## Basic Course in Ornithology Prof. V V Robin Department of Indian Institute of Science Education and Research, Tirupati

#### Lecture-43

# Understanding Evolution With Molecular Techniques: a Hands on, Interactive Session

So, Mr Muniram, if we can make 6 breakout rooms. I think Durga Rao is here. Robin whom did you talk to from NPTEL. I think Durga Rao is the one who is right now making the co-host. Tell me ma'am. We need to make 6 breakout rooms. I think it looks like I can do that. So, we will assign automatically. So, before we go there, I want to share this link. So, I will also just share my screen so you can see what I am talking about.

### (Video Starts: 03:15)

So, hopefully what you all should see when you click that link is this. And what we are going to do is just simply click this launch interactive. It will open a new window which you cannot see but if I share my desktop you will be able to see it. So, it will open a window like this. So, this is a virtual lab. It is created by Howard Hughes medical center HHMI, and it is a fascinating you exercise. It is a virtual lab. So, what it does is that it is going to talk about lizards. And we are in an ornithology class.

But it does not really matter. We are interested in looking at the concept. The exact traits that we work with will be different if it is a bird. But I think you will understand the whole process once you follow this through. What we need to do is first we just listen to this 50 second story. That is not much of a story but essentially the idea is that there are different kinds of anoles. And what we are going to do is to try and understand what is called the ecomorphs which means that in terms of morphology what organisms are more similar.

But we are going to collect this data and assess this quantitatively. We are not just going to make a guess. And this is what we would do if we were working on something. Even if it is a bird or something else this is how we would proceed. I know this is part of the module that looks at genetics which actually comes in the second module here which is phylogeny. But for the phylogeny to make sense you know to put it in context you have to be able to do the first module first.

So, you have to kind of bear with me while we do the module 1. And then we will move to the module 2. And the way this is going to work is that we are going to kind of have these breakout rooms. And there are TAS who know quite a bit of these modules. And you have to still work it out yourself. But despite that if you run into any problem then the TAS will be able to help you. And there is a lot of materials to help you here whether there is a tutorial and so on.

But for now, just go through the module 1 which lets you collect some data on these different types of anoles that are there. And once you have that then we will move to the next module which looks at the genetic data from these lizards. And the question that we are asking is that if you saw that video there were all kinds of anoles. So, there are different forms of anoles. There are some that live on the trees.

Some that live on the ground and some that live in the canopy and so on. The question is how did they evolve? And this is in the different islands in the Caribbean. So, did they all evolve once? And then they moved to these different islands. Or did they evolve independently in each of these islands. So, I will stop sharing the screen.

So, you only need to log in if you are going to come back and get your restart at the same point. And if you have a Google chrome or something like that it will remember it based on that. But that is really up to you. So, I am going to create 6 breakout rooms which will assign people automatically. Hopefully, the TAs should be spread around equally. And they will help you if you get stuck but essentially you have to do the exercise yourself.

They are just there to help you, if you have doubts. At the end of the session, once you finish the module 1 and module 2 then what we are going to do is just have a small discussion among your group in your breakout room about what are the main things that you learned from this experience. It is just like 2, 3 points. But do discuss. And then make those 3 points. And then we will meet in the common room. And if everyone can kind of synthesize what they learned that will be useful.

Any questions at this point? I am using mobile. It may not work very well. But you can give it a shot. I know that a lot of people have tried it out with mobile. We can give it a shot and see how it works. Excuse me sir, I have just joined the class with another device and my laptop. I do not have the link which you just shared. Can you please resend? Jobin, can you just resend? I think Jobin is going to send the link again.

And once you are in the breakout room again the TAS will copy and paste the link for you. So, that it is with you in the breakout room as well. So, do not worry about that. So, the idea is let us give us for module 1 maximum about 15 to 20 minutes. So, you have to kind of rush through this a little bit. So, try and be very quick by around 3:30 3:40 we should be done with module 1. Then we go to module 2.

And then max around 3:45 or so we finish all your activities. And then get back to the main room. So, over to breakout rooms. Hi, so those of you who are still in the main room, if you have any questions, please tell me. Otherwise, you have to kind of get into the breakout rooms. Devica, can I request you to just assign unassigned people as they come. If you can request them to or you can assign them to some room.

Yes absolutely. Thank you. So, now I will head over to these rooms one by one. Prasad Kamath, are you here? Anu, is Anu here or FK? Viraj, did you just join? I am going to assign you Viraj to some breakout room. So, everyone is in separate breakout rooms. And they are doing some hands-on session there. So, I am just going to assign you to a breakout room. Let me see how I can do it?

Not sure of how to do it actually. Durga Rao, do you know, how we can put Viraj in our breakout room? Ma'am. So, we need to put Viraj in breakout room number 2. Do you know how we can do that? Viraj just joined. He joined late. You can put him in room number 2 but how do we do that? Tell me ma'am. Can you put Viraj to room number 2 Durga Rao? Viraj, I am not able to do this.

I am just gonna Google it how to do it. Punil Gajjar, did you just join? You can unmute and talk actually. Ayesha, so let me tell you that everyone is in different breakout rooms at the moment

doing some hands-on work. So, I am just going to put you in one of the rooms. Madam, actually I am Archana. And I could not see anything on the screen. Actually, earlier sir was there. But now it is showing meeting topic, which room were you assigned to? I think room number 3.

You were assigned to room number 3. Yes. I think I should copy the link. Which link? On my screen it is. I can see the meeting topic then host, password, invite link and participant Id. So, instead of the zoom meeting. Can you just try joining room 3 again? I do not know how to join room number 3. Because I cannot assign you, because that room has already been assigned to you. I am trying to join.

Yes. Ahana and Bina, I think you have just joined. So, everyone else is in different breakout rooms doing some hands-on sessions. I am going to put you both in room number 4 Ahana and Bina. Hello, I was already in group number 1. Actually, I have joined from another device. But I cannot assign you to any other room now because a room has already been assigned to you. Just try joining in again in room number 1.

Musamballu, I am just going to put you in room number 4. Deepak Sinha you just joined or you just got out of a breakout room. Deepak Sinha are you there? Mrinal, did you just join the meeting or did you just get out of a breakout room? Mrinal, are you here? Yes madam. Did you just join or did you just come out of a breakout room? No, I just joined madam. So, everyone else is in different breakout rooms. I am just going to assign you to room number 2.

Thank you. Krishnendu Das, did you just join or did you just come out of a breakout room? Please feel free to unmute yourself today and talk to me. Pushpa Suresh, did you just join the meeting? Pushpa Suresh are you here? Yes madam. Did you just join the meeting or did you just come out of a breakout room? I just joined the meeting madam. So, everyone else is in different breakout rooms doing some hands-on session.

So, I am just going to assign you to one of the rooms. Hope everyone here is on their laptop and not on your mobile because it will require you to do some hands-on work. So, if you are on your mobile, please try and get on your laptop. Adil A, are you here? Adil A did you just come out of a

breakout room or did you just join the meeting? Someone called user has just probably joined the meeting.

Can you please unmute yourself and talk to me? Can you please rename yourself? It says user at the moment. Wildlife of DJ, I guess you have just joined the meeting. Or did you come out of a breakout room? You can unmute yourself today. I am going to just put you in one of the rooms. So, user and Wildlife of DJ, I think you have just joined the meeting both of you. Everyone else is doing some hands-on session in different breakout rooms.

So, I am going to assign you to 1. I hope you are on your laptop and not on your mobile. Because it needs some clicking of links to do. Dipak Sinha Are you here? I cannot hear you at all. Hello Devika I guess you just joined the meeting. Can you hear me? Hi Devica. Hi Robin. So, I was thinking that you know if new people join at this point maybe do not send them into the room. Make sense.

Because I just noticed that some people come in. And then we have to start all over again. So, it is a little disturbance. I guess those have joined in right now are already almost 40 minutes late. So, you can just give them the link. You know that is this link and they can do it themselves later. We will do. Cool, I will just head out to the breakout room again. Sure. Rekha you just joined and you heard that.

So, I cannot put you in any breakout room now. But Robin just shared a link on the chat. So, you can just explore that link. Basically, that is what everyone else is doing in the rooms. You those who have just joined Govind and Vishwanathan everyone else is doing some hands-on session and hands-on work in their respective breakout rooms. But if you can see the chat there is a link there shared by Dr. Robin.

You can just check that link out and explore. If any questions please go ahead and ask me. Govind Girija, actually this session cannot be recorded because everyone else is doing some hands-on work like in the breakout rooms. So, I am not sure how much uploading the video is going to help.

But I will share the link. I will join there. I have just shared the link which you can explore on your own. Thank you.

Please share the link again. Just shared it right now again. I will do it later. Because I tried, I am not able to do it. We have done the calculations and the videos, it is the continued tab lead meet your video, is that all after that sorry was someone here talking to me. After the measurements it went to at measurements table. Is this about the link that has been shared? The breakout room once we started.

Sorry, I do not use that software so I am not aware of it, but probably someone among the TA's can help you or can you just give me a second, I will figure something out. Once the measurements have been done, I have been led to the table to fill the relative lengths. So, after that the tab led me to a video. Then you have to say did you say launch, just a minute I will just open it myself. Did you say launch interactive after the video?

I started with the link which went for launch interactive, then I was given images of the lizard to grew. Once, I grouped them then it went to the continue button took me to the measurement. Robin should be here in some time to help you with it. So please tell me after you did the measurements what happened? After I did the measurements, it led me to your table where three-fourth of the table was auto filled and the balance was left me to fill by calculating.

So, I did that and I went for continue button and it went to a video straight away. So the video is like a synthesis that it tells you know from the data that you have collected, what it can answer about your question. So, you do not need to watch that video fully you can switch back to module two after that. Thanks Robin, no problem essentially what this is trying to do is that you know initially this module asks.

So, let me just since all of you are here, I will just share my screen and show you also what this is about what is happening.

### (Video Starts: 1:00:48)

So, we have a module which is like this, so initially there are all these anoles this is the first module Ecomorphs and it asks you to group the lizards that you see. So and they do not say how you should group it is totally left to you so you say this is Green and you know these two are green so I am going to keep them together and then these are kind of Brown. So I will keep the Brown ones together and something is like slightly different color or something else.

You know I mean whatever you feel like and then you have to kind of make a label and you can say these are Brown lizards and I put the Brown lizards here then I add another label which is maybe twigs and then I do that I put that here another is Green lizards, so I put that here. So basically, after that you have to start measuring these. So you know what you have done is you have looked at what it is for you what it seems like the groups.

But then you have to collect data on this and for that they already have some data on like they have x-rays, so if you click on any of those species, then it lets you collect data you know you can say this is the you know the body length by measuring this and then this is the hind limb length and so on and then this is the tail and so on. I mean you have to do it carefully, I am just doing some random thing and once you record the measurement which is not this.

But I hope you get the idea that you can carefully collect all of this data, see actually this is how it should be. Something like that so that is a hind limb length and then you have to collect the body length which is this and then you measure the tail length. So similarly, you just have to do I am just trying to you have to do multiple species and then what happens is that you can look at the results table.

So, until I do all of those it would not show the results table. So, I just have to look this click, click, click thing through all of them and record this measure and then I got that and there is one more remaining actually there are two more remaining. So, the first is the hind limb. So the point is that you are now measuring the x-rays of all of these animals which gives you some data of them.

So there are a few more actually and if you have doubts there is something called tutorial which tells you where you have to measure from. It is supposed to include the head I think I did not include the head when I was measuring the body. So essentially I did the hind limb right almost but the body length is actually from the tip of the head. Robin so mine, the exercise is done with my group. So, should we all get to the main room now? Yes, please.

So unfortunately, this is a 15 minute exercise and I am trying to get it done in a few minutes so it is a little bit I think we are mostly done. And then there is this thing called toe pad. So what this does is you have to measure the lamellae, you know on the toes and again if you are not sure how to do here is how you do it so you have to click on each of these and it gives you a count of how many there are.

So technically you are supposed to kind of you know click on each one terrible. So basically, what it is telling me is that I did it badly, it is a little bit easier and then once you have done this you essentially get a table and this table summarizes your measurements and then the reference measurements. So, this is what it is supposed to be like and of course I have made a lot of mistakes;

But that is okay and then you have to kind of calculate this relative hind limb length, you know and you have to put that number here. I hope it would not let me but we just move on and then there is a two minute video to watch which you do not have to watch right now you can watch it later, but please do watch it. But essentially what it tells you is that the grass bush anoles have long tails and you know there are these different ecomorphs that are associated with different morphological features.

And then you can say oh now do you want to regroup them and you do not have to do the quiz for now but it will give you the graph and this graph has relative hind limb length to relative tail length and what it tells you is that these are these ecomorphs, so this is actually not the exercise what they want to do, what we want to do is module 2 which is I think here and what this module does is that it you know you are asking; Maybe I will just let everybody join in and then we can finish this up. **(Video Ends: 1:10:35)** 

So, all the room should have closed now, is that right? Yeah. I think there are more people in the room as well right now, even I can see 7, 8 somewhere but I closed all the rooms. I think they are closed they just to be or just delete all the rooms. So now no rooms are open. So, I hope I realized

that you know this may have taken a little bit longer. Maybe you can continue this exercise later as people were suggesting.

#### (Video Starts: 1:13:43)

But I just wanna share my screen and just take you through what you know what we really wanted to get to was this, which is this module 2 and I will just play this video I think it gives a good idea. "On each of the Caribbean's 4 largest islands Puerto Rico, Jamaica Cuba and Hispaniola. We find the same distribution of similar looking lizards. Each island has slender grass bushnells with long tails, long-legged trunk ground anoles

Short-legged twig anoles and canopy anoleswith large toe pads. Did each body type evolve once and then spread to the other islands? Or did each type evolve independently on each island?"

#### (Video Ends: 1:15:07)

So I know that you know some of you may be a little bit disappointed that this is you know that this has lizards not birds and we are in an ornithology class, but you can imagine any other bird you know you could be thinking about Darwin's finches or any of those the idea is the same you know Darwin's finches also have different weak shapes and you could ask the same question did they did they evolve once or did they evolve multiple times.

So the question is the same just the organism that with which we are studying them and the traits that we are studying a change and are different a little bit. So do not worry about the specific organism or that you do not know you know how lamellae matter for lizards, but it you know it is not something that you would measure for birds. Can you hear me now? Can somebody else indicate if they can hear me? Yes, Sir you are clear, Thank you. So, the idea has been that you know we have these ecomorphs and we are trying to understand how they evolved and those are the kinds of questions that that scientists ask frequently.

So, now I was just wondering if we can have a few volunteers to summarize what they learned from you know different groups, so if group 1 if anybody wants to say something. I mean which is the group 1? I thought everybody knows I was in group 1, I think there are people from my group here. Please, Is I am audible? Yes you are. So firstly, I must thank the faculty for you know such a fantastic module been presented to us.

As far as I am concerned when person it really actually did not matter to me that I was working on lizard, though I am into ornithology. Because you know this is a fantastic example wherein four lizard are on an island and each of them having a different habitat which speaks in parallel to the avian also, where the course went down you know where the birds carved their own niche in their own habitat.

So maybe because of competition and for various things, so I would not take much of time Sir, thank you this is something which I could draw in one line that this was very interactive and very beautiful way to present it, thank you sir. So, my question now maybe the second team could say what you know what were the three main things that you looked at you know in this module and how does it affect your thinking about species?

Actually, let us not do it by teams if anybody wants to you know. I just understood that the habitat division is based on the tail which is longer short, leg which is longer short and if the result is having a number of larger toe pads they were higher up with the trees and in each floor they were having different evolutionary applications in there excellent. So that is what we can that is what we can tell by quantitatively looking at these characters.

I mean first you just looked at the animals so this is the equivalent of bird watching where you just see the bird and then you are trying to kind of get some ideas and then you go to the next step which is you collect a little bit of data and that is your ecomorph which tells you how they are kind of adapted and then there is a next level of data which is the genetic data, so can somebody tell me what did you learn from the genetic data?

Some maybe a group that finished tell me please. S,o I think the phylogeny data kind of indicated that evolution can happen not on the genetic tree but also on the on the habitat, so I think that determined how the species could evolve independently like the convergent evolution concept that was you know explained. Nice anybody else has anything else to add to this? Yes, this is an example of radiation like Darwin's finches what was shown in the module.

And I thought it was very interesting that when we measured the highlights and afterwards when they were compared the hindlimb ratio was taken with the body so I thought that was very interesting. I know what you are talking about and let me just maybe I can just share my screen and show that I am not sure if everybody got to this. (Video Starts: 1:20:35)

But in the ecomorphs module at the very end there is this graph that you that people reach to and this is relative hindlimb length and relative tail length and it this is these are the eco morphs that people are talking about if those of you who did not manage to reach this phase, so there are some that are that have you know relatively larger hindlimb and large tail. So, if you have long tail and long limbs this is where you are and then some are really squat and short.

(Video Ends: 1:21:21) Thank you Mukta, I think for that summary, anybody else wants to add something to that? So, I just I want to add a point related to phylogeny sir which is quite interesting the exercise and thank you to the TAs and like very good lecture actually I would just come across your lecture also. They are related to ornithology it is so nice sir. Actually, through phylogeny we all of us we come to know the common ancestor and what way the grouping that was too fantastic;

But basically, I have a question in my mind and though we have a conventional mode of classifying our taxonomically classifying organism it is not only birds or animals whatever we are studying we have a very good tool nowadays molecular based work in that case should we do experimental analysis in both the way, conventional as well as molecular base or can we restrict ourselves to molecular bases alone. This is what the question I had in my mind will you please clarify sir?

It is a good question and I think some of it is philosophical you know. The thing that we are always trying to do is trying to understand the truth and a long back what people thought the truth was by measuring you know you collect specimens and you look at the morphology and you think that this is what it is and today the idea of truth has changed a little bit and maybe you know a decade back people thought that genetic.

Once you have genetic data you know they think that that is the end of it like you have genetic data so this must be the truth and then slowly people start realizing that it is not just genetic data

but the number of genes also matters so it is not enough that you have one gene, because that gene probably tells you a different story. So, you want more genes so then people started saying you need like 6 genes or 10 genes or something like that.

But now you are looking at like whole genome level data so people are saying no that is not enough you know you have to look at the entire genome. So, what is truth is you know it depends on the times that you live in the kind of data that you collect, but as of now what it looks like is that people say that genetic data by itself is not enough. You need to have an integrative approach.

So, you will find more recent birth taxonomy papers which will have some amount of genetic data some amount of bird song data acoustic data that is it can be calls or songs, they may have morphology data most they mostly always do and there is an integrative approach that is taken to that. People also add other ecological traits to this depending on what species it is. So I think taxonomy is kind of you know is because it is an old field they are a bit hesitant to accept change.

But the truth of the day is changing so we need to adapt and include all possible evidences I would say. So, thank you sir, so one clarification sir is there anyway a cloning approach was employed in ornithology thing? I do not think I am not sure right I do not know there we have a lot of, does anybody know of any cloning? Anand Krishnan is my go-to person for bird trivia, I am not aware of too much of the cloning work in birds.

In mammals quite a lot has been done there is a whole lot that is done with pets. In China there are companies that will clone your dog for you and so on. But with birds I have not seen this too much, but I have not followed the literature as much. So but cloning does not give you too much information about evolution you know in that sense you just create another copy of the same individual, thank you.

That is for the curiosity. Sorry Badri has his hand up can I ask him to go first? I am sorry, I could not complete even for the first step of the exercise but the initial part of the exercise was to measure the lizards and I am not sure whether there was any information on the habitat which each type

each one of those eight lizards preferred? Should not the way one be going over it be the first to see the lizard in what sort of habitat does it live?

And what sort of tools does it need to live in that habitat and then does it have those particular tools. Instead of rather jumping into a measurement first of all. No, I think I understand what you are saying and that is very true I think that is what an ecological research normally that would be the way that they go about it. In this case, there was a point that they are trying to make which is that you can infer the habitat just by looking at their morphology.

So let me give you a bird example, so you know tarsus length is a very good indicator so birds with long tarsi are often not perching birds they are ground-dwelling birds you know whether you take I mean pretty much owl or even pipits or you know but as you go more canopy dwelling more perching birds they have shorter tarsi. This is similar for the wing length also the more pointed wings birds are the ones that are mostly on the air for long periods of time.

But rounded wings and those birds do not move as much so I think what this exercise was trying to do was to tell you that if you have just the morphology information you can infer the ecology of the species. So that you can go ideally you should go from ecology and then do morphology, but the association is so strong in some cases that you can you know infer one thing from the other, thank you that is very good observation.

So can I have Sarkar Krishnandu, good evening once again so I will request if you can be a bit more elaborate on your work on the sholakili and rather the laughing thrush where it actually came into a different genus Sir what was the finding actually can you be a bit elaborate because in my personal capacity I was not a bit satisfied with that lecture part, you know I could not make out what brought them into a different genus if you will be kind of it Sir?

I can also share a link of a talk that I gave that talks more in detail about this, but essentially what we understand today about these birds is that they probably came in from somewhere in the Himalayas and with as the climate change they moved up the mountains and they got isolated, and they are tied to this habitats which are found on mountain tops. So when they move you know when the habitat moves they are able to move down the mountain, otherwise they do not they are stuck on the tops;

And in fact the sholakili and the laughing thrush seem to be stuck across one particular barrier for over five million years they have not crossed that 40 kilometer gap and that is why they are different species. So, the use of molecular tools which was the I think the lecture that this was under, so we had two species earlier of laughing thrushes but because we had this molecular tools we are able to kind of look at these differences between the populations and we know that they are they are actually supposed to be seven species.

So, I think that is a quick summary, thank you sir. Krishnandu, you had a question you can go. Yes I wanted to ask my question will be rather straightforward. You mentioned three things in the synthetic approach you take genetic data, you take acoustics into concentration and you also take the morphology part. So, my question is in one of the earlier lectures in the course on there was an example discussed about greenish warbler which was thought to be a single species?

But later on details studies on acoustics and non-genetic analysis founded to be six different species. So my question is can two species have a more or less similar genetic data but still differ by their bird songs and maybe is it possible? So, see the thing is that, so first I will give you a bigger picture answer. The question goes back to what is a species and you know we are trying to kind of group birds into these different groups.

And you know birds whether they follow the rules that we are following to group them that is the question that needs to be asked and what the way people do that is by you know doing playback experiments see if they respond to each other and so on. I mean the older ideas were to kind of actually put them together physically and see if they breed but that is not considered necessary anymore.

I would say that you know there can be isolation between species, but there are some thoughts that say that you have to be at least two million year old divergent for to be called a good species. So but this is also now considered arbitrary because there is something called speciation with gene flow, where some amount of gene flow is possible and still you may find speciation which is keeping populations apart in somewhat the kinds of ideas that you talked about.

You know where maybe other traits are different, so ecologically and you know acoustically they are divergent. So at least theoretically there are some ideas that have kind of pushed that forward so this is something that is ideas of active research today. Thank you, there is a lot of questions so maybe I should go to Google form and one thing I just wanted to mention the reading materials that is being provided, sometimes those papers are from journals which are not accessible to us.

When we cannot access this so is it possible to provide the link of the article directly? So, I think that so we will have to try out how we have to try and figure some alternative for that I have at least made sure that we have at least the articles are available through research gate. I mean there is a legality complication about this, but I think if those who are interested in the paper can write to Devica.