Basic Course in Ornithology Dr. Suhel Quader Nature Conservation Foundation

Lecture -12 Methods of Science and Asking Research Questions

Welcome to a discussion on the methods of science and some thoughts on how to approach the rather difficult job of framing research questions. Let's start by understanding what some main goals of scientific research are.

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Broadly speaking, most scientific research falls into one of three categories of exploration. We may seek to describe the world asking, what exists in nature or we may wish to explain why things are the way they are or we may be interested in prediction what will happen in the future under similar or change circumstances. Let's look at these three broad goals one by one with some examples.

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First, description - we might ask questions like how do species numbers change as we move from the equator to the poles or how do Baya Weavers choose from among a number of possible mates or how are species populations changing over the years? In all these questions we are describing what certain aspects of the world are like.

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When seeking explanations, we typically ask why the world is like this. In other words, what explains it. So, we may ask why there are more species close to the equator than far away or why female weaver birds prefer nests that are higher up in trees? or why vultures are showing population declines.

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We often talk about descriptive studies as documenting patterns in nature and explanatory studies as uncovering the underlying processes that lead to the patterns that we see. Processes can also be thought of as the causes behind the outcomes that we see in the world. We won't talk all that much about prediction but it forms an increasingly important part of scientific research. Examples include including questions like how birds will be affected by global heating or by increasing urbanization or what will happen if oil palm plantations replace existing habitats in certain parts of the country.

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So, having understood a bit about these three main goals of scientific research, let us move on to exploring what methods are available to us to tackle them. Perhaps the most obvious method available is simple observation to see what the world is like. We can also go beyond this to experiment with what we can learn by altering things and seeing what happens. We can also put our thoughts into verbal graphical or mathematical form creating abstract models of the world and how we think it might work. These can be used to hone our intuition about how the world works and give us new ideas for making key observations or conducting key experiments.

And an important method available to us is that of synthesizing existing evidence on a particular topic in order to come up with an overall understanding based on all the information gathered so far. This synthesis can be done verbally and qualitatively but increasingly is being carried out statistically and quantitatively. For example, to understand the rate of breakthrough infections of Covid even after vaccination or to aggregate multiple studies of population change in birds to come up with a single index of how the world's birds are faring.

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In practice the way in which many research projects start is for you the researcher to think of an idea. Then, to collect data to provide evidence related to your idea and finally to assess whether the data supports the idea or not.

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One specific manifestation of this broad scheme is the hypothetico-deductive method sometimes called the "Sherlock Holmes" technique after the famous fictional detective. But I have put in the quotes because it is arguable whether Sherlock Holmes actually used this technique all that much. We first start with some observation or puzzle or question. We then think of a possible explanation. In science we like to call such an explanation a hypothesis.

We would ideally think of multiple such explanations or hypotheses for the puzzle at hand. For each hypothesis we have thought of, we derive predictions. A prediction takes this form, if this hypothesis is true then we should see this in the world. The prediction is then compared against information gathered from observations or experiments that have been specifically set up for this purpose.

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If the evidence matches the prediction, then we say that we have found support for the hypothesis. If the evidence does not match the prediction the hypothesis is not supported. And we carry this procedure out with predictions from other hypotheses as well not just a single hypothesis. **(Refer Slide Time: 05:12)**



Now, this word hypothesis is enclosed by a fog of confusion about it what it actually means. Different researchers appear to use it in quite different senses, so, let me try and clarify the meaning of the word. The main thing to understand about a hypothesis is that it is a statement about something that cannot be observed directly. For this reason hypotheses are often about a process rather than a pattern. For example, the ecological process leading to the decline of vultures cannot be directly observed.

Neither can the evolutionary processes leading to a parent species splitting into two daughter species, since this has happened far in the past. So, if we are unable to directly observe, we have to derive predictions that are observable from the hypotheses we have. But what if the phenomenon we are interested in is directly observable. Well then, we can directly observe it and therefore we have no need to pose hypotheses. Patterns are most often directly observable and measurable.

And so we most often have no need for hypotheses in descriptive research. In fact, although you may have read that the hypothetical deductive method is the key to all science, this is simply not true. A lot of research has no need for hypotheses including those involved in description, classification and more. Now all this has been rather abstract for now but we will have a concrete example or two a little later.



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Before that, let us talk about another key concept you should understand thoroughly, which is the difference between correlation and causation. Let us illustrate with an example. Let us imagine there is a primatologist pictured above let us call him Rana. Rana wants to study Bonnet monkeys he finds a nice field site and begins to study them. After some time, the local people get annoyed they say that wherever Rana goes the monkeys gather and so they want him to stop coming here.

The reason the local people say this is that they have picked up on a pattern. They have noticed that there is a positive correlation between Rana's activity pattern and that of the monkeys. In locations where Rana spends a lot of time monkeys tend to gather in large numbers. The pattern is undeniable the data are shown here. The question is whether the conclusion is correct that Rana's behaviour influences where monkeys gather.

Can we think of other explanations for this pattern? One obvious explanation is that in fact it is the other way around? It is Rana's behaviour that is influenced by the monkeys not vice versa. Conventionally, in a graph like this we place the supposed causal variable on the x-axis and the outcome variable on the y-axis by making a plot like this on the right, we are implying that monkeys are affecting Rana's behaviour.

So, this seems reasonable but for completeness we need to consider another possibility, which is that there is something else entirely that is going on. Imagine that the monkeys in this location like to eat discarded banana peels and pieces of biscuits that have fallen to the ground and for this reason they like to hang around tea shops which sell both these items. So, where there are more shops you find more monkeys.

Entirely independently, our primatologist is fond of tea and likes to spend more time where there are little clusters of shops so that he can drink tea and gossip with the people around in this scenario there is no connection between Rana and the monkeys whatsoever. But the apparent relationship between them is driven by a third factor which is tea shops. So, here we have two alternatives to the first interpretation - reverse causation and the possibility that there is an unknown and unmeasured third variable.

Now in this example given that Rana is a primatologist perhaps we can all agree that the situation on the top right is likely to be the correct one, Rana goes where the monkeys are but in other situations it may not be all that clear.

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Here is an example where researchers have found a negative correlation between the density of Lantana and the density of native shrubs in a location in southern India. Because Lantana is an exotic invasive plant the first thought we might have is that the presence of Lantana has a negative effect on the native plants but we could just as easily flip the axis to see whether reverse causation might be at play perhaps Lantana has no effect on native plants rather it is the natives that have a negative effect on Lantana and reduce its numbers.

And of course, as discussed earlier maybe Lantana and natives are completely neutral to each other and it is some third variable that is creating the supposed relationship. Here, perhaps lantana does best in drier areas and native plants do best in wetter areas and their separate and opposite correlation with rainfall is what generates a pattern that looks like they are competing with one another when in fact they are not. So, whenever you are tempted to conclude something about causation from a correlation you should always carefully consider these alternatives of reverse causation and unmeasured third variables.

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How do we deal with this problem? We don't want to be left with no option but to say "well we think x causes y but it is possible that y causes x and it is also possible that there is some underlying z that we do not even know about". What can we do to draw strong conclusions about cause and effect? Well, often the most powerful approach is this if you as a researcher can change the supposed cause then the outcome should change in response.

If it does change then we have identified cause and effect, if it doesn't change then there is no causal relationship. Let's illustrate this with our two examples. If Rana's presence causes monkeys to gather then if we are able to change Rana's usual behaviour and make him go to a place where he normally does not go then monkey should start going start going there too. To assess reverse causation, we might change the monkey's natural behaviour.

Perhaps shooing them away from places they normally gather as a consequence Rana should stop going there too. And if we have some idea about what other factors might be in play, we could do something like ask tea shops to temporarily close and see if both Rana and monkey stop coming there. Finding the corresponding change for each experimental manipulation would bring supporting evidence for that particular causal possibility.

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Similarly, we could assess possible competition experimentally. We could remove Lantana and see whether native plants increase in abundance or we could do the converse to assess the reverse possibility or we could try and artificially increase or decrease moisture in the soil to mimic alteration and rainfall and see what happens to both Lantana and native plants.

This was just an introduction to the logic of experiments. When actually conducting an experiment to uncover cause and effect there are many more aspects to consider but that is a subject for another day. So, this brings us to the end of this brief introduction on the methods of science. Asking a question is a fundamental part of doing research, it's only once you have a question in mind that you can proceed with the further thinking and planning needed to carry out a research project.

In a world full of strange wonderful and fascinating things to investigate it is no wonder that many young researchers find themselves stuck at this initial stage of framing a question. How can one choose from among all the possibilities? Once you have chosen how do you proceed? Let'sstart with discussing how to choose a research question.

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What Motivates/Interests You?



'Pure' vs 'Applied' questions

Level of organization: Physiology, Behaviour/Morphology, Life History, Populations, Species, Communities, Landscapes, Ecosystems

Focus of interest: Species, Ecosystems, Places, Processes/Phenomena, Conservation, Tools/Techniques



To my mind, the first thing to ask yourself when searching for a research question is what motivates you. Different people have different motivations and the same person might have different motivations at different stages in their life. Let's explore some possible motivations. One way in which people divide up possible scientific research is into pure versus applied motivations. So called basic or fundamental research is typically driven mostly by curiosity and a desire to understand how the world works.

By contrast applied or translational research usually stems from a desire to find ways to change the world for the better. There is a lot of debate about which of the two pure or applied research is more valuable but that debate is irrelevant here. The point is if you examine your motivations deeply you might discover which kind of research interests you most in this crude dichotomy and that is the first step towards narrowing your thoughts to a specific research question.

Another way in which we could divide up possible research topics in biology at least is by level of organization. At one end of the scale some people are fascinated by what goes on within individual organisms and for them physiology, biochemistry and molecular biology are the most attractive. At the other extreme, you might want to uncover the secrets of how ecosystem as a whole function, with their myriad species even larger number of species interactions and all the biogeochemical processes that happen at large scale. And of course, there are many fascinating things to study at intermediate levels of organization. Some researchers are obsessed with specific interests one may love a particular species or taxon say big cats or butterflies, others are drawn to particular ecosystems such as rainforests or grasslands. Yet, others might be fascinated by particular phenomena perhaps animal behaviour or mutualistic interactions or competition.

For some starting point, may be a desire to conserve biodiversity others may begin with a particular tool or technique that they know well like molecular techniques in a lab or a particular field skill like mist-netting or a computational skill like agent-based modeling. All of these are good places to start in your search for a research question. Although personally, I feel it is best to not start with a technique that you know and then find a question you can answer with it.

Ideally you would find a question that is meaningful to you and then search for the tools and techniques needed to answer it. So, once you have thought sufficiently about your broader motivations and interests it is useful to think of the next steps in the form of a sort of hierarchy.

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A	Hierarchy ALLAN			
Motivation		A world in which humans and wildlife can coexist		
Goal		Assess sustainable levels of resource extraction		
	Objective 1	What are overall levels of extraction?		
	Objective 2	What is the relationship between extraction and seedfall?		
	Objective 3	What is the degree of density-dependence in seed and seedling survival?		
	Task 1	Observational study of density-dependent seed survival rates		
	Task 2	Experimental study of density-dependent seed survival rates		
	Task 3	Exclosure experiment to assess rodent predation		
()	Task 4	Spraying experiment to assess fungal disease effects		
NPTEL	Objective 4			

The sort of hierarchy I am about to describe starts broad and overarching and ends up narrow and specific. We start with a statement about our broad overall motivation in doing research. Let's say that motivation is to work towards a world in which humans and wildlife can coexist that motivation might lead us to identify a goal that we wish to reach in this particular research project.

For example, we know that local people depend on the harvest or of a forest fruit say Amla but the concern is that over harvesting might cause a negative impact.

So, we articulate a goal for ourselves to understand what levels of Amla harvest would be sustainable in the long run without negatively affecting biodiversity. To reach this goal we need to find out one or more specific things let's call them objectives. I have put in a few objectives that might be needed to reach the goal in this example. The first objective might be to assess the current levels of Amla extraction. A second objective could be to find out how extraction levels affect seed fall under the parent trees.

Since the more extraction there is the fewer seeds we might expect to remain for germination. Now, more seed fall may not lead to higher recruitment into the population since we know that disease, seed predation and herbivory can all act in a density dependent manner. Such that where there is high density of seeds, seedlings or saplings there might also be high mortality rates. If this is true then in nature it might not matter whether we start out with 20 seeds or 100 seeds below a parent tree ultimately only one or two might survive.

And this has implications for how much harvest is sustainable. So, our next objective might be to assess the degree of density dependence in seed germination, seedling survival and so on. Let's look at this objective a little more carefully we know that density dependence might act differently at different life stages and might act through different causes. We can examine it observationally or through experiment..

This then gives us an idea of a number of specific tasks that we might need to carry out to be able to answer the question articulated in objective 3. Here are some possible tasks we might find locations with different seed densities and track how many survive at different densities. Alternatively or additionally, we could devise an experiment laying out different numbers of seeds and plots to see how survival changes with density.

We might also be interested in the causes of density dependent seed mortality and could devise studies to look at the intensity of rodent predation or of mortality due to fungal attack. There may

be more tasks under each objective and more objectives under a particular goal. As you can see each task is a distinct study, you can think of each task as being that unit of work that contributes a single graph or a single table to your research paper or thesis. Taken together the tasks are meant to end to answer the objective level question. And the answers to the objective level questions taken together are meant to answer the goal.

You'llnotice that in this hierarchy we start very broad and step by step by step we break down larger problems into smaller problems that can be both stated with more precision can also be tackled with a specific set of activities. I have called these levels motivations, goal, objectives and tasks but it does not really matter what you call them the important thing is to practice moving your thinking along from broad to specific.

So, that you can work out what actually needs to be done and conversely also from specific to broad so that you can assess whether your detailed plans are sufficient to answer that larger overarching question that you have.





It's often helpful to think of research on a particular topic as being carried out as a cycle. We start with motivation and a goal that flows from that. The goal leads us to frame one or more objectives. To meet an objective, we may have to measure something directly observable or we need to understand we may need to understand a process that is unobservable and if so we will need to articulate one or more hypotheses and deduce observable predictions from them that leads us to the specific things we need to do on the ground.

And how we will plan or design them and then we go ahead and collect the data after which we analyze the data and the interpretation of these analyses then let's say something about each objective that we started out with. So, all this I know is very abstract, let me illustrate with an example.



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Let's say we are interested in the phenomenon of brood parasitism as an instance of a common type of species interaction in which one species benefits and the other species suffers a cost. As you know the familiar Koel is a brood parasite, it does not build its own nest rather it lays its eggs in the nests of other species very often House crows. The House crows then act as foster parents to the Koel eggs incubating them and feeding the chicks alongside their own after hatching.

Now Crows are supposed to be smart, how come they do not recognize the Crow eggs and throw them out of the nest? In general, we expect that host species should evolve egg discrimination such that they can recognize and reject parasite eggs. In turn, we would then expect parasites to evolve egg mimicry such that hosts find it difficult to distinguish between the two kinds of eggs. This co-evolution of strategy and counter-strategy has been termed an evolutionary arms race.

And one can see examples of fine egg recognition and discrimination in hosts as well as almost perfect egg mimicry by brood parasites. So, what is the situation with Koels and Crows?

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In the photo on the top right, you'll see that although Koel eggs resemble Crow eggs in some aspects, particularly the blotching pattern they are different in other respects including size and background colour. Although ornithologists have looked at the eggs and have declared that Koel eggs mimic crow eggs this has not actually been formally tested. So, let us follow through and see how this question can be investigated using some of the approaches we have talked about so far.

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F	Example: B	roodParasitism	111111	
Motivation Goal		To understand the co-evolution between hosts and brood parasites Assess outcome of arms race between parasite		
	Objective 1	Do koel eggs mimic the eggs of their hosts (crows)?		
	Task 1	Measure and compare colour and patterning of koel and crow eggs		
~	Task 2	Observe rejection rates of eggs of naturally differing colour and pattern		
(*) NPTEL	Task 3	Conduct egg mimicry experiment with artificial eggs		

We start with our larger motivation to tackle these sorts of questions which might be to better understand how co-evolution works between hosts and brood parasites. From that broad motivation we need to articulate a more specific goal, something that potentially can be reached within the scope of a research project. So, we say that our goal is to assess what the outcome might be in this arms race between egg mimicry by brood parasites and egg recognition by hosts. More specifically, we have as one of our objectives the question do Koel eggs mimic Crow eggs? To answer this objective level question, we may decide to do several things one might be to just compare the colour and patterning of eggs and come up with some kind of measure or index of how similar they are.

Another could be to take advantage of natural variation in Koel eggs to see whether those that happen to be more similar to crow eggs are rejected less often and those that happen to be more different are rejected more often. And finally, the definitive thing to do would be to conduct an egg mimicry experiment to see how crows respond to eggs of different kinds. Let's take this last task and explore it further.

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The first thing to recognize is that when we ask are Koel eggs mimetic, we cannot observe the answer directly no matter how different or similar the eggs look to our eyes. The mimicry is to be judged with reference to the eyes and mind of the host species. Now since we cannot directly ask

crows to tell us whether they find two eggs to be identical or not we have to find other ways of getting them to answer this question.

So, our hypothesis here is that Koel eggs are mimetic from this we can derive the prediction that if crows cannot distinguish between their own eggs and Koel eggs then if we were to place a Koel egg in the nest the crows would accept the new egg in all cases. So, in our experiment, we need to place a Koel egg in a Crow's nest. But here is an objection perhaps Crows do not pay any attention to what is in the nest and except anything that is placed inside.

We need to account for this possibility, only if Crows do discriminate against odd looking objects does the result mean anything. To check for this, we can add one or two additional treatments here they are eggs painted to look very different either fully black or with large circles or polka dots. These act as a sort of control if crows do not discriminate against them then the experiment does not work.

Now the eggs used in this experiment are not actual bird eggs rather they are artificial eggs made in a mould and then painted. It is possible that there is something about these eggs perhaps the texture or the smell that crows do not like and so they might throw anything out. We need to check whether our method of making artificial eggs is convincing which we do by adding a fourth treatment. An artificial egg of the size and colour of an actual Crow's egg.

This treatment acts as another kind of control, if the Crows do not accept an artificial egg made and painted like one of their own then we cannot conclude anything from this experiment. So, to the answer the question of whether Koel eggs are mimetic we hope that Crows accept the Crow type eggs we hope that they reject the polka and black eggs and then we can see what happens to the Koel type eggs.

If they accept the Koel type eggs then that supports our hypothesis that the eggs are mimetic, if they reject the Koel type eggs then that goes against the hypothesis. Now, you might also be wondering how accurately these eggs are made. Sumit Sinha who made these eggs and carried out this experiment did so, brilliantly. At least to our eyes the artificial eggs marked with a red arrow here look indistinguishable from real eggs. So, so far so good what happens in the actual experiment.



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The first thing to check is whether Crows accept Crow like artificial eggs and the answer is yes they do. They accepted those eggs in 12 out of 13 cases. So, that gives us some confidence that the artificial eggs are working, they are able to fool the crows. Next crows discriminated against clearly odd looking eggs rejecting them in over 80% of cases, this tells us that crows do not just accept anything vaguely egg-like that they find in their nests.

So, indeed there should be evolutionary pressure for Koels to evolve egg mimicry. Now the big question of course is how the crows respond to Koel looking eggs. And the answer is somewhere in between. Crows do not accept them quite as much as they accept their own eggs nor do they reject them as much as they reject completely odd looking eggs. What we can conclude from this is that Koel eggs are mimetic but not perfectly so.

Although this answer might seem somewhat unsatisfactory to you, the main thing is that the experimental logic works. I in fact come to think of it perhaps looking at actual Koel and Crow eggs we might have guessed as such an intermediate answer in the first place. So, this example was not to describe the details of the interaction between Koels and Crows but rather to illustrate

how one might pose and answer research questions. So, let us look once more at the research cycle illustrating it with what we have just discussed.

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We started with our goal of assessing the arms race between brood parasite and host as manifested in egg colouration. We pose the specific objective level question of whether parasite eggs mimic host eggs. There could be other objective level questions as well but we will follow just this one in the cycle. The objective level question leads us to devising various tasks to answer the question one of these tasks might be to conduct an egg mimicry experiment which is designed to assess whether Crows are fooled by Koel eggs.

We realize that we cannot directly observe the answer to this question and. So, we articulate the research hypothesis that Koel eggs are indeed mimetic and from the crow's point of view it cannot distinguish between its own eggs and Koel eggs. From this hypothesis, we deduce the prediction that if crows cannot tell the difference then they will accept Koel eggs at the same rate as they accept their own eggs.

This prediction is directly observable unlike the hypothesis. Of course, if the phenomenon we are interested in is directly observable in the first place, we have no need for hypotheses and their predictions and instead we can proceed straight to the next step. And that step is to design the study

including deciding what kind of eggs we need to use, how we will measure acceptance or rejection and so on.

We then need to carry out the study in this case an experiment. Once we have conducted the study we analyze and interpret the data and decide that our results are consistent with the conclusion that Koel eggs are indeed mimetic but not perfectly so. And this is what we can take back to our original objective level question about whether brood parasite eggs mimic those of their host.

So, in this session we covered some basics of both the methods of science as well as some ideas for how one might approach the rather tricky and often confusing task of framing research questions in a way that is most productive. There is a lot more to explore and understand in these topics but I hope this discussion has provided a fruitful start.