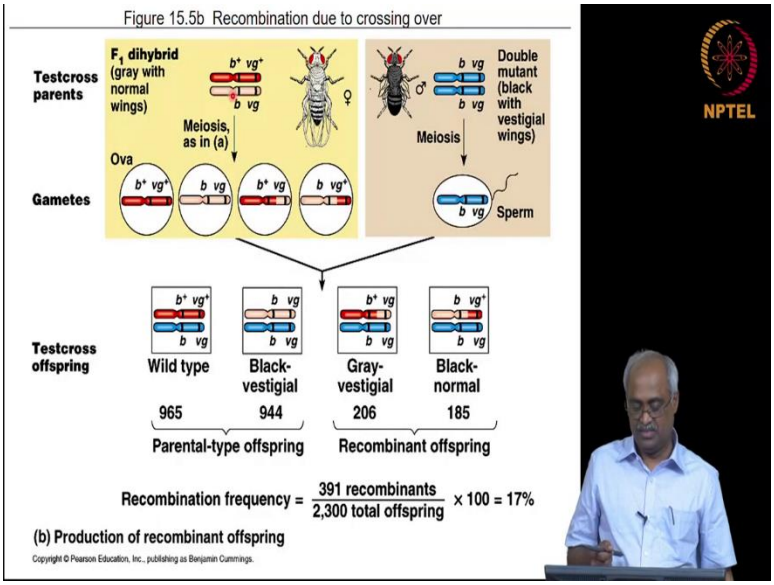

So, by proposing that model Morgan could explain how he got this ratio. So, in most of them, the swapping did not happen between the **b** and **vg** loci, and as a result, most of them ended up being like either one of the two parents. And in some of them where that crossover recombination happened, ended up having recombinant offspring. So, in this particular example you can determine the recombination frequency by calculating among the testcross progeny.

You total the progeny you got which is 2300, and then you look at how many that are recombinants that are the black body with the normal wing or grey body with the vestigial wing, and you total them. In this particular example,

$$\frac{\text{391 recombinants}}{\text{2300 total offspring}} \text{ X } 100 = 17\%$$

So, the recombination frequency in this case is 17%.

So, Muller and a few other Morgan's descendants did not stop there they proposed if the genes are closer together then the recombination frequency is likely to be less. The probability of assuming that the crossover this; the parts can be swapped anywhere along the length of the chromosome. Now if you take loci that are farther apart, then the probability of a cross happening between the two is higher than when you have chosen them closer together. So that means the recombination frequency is a function of the distance between the two loci. In other words when I take two different phenotypes and cross them and based on recombinant frequency, I can determine the distance between the two loci on the gene, so this is the basis for genetic mapping. So, the recombination frequency indicates the distance between the two genes on the chromosome.

So, this way of determining the distance between the two genes is not precisely physical distance. So, the physical distance is measured by the number of DNA base pairs; this is based on the frequency of crossover. It is not always directly proportional to the physical length because due to the chemical nature. For example, the structural uniqueness for different sequences could have some parts of the chromosome where recombination readily happens in a particular locus, and in other loci, you will have variations. Except those hotspots where the frequency of recombination is higher than an average locus, mostly this holds true, and this is the basis by which genetic maps are developed.

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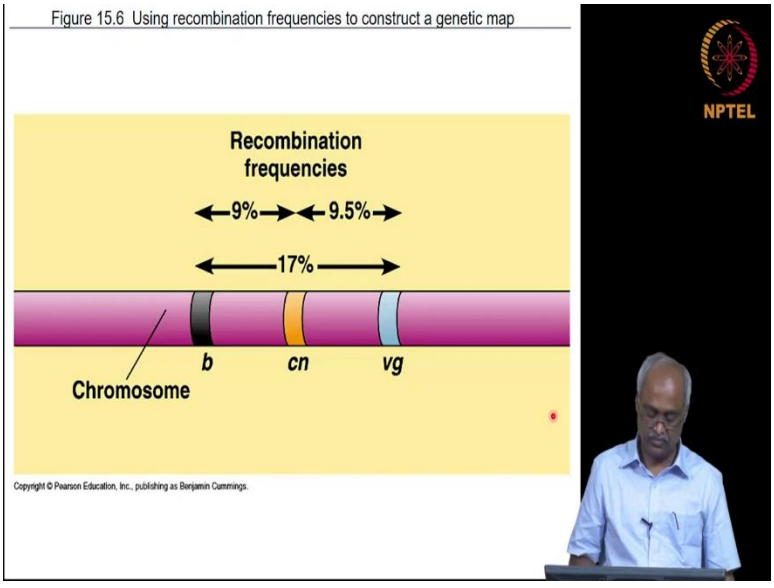

So, in this slide, if you take another locus here this **cn** and then when you do the same experiment by finding out the distance between **b** and **vg** loci, these people were able to tell **vg** is actually on the left side of **b** on the chromosome. But the main point here is the recombination frequency reflects the distance between different loci on a given chromosome.

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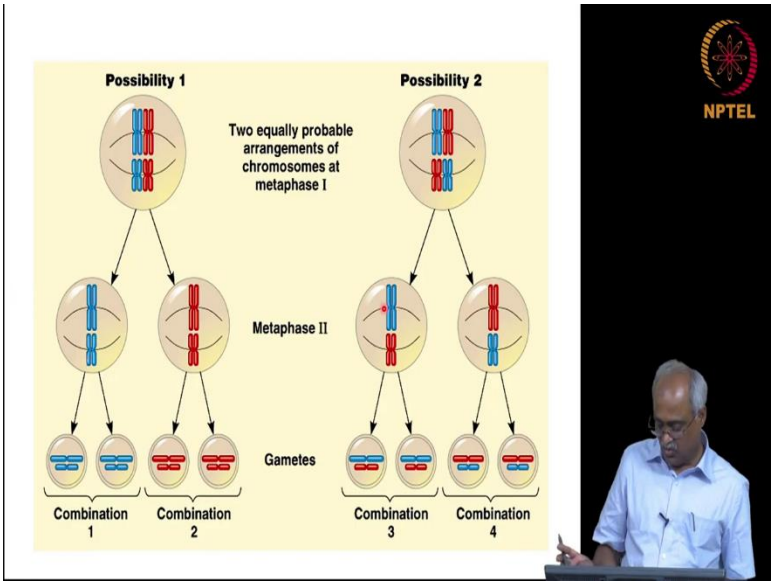

So, the variations always do not arise due to new mutations; it can arise due to simply allelic shuffling during the crossover. For example, you look at two individuals; there was no new mutation that happened in either one of them. No base change happened during DNA replication; it is just the allele of one gene, and allele of another gene came together. Because of the crossover, this variation is generated. This is important because most variations in nature on which natural selection works are based on that. So, the variations among us here are not purely due to each one of us generated a new mutation as we began as a zygote. It is simply by which one of your two grandparents' chromosomal regions during recombination, during gametogenesis in your parents, was swapped and brought together; we call that as allelic shuffling. So, remember this, in future I might use that word just commonly without explaining. Allelic shuffling creates variation. You do not even need that to create variation particularly if you consider an organism which has 23 pairs of chromosomes.

So now, we are going to look at two chromosomes here in the slide. So, the chromosomes are duplicated; therefore, two sister chromatids are present together here. And let us say this blue chromosome came from your mother, and this red came from your father; both are duplicated. And now, in metaphase, they could be randomly present, the maternal copy is here on the right side, and the paternal copy was on the left side or it could be this way. There are no selections here; the force that operates here is bringing all chromosomes to the metaphase plate and making sure one of the two homologous chromosomes go to one daughter cell after meiosis I. In contrast, the other one goes to the other daughter cell of the meiosis I. It does not worry about which one of the two allelic chromosomes were taken. As shown in the cartoon, you could get both these possibilities, after sister chromatids gets separated you will have either combination one where both chromosomes are of maternal copy, or combination 2, where both chromosomes are of paternal copy.

So, you could already have four different variations generated in the gamete, even without mutations and allelic shuffling coming from the crossover. It would be best if you remembered this because these are all fundamentals to follow what happens in biology. I do not worry about what happens in genetics because this now governs everything else.

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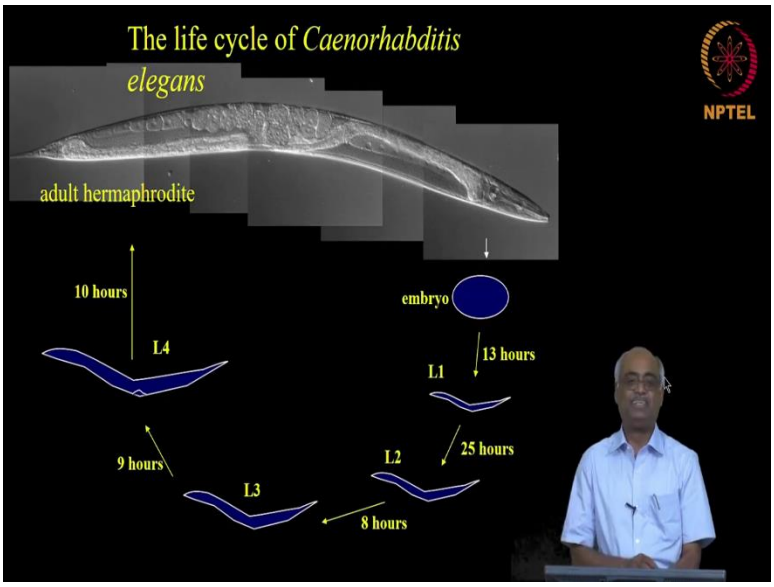

So, for the rest of genetics, I am going to take one model organism and use a genetic screen done in that organism as an example to understand some of the fundamental concepts. It could be done

using any one of the genetic models. So, I am taking a model where I am familiar with the original literature. So, that it is going to be *C. elegans*. So, this topic can be taught and understood using Drosophila or Arabidopsis as an example too.

So, in this particular genetic screen, you are going to find all different concepts found that one screen itself. So, before we go into that, I will give a brief introduction of model organisms. So, when we choose a model organism, we select an organism where its genome size smaller, why? For example, if I want to look for a gene involved in germ cell development in *C. elegans*, having only 100 million bases versus finding a gene that is involved in the same germ cell development in 3 billion bases, I need to screen a minimum of 30 times more individuals after mutagenesis in that the second organism which is the Homo sapiens. So, you need to know this, many times people just say that a smaller genome is better but they do not understand why smaller genome. And the second one which is illustrated here is an adult this is about one millimeter long; it is not this big as shown in this image. This adult produces an embryo that will go through the larval stages at these hours mentioned here and becomes an adult that reproduces less than three days little more than two and a half days but certainly by third day. So shorter generation time helps in obtaining the required results in a shorter time span. So, these are some of the advantages with *C. elegans.* There are more but I am not going to get into all those details we will learn them as we go.

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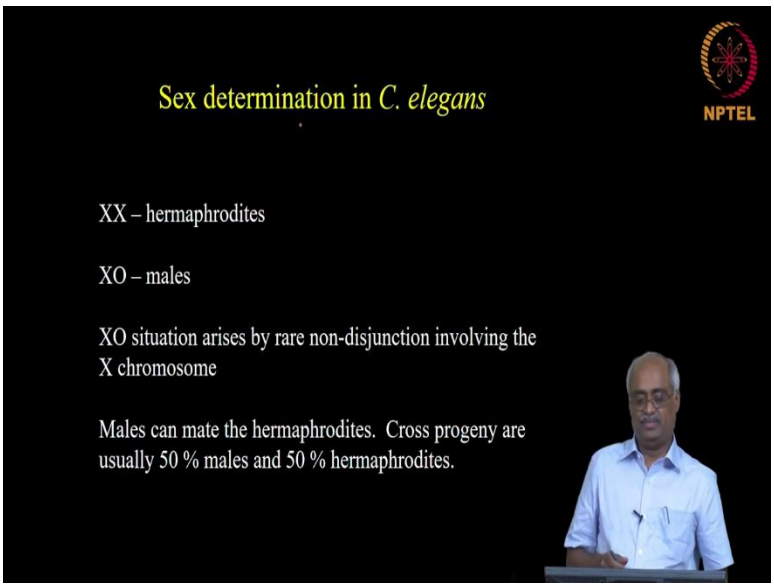

So, this is the necessary information that we need to know before we go forward. Sex determination in *C. elegans* happens like in any other sexually reproducing organism, *C. elegans* is a hermaphrodite. So, two X means it is a female, if it is X0 then there is no Y chromosome. So, it is the number of X that determines sex in *C. elegans*.

People usually say X to autosome ratio so I will simplify that as the number of X chromosomes. 2X chromosome means female and 1X chromosome means male and this X0 arises usually due to defects in meiosis called non-disjunction where both the homologous chromosomes go into one of the daughter cells after meiosis I, therefore the other daughter cell lacks that chromosome entirely. After meiosis, the gamete will not have the X chromosome if non-disjunction happened with the X chromosome. So, if that gamete fuses with a normal gamete having one X chromosome you get the X0 situation and those zygotes will eventually end up becoming males. Interestingly these females during their sexual maturation the first 40 germ cells go through spermatogenesis and give rise to sperm. Those sperm are stored in a pouch and then the germ cells switch to oogenesis and they behave like a female. So, it is called female because her anatomy is female anatomy. She has the uterus and the vulva to lay the embryos out. So anatomically it is a female, it is just that she can make sperm as well but she does that only when she's young.

Since she makes both the gametes, we call her hermaphrodite, but anatomically she is a female and reproduces like other sexually-reproducing organisms, where the germ cells undergo crossover and the homologous chromosome duplicate and separate during meiosis I and form gametes. So, the two gametes will have to fuse to make a new zygote; so, it is a perfect sexual reproduction just that it can make both the gametes.

So, males are like any other sexually reproducing male. Anatomically they are differentiated as a male, they have vas deferens that make a lot of sperm and they have the copulatory organ to mate with the hermaphrodite and they do not have a uterus or vulva. So, this gives us a big advantage for genetics so that is one of the reasons why I need to introduce it at this point.

Since males are X0, now through normal meiosis with no defects, half of this sperm will have X, and half of this sperm will have no X because there is only one X and therefore when a male mates with a hermaphrodite 50% of the sperm will have an X, 50% will have no X. So, as a result without looking for any mitotic defect, the progeny will be 50% male 50% hermaphrodite. So why is this

advantageous for genetics, you can do selfing without going through the laborious process that Mendel went through.

All you need to do is take the hermaphrodite put in one plate she makes both the gametes there is no need to cut open the flower and put a cover and take a paintbrush to take pollen, none of that is required. So, when you want to cross you take the male and hermaphrodite of the right gene and put them together and they will produce the hybrid progeny.

So, in the next class, we will continue from there and we will look at actual experiments and what can be learned from them. So, the main point I am driving home is you can learn a lot about molecules and their functions without ever actually knowing that DNA sequence or purifying the protein and determining what that protein does. So, that is what we are going to learn at the end of this series of genetics classes, thank you.