
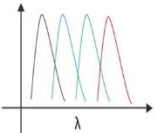









Basic Course in Ornithology
Dr. Anand Krishnan
Indian Institute of Science Education and Research, Bhopal

Lecture -8
Colour

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Pigments	Structural										
<p style="text-align: center;">HPLC, NMR, LC-MS, RAMAN</p> <table style="width: 100%; border: none;"><tr><td style="width: 50%; vertical-align: top;">CAROTENOID 40C TETRATERP NON/POLAR DIETARY PRECURSOR</td><td style="width: 50%; vertical-align: top;">MELANIN FUNCTION EUMELANIN PHEOMELANIN</td></tr></table> <div style="text-align: center;"></div> <p style="text-align: left; font-size: small;"></p>	CAROTENOID 40C TETRATERP NON/POLAR DIETARY PRECURSOR	MELANIN FUNCTION EUMELANIN PHEOMELANIN	<p style="text-align: center; background-color: #f0f0f0;">SCATTERING - RI, USE TEM</p> <table style="width: 100%; border: none;"><tr><td style="width: 50%; vertical-align: top;">MELANOSOMES LAMINAR </td><td style="width: 50%; vertical-align: top;">KERATIN + AIR BUBBLES BARS-NETWORK</td></tr><tr><td style="vertical-align: top;">CRYSTAL </td><td style="vertical-align: top;">SKIN-COLLAGEN</td></tr><tr><td style="vertical-align: top;">QUASI-ORDERED</td><td style="vertical-align: top;">IRIS-PTERINS COMBINED (BUDGERIGAR)</td></tr><tr><td style="vertical-align: top;">DIRECTIONAL = IRIDESCENCE</td><td></td></tr></table>	MELANOSOMES LAMINAR 	KERATIN + AIR BUBBLES BARS-NETWORK	CRYSTAL 	SKIN-COLLAGEN	QUASI-ORDERED	IRIS-PTERINS COMBINED (BUDGERIGAR)	DIRECTIONAL = IRIDESCENCE	
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Welcome to the class on bird colour, my name is Anand Krishnan and I am an assistant professor at the Indian Institute of Science Education and Research in Bhopal. So, I am going to be teaching you today about bird colouration in today's lecture. And this lecture is going to be done primarily on the chalkboard as you can see, I am going to be writing on the board and I am going to be taking you through an outline of the lecture.

As I give it and this lecture will require you therefore to follow, what it is I am saying to make notes for the same and then refer back to the books. As of now I am going to begin by asking you the question, what does bird colour actually mean? Colour in general refers to what wavelengths of light we get back to our eyes when something light reflects off of the surface.

So, if light reflects off of something that we see as red that means it is absorbing all other wavelengths of light and reflecting only red light back to our eyes. So, as a result the object appears red. Now as you know human beings have three different colour channels the blue or short

wavelength, the green or medium wavelength and the red or long wavelength colours. Birds on the other hand differ from us in actually possessing four colour channels they are what you call tetrachromatic.

And to illustrate that I will point you to this graph here this graph on the y-axis indicates the percentage of light absorbed by the photoreceptor and the x-axis indicates wavelength. So, birds have a violet or ultraviolet receptor a blue a green and a red or long wavelength receptor. And these receptors have slightly overlapping but mostly non-overlapping sensitivities. As a result, if we can see a number of different shades by the combinations of our three colour channels just imagine what diversity of colours a bird can see with its photoreceptors.

And as a result of this, birds have evolved a diverse array of colours. So, that leads us to the first problem how do you actually measure colour right. And in particular you want to measure colour as the bird would see it not as you would see it. Most common places use what is called a spectrophotometer. So, you have a fiber optic probe, you shine light onto a feather and include UV because birds can see UV and you measure the percentage of light that is reflected back.

So, if you're looking at reflectance from a blue feather you get a peak somewhere around here. So, imagine this is an entirely ultraviolet feather you will also get reflections like this. So, the photoreceptor absorbs the most light at that wavelength and a feather of that colour also reflects the most light at that wavelength and the same goes for blues, greens, reds and all the possible combinations of these that you can get okay.

Now colours in birds are due to a number of factors. And birds as you know span the gamut from blues and violet colours all the way to bright oranges and bright reds across the spectrum. So, what causes birds to actually have these colours. Well! we are going to discuss that in this lecture the diverse ways in which birds have evolved these amazing colours. And most notably I am going to tell you right at the outset when most of us think colour we think there is a pigment that causes colour, our blood is red

because of a pigment hemoglobin that is not always the case for birds as we are going to see a lot of the shorter wavelength colours that birds exhibit are a result of completely different phenomena but birds do have pigments in fact a number of colours in birds are due to pigments and we are going to discuss some of the major classes of that. Now, how might you identify a pigment? Well, first of all you have to mash the feather and extract the pigment. Once you extract the pigment you can use a number of tools.

You can use high performance liquid chromatography HPLC and more polar compounds will elute first. So, you can identify whether your pigment is polar or nonpolar and then you can identify what the structure of that compound is using a number of techniques. Mass spectrometry, nuclear magnetic resonance, and Raman spectroscopy even Raman is being more recently used to identify pigments in fossil feathers from way back.

So, it actually has a lot of use it was also the most recently many of you from India will know that the name of the Pink headed duck, it was recently used to identify the compound that caused the pink in the head of the Pink headed duck. So, these techniques can give you a lot of information and thanks to those we've identified the major classes of pigments that make up bird feathers and because of this we know that birds that have bright yellow, bright oranges reds and pinks are more or less always coloured by a group of compounds called carotenoids.

Now, I do not expect you to remember this but carotenoids are chemically what you call 40 carbon tetraterpenoids. That is what they are structurally they form a number of different classes. So, you can have non-polar substances like beta-keratin which many of you probably know and polar carotenoids like the xanthophylls they are polar because they have a hydroxyl group or a keto group attached to them.

And that is all you need to know. The most important thing you need to know about carotenoids are twofold one they are not made directly by the bird, they have to be obtained from the diet this is true of many species including Flamingos many of you will have heard that Flamingos in captivity lose their pink colours that is because they do not have a dietary source by which to put carotenoids in there.

The other factor in carotenoids being dietary is that they cannot form patterns. So, feathers are either uniformly coloured with them or not at all. Remember these two things. If you want to feed a bird carotenoids it will put them into its feathers if you do not feed a bird a sort of carotenoids it will not be able to put them into its feathers and the feathers will become white and lose the colour that is very important to remember.

This has been shown by a number of studies. Now what are these dietary sources of carotenoids? They can be insects, they can be fruits, they can be leaves and other plant material and sometimes they can be extremely bizarre. The Egyptian vulture, its yellow face is because of carotenoids and it gets these from dung. So, the Egyptian vulture actually eats animal dung and it has been shown to assimilate carotenoids through there.

Now typically you do not get carotenoids in their pigment form. It is not that you just eat the pigment and put it in your feathers and you are orange or pink or whatever it is. Most commonly you get these pigments as precursors, the most common precursors are called lutein and zeaxanthin. This is just for interest you, do not need to remember. These names these precursors go through metabolic modifications to form other compounds.

And that is how you get all the variety of carotenoid colours. I will give you a cool example of that there is a bird called the Scarlet minivet which many of you will probably know from India the male is red and the female is basically yellow. Now what happens is, the female just they eat the same precursors the female takes these precursors and deposits them directly into her feathers the male actually does a little metabolic trick which is sex specific and converts them into a red compound that makes his feathers red.

So, even within sexes of the same species you can have differences in how carotenoids are metabolized and that is how they provide the diversity of reds, oranges and pinks that you see in the bird world. The other major class of pigments in the bird world are melanins. Melanin as you know are compounds that we possess too they are what give our hair and skin their colours. And

melanins are valuable photo protectors they protect you against UV light they are valuable antioxidants as are carotenoids which is why these pigments are so important to birds.

And they can be used therefore to colour feathers and the most important thing about melanin is they can be patterned. So, if you see a feather with patterns, bars, flex, mottling that is probably a melanin. And melanins come in a number of forms normally but the two most common melanins in the bird world are Eumelanin. So, Eumelanin gives birds feathers their black, grey and other colours and you have Phaeo melanin which gives birds light browns, ochre and rust colours.

And you can have eumelanin and phaeo melanin in ratios in a feather to give it a whole range of melanin pigmented colours all the way from black to pale yellow brown and that is very important to remember because if you actually look at a dark brown or a rust brown colour that is typically caused by a melanin and not a carotenoid but deep maroons can be caused by a carotenoid. So, how do you tell these apart? That is how.

You take the tools that you have biochemically and tell them apart. Are these the only pigments known in birds? No, but they are by far the most common ones. Some examples though, Parrots possess a whole range of yellows and reds etc. They do not actually have any of these compounds, they do not have carotenoids anyway they actually possess their own set of compounds called Psittacofulvin.

You do not need to know what the what that is in any detail but basically remember that parrots have special compounds. In most birds, green is not formed by a pigment I will come to green in a minute, but green in one group of birds actually comes from a pigment these are the African Turacos. The Turacos are one of the few birds that have a green pigment and those pigments are porphyrins. Okay.

They are called the Turacoverdin, never mind they are porphyrins just like hemoglobin hemoglobin itself is a pigment that some birds use to colour their eyes and there are a number of other such mostly minor pigments that are found in specific areas of the body but by and large bird feathers are typically coloured by either carotenoids or melanin. You will notice again that I told

you to hold on for a minute when I talked about green and I did not talk to you at all about blues violets and ultraviolet colours.

That is because as I mentioned earlier birds do not possess pigments for those colours with the exception of the Turacofor green. By and large, blues and violets are obtained through a completely different mechanism in feathers. They are referred to as what are called structural colours and structural colours can come about through a number of different mechanisms. But broadly, they are based on one very simple principle.

If you have groups of objects arranged in a specific order, when light falls on them, they will function like a diffraction grating and scatter light and if they are arranged in a specific way, they will not scatter all wavelengths of light equally. As a result, some wavelengths of light will be scattered more than others giving that substrate a particular colour. For that to happen all you need to remember is you have to have two materials of different refractive indices next to each other.

So, there has to be an interface because the scattering happens at the interface of materials of two different refractive indices. If you disrupt this boundary the scattering will fail and the colour will be lost. In most birds that interface is. So, feathers are made out of keratin beta-keratin just like nails in our hair the interface between beta-keratin and a melanin granule that is called a melanosome is one such place where scattering can occur.

The other is the interface between beta-keratin and air and I am going to talk to you about both of those today. How do we know that these scatterings actually occur one way to do it is these structures are really small they are microscopic. You take little sections of the feather and look at a transmission electron micrograph. You can look at that and identify periodic structures, then you can use computational tools to estimate what wavelength of light it is going to scatter and you can actually mimic these scattering patterns to show that these are in fact scatterers.

They scatter through a number of phenomena which may be familiar to students of physics thin film diffraction scattering by Bragg's law. So, if you have crystal-like structures they scatter using Bragg's law there is a reason I have not written these down on the board because the math is

unimportant here. It is just important to know that if you find ordering, typically your light is scattering coherently.

So, some wavelengths will scatter coherently and give the feather its colour that is all you need to know let us go into some examples of how this might be achieved. Thanks to studies that use TEM, we now have a number of examples of how structural colours are achieved. Let us go into some of them as I mentioned feathers, if you remember from other classes have barbs which then have barbules.

So, scattering structures can be present either in the barbs or the barbules. And they can take number of a number of forms, in the barbules typically the scattering is between keratin and melanosomes. And the melanosome arrangement can be either laminar and I have drawn an approximate laminar arrangement here one such example is the Superb Sunbird of Africa that has laminar arrangements such that they scatter light like a thin film of oil on a water surface this is what you call thin film diffraction, that is why oil on water appears colourful.

The same phenomenon happens here. The second arrangement you can have is in you, you can have melanosomes arranged very ordered like like a crystal, so you have a square or a hexagonal array of melanosomes that scatter light like a crystal according to Bragg's law alright. So, the spacing between scatterers will determine what colour you get because of Bragg's law. An example of this is a bird called the Velvet asityt in Madagascar.

The examples are unimportant just to know that there are many ways in which you can achieve coherent scattering of light there is also now a lot more evidence of a form called quasi-ordered and you can look up the literature on this means that if you look under a microscope it does not appear to be any order it seems very random okay but actually there is order it is just that it is higher order. So, you can not really pick it up and when you look at this higher order structure it actually still scatters light coherently.

So, quasi-ordered structures are found in for instance some Parrots like love birds and others have this sort of arrangement of scatterers. Now here, I have talked to you about how these cameras are

arranged at the microscale and if they are arranged throughout the barbules they scatter like uniformly in all directions. As a result, for instance if you look at Kingfisher's back it will appear blue no matter where you are viewing it from. That is because the scatterers scatter light in all directions not all feathers do that.

Hummingbirds for instance have arrays that are not uniform they are arranged directionally, as a result what you get is scattering only in some directions but not others. I hope you see where I am going with this. If you are standing in the path where it is being scattered you might see an intense blue colour. But if you are off that direction all you will see is black because of melanosomes you will just see black that kind of highly directional scattering is referred to as iridescence.

I am sure I do not need to tell you what iridescence are is if any of you have Purple Sunbirds in your backyard, go and look at them and you will understand exactly what I am talking about. So, remember all of these can result in either iridescent or non-iridescent structural colours depending on how the scatterers are arranged within the feather. As I mentioned earlier, you can also have scattering structures in the barbs in the larger barbs of the feathers.

And there obviously the scatterers have to be much larger because the barbs are larger. So, you can't have melanosomes there. There typically you have air. So, air structures interspersed with skeleton serve as the scatterers here. You can have bubbles of air in the keratin so that it looks like swiss cheese that is what Barbets, Motmots and many other birds have or you can have a more ordered arrangement where you have bars of keratin with air bubbles forming interconnected networks with each other like.

So, with connections of air bubbles many Parrots have this and that is why they are able to exhibit overall uniform bluish colours. You can see that in many parrots in fact now. So, far as I mentioned I have only talked to you about scattering structures in the feathers but those are obviously not the only parts of birds that are coloured. I mentioned that the Egyptian vulture is coloured in its facial skin using carotenoids. Many birds can have carotenoids in their skin as well they can flush their skin with blood.

So, that the hemoglobin gives it a red colour I am sure I do not need to tell you any examples of birds that do that you probably know quite a few yourself. But skin can also be structurally colour and that is important to remember. Now obviously skin has to have a light scatterer in it to get these structural colours. Now that cannot be a melanosome and it certainly cannot be air none of us have air bubbles within our skin.

But we have collagen environments and collagen fibers can form diffraction gratings because scattering and therefore blue and green colours in the skin. Again remember, green is a very special case because it is only in Turaco's do have a green pigment otherwise it is always light scattering or another phenomenon that I will get to in a minute. The eyes of many birds are also coloured and that colouring happens because of crystals in the iris that serve as scatterers.

For instance, tearing crystals or guanine crystals can serve as light scattering structures to colour iris is blue or bright white or any of the other colours that you see in bird irises. And the last thing I want to leave you with is the idea I hope I have given you a sense that birds not only have a diversity in their visual systems but they have a diverse array of colours that come from different necklaces.

So, if I were to give you a colour and say what is likely to cause it, it is probably one of the three main classes and how might you find out what is causing the colour you could do investigations biochemically to find out what is causing it you could measure the reflectance of the colour you could also use a TEM and look at both the barbells and the barbs and explain what sorts of scatterers you might find.

But that is and that is what I want to take away want you to take away in terms of what sorts of colours birds exhibit. But not all colours are as exclusive as this and I will take you to the example of the Budgerigar which is a common pet bird in many households the wild type Budgerigar is green but as many of you who own them might know you also have blue Budgerigar and yellow Budgerigar.

And that is because the green of the Budgerigar is a combination of a structural blue colour and a pigment psittacofulvin that gives the Budgerigar a yellow colour. If you mutate the feathers such that they do not deposit melanosomes in them you lose this and you get a yellow bird and that is why all the black markings also disappear in the yellow bird. If you mutate them on the other hand so that they cannot deposit pigment in their feathers, you get a bird that retains the black markings

but is otherwise blue and the combination of blue and yellow is what gives the green of the white Budgerigar but it is a combinatorial colour. And that is the last dimension I want to leave you with if you for instance get an assignment that birds have different kinds of mutations giving rise to different colours, that is your answer. That is how to think about it and that is how to design an experiment around it.

And with that I will end my class on bird colour and leave you guys to go and read more. Please look at the readings that your TA's will prescribe you and try to learn from them a little bit more about the experimental approaches used I have not gone into great detail since this is still a beginner's class. But for those of you interested in biochemistry there is a huge literature on this, for our physics and engineering students there is a lot of literature on newer and more fancy tools that you can use to quantify structural colours. Please go look them up and as always ask if there are any questions that you may have.

Thank you.