Introduction to Developmental Biology

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Lecture No – 28

Evolutionary Developmental Biology (Part 3 of 3)

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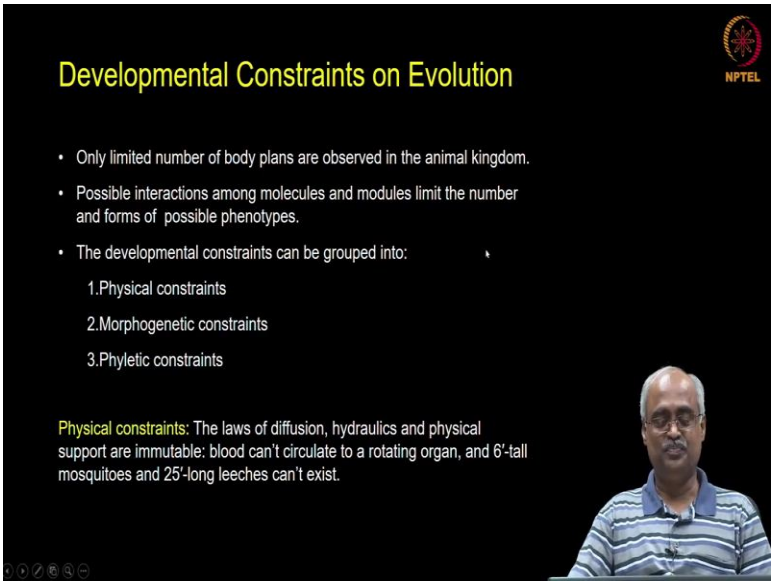

Welcome back to the developmental biology course; for this session, this will be our last class. Until the previous class, we discussed the various mechanisms that generate phenotypic variations. We learned that various phenotypes could be generated from a common lineage by independently varying the developmental modules and the genetic module through those mechanisms. Now we got an impression that there is no limit to the varieties of phenotypes an organism can generate. But the reality is different, although we have a remarkable diversity among biological structures. Initially, it was hard to believe there is unity among all organisms, and they all came from a common ancestor. But if you look closely, you will realize there seem to be a limit to the body structure variations that can be generated.

So we do not find any organism that uses a bicycle wheel to move around instead of using a limb movement. We do not see any organism with a wheel-like structure, a moving organ, but if you look at the human inventions, almost all of them seem to depend on circular motion. So, when you think in these lines, you will soon realize there appears to be a limit to the types of structure and shapes that biology can create. And those limits are imposed by the body plans initially laid out.

So the mechanisms that we went through impose certain restrictions. So that is going to be our current theme, developmental constraints. Therefore, these constraints will limit the kind of variations possible, which will determine the variety from which natural selection can choose. Thus, evolutionary adaptation, therefore, is going to be restrained by the developmental possibilities that exist.

We are going to look at what those constraints are and how do they impact organism development. So the first constraint we are thinking about is the physical constraints. For example, one cannot disobey the laws of physics and chemistry. Molecules diffuse only at a specific rate and no faster than that. Similarly, the movement of fluids against gravity or towards gravity will be controlled by the laws of fluid dynamics. Also, physical support, what weight can be borne by what kind of a structure, and so on.

One of the very easy things to understand is mentioned here; blood cannot circulate to a rotating organ. Imagine setting up a plumbing work for something rotating all the time; how do you send fluid to it, and how do you take fluid out from it. And think about a mosquito that is 6 feet tall or in the leech's body plan making it 25 feet long.

These are not going to be readily permitted by the basic body plants, and these are the physical constraints here; the laws of physics determine these. So we will look at more such constraints as we go along.

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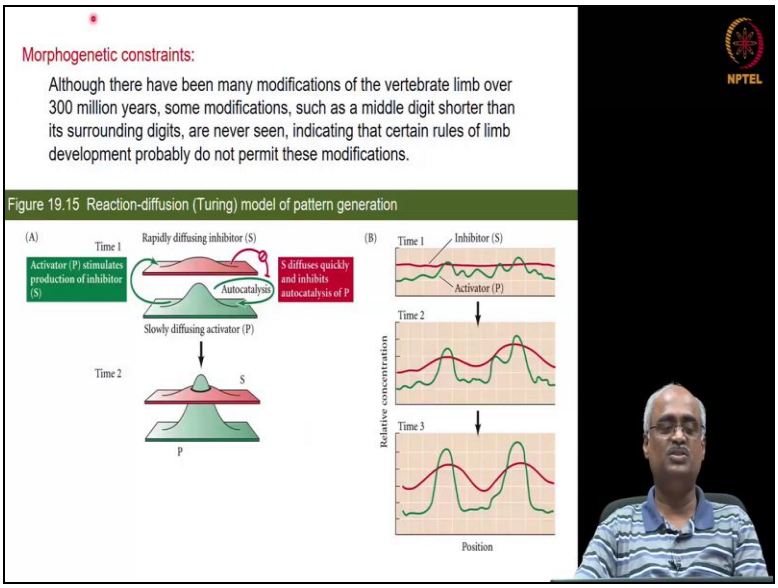

Next, we will look at morphogenetic constraints. So morphogen is a molecule whose concentration determines what genes it will turn on or turn off. It will regulate a different set of genes at a different concentration, so the concentration is critical here. So the morphogen usually forms a concentration gradient, and in that field of that gradient, you have different concentration levels due to which different genes will be regulated. That is called a morphogenetic field, and that itself provides a certain constraint.

The rate of production of a molecule and its diffusion rate and the effect of inhibitors that would inhibit its production or its activity altogether will set up a framework that will govern how a morphogenetic field is going to behave. And due to that, the kind of structures that morphogenetic field will permeate will be limited.

For example, if you look at your foot, the middle toe is longer than the one towards the end from the biggest one. So you will never find an organism where the middle one is shorter. That is probably is governed by the morphogenetic field that sets up the growth rate of those toes. And similarly, many examples exist. Before we look at an actual example, let us look at a mathematical model that explains a morphogenetic field's boundary conditions.

So the famous model that explains this is called a reaction-diffusion model proposed by Alan Turing. He is a computational biologist, but he was interested in many areas. His major contribution to biology is this reaction-diffusion model. So this model explains how two homogeneous chemicals would behave in a solution with certain properties. So let us take two molecules, here one is called P, and the other one is S. Let us say P is an activator of a certain phenotype, and then this, P has a certain diffusion rate. So it diffuses from its point of production if you see this greenish graph here. So it diffuses rather slowly compared to another molecule called S, which diffuses rapidly, and as a result, it forms a shallow peak compared to the other.

Let us assume P activates its production and S's production, an inhibitor of P. If these were the properties of these two molecules now, so let us see what happens over a while.

Let us slowly go through this, if P produces S, S will also increase wherever P increases. S will diffuse faster. Going away from the P peak and suppressing P then slightly around the P peak S will prevent the P peak from forming further because this S concentration would suppress P coming. As a result, smaller shoulder peaks around P's initial mound do not happen due to S's property.

So if you leave this homogeneous solution of P and S for some time and based on their diffusion rate and effect on each other, you will generate this sort of pattern that you see in the slide. Initially, you will have multiple P's because P is an auto activator. And therefore, where there is a P, you get more P, then you will get S. If S is distributed uniformly, then S will uniformly suppress P, and that would lead to this sort of a change in S because of P, wherever P is more S, is going to increase.

The S will diffuse quite rapidly, suppressing these smaller P peaks, which would eventually lead to this condition. And suppose this morphogenetic field will be set up this way; in that case, only a certain type of development will be possible. Every possible variation is not going to be accommodated by this kind of property of this morphogen. This is just one example of a model that does not cover all the morphogens. This is just an example to make us understand how there will be limitations in a morphogenetic field. And these sort of constraints provided by a morphogenetic field will limit the variations in the possible structures.

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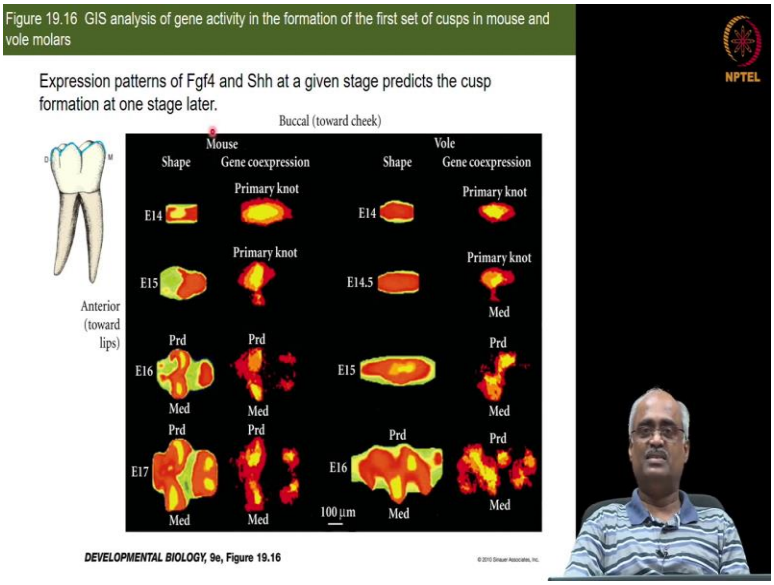

For example, this reaction-diffusion model was famously used to explain the cusp's formation in teeth. If you look at your teeth, let us take one of them, a molar tooth. On the surface of the tooth where you are biting, the visible part, you have these shapes, called cusps. And the mound-like shape is what is a cusp. And the pattern of this cusp is determined by a morphogenetic field that follows the reaction-diffusion model.

Therefore, from the reaction-diffusion model, how the morphogens ultimately responsible for this cusp pattern can predict what kind of cusp pattern will form. And that is what is explained by looking at the cusp pattern in mouse and another rodent, Vole. In these two animals, the initial production of FGF4 and Sonic hedgehog, their diffusion inhibited another molecule, a BMP.

Based on that, scientists could predict a little ahead of the cusp pattern by looking at the gene expression. For example, the gene expression pattern of embryonic day 14 helps you predict the cusp pattern that will be formed on embryonic day 15 in both these organisms. These two organisms' expression patterns and their variation help us explain the final cusp pattern variation in these two organisms.

So this is the gene expression pattern, FGF is in one color, and SHH is the other color, and based on that expression pattern, you can predict the structure that will develop. So this sort of a structure formation you see it here, and then this variation ends up becoming these two and so on. And here in the original Turing's model, all that they needed to incorporate is that as the development progresses, the two molecules' diffusion rate does not remain the same because extracellular matrix forms will affect that. So a correction factor for such changing behavior of extracellular matrix, which changes the diffusion, had to be incorporated.

And another thing was the binding strength of the inhibitor varies again, and by changing these two, scientists were correctly able to predict the cusp pattern in the molar teeth of these rodents.

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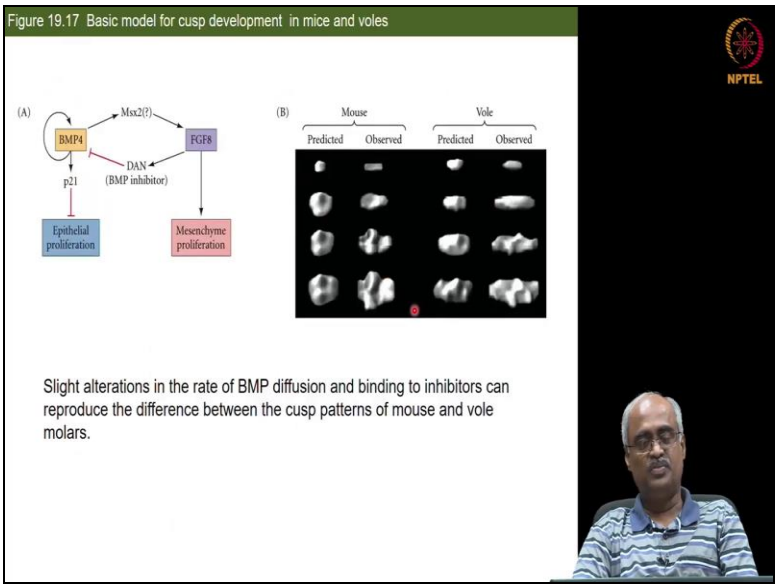

So here is the way the reaction works. So BMP4 promotes epithelial proliferation; this is the cell that is going to deposit the enamel. In contrast, FGF8 promotes underneath mesenchymal cells, which form the dentin, the layer towards the inside from the enamel side. BMP4 and FGF8 have a relationship, as you see in the slide. In addition to promoting its expression, BMP4 also promotes the expression of a gene that will ultimately produce the FGF.

So BMP4 is like our P in the reaction-diffusion model, and FGF8 is like the S. So the S, as we saw earlier in the model, here again via DAN, ends up inhibiting BMP4. This is the kind of molecularly determined relationship, and then when you use those molecular parameters in the reaction-diffusion model, you can predict. So this is the predicted cusp pattern based on these relationships and these two molecules' phenotypes.

So you see the predicted pattern and observed pattern in the slide. They are more or less the same in both organisms. So slight alterations in the rate of BMP diffusion and binding to inhibitors can reproduce the difference between these two. All you need to do is just the diffusion rate because of variations in the extracellular matrix and the allelic difference coming into play at the level of fine binding affinity of these inhibitors.

And these two could explain the difference between the two, which could be predicted based on the expression pattern or observed at an earlier stage.

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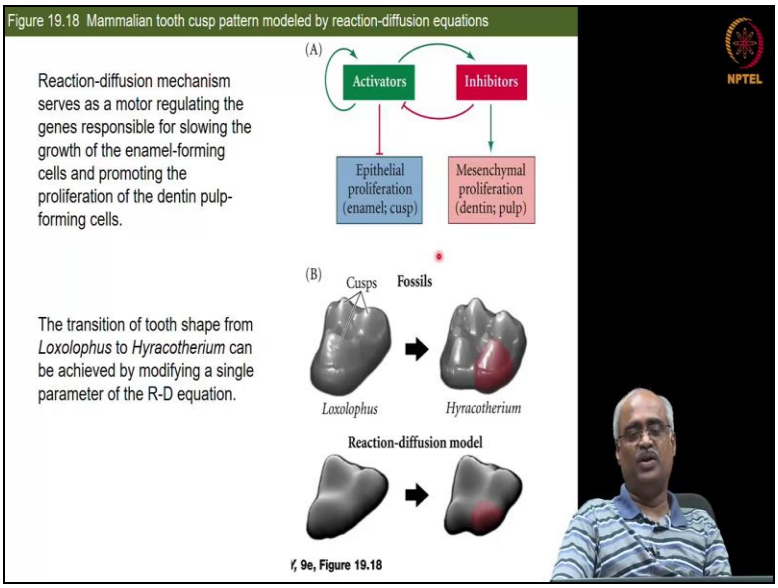

This also has been useful in tracing the evolutionary history of tooth development among horses. So that is shown in this slide; here you see the summary of the previous one. So this acts as a motor; the activator activates itself and also activates the inhibitor. The inhibitor inhibits the activator. This relationship, which acts as a motor, controls these two tissue formations; the rate of proliferation of these two is regulated by these structures, these genes or molecules regulating themselves.

By looking at an ancient horse's expression pattern like this Loxolophus and by inducing certain BMP and SHS changes, people can predict how this structure would form in the modern horse. And by looking at the concentrations in this kind of structure and predicting using the reaction-diffusion model, you explain how this fourth cusp forms in modern horses.

Here we see reaction-diffusion in how this will have certain restraints and, therefore, only a certain limited variation in the possible structures. This is why you do not get the middle toe or the middle finger in your hand being shorter because the morphogenetic field that sets up the field is such that the genes expressed only in a certain way, which ends up producing only these lengths of the toes or fingers.

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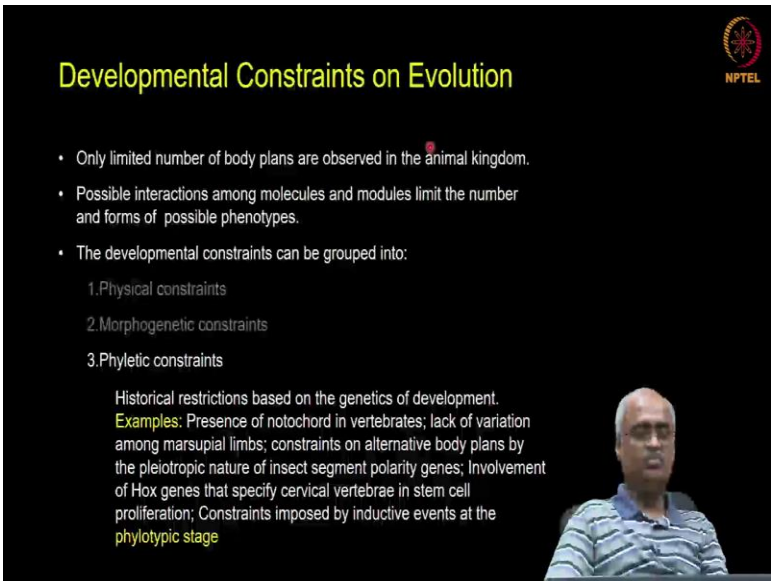

The principles that govern the diffusion here end up governing the length of the fingers. We saw two of them, the physical constraints like you cannot break the physical loss; for example, blood cannot be supplied to a circulatory organ. Then we saw that another morphogenetic field would have certain principles that govern them, which will impose certain constraints on development. Third, we will look at the evolutionary history of specific development; a body plan formed in one particular way now cannot be reworked because evolution works on what already exists. You cannot go back to the drawing board to say and then redraw the whole thing from scratch that does not happen in biology. So this is one of the strongest evidence that people put forth to prove that modern-day organisms came from ancestors. Therefore, the organisms are not perfect machines.

There are many examples; I would urge you to read a book by Richard Dawkins called the greatest show on earth. In that book, he lays out many examples to illustrate how our body is imperfect from an engineer's point of view of designing and building an efficient machine. So that evolutionary history of a certain body plan lays certain constraints, which we call Phyletic constraints. So the word phyletic is coming from phylogeny.

Let us look at some of the examples. Notochord in vertebrates like us is only a transient organ in the embryo. Still, we are dependent on it for that particular stage of embryonic development to instruct the neural crest formation and the somites. But if you look at the evolutionary history, this particular way of making neural crest and somites formed in earlier chordates where the notochord exists in the adult and functions. Due to that evolutionary origin, although we do not need it in the adult, it remains as our important crucial structure in our embryonic development.

And another interesting example is the lack of variation among marsupial limbs. Suppose you look at other vertebrates like eutherian mammals. In that case, you see variations like the hand,

batwing, flipper, and then your claws. All those variations have not happened in marsupial limbs primarily.

The first thing the fetus needs to do once it comes out is to find a way to crawl and climb on into the mother's pouch; so that means the limb development, to be able to grasp and crawl has to happen first. There you cannot experiment because life depends on it; its safety depends on that. As a result, those limbs did not vary much over a long period. So that is a lack of variation, and then constraints on adult alternative body plan by pleiotropic nature.

So if you have a molecule, let us say a protein, for example, the segment polarity genes. Initially, they are required to identify a particular segment, provide segmental identity but then later on, that molecule has been adopted for multiple functions. And once a molecule is involved in doing multiple works, you cannot get rid of it because then the whole thing will die. It becomes so vital it is going to have multiple phenotypic problems.

That pleiotropic nature of certain molecules ends up constraining possible variations, for example, in insect segmentation. An excellent example in vertebrates is the hox gene, a particular hox gene that specifies the cervical vertebrae. So we saw that the cervical vertebrae are more in chick than in mouse. Why cannot we have more in our neck? Why can I not have a longer neck?.

So that is because this hox gene that specifies the cervical vertebrae got involved in regulating stem cell proliferation as well. So if I want more of it to make a longer neck, then I will end up promoting an unnecessary excessive proliferation somewhere causing a tumor, and not surprisingly, this is not simply a prediction for an argument. This has been proven to be true, and that is shown in the next slide. And then, we will come back to this phylotypic stage, which is another important constraint.

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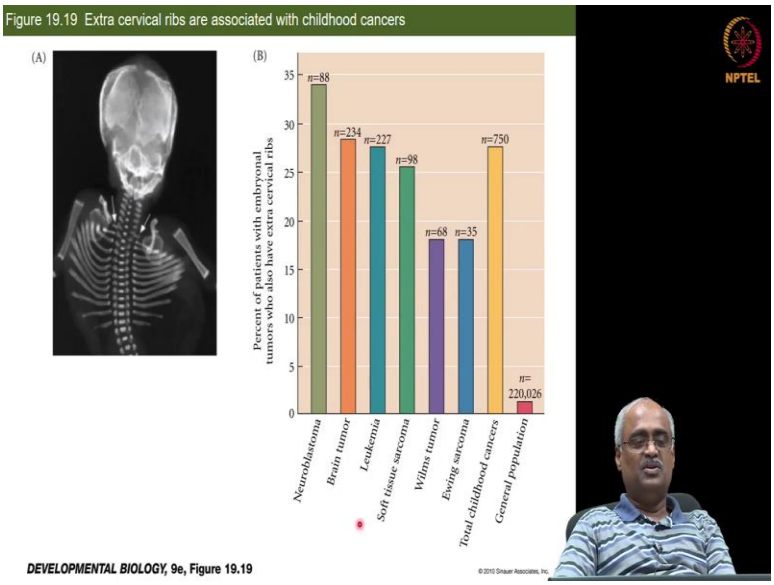

So, here in the slide, this particular child as an infant had one extra pair of cervical or a vertebra. As a result, it ended up having embryonic tumors and died. So scientists looked at the children's embryonic tumors and what fraction of those children had this extra cervical bone. So that is shown in this graph.

In the general population, the percentage of patients with embryonic tumors also had the cervical ribs a very small percentage, probably 1 or 2%. But if you look at the total childhood cancer in all of them, this extra rib frequency was a lot higher compared to this general healthy population. So indicating a direct connection between this extra rib and then the cancer is because the hox gene involved in this extra rib formation is also involved in stem cell proliferation. So, due to this, you are going to have a fatal condition.

Therefore, these extra cervix-producing vertebrae will not be tolerated in evolution, so this is what you call a phyletic constraint. So the other one is this phylotypic constraint so that we will look at it in some detail.

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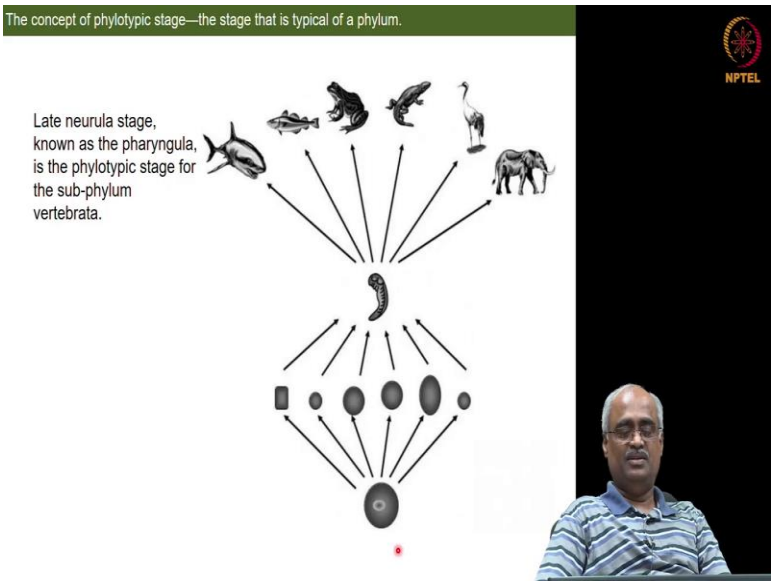

So we would intuitively think that the early embryonic development probably is not very flexible, and we cannot have any alteration that will mess up the entire embryo. Still, surprisingly that does not seem to be the case. So many members of this subphylum Vertebrata can go through a variety of cleavage and gastrulation patterns. For example, while we talked about early mammalian development, the mammalian embryo is unique in many ways. I told elaborately about this rotational cleavage, asynchronous division, blastocoel formation compaction, and so on. They do not happen in other ones because that is the hallmark of mammals. Variety of these early embryonic steps, like cleavage and gastrulation, all come to a certain common structure during embryonic development. In this particular case is the little later stage of the neurula stage, which is called pharyngula. So this pharyngula stage, all these members of this subphylum vertebrate all produce this structure. And such a structure is called the phylotypic stage meaning this structure is typical of this phylum.

So that is why it is called the phylotypic stage. This stage of embryonic development typifies this phylum, and it is unique to this phylum regardless of other subgroups within this phylum. So we call this the phylotypic stage. Once the body plan is laid out, then that seems to be fixed. It can only generate these varieties, and you cannot meddle with them. Once you have come here, you will be a vertebrate; you will not be something else. And that is the bottleneck created by the phylotypic stage. So this is another example of phyletic constraint, and this is explained in some detail in the next slide.

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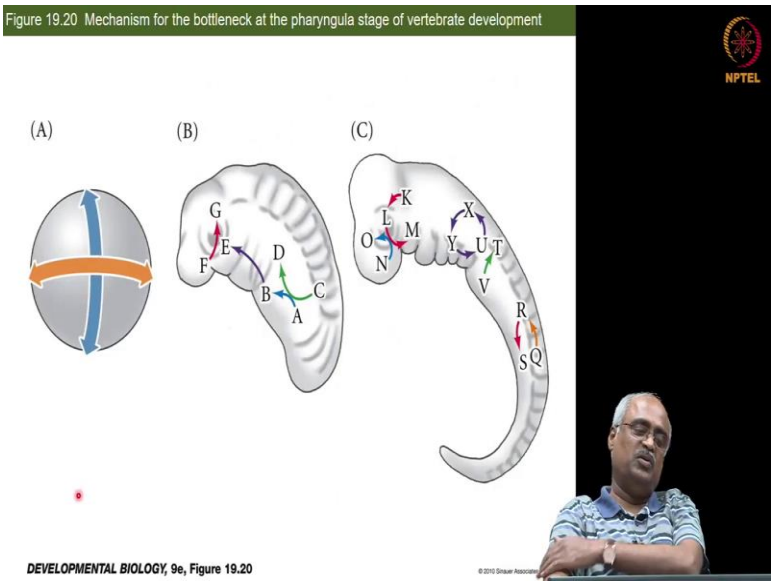

During the early embryonic development, you do not have many inductive activities, and even those that exist are global, affecting the whole embryo. That is primarily in setting up the axis anterior-posterior or dorsal-ventral axis. And small variations in those morphogenetic fields are usually accommodated. But when you get to the later stage, there are local developmental modules and many inductive events, as shown by these arrows here. These are local interactions. For example,when the induction signal between optic vesicle and lens is affected, only the eye will not develop but not the rest. Here, the interactions are within the modules, and therefore, only a local structure will be affected. But if you look at the phylotypic stage, you have the modules interacting, and induction happens here. But here, these inductions are among the modules themselves, not within the module.

Meaning here a module tells another module where to form a certain organ, like where to make the kidney, heart, eye, and so on, and there you cannot afford to mess up. Then that will not be a functional adult. And due to that, in the phylotypic stage, that particular structure needs to be formed to make a vertebrate. This imposes constraints about how many varieties are possible in that basic body plan, which is an example of a phyletic constraint.

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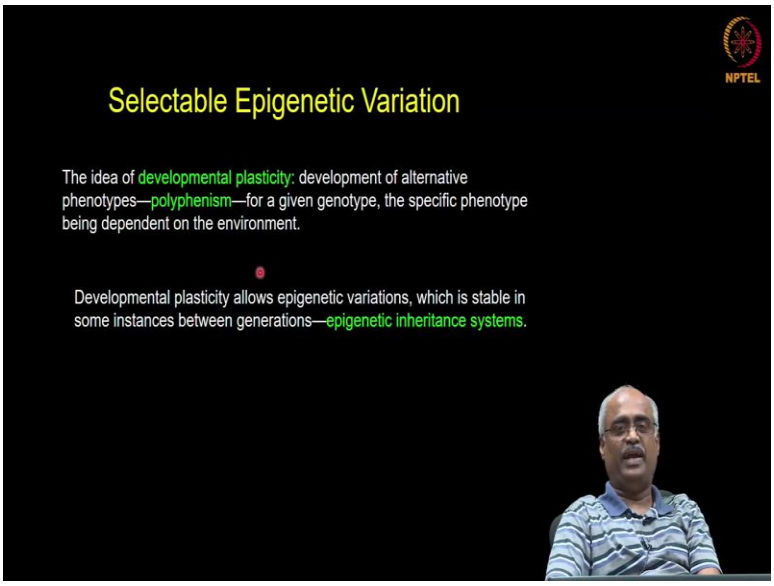

These are examples and the types of constraints that stem from the developmental mechanisms of certain body plans. Now we will move to a different theme where we will look at how sometimes we could have a range of phenotype for a given genotype. And within that range, you may select a certain position; let us say I arbitrarily say for a genotype, you could have skin color ranging from black to albino, let us say for the same genotype. And whether it is going to be black or albino is dependent on the environment. Such a situation is called polyphenism, multiple phenotypes for a given genotype. And this indicates certain flexibility in the development of the same molecules. We call this developmental plasticity the development of alternative phenotypes for a given genotype, and within that multiple phenotypes, what will be decided is dependent on an environment.

So here, I have introduced two important words, developmental plasticity and polyphenism. Here, the important thing is genotype is identical; there are no allelic variations, there are no enhancers or anything changes. The DNA sequence is identical, but phenotype has a certain range of alternative forms. It may not be range always; sometimes, it is alternative forms, and those alternative forms where a given environment determines each form is what we call polyphenism.

This development ability is what we call developmental plasticity, and this phenomenon again contributes to selectable variations. So you need to have variations in the population where you can select and produce those selectable variations. We already have learned that everything is controlled by changing the DNA sequence. Now we are looking at a situation where we are not touching the DNA. Still, then we recognize that multiple phenotypes may be allowed by the genotype and which phenotype will be selected is probably going to happen in a non-genotype-dependent manner, and that is why this word epigenetic variation comes into the picture.

So does such variations exist? And do they help evolution ?. That is what will be our topic now. So this plasticity lends itself to such a selection, and that is what we call epigenetic inheritance. So this somewhat supports the Lamarckian idea, but it is not really. Lamarckian idea talks about used is used, but we are not talking about used is used here. The Lamarckian idea is wrong; for example, if someone is a bodybuilder, there is no guarantee that that person's child will be very muscular. As the textbook says, accident victims who lost a limb can be assured that their children will be born with normal limbs.

If an environment induces certain changes in the somatic cells and supposes if those factors that are originally made in the soma could find a way into the germline, it would not get inherited. They do get inherited, and that is what we call epigenetic inheritance systems. And we will see examples of that; then it will all become clearer.

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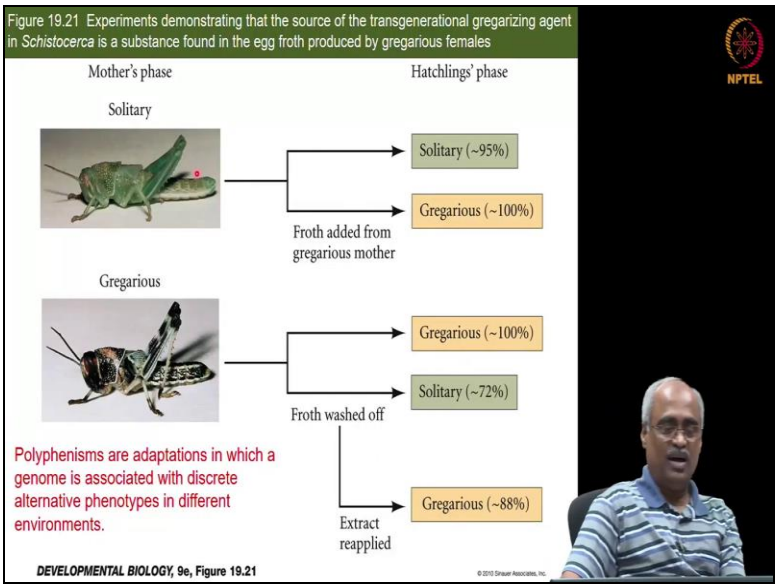

Here is one example, here you have this locust. So this is a solitary locust, greenish, and it just forages on its own; it never becomes a group. So it is solitary of existence. It produces progeny that is again will be solitary. They are not going to be gregarious, whereas, under certain conditions, they end up being in a large population or producing gregarious progeny. Although the genotype is the same, and this gregarious progeny, even when it is not in a group in a solitary condition, produces gregarious progeny.

So in earlier generations, the environmental experience, here being in a crowded population, produces an inheritable property characteristic. So a subsequent generation that is not in a crowd also ends up producing this gregarious variety. So that is an example of polyphenism. Here, these two are two different phenotypes possible for the same genotype. It depends on a chemical produced by the oviduct, and it is deposited in the form that protects the egg.

And when you take that gregarious locust, the foam and coat it on the egg produced by the solitary, and the solitary one ends up producing gregarious. And if you wash off that foam, then this ends up producing solitary and if you reapply after washing, it becomes gregarious. So this is how people showed that it is a chemical that is coating the egg ended up deciding this phenotype. After its original experience in subsequent generations, even when that experience is not there, it again produces the same phenotype. That is how it becomes epigenetic inheritance; it is inherited, the initial stimulus is not required anymore. So now, what are the mechanisms by which this happens? There are three different mechanisms, and that is what we are going to see in the subsequent slides.

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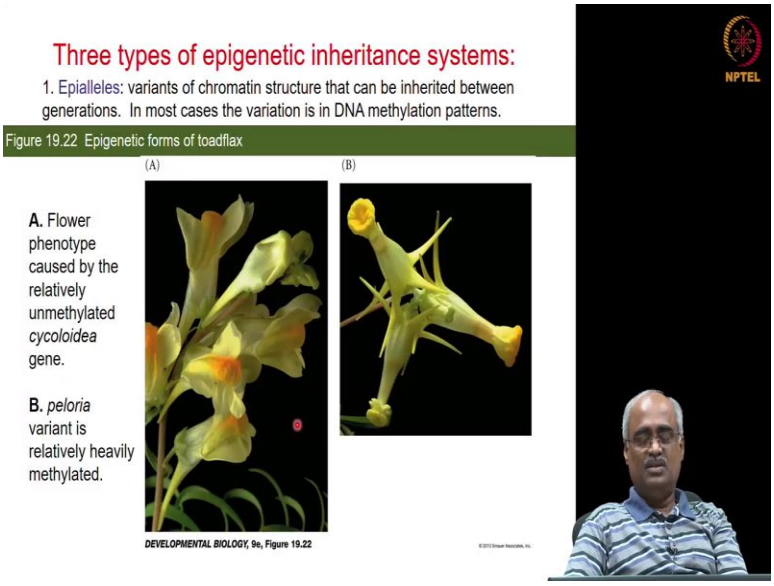

Firstly we will talk about epi-alleles. We know alleles are having differences in the DNA sequence for a given locus on the chromosome; for a given genetic locus, you have some variations in the DNA sequence without altering the total property of the encoded protein or the RNA call alleles. Epi-alleles are again variations but here not on the DNA sequence but on the chromatin structure itself.

One chromatin structure you can readily think of is DNA methylation. A certain part of that gene, let us say promoter, is methylated or not methylated or hypermethylated. And if that methylation pattern can be passed on, let us say a certain environmental influence in the fluid caused that kind of methylation on a certain somatic cell in one particular generation and if it can impact the chromatin of the germline.

Then it can go to the next generation, so it does not matter whether the variation is in the DNA sequence or the chromatin structure if either of them can pass down through the germline. If they are going to have an impact on the gene, it is going to have an impact. So that will not be

distinguished by whether the variation is on the chromatin structure or the DNA sequence itself. So when you have that kind of a variation based on chromatin structure, you call Epi-alleles.

So here is an example of Epi-allele. Here you have this normal flowering pattern in certain toadflax. Figure A is an unmethylated version of a particular gene. When this gene is hypermethylated, it produces this flowering pattern (B) inherited stably. So if you look at the sequence, you will not find variation, but if you look at the methylation, you will find this allele is hypermethylated, and this allele is not, and both are stably inherited.

And this is one mechanism by which you have epigenetic inheritance, which is determined initially by an environment that induced this methylation.

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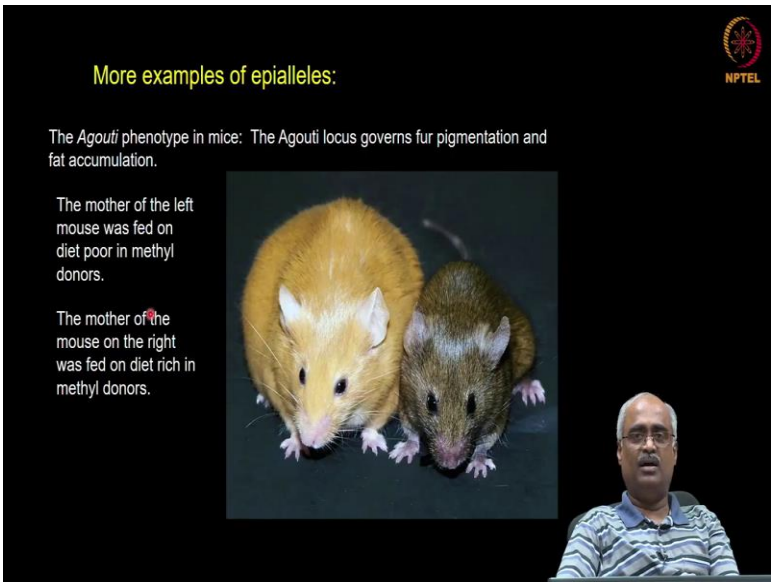

There are many examples in the animal kingdom as well, and so in the interest of time, we will only look at one example. Agouti phenotype in mice is a grayish-white fur pigmentation pattern that is often connected with obesity. A mother fed in a poor diet in methyl donors then ends up not developing that fur pattern and becoming obese.

The availability of methyl donors in the mother's diet affects the progeny and progeny and the grand offspring. So if you have a proper diet rich in methyl donors then it develops the normal phenotype. Here in one generation, the environmental influence, in this particular example, is the diet; whether it is rich in methyl donor or poor in methyl donor determines whether it is going to be obese and also have this fur pigmentation pattern. And this gets stably inherited in subsequent generations as well, so this is another example of the epi-allele.

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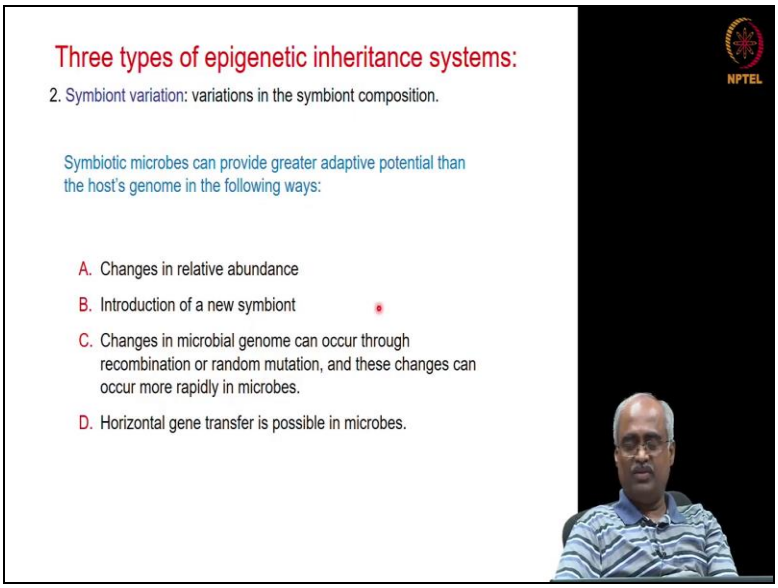

Secondly, we will look at symbiont variation. Symbiosis is one organism setting up a relationship with another organism in which both of them mutually benefit. A good example is our intestinal microflora, where many bacterial species colonize our digestive tract. And these being bacteria, they can readily generate variations. If each of those variations will influence the host organisms of phenotype, you can easily generate many variations in the host. So that is the symbiont variation-based example of epigenetic inheritance.

Let us look at it in some detail. Symbiont variations can readily cause these variations because of these four characteristics of the bacterial population. One of them can be relative abundance; you could have let us take bacterial population A, bacterial population B; let us assume there are two populations in our gut. Now population A to B ratio can vary, maybe initially 50 50 that is in equal fraction. But then, due to changes in the food or environment, you could end up having variation in them. A being more or B being more can readily happen, which could readily affect the phenotype that is selectable at the host level.

And you can easily introduce another bacteria. You will not experiment with changing the host's genetic makeup, which will require more effort and time than introducing one more microbe into the symbiotic population. So that is another feature of the bacterial population, and the third is they can easily undergo mutations. So the host cannot undergo genetic mutation and generate a phenotype that is selectable at the given generation itself.

One needs to go through several generations of that host, but instead, bacteria have shorter generation time and large numbers to undergo mutations. They will not have two alleles so that we can generate mutations easily through recombination and random mutation. And these changes can occur more rapidly in microbes, and lastly and equally importantly, horizontal gene transfer happens among bacterial species in prokaryotes. Genetic transfers from one species to

another species happen readily, which does not happen in vertebrates like us from one vertebrate having a transfer to another vertebrate. But among bacterial species, this readily happens. Due to these characteristics of bacterial species, the symbiotic variation can readily happen and could cause selectable variations in the host itself.

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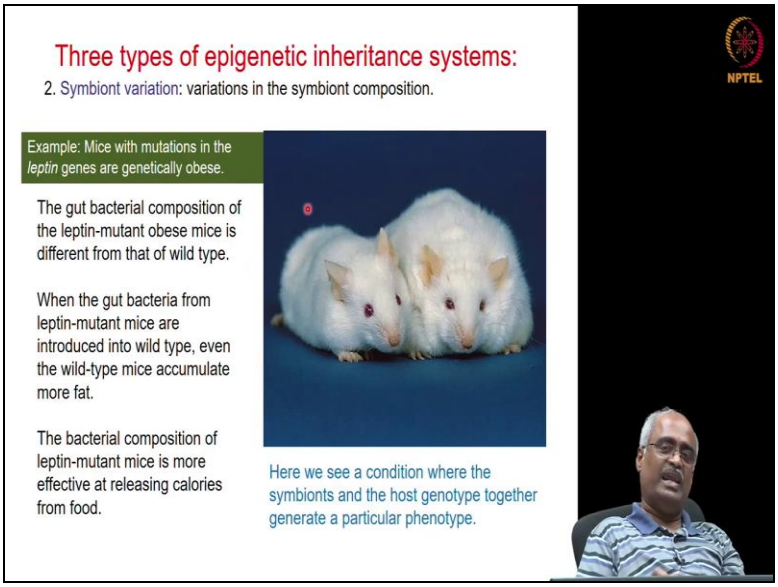

One example is shown in this slide, where we see an interplay between the host genotype and its inter digestive tract's bacterial composition. So the gene here is the leptin; here, you have this wild-type mouse and the leptin-mutant one. So you find that leptin-mutant is obese that is caused in a complicated way by leptin mutation and the gut microflora. So this has a certain bacterial composition, but this has a different bacterial composition that is very efficient than this in releasing calories from the food. As a result, it does not need a lot of food to put on weight.

And what influences this particular bacterial composition is the leptin gene. So the host genotype influenced the bacterial composition in the gut, which influenced the host's phenotype. If you take this bacterial mix from the leptin mutant mice and introduce it into wild-type mice, their progeny ends up putting on weight. However, they do not carry the leptin mutation, so this bacterial composition affected in one generation by the genotype ends up causing the phenotype in subsequent generations, even in a wild-type genotype.

So this is a good example of a symbiont variation. Here we see that the composition of the symbiont and the host genotype together generate a host phenotype. So that is why I said this is a complicated situation where you have symbiont composition varying in a manner that is dependent on the host genotype.

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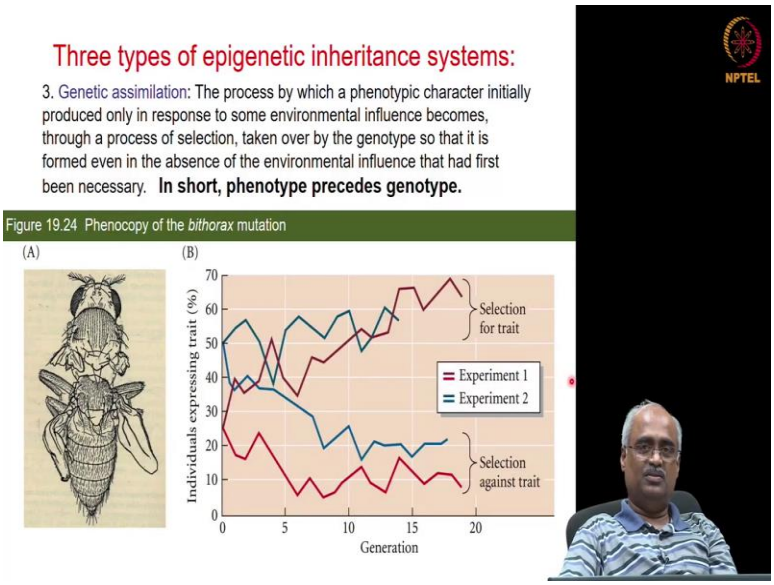

So far, we saw Epi alleles with two examples and then how symbionts can readily influence variation. The last one in this epigenetic inheritance system is called genetic assimilation. So this will look a little complicated as we go along, but I will tell you in a nutshell what it means. In the previous examples, we saw an environmental factor that induced certain phenotypes allowed by that developmental plasticity inherited in subsequent generations.

But I did not mention for how long. Suppose a certain phenotype has a increased frequency among the alternative phenotypes; it eventually gets taken over by the genotype and becomes stably passed on without requiring the environmental trigger. So that is known as genetically assimilated. A phenotype thrown out by an environmental trigger eventually gets genetically assimilated; it no longer requires an environmental trigger.

So you will be wondering and greatly confused how this ends up creating variation in the genotype without the trigger anymore. In a population where you have multiple alleles existing in certain frequencies, the phenotype range is not visible due to the lack of that environmental trigger that is required. Once triggered repeatedly triggered for some time, it increases the frequency of certain allele. that is how it becomes genetically assimilated. So when we see the examples, it will become clearer.

So here is an example of fly population, the drosophila. In an experimental population in the lab, scientists noticed that when you expose developing flies' embryos at a certain stage to a organic compound called ether, they end up producing a bithorax mutant-like phenotype. They develop four wings. Drosophila is a dipteran, and it has only two wings produced by the middle thoracic segment. In this particular image in the slide, these regular wings are cut to expose this segment. This third thoracic segment that usually produces a balancing organ called haltere is now expanded into a wing-like structure. So this is the four-winged phenotype. So this four-winged

population came out in the same genetic stock when exposed to ether. Now the scientists allowed the four-winged flies and two-winged flies (ones which even after ether exposure did not display the four-winged phenotype) to mate among themselves. Offspring of those flies were allowed to develop to adulthood and mated among themselves. After a certain generation, the four-winged ones remained four-winged ones even without the ether exposure. So, how is this possible? It turns out that when they looked at the bithorax gene, the sequence of the original stock had four alleles that were present, but these alleles did not cause the four-winged phenotype. Only when an environmental queue was there, here exposure to ether, some of these alleles produced the four wings. Initially, these four alleles were there in the population, and in the absence of any selection, they were all equally present; there was no need for one allele frequency to go down or one allele frequency to increase. That will happen only if there is a selection force. So there was no selection force. When an environmental change ended up throwing the range of phenotype here, let us say polyphenism has two alternative phenotypes: two-winged or four-winged.

When you are artificially selecting the four-winged one, you have increased the frequency of the four wings causing allele. So that is how the genetic assimilation has happened, so the underlying genotype already existed, just that the phenotype was not exposed. When the phenotype was exposed for that particular genotype, and now there is a selection for this phenotype, it gets selected, so this is genetic assimilation.

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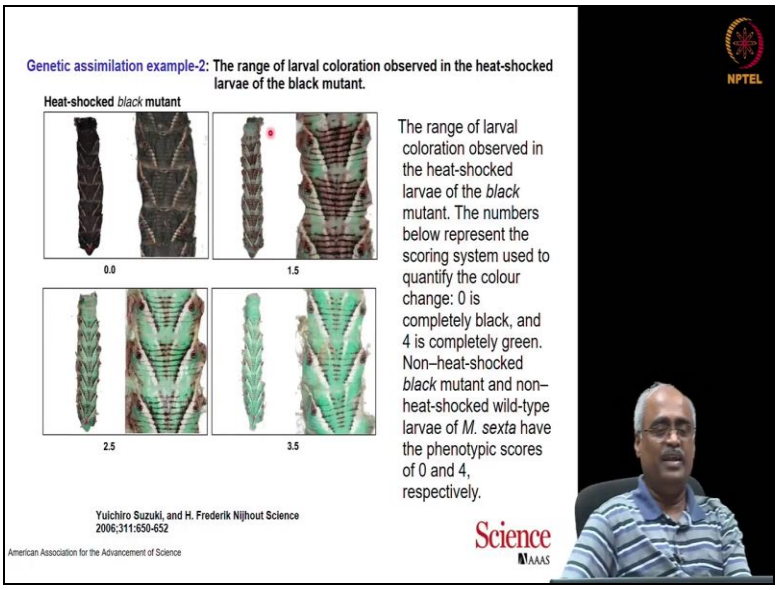

This has been seen in tobacco moth as well. Here you have a black mutant that, when it is heat-shocked, can either not change the color, or the larvae may change color a little bit greener or some more green or really green. So the one that does not respond to the heat you score zero, and the one that responds to the other end of the range of the phenotype here green you score four. So here, the first color is assigned 0, and the last one 3.5.

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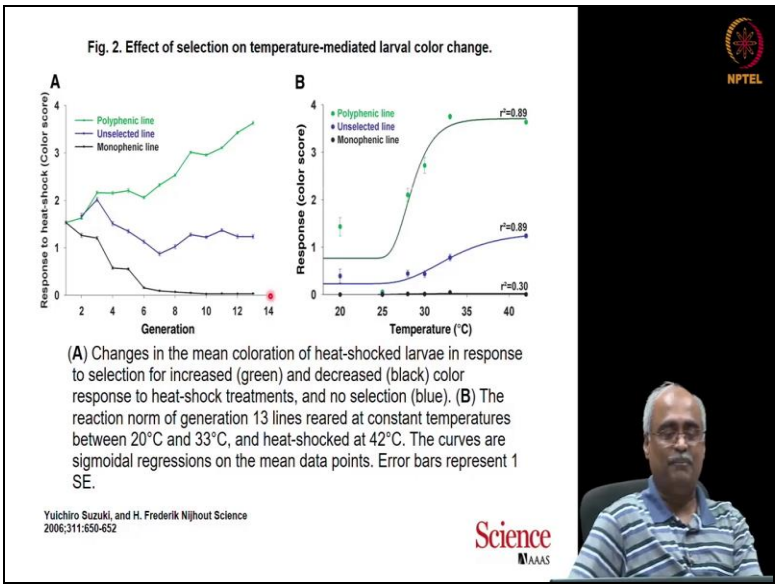

So now you select them and allow them to mate among themselves for a period of multiple generations and see what happens. So here you have a situation where no selection is made. In this blue graph, the x-axis is the number of generations; the y-axis is, in a given population generation, like shown in the previous slide, how many become green or black or an intermediate thing (the score is plotted in the y axis).

So only a certain fraction responds to the color change in the unselected population. So when you select the one that scored high and allow them to with another that also scored high and repeating it for certain generations, you will see many members of them very readily change in response to the heat. While the one you selected in the opposite direction, where it scored the low and mated among other low scored ones, eventually became monophonic. It is black whether you heat shock or not heat shock, and here you end up seeing the other end of the spectrum of the polyphenic or thing. And at the 13th generation, people looked at how well they respond, like the phenotypic range possible for the environmental variation. Here the environmental variation being heat shock.

So that is called a reaction norm. When you look at this reaction, these graphs are called the reaction norms, that is, the phenotypic variation that is possible. This selected one very readily changes to a larger extent when exposed to the heat shock. When the 13th generation progeny reared at the temperature, let us say around 28, it dramatically responds to the heat shock change. The general population does not have that kind of a reaction norm, and the other one selected against does not have any effect. So these are examples of how genetic assimilation happens. Just like there, bithorax had multiple alleles and one allele we ended up selecting by experimenting.

So the main point is that many phenotypic possibilities exist. When an environmental condition exposes the variations and selection pressure to select any of them, they are selected. So they do not exist only in the experimental settings but also in nature.

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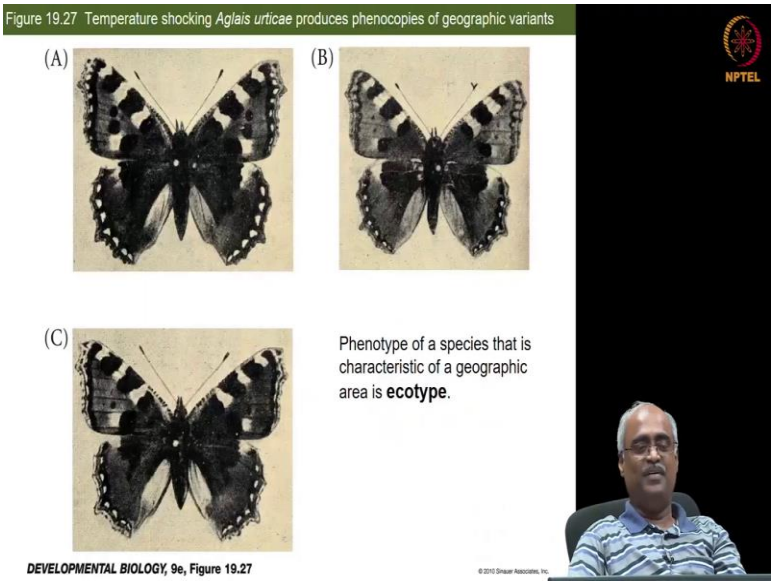

So here is an example of a butterfly species. It has a big coloration pattern on the wings, and this coloration pattern is temperature-dependent. So you can take the butterfly population, let us say, in a colder climate, you heat shock the butterflies; thus, they produce a variety of variation in the coloring pattern. Now, if you go to a warmer place and look at the same species, you will find the phenotype you produced by heat shocking the fly grown in a colder environment. And the opposite also is true.

So that is this example. So the phenotype of a species that is characteristic of geography is what is called ecotype. The same species in certain geography will have a certain phenotype in certain other geographies will have a different phenotype. And these have been selected by those geographical conditions.

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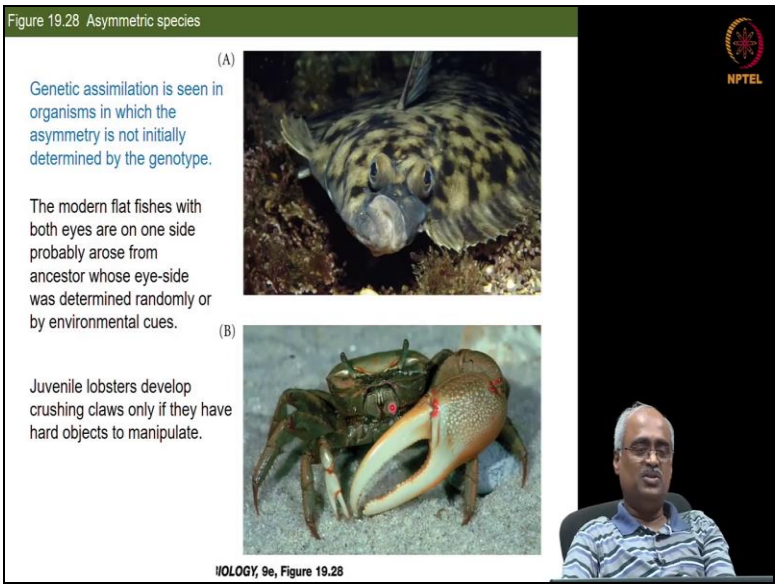

And it works in situations where asymmetry is initially not determined by the genotype. So here is one good example you look at this fish in the slide. It is a flatfish, and it benefits by having both eyes on one side, so during embryonic development, one of the eyes moved over the skull to the other side to have both eyes on the top. This probably arose from an ancestral species where this asymmetry was not genetically determined, and it probably happened by random or due to an environmental cue.

And eventually, then it got selected probably because that fit its new adaptation in a new environment. Another good example is these lobsters. These lobsters, in their juvenile stage they make both the claws the same way. If they do not have any hard objects to crush and break, then the claws develop symmetrically. But if they ended up grabbing something with one of the claws and used a lot of effort to crush and crack, that creates an asymmetry and that particular claw becomes big.

So this is permitted by the genotype again. I want to highlight the point that the genotype enables this much flexibility. When a given environmental condition gets selected, and if that is the fittest to that environment, then that early life frequency changes. And so, this is how the environment can bring out the phenotypic possibilities of a given genotype. And this has at least two definitive advantages in evolutionary adaptation, and that is explained here.

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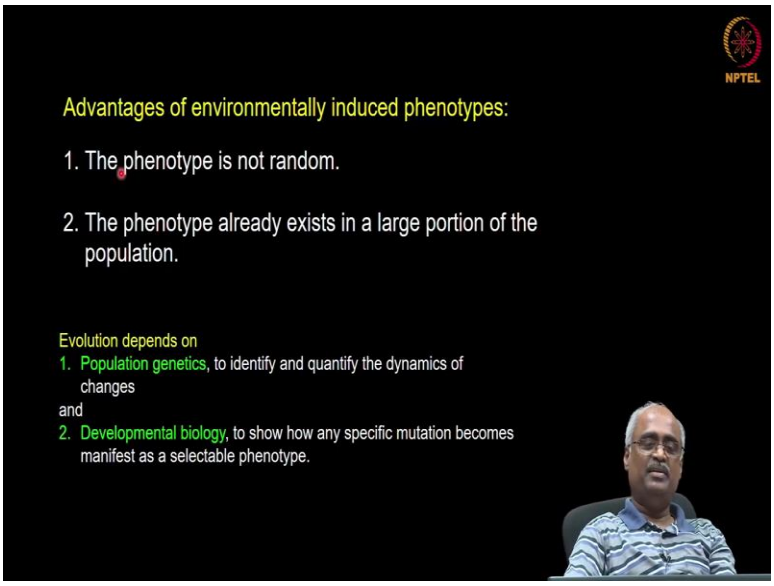

When you do not experience random mutation, you are not experimenting with generating a phenotype that fits in an environment. You already have that phenotype in the population, which means it has not been detrimental. So it has already been tested, and it is to be there, and now when the environment changed, you could readily bring out that variant of that given structure, and it can readily adapt. So this is preferable over newly generating a mutation and selecting.

And a new mutation that may be better for an environment will be one random monster that will not be in a population. It will take a lot of generations before that a new mutation can be assimilated. But to survive in a given environment in a given generation, if it is already there in multiple members in a large fraction of the population, many members can display the phenotype. There is a good chance of getting selected.

So this is why one should not only look at the genetic sequence itself. But we also need to look at the developmental plasticity that allows these variations and, therefore, how this could help in developing. To sum it all, what we end up understanding is this theory of change, that is, evolution is dependent on two pillars: one of them is population genetics, where you identify and quantify the dynamics of these phenotypes But then you need another pillar to support this that is the developmental mechanisms, a way to explain how any specific mutation can become manifest so that it is a selectable phenotype. Therefore, the mechanisms that construct the body are equally important, along with population genetics, to help us understand evolution. So with this, we end not just this class this course itself.

So I hope you guys learned and developed a flavor for developmental biology, and in due course some of you pick up this field for research and become a famous developmental biologist. I wish you all the best.