Plumage colour assessment by reflectance spectrometry

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Avian coloration

Birds are the most colourful and spectacular creatures in the Animal Kingdom [22]. Their plumage has a great variety of functions such as: flight, isolation, protection, camouflage, and inter- and intra-specific communication. Moreover, from all vertebrates, they are the most visually dependent class. They rely primarily on their visual capabilities to collect information from their environment and have the most elaborate colour vision system of all vertebrates [24]. Considering that visual communication is the principal mean of communication between individuals, plumage coloration, as well as of other bare parts (e.g. bill, eyes, feet, legs), is associated with mechanisms of exhibition and ostentation. These mechanisms support identification, signalling (e.g. attack/escape; minance/submission), communication of intention (e.g. to play, to mate, to invade a territory), or simply action intentions [6]. Bird coloration could be generally grouped in two categories: (1) cryptic colours, (2) conspicuous colours. Cryptic colours provide camouflage allowing birds to "disappear" in its environment. This cryptic coloration is often more efficient when associated with corporal postures or assuming different positions. Conspicuous colours, that make the bird extremely well noticed, associated with exhibitions displays and feather fluffing, transmit quality indicators and frames of mind [22].

Avian colour vision

In 1972, the first behavioural evidence for the ability of the Hummingbird (*Colibri serrirostris*) and the Pigeon (*Columba livia*) to see in the ultraviolet (UV) was given [10] [31]. Two decades later, the UV pigment by microspectrophotometry was characterised [15]. Since then, there has been growing evidence, based either on behavioural, microspectrophotometric or electrophysiological studies, for UV sensitivity in many other diurnal avian species. Colour vision is achieved by comparing the inputs from retinal photoreceptor neurons that are sensitive to different portions of the spectrum. In the avian retina there are two types of photoreceptors: rods (which are responsible for the scotopic vision), and cones (responsible for photopic vision and involved in colour vision). Birds have, at least, five types of cone photoreceptors: four single cones and one double cone. Each photoreceptor contains visual pigments, which consist of one chromophore (11-*cis*-retinal), and an opsin (transmembrane protein). Based on physiological studies of the visual pigments by microspectrophotometry, the characteristics of these single cones are well defined: ultraviolet/violet sensitive (UVS/VS) with a maximum sensitivity between 362-426 nm; short wavelength sensitive (SWS) with a maximal absorption around 430-463 nm; mid wavelength sensitive (MWS) with an absorption peak at 497-510 nm; and long wavelength sensitive (LWS) with maximal absorption at 543-571 nm [8]. The double cones, with sensitivity similar to the LWS cones, are apparently involved in the detection of brightness and not in the detection of chromatic components [15] [29].

With each class of cones there is a particular type of oil droplet associated. Oil droplets are lipid globules situated in the distal part of the cones inner segment. They act as cut-off filters absorbing wavelengths bellow a critical value and, thereby, narrow the cones' spectral sensitivity and reduce the quantum catch [28]. The overall effect of this spectral filtering is a reduction of the overlap of adjacent spectral cone classes [8] and, therefore, enhancement of discrimination between spectral classes and improved colour constancy [30] (Figure 1). The combination of the

absorption spectra of the photopigments and the oil droplets defines the absorption function of the cones and the cones' effective sensitivity. Colour vision in birds is, thus, highly regulated by coloured oil droplets. However, in most bird species the oil droplets from the V/UV photoreceptor have no significant light absorption. As a result, ultraviolet vision is based exclusively on the visual pigment's sensitivity [4].

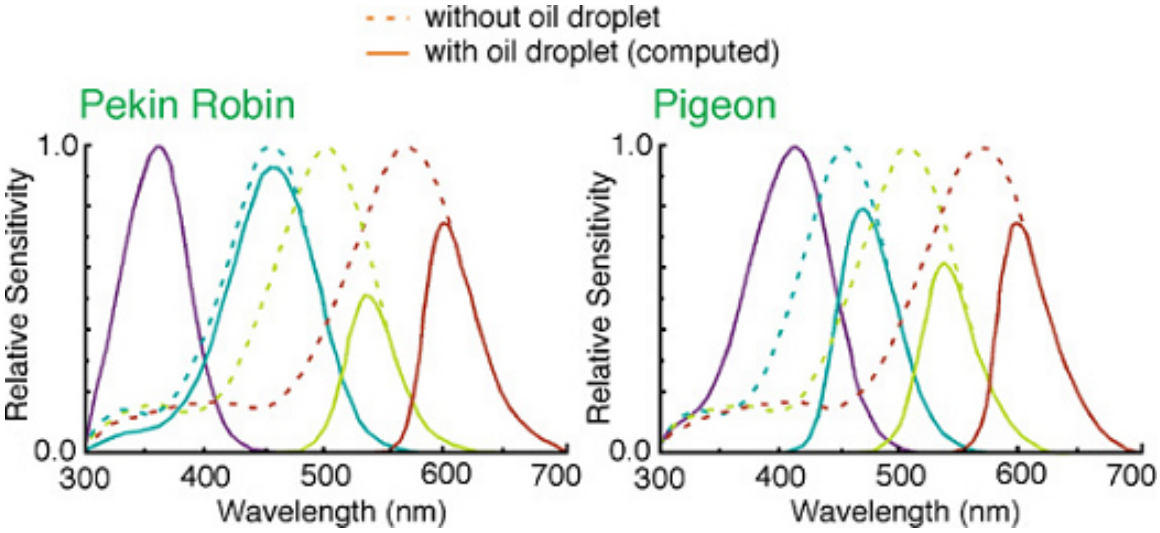

Figure 1. Receptor relative sensitivities of four cone types in a Pekin robin (*Leiothrix lutea)* and a Pigeon (*Columba livia*) with and without oil droplets. To each class of cones, illustrated with different colours (UVS/VS in purple; SWS in blue; MWS in green; LWS in red), there is a particular type of oil droplet associated. Oil droplets act as cut-off filters absorbing wavelengths bellow a critical value and, thereby, narrow the cones' spectral sensitivity and reduce the quantum catch [28]. The overall effect of this spectral filtering is a reduction of the overlap of adjacent spectral cone classes [8] and, therefore, enhancement of discrimination between spectral classes and improved colour constancy [30]. The combination of the absorption spectra of the photopigments and the oil droplets defines the absorption function of the cones and the cones' effective sensitivity. Adapted from [30].

The VS type of colour vision is the most common system among birds. The presence of the UVS/VS cone type in birds is not limited to a particular taxonomic order. Even though, there is some indication that the UVS type is present in Passeriform and Psittaciform species [8]. Conversely, there are some exception [18]. Therefore, to date (to my knowledge), there is not enough information about the UVS and VS cones in order to ascribe a certain cone type to a specific group of birds and more research is needed in this area to make further conclusions.

The molecular properties in the chromophore binding site determine the maximum sensitivity wavelength of the photoreceptor complex. In the SWS1 opsin, six amino acid positions seem to be responsible for the wavelength sensitivity. The origin of avian UV vision can be traced to a single amino acid replacement, from serine to cysteine at position 84 (S84C [32]). This amino acid (C84) is distinctly associated with the UV pigments and cannot be found in other SWS pigments, not even in the violet pigment. This suggests that the UV pigment in this class may have evolved from an ancestral violet pigment by a single amino acid replacement. There is a strong indication that UVS character has been acquired independently in each taxonomic group and that it does not reflect the degree of relatedness between avian species. Therefore, instead of a phylogenetic explanation, the distribution of the UVS/VS character may have an adaptive explanation [18] [26]. However, further research is needed to confirm these assumptions.

It is tempting, although erroneous, to assume that the number of photoreceptor types directly indicates the dimensionality of colour space. If so, birds would have a hexachromatic colour space (four single cones, one double cone and a rod). In order to demonstrate the colour vision dimension, extensive behaviour colour mixing and colour matching experiments have to be conducted. Hue discrimination and spectral sensitivity experiments in birds indicate that only single cones are involved in colour vision and birds have, therefore, a potential tetrachromatic colour visual system [7] [15]. Evidence for a tetrachromatic visual system has been given for few bird species such as: Pigeon (*Columba livia*), Pekin robin (*Leiothrix lutea*) [29], and Chickens

(*Gallus gallus*) [20]. Although it is general assumed that all diurnal birds possess tetrachromatic colour vision, thus far, to my knowledge, no more studies have provided such evidence.

Ever since Darwin, avian plumage colours have been an object of study for behavioural ecologists and have had an important role in the development of theories on sexual selection, communication and signalling [24]. Nevertheless, Darwin and his successors initially did not realize that birds have different colour visual system than humans. Human perception of avian plumage characteristics can be considered, therefore, deficient. The main difference between the avian and human visual systems is that birds are able to perceive light in the near-ultraviolet spectrum (to which humans are blind). It is of great importance, therefore, that this part of the spectrum is somehow considered for the assessment of plumage colour and all related studies [24].

Although there is still some controversy whether UV plays a special part in avian communication, the use of UV as a cue in foraging and mate-choice has been well demonstrated (for discussion see [24]). The fact that most species' plumage reflects considerable amounts of UV is also an established statement. However, this is no guarantee for its role in signalling. The UV reflection can be simply an unselected consequence of plumage physical or chemical structure [1]. The sexual dichromatism in this particular part of the spectrum (UV) is, however, a much better indicator for the signalling role of this characteristic. UV sexual dichromatism has been proven in many bird species such as Blue tit, European starling, Yellow breasted chat, Black-capped chickadee, Piccui dove, Eastern bluebird (for references see [24]).

Plumage characteristics

A bird's appearance is largely a function of its feathers. Feathers aid in body temperature regulation, skin protection, camouflage, communication, swimming, sound production, cleanliness, water repellence, water transport, tactile sensation, hearing and balance [6]. Feathers alter the appearance of the bird by forming both individual and collective (feather coat) patterns. The structural and chemical constitution of individual feathers contributes to the bird's overall appearance. Additionally, an alteration in feather arrangements creates a variety of contour shapes and patterns.

Individual feather structure

Feathers are basically constituted of keratin (a tough, inert, insoluble protein substance of microscopycal microfilaments) immersed in an amorphous protein matrix. Keratins are long-lasting biological material resistant to attack by protein-digesting enzymes of microbes or fungi [6]. A feather is a structure composed by a main shaft and a hierarchy of fine branching structures extending from either side. The main shaft is the calamus (at the base, where there are no branching structures) and the remaining portion is the rachis. The barbs branch from the main shaft and extend angularly towards the tip of the feather forming a vane. Barbules branch from the closely arranged parallel barbs and from these stems, even smaller structures - the barbicels. The feather appearance is defined by the number of levels of branching microstructure (Figure 2).

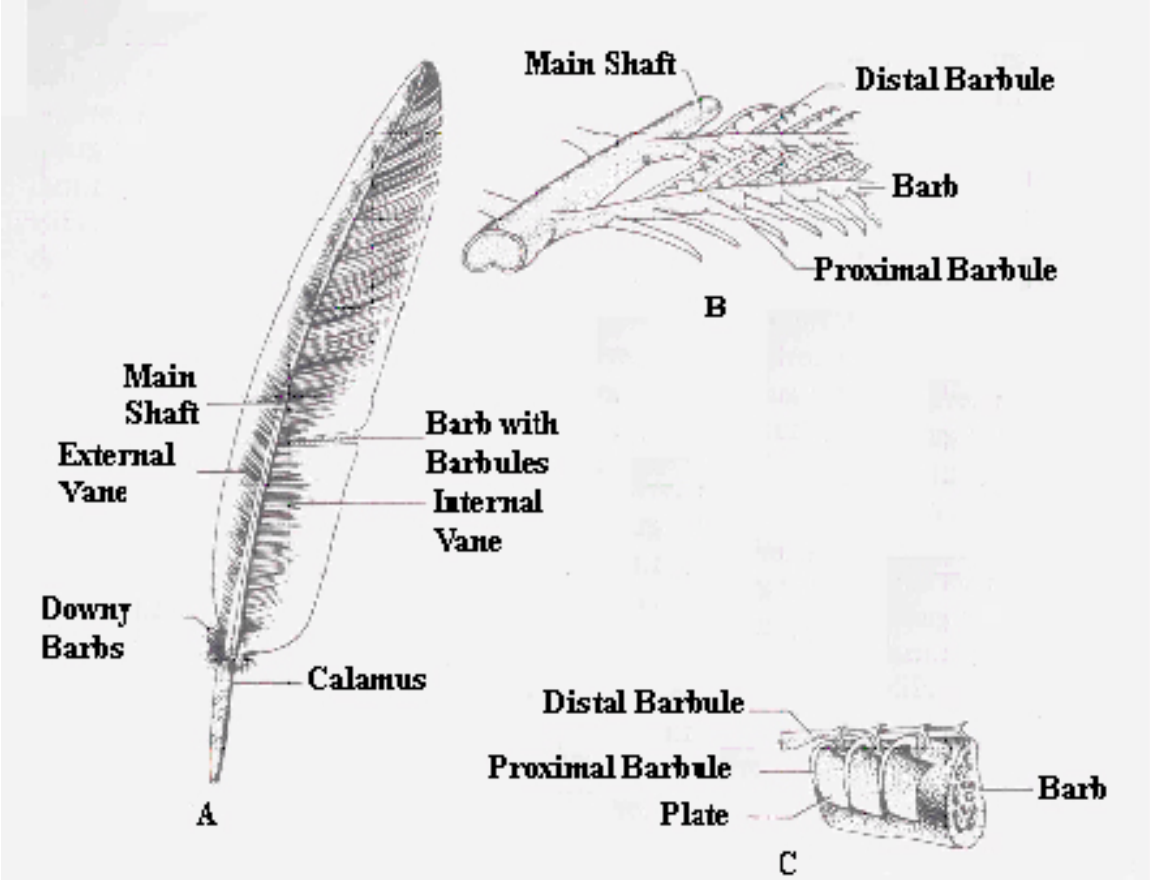

Figure 2. Feather constitutions. A feather (A) is a structure composed by a main shaft and a hierarchy of fine branching structures extending from either side. The main shaft is the calamus (at the base, where there are no branching structures) and the remaining portion is the rachis. The barbs (B) branch from the main shaft and extend angularly towards the tip of the feather forming a vane. Barbules (C) branch from the closely arranged parallel barbs and from these stems, even smaller structures - the barbicels. The number of levels of branching microstructure defines the feather appearance. Adapted from [11].

Feather taxonomies (based, exclusively, on the feather structure) exist to classify the wide variety of feather sizes, shapes, and types. There are five different types of feathers: contour feathers, down feathers, filoplumes, bristles and semiplumes (Figure 3 A and B). The most common type of feathers is the contour feathers (Figure 3A). These are found on the outer surface of the feather coat and are the most conspicuous formations and, therefore, the main responsible for the plumage coloration. The contour feathers can be subdivided in (1) Remiges – wing feathers that can be primary or secondary (depending of their location, hand or arm, respectively) (Figure 4A); (2) Tectrices - that cover most of the bird's body (Figure 3A); and (3) Rectrices - tail feathers that, together with the remiges, are responsible for the bird's ability to fly (Figure 4B) [11] [22].

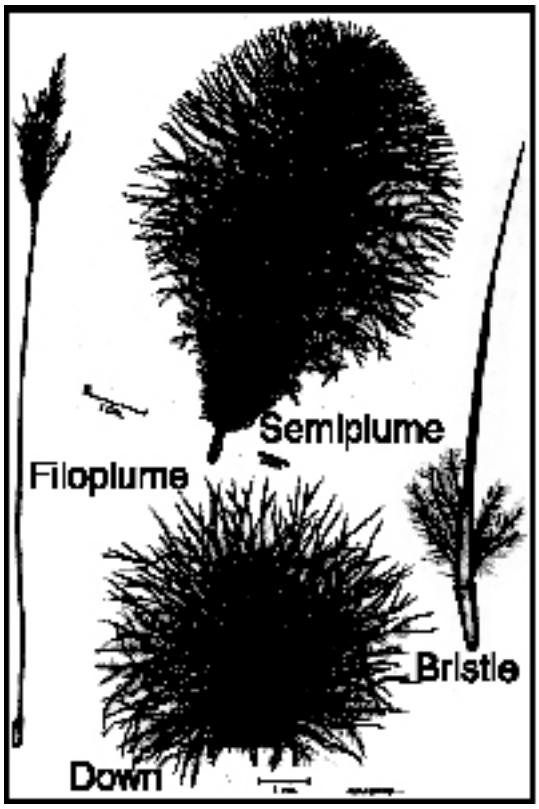

Figure 3. Feather types: Contour feathers (3A) and non-contour feathers (3B). The most conspicuous feather type is the contour feather. This is due to the fact that this is the main type of feather responsible for the plumage colouration. The most defining feather characteristic is the type of vane - ranging from plumulaceous (fuzzy) to pennaceous (firm and stiff). This variation is due to the presence or absence of barbicles, which aid in interlocking adjacent barbs. Down feathers and semiplumes (3B) are entirely plumulaceous, while flight feathers (wing and tail feathers) are entirely pennaceous. Contour feathers are pennaceous at the tip and plumulaceous at the base. A second defining feather characteristic is the ratio between barb length and rachis length. Except for the down feathers, all feather types have a rachis longer than any barb. Specialized and less common feathers include filoplumes and bristles (3B). Finally, the presence or absence of an afterfeather is another defining feather characteristic. Adapted from [11].

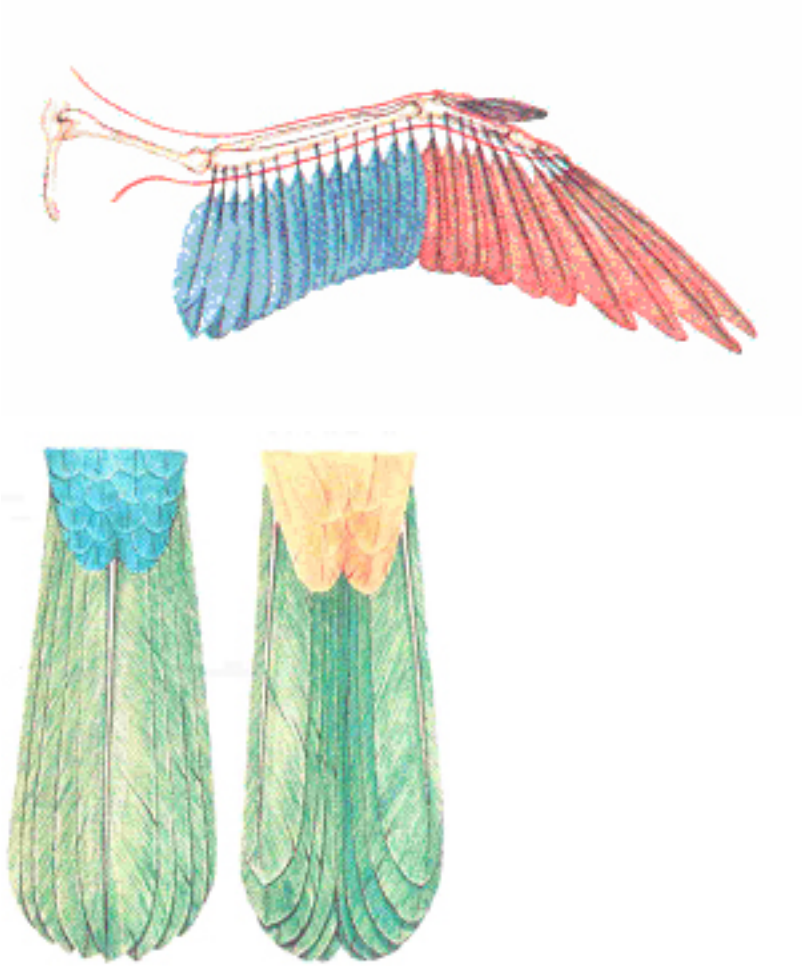

Figure 4. The contour feathers can be split as remiges, tectrices and rectrices. Remiges (4A) are wing feathers that can be either primary or secondary (depending of their location, hand or arm, respectively); Tectrices are contour feathers that cover most of the bird's body (see Figure 3A); Rectrices (4B) are tail feathers that, together with the remiges, are responsible for the bird's ability to fly. Adapted from [11] [22].

Feather coat

Feather coat is defined as the set of feathers on the bird at a particular time. Feathers do not grow equally along the bird's body. Instead, they distribute in different tracts called *pterylae* that alternate with apterylae regions (where only semiplumes are present). Due to moulting this may mean that in a particular coat there are various types of feathers from various different plumages at various stages of growth. The shape of these feather tracts varies with species and is actually used for species taxonomic classification (Figure 5).

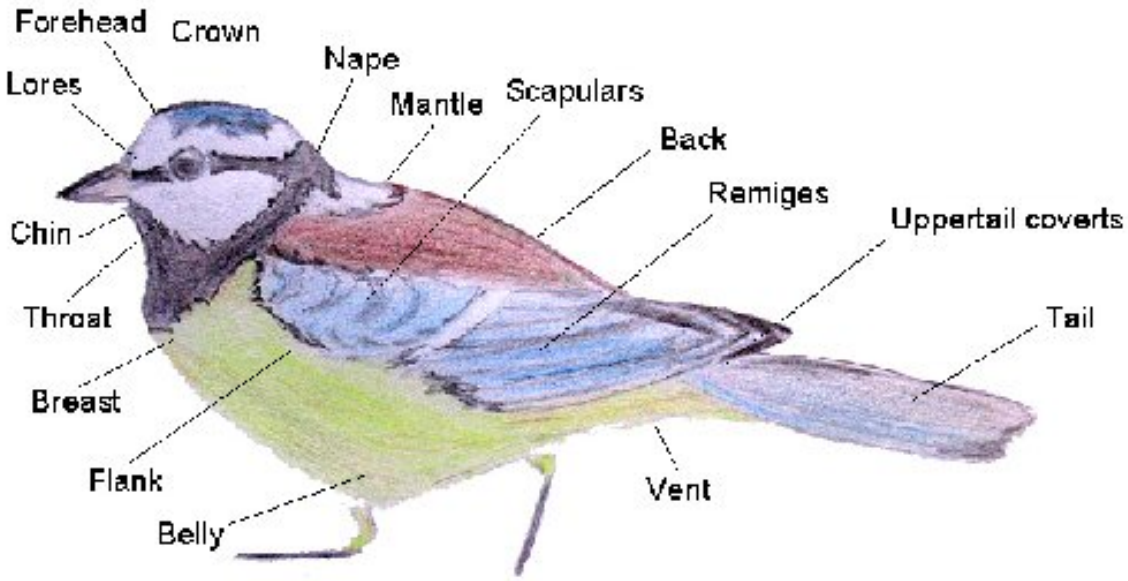

Figure 5. Bird topography. External features of a bird's anatomy (feather tracts, limbs etc.) that are useful in describing a bird's appearance and are used in species identification. Illustration by Nuno Meira.

Plumage colour origin

The complexity of the feather's microstructure associated with the presence of pigments generates a splendid manifestation of colour in the birds' plumage. Colorations and ostentatious patterns of some species, contrast with more discrete and homogeneously coloured species. The evolutions of these plumage colours are in accordance with the species behaviour and its natural habitat [6]. Great part of these combinations and variations in plumage colour are genetically determined, however, some of these colour manifestations have origin on transitory responses to environmental variations.

The diversity of bird feather coloration is due to several different chemical and structural mechanisms, inherent to the feather microstructure, which can act either separately or combined [24]. Pigmentary colours result from pigment molecular absorbance and emission of light, while structural colours result from physical interaction of the light scattered at the interfaces of materials with different refractive indices [23]. Feathers pigmentation results from granules of biochrome pigments in the feather microstructure that absorb and reflect the energy of certain wavelengths of light. Pigment-based coloration results from three major types of feather pigments: melanins, carotenoids, and porphyrins [24]. This type of plumage coloration, especially carotenoid-based, has been receiving much attention in behavioural ecology studies since their expression can be an indicator for nutritional and general fitness (for references see [24]).

According to Prum [23], there are three structures responsible for the structural coloration in feathers. Unspecialised, unpigmented feather keratin, which produces white by incoherent scattering of all wavelengths; structurally coloured feather barbules that produce iridescence by scattering from arrays of melanin granules and/or air vacuoles suspended in the keratin; and specialized, spongy medullary barb cells. Some feathers combine the effect of coloration by pigments with the effect of the structural colours [22]. A good example of this kind of coloration is the green colour that is frequently produced by a combination of the structural blue with the yellow pigment.

Plumage colour assessment techniques

Colour assessment techniques are a common procedure in many ornithological studies. Methodologies such as the colour chart systems e.g., digital photography, digital colorimetry and

reflection spectrometry are currently used for plumage colour assessment.

Colour is a property that a certain visual system awards to the object. It is not, therefore, an inherent property of the object. Accordingly, the perception of spectral composition is a process that engages both physical, physiological, and psychological processes. The perceived colour of an object is a function of (1) the colour of the object itself, (2) the colour of the light source, (3) the colour of the surrounding area, and (4) the visual system of the observer. Hence, colour is a very complex subject to investigate. In order to fill the gap in knowledge about limitations concerning the avian visual system, recent studies have been focusing their attention on objective methods for colour assessment, which are independent from the observer's visual system [5] and, more recently, that consider the avian visual abilities in the UV. In many ornithological studies, unidirectional reflectance spectrometry in the avian visible range (300-700 nm) seams to be, currently, the method of choice (for references see [24]). Hence, in the next section we will be looking into the physical and reflective characteristics of plumage surface and the bird's body shape. Both these characteristics seam to affect the perceived plumage colour.

Reflective characteristics of feather-like surfaces

Everybody is familiarised with the iridescent or "metallic" feathers of the astonishing Peacock's (*Pavo cristatus*) tail feathers (Figure 6). Other examples of iridescent coloured feathers are the head of the Mallard duck (*Anas platyrhynchos*) (Figure 7) and the neck feathers of the Domestic pigeon (*Columba livia*) (Figure 8) (commonly seen displaying its gorgeous neck feathers to the females in our European cities).

Figure 6. The most notorious example of iridescent colouration in birds, the Peacock's (Pavo cristatus) tail feathers. In the Peacock's tail feathers the "metallic" look of the feathers becomes prominent during the sexual displays.

Figure 7. The head of the Mallard duck (*Anas platyrhynchos*) is also an illustrious example of iridescence in plumage. It appears blue, purple or green depending on the illumination and the position of the viewer (in this case humans).

Figure 8. Neck iridescent feathers of the Domestic pigeon (*Columba livia*). Iridescence of feathers on the neck of male pigeons is a commonly seen trait when they display their gorgeous attributes to the females in our European cities. During these displays, it is noticeable the transition of purple to green (and vice–versa) metal-like colours.

In an iridescent coloured object the change of colour is clearly noticeable when the angle of incidence or observation are changed (Figure 9). "Colour", in this sense, is equivalent to hue

(refers to the gradation of colour within the optical light spectrum, or human visible spectrum; it is defined by its dominant wavelength, or the central tendency of its combined wavelengths).

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Figure 9. Example of an iridescent coloured feather (a secondary remex of a Mallard duck). In this feather it is clear the change of colour occurring when the angle of incidence (illumination) or observation (observer) are changed. This is more obvious in the coloured vane. "Colour", in this sense, is equivalent to hue (which refers to the gradation of colour within the optical light spectrum, or human visible spectrum; it is defined by its dominant wavelength, or the central tendency of its combined wavelengths). In this plain illustration the feather was photographed both by rotating the camera while keeping a steady illumination or by shifting the illumination while the camera is steady. In both situations the change of hue from different shades of purple - blue - green is evident. The need for more than a single angle to assess the colour of this feather (and, therefore, of the plumage region that this feather belongs to) by reflectance spectrometry is evident. This simple setting was achieved with the use of a digital camera (Nikon coolpix SQ) on a tripod, a regular halogen light source and one feather.

From reflectance spectrometry studies made in iridescent, and few other structural, coloured feathers it was concluded that there is a need for more than one angle to characterise the colour of these feather type (for references see [24]). In fact, this can be manifestly understood considering the apparent change of colour in the above presented photos. In the case of non-iridescent colours, there is, apparently, no need for other angle geometries to quantify the plumage colour. Pigment colours in feathers have been regarded as not being dependent on incidence and observation angles, since changes in these angles have been assumed not to influence the location of their spectral peak [5] [21]. However, changes in colour are not limited to hue changes since angle dependence in brightness and saturation can also occur, although not so pronounced as hue changes in iridescent feathers. Moreover, the above mentioned assumption only applies for uniformly pigmented flat surfaces. Neither feathers nor plumage surfaces are characterised as uniform and flat. A simple example illustrates the changes that may occur on few feathers (pigmented and structurally coloured) with the change in incidence angles (Figure 10 A) and with change in position of the observer (in our case, a digital camera) (Figure 10 B).

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Figure 10. Simplified illustration of "colour" changes on non-iridescent feathers with the change of incidence (A) and observation (B) angles. In (A) the feathers and the camera are set constant and the only thing that varies is the illumination source. In (B) the feathers and the light source are set constant and the camera rotates the feathers. In both cases, there is a clear change not only in hue (very evident in the blue and yellow feathers) but also in glares and in the way the macrostructure of the feathers is perceived. This simple setting was achieved with the use of a digital camera (Nikon coolpix SQ) on a tripod, a regular halogen light source and four different feather types. Camera settings were: flash off; F/4.8; ISO 70.

Angle dependence of reflectance spectrometric results in non-iridescent feathers have been acknowledged before [1] [5] [21]. Nevertheless, little attention has been given to this feature and to the possibly severe implications that it may have in related fields in biology. There is no agreement on the optimal angles of incidence and observation for plumage colour assessment. Moreover, no justification is made for the use of a specific angle geometry (incidence/observation angles) in spectra recording, nor is reference made to alternative approaches. Yet, different angle geometries are being used indiscriminately in different studies for the same purpose. Structural components and surface roughness are features, which can be found in every coloured feather type. These two components are very likely to influence colour and to cause angle reflectance dependency. Whichever principle accounts microscopically for the colour of the feather/plumage, surface characteristics ought to be considered in the colour assessment. A study showed a clear example of this trait in an iridescent Peacock tail feather [33]. Peacock's tail feathers are one of the most studied iridescent feathers. Nevertheless, this study revealed an additional influence of the macroscopic structures, as surface curvature and barb configuration, in their reflection properties.

The visual assessment of any surface is associated with the geometrical and physical properties of the object in question. Colour is a visual cue as important as shape, size and surface physical characteristics (e.g. if a surface is shiny/matt, rough/smooth). The light reflected from an object's surface can be approximated as a linear combination of two reflection components: diffuse and

specular reflections (Figure 11A). In a perfect diffuser (Lambertian surface - perfect matt surface) the incoming light ray penetrates the surface and it is reflected in every direction in the same amount. Studies have shown, however, that when the surface has high macroscopic roughness the diffuse reflection becomes angle dependent [19]. Nonetheless, to date, no perfect matt surfaces (Lambertian surfaces, see [12] have been found (e.g. [19]). In the specular reflection the light is directly reflected at an interface between the air and the surface and has a spike in the perfect mirror direction with relatively weak intensity spread (a lobe) around the perfect mirror direction [17] (Figure 11A). When the smoothness of a surface changes (increased texture, for example) the light reflection characteristics from the surface in question become more complex (Figure 11B). Therefore, the perceived colour is strongly influenced by the characteristics of the surface in question.

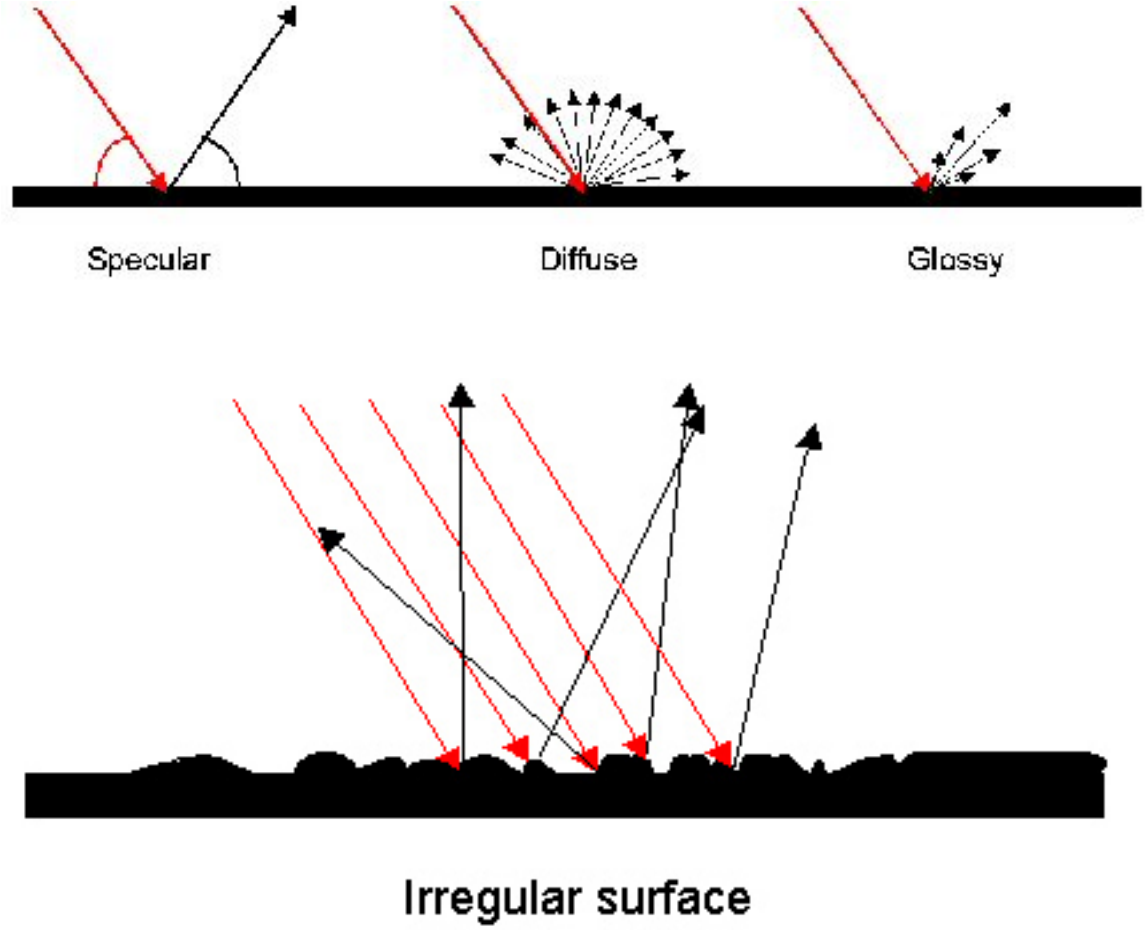

Figure 11. Reflectance geometry. The red arrows represent the incident light and the black arrows the reflected light. From left to right, respectively: (A) shows reflection in a mirror-like surface (specular); a perfect diffuser or lambertian surface (diffuse); and in a semi-perfect mirror with small irregularities (glossy). (B) illustrates a possible scenario of an incident beam of light when reaching an irregular surface (instead of flat). In this case, each ray of light has its angle of incidence and its angle of reflection. Thus the light striking an irregular surface gets scattered in all directions upon reflection. This irregular surface could represent the structure of a feather/plumage surface.

To an average observer a high gloss specimen appears to be darker than an identically pigmented specimen with lower gloss or increased surface texture (Figure 12). Both feathers and plumage surfaces are far from being uniformly smooth and perfect matt surfaces (Lambertian). Therefore, angular dependence (change in reflectance spectra with change in incidence and observation angles) is expected [12] [19] in any feather, independently of its colour origin. Limiting

measurements to just one angle of incidence and observation, therefore, may reveal only partially the plumage colour.

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Figure 12. How surface characteristics can influence perceived colour. Are these squares all the same colour? If we look at all these squares we could almost guarantee that they are undoubtedly of different colours. Nevertheless, if we place them side-by-side we can see that they are actually made from the same piece of plastic and, therefore, exactly the same colour. The only thing that varies within these squares is the surface alteration.

Colour plumage assessment is a much more complex task than it may seem and there are many factors to consider, such as: dimensions and geometry of the surface corrugations, local reflectance properties, specular highlights, not to mention dynamical properties (e.g. during display movements). Colour quantification of any anisotropic surface (of unequal physical properties along different axes) should be done, ideally, the by measuring of the Bidirectional Reflectance Distribution Function (BRDF) [16]. BRDF is a fundamental optical property that describes directional dependence of the reflected energy. It characterizes the energy scattered into the hemisphere above a surface as a result of incident radiation. The geometry of the BRDF definition is illustrated in Figure 13.

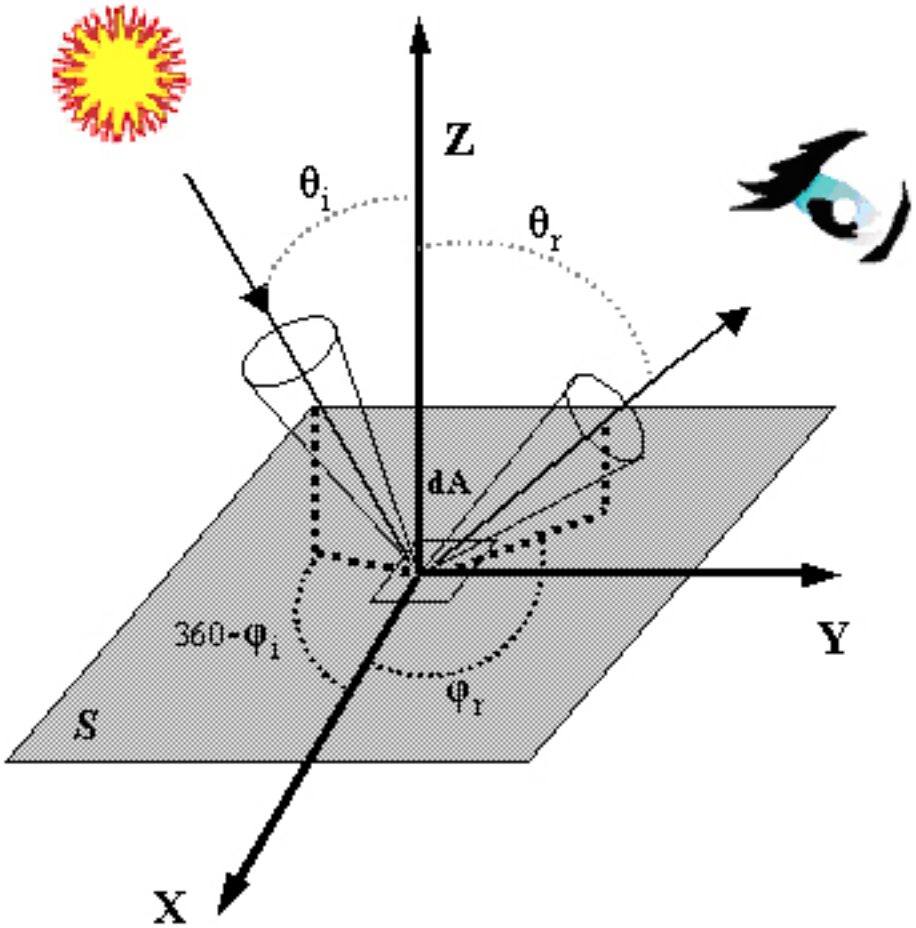


Figure 13. Coordinates system to define angles and terms for Bidirectional Reflectance Distribution Function (BRDF). The BRDF is defined as the ratio between the quantity of light reflected as per direction (r) and the quantity of light arriving from direction (i). In other words, the BRDF is the ratio of the radiance of the exit beam to the irradiance caused by the incident beam. A surface (S) element (dA) is irradiated from direction (θi, φi) and observed by a sensor in the direction (θr, φr), where θ and φ denote polar and azimuth angles, respectively. E(θi, φi) is the irradiance (radiation flux incident per unit surface area) [14].

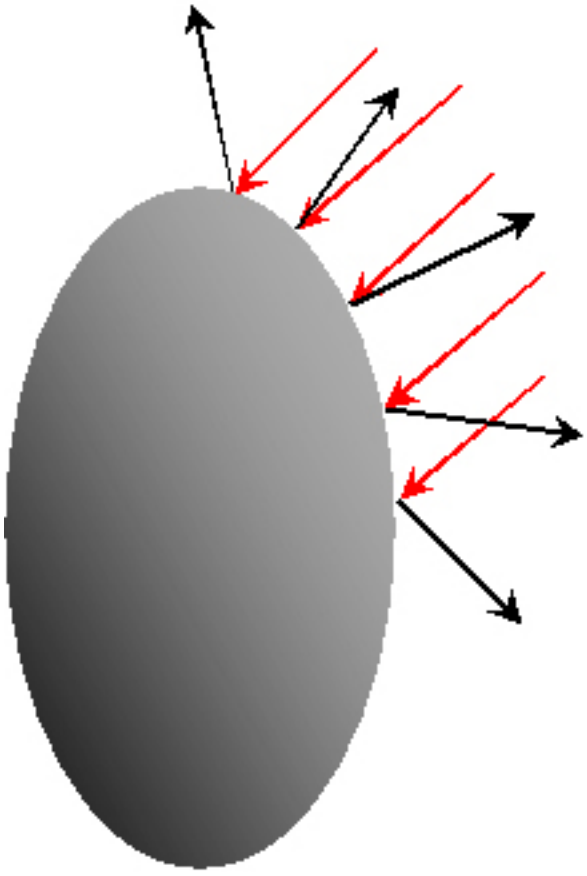
Simply, this mathematical function indicates the fraction of the incoming (illumination) direction and outgoing (observation) direction relative to a local orientation at the light interaction point.

Only when a priori knowledge about the surface's BRDF exists can few parameters, which characterise a class of BRDF's, be elected and then used for colour assessment.

A surface (S) element (dA) is irradiated from direction (θi, φi) and observed by a sensor in the direction (θr, φr), where θ and φ denote polar and azimuth angles, respectively. E(θi, φi) is the irradiance (radiation flux incident per unit surface area) [14].

Birds' body shape

In a natural situation, the observer (in this case the bird) samples the BRDF over large parts of the domain of incident and exit directions (incidence and observation angles) of the other bird's plumage. This is due to the bird's body shape that is approximately spherical (Figure 14A). In this way, two observers positioned in different locations wont have the same perception of the colour of a certain body region (or spot in the plumage) (Figure 14B).



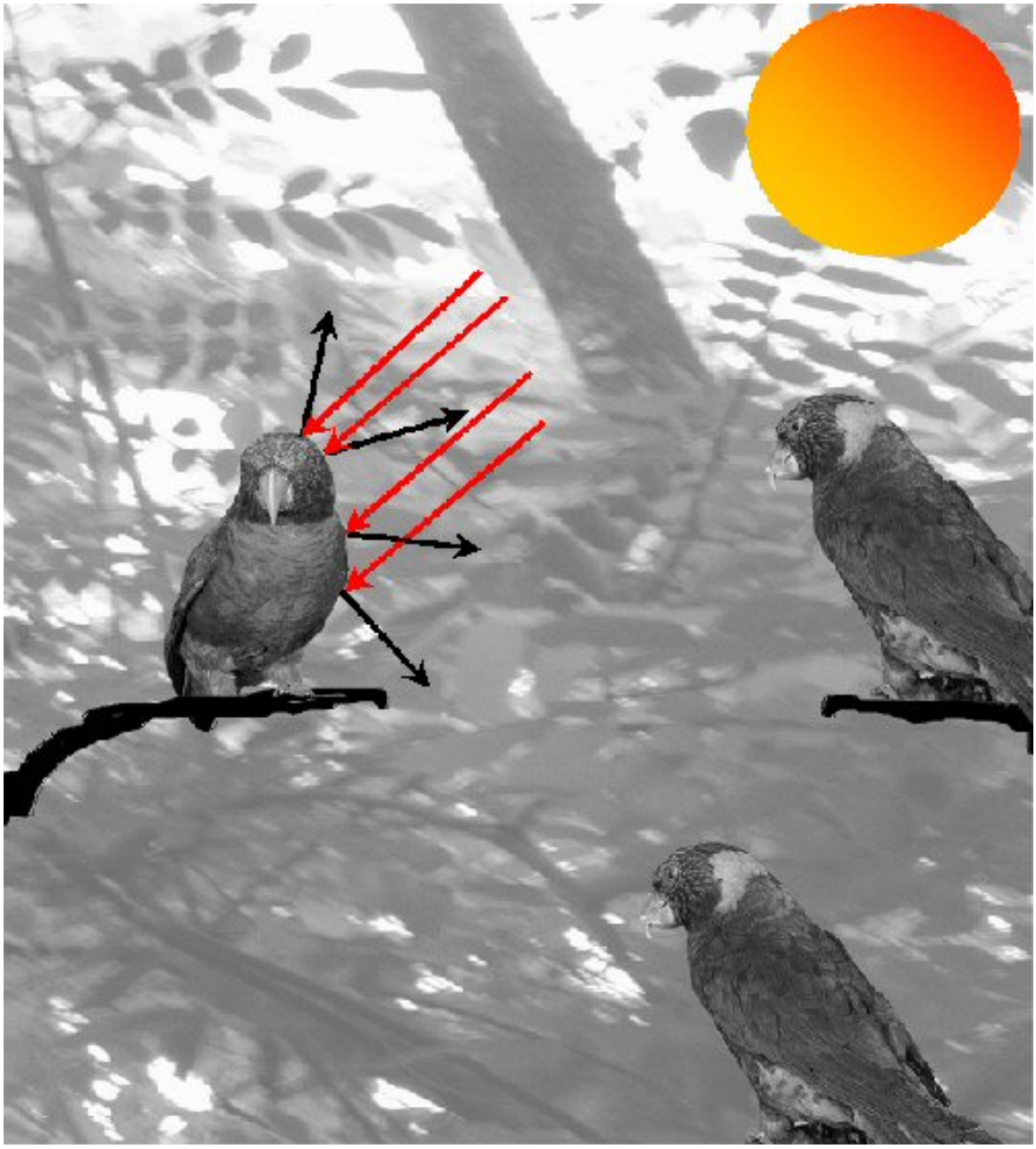


Figure 14. (A) Oversimplified illustration of a bird's body shape (elongated spherical object) illuminated (red arrows) and observed (black arrows) from particular directions. In this particular case, the shading representation was assumed to be close to a perfectly diffuse or Lambertian surface (for which the brightness is independent from the viewing angle or, in other words, the BRDF is constant). It is, therefore, under natural conditions, an "impossible scenario"; (B) Oversimplified scheme representing a situation where two birds (on different locations) may perceive differently the plumage of a third specimen. This illustration is simply based on the body shape and position towards the light source (sun) of the third (observed) bird. The two observing birds will perceive the colour in some areas of the plumage in a completely different way. The red arrows represent the illumination direction and the black arrows the reflection.

A practical example of this mechanism, now in a more scientific sound approach, will be given in the following. A euthanasia specimen of a Long-tailed finch (*Poephila acuticauda*) was photographed under collimated illumination (which is similar to direct sunlight). The set-up consisted of a collimated xenon arc source and a digital camera (Olympus E-20N). The light source could be rotated around the object (in this case, the bird) on a heavy framework placed on a levelled floor. The exposure and F-stop were adjusted manually and kept constant during the whole session, in order to allow for direct comparison of the resulting images. We chose to

maintain the bird and the camera positions constant, and just rotate the light source around the bird. The bird was positioned and five photographs were taken for the following phase angles (the angle between the camera and light source): 15º, 30º, 45º, 60º and 75º (Figure 15).

Figure 15. Set of photographs of a Long-tailed finch (*Poephila acuticauda*) specimen in a specific position (side) with the phase angles (angle between light source and camera) changing from 15º till 75º. This specimen was photographed under collimated illumination (which is similar to direct sunlight). The set-up consisted of a collimated xenon arc source and a digital camera (Olympus E-20N). The light source could be rotated around the object (in this case, the bird) on a heavy framework placed on a levelled floor. The exposure and F-stop were adjusted manually and kept constant during the whole session, in order to allow for direct comparison of the resulting images. We chose to maintain the bird and the camera positions invariable and just rotate the light source around the bird. The bird was positioned and five photographs were taken for the following phase angles: 15º, 30º, 45º, 60º and 75º. These are the original photographs (8-bit RGB image with 16.777.216 possible colours; the usual "millions of colours"). In this set of photos it can be seen how different incidence angles can influence colour.

Since human observers are bad at judging smooth colour changes, it is hard to judge the colour differences between the different angles from the original image. Therefore, we have resorted to an image "manipulation" technique (the posterization) that allows visualization of these colour changes. Posterization occurs when a region of an image with a continuous gradation of tone is replaced with several regions of fewer tones, resulting in an abrupt change from one tone to another. The results of two posterizations of some of these photos can be seen in Figure 16. From visual comparison of the upper and the lower sets of picture (that is, of results with illumination at 15º and 60º, respectively) we can see obvious colour differences. There is a clear colour variation within the bird with the change of angle. Not only in differently coloured body regions, but also within a certain body region with the same coloration (see wing coverts, for example). This is due to the variation of the local surface attitude with respect to the light source and the viewing direction. The colour variation can be more or less pronounced depending on the shape of the body region in question and of the characteristics of the surface. These visual differences were verified through comparison of different colour parameters, such as hue, saturation and brightness (HSB colour), in different pixels of different body regions.

about

Figure 16. Photographs of the Long-tailed finch taken at two different phase angles (15º and 60º) and two correspondent "posterized" photographs subdivided into 64 and 27 colours (upper and lower photo, respectively). Posterization is an image "manipulation" technique that allows visualization of smooth colour changes. Hence, when posterization occurs, a region of an image with a continuous gradation of tone is replaced with several regions of fewer tones, resulting in a more abrupt change from one tone to another and allowing easier judgment of colour differences. Visually comparing the upper and lower sets of the picture (that is, results with illumination at 15º and 60º, respectively) it is obvious that there are some drastic colour differences. There is a clear colour variation of the bird plumage when changing the angle, not only in differently coloured body regions, but also within a certain body region with the same colouration (see wing coverts, for

example).

The explanation of these results is that up to date no perfect matt surfaces (Lambertian surfaces, see [12]) have been found. Accordingly, the BRDF [16] is not expected to be a constant but, instead, the reflectance will depend on the angles of observation and illumination (therefore "bidirectional"). Furthermore, the light source colour is different from the object's colour, as it generally occurs in natural situations. As a result, altogether, this means that colour variations are to be expected for a three dimensional object (e.g. a bird) for which the local attitude changes throughout the object and, thus, the local incidence and observation angles changes throughout the object. These variations can only be captured using photography or multiple bidirectional local measurements. It is important to notice that the plumage of the Long-tailed finch used in the previous experiment was non-iridescent. This comes, once more, in corroboration with the idea that there is more than one single angle spectrometry needed to quantify a plumage like surface. As a reasonable "alternative" to BRDF in live birds, we decided to use multiple angle spectrometry in an attempt to measure a subset of data, in one single plane of incidence.

Multiple angle reflectance spectrometry

Considering reflective characteristics of feather-like surfaces and birds' body shape, and as an attempt to assess part of the BRDF of such complex structural surfaces as the feathers/plumage, we developed an expedite methodology to assess plumage colouration. We named it, for the obvious reasons, multiple angle spectrometric assessment. Thus, we assess plumage/feathers surface colour by reflectance spectrometry using several different angle geometries (incidence/observation angles). Different angle geometries are allowed by the multiple angle fibre holder. In this mechanical holder, the illumination and measuring optical fibres are separated and can be fixed in several different geometries with intervals of minimal 15 degrees (Figure 17). Additionally, this device allows plumage colour assessment without any external influences since it can be positioned against a surface and it prevents any light entering from the exterior while maintaining a certain fixed distance from the sample.

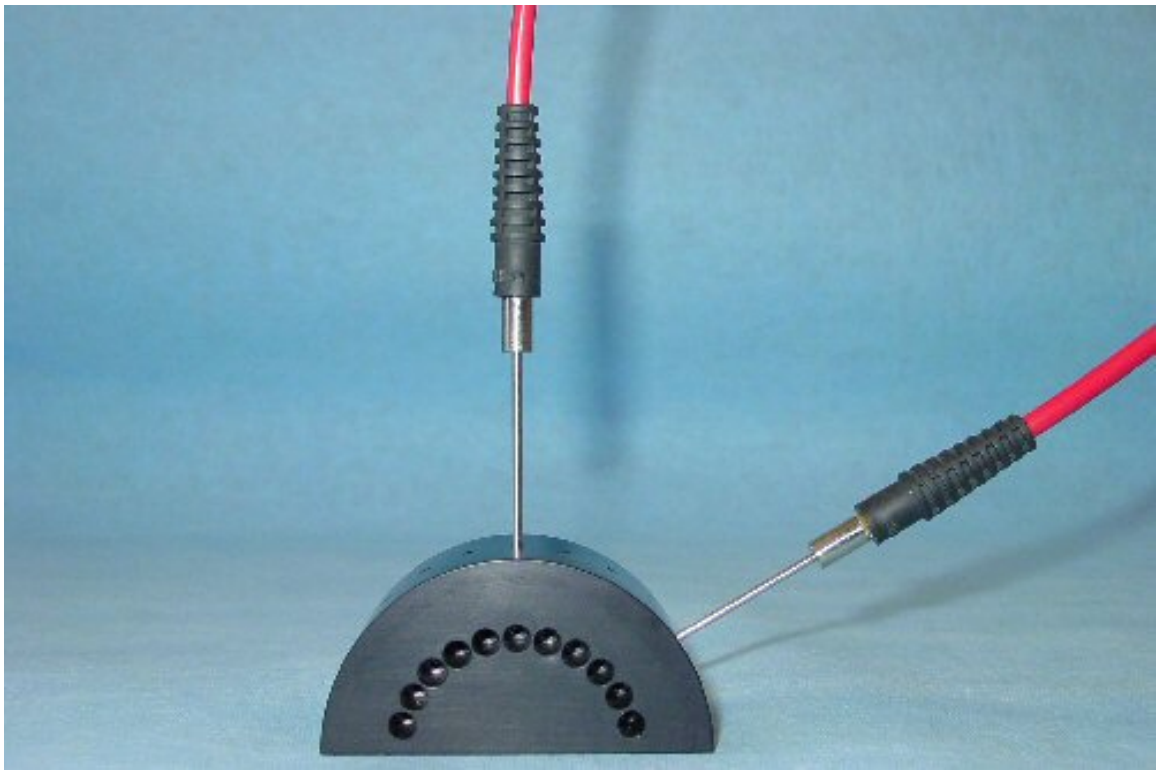


Figure 17. Multiple angle fibre holder. This mechanical device allows the separation and the fixation of the illumination and observation optical fibre probes with 15º angle steps. Moreover, it maintains a fixed distance between the fibre ends and the measuring surface (approximately 2 mm) and prevents any external reflection/illumination.

Multiple angle spectrometric assessment of feather colours. Angle geometry and feather barb orientation affect feathers' spectral reflectance, a pilot study

In what was going to be the demonstration - by plumage reflectance spectrometry - of sexual dichromatism concealed to the human eye in, so-called, "monochromatic" avian species, poor reliability of spectrometric measurements was observed. Moreover, it was not clear which angle to use for the illumination and observation in spectrometric plumage assessment. Changes in reflectance spectra with the varying of the illumination and observation angles could be visually observed and literature information was contradictory in that different research groups were using different angle geometries. In this study we've used five different feather types (with different colour origins), and we've assessed their reflectance using different angle geometries (incidence/observation angles) and using different positions of the feathers in relation to the fibre optics.

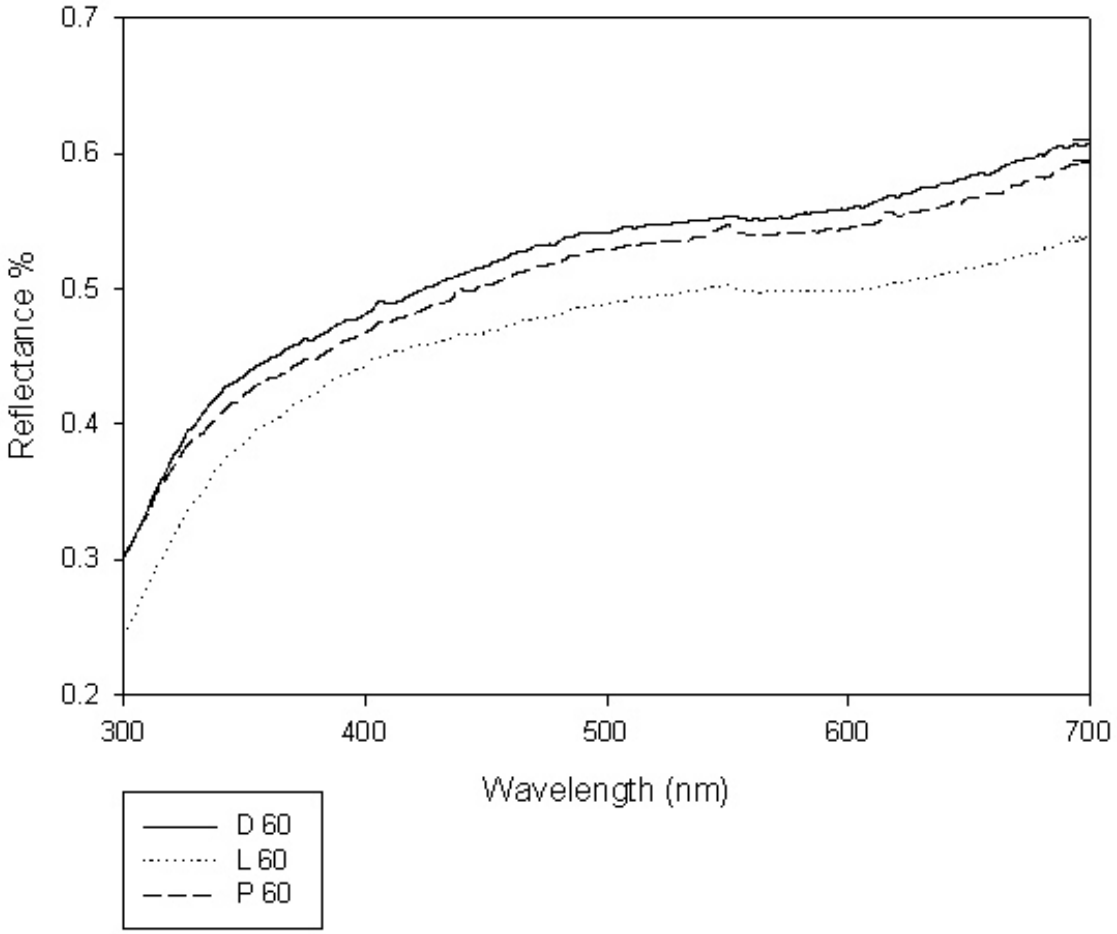
An iridescent secondary remix from a Mallard duck (*Anas platyrhynhos*) and four examples of non-iridescent feathers: yellow, light blue, green and white pectoral contour feathers from Budgerigars (*Melopsittacus undulatus*) were used in this study. In each feather, reflectance spectra were measured on an approximately 2 mm diameter area of the feather vane (*vexillum externum*) using different feather orientations and different observation angles. The incidence angle was kept constant at 0º, relative to normal (i.e. perpendicular to the plane of the feather). Feathers were positioned (in relation to a plane defined by the direction of the detector and the light source) longitudinal, perpendicular and diagonally in relation to the feather barbs. Reflectance was measured with the detector directed distally away from the calamus, except for the diagonal measurements (which were made at an angle of 45º and 225º in relation to the barbs). The angles used for measuring reflectance were: 20º, 30º, 40º, 45º, 50º, 60º and 70º, all relative to normal. In each observation angle, both in the longitudinal and the perpendicular position of the feather, nine spectral measurements were made over the same spot of the feather (distal pennaceous portion of the vane) by replacing the feather for every recording. In the diagonal position (D1 for 45º and D2 for 225º), four measurements were made in each position (with the detector positioned from different sides of the feather barbs) changing the orientation of the feather. All measurements, in different angle geometries and feather positions, were made randomly.

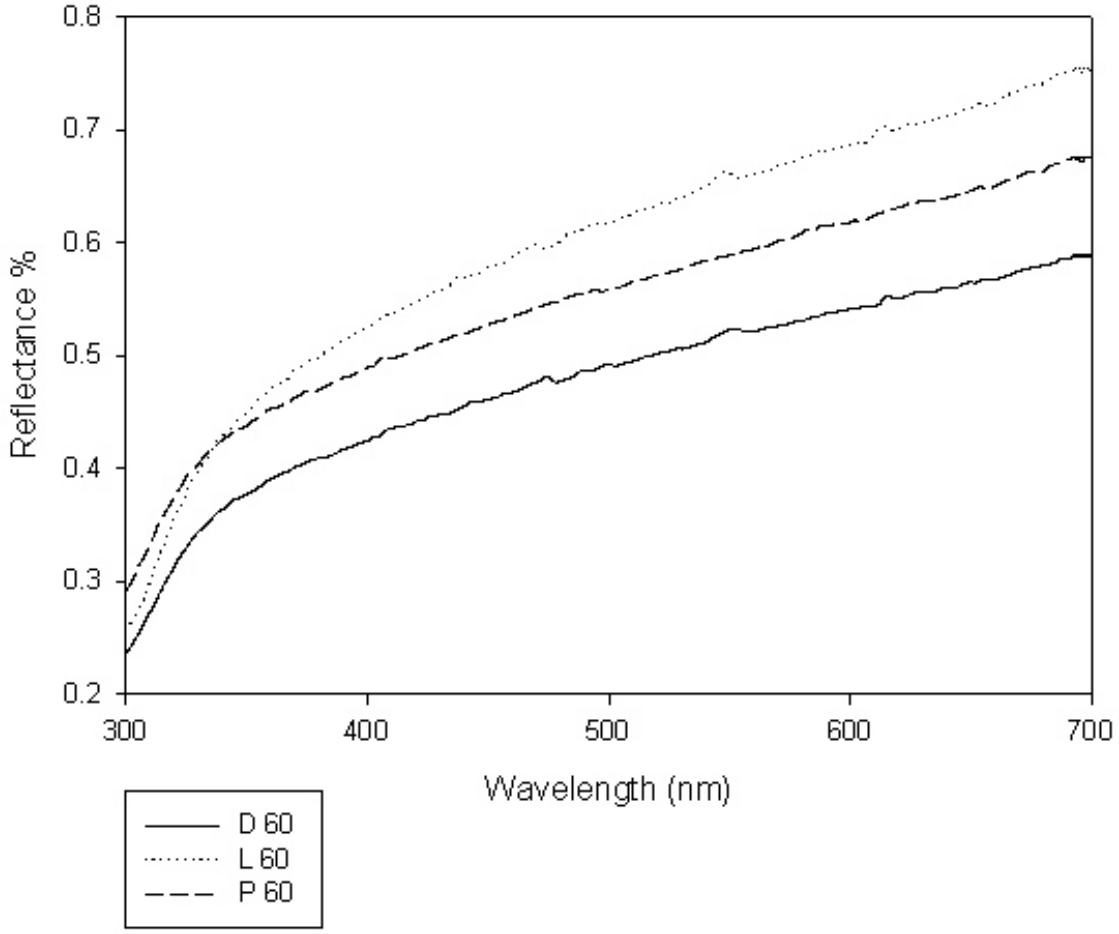
All spectrometric measurements were made with a Diode Array Spectrophotometer X-Dap (IKS Optoelektronik GmbH, Waldbronn. Germany) with a wavelength range between 220-820 nm, attached to a 150 W Xenon Lamp through a set of two optic fibre cables XFF1-Q (IKS Optoelektronik GmbH, Waldbronn. Germany). The recordings were made in wavelength intervals of approximately 0.63 nm, ranging from 300 to 700 nm. A fibre holder, with an angle scale, kept the fibres in position in front of a feather stand with an aperture for the illumination and recording. The feather was held in position, with the holder at the feather surface, without altering the feather shape and the barbs original structure. The spectra were expressed relative to a reference – White Standard - "Teflon" (97%) (IKS Optoelektronik GmbH, Waldbronn, Germany). Standard reference measurements were taken immediately before measuring each feather to minimize any error associated with the drift of the light source and detector. The resulting spectra were stored through software included with the instrument – XLAB, 3.11 (IKS Optoelektronik GmbH, Waldbronn, Germany). For each feather type, 182 spectra (each one of them with 634 data points) were taken. The original spectra were compressed, reducing the spectral resolution by a factor of two (mean of two reflectance values), to facilitate calculations. The compressed data were analysed in two different ways. The first analysis comprised principal component analysis (PCA) of the raw compressed spectra; and the other spectra were standardized by subtracting, from each data point, its mean reflectance (across all wavelengths). Analysis of raw compressed data allows mainly comparisons of brightness between spectra while the transformed spectra allow comparison of the spectral shape (that describes hue and saturation). The PCA was performed using the correlation matrix of the data. For each feather, two PCAs were performed: one on the series of data in which the feather position (barb orientation) was

different at fixed angles of observation and one in which the angles varied at fixed feather position. In both cases the significance of the effects was tested on the PC–scores by one-way analysis of variance (ANOVA).

All the feathers showed reflection both in the near ultraviolet region and the human visible region. The observed reflectance spectra varied with the feather barb orientation (Figure 18) and with the observation angle (Figures 19, 20 and 21). In the iridescent feather a shift of the peak (change in hue) was also observed by changing the observation angles (Figure 20 and 21). Different feather barb orientation caused differences in brightness but could also cause considerable differences in the spectral shape. A small difference either in the observation angle or in the feather barb orientation could lead to a large effect on the spectral shape (Figure 19 and Figure 21, respectively).

ANOVA of PC scores, result of the PCAs, showed that there was a significant difference between spectral curves obtained with different observation angles in every considered feather type (Table 1). Moreover, the positioning (feather barb orientation) of the feather during measurements (in relation to a plane defined by the direction of the detector and the light source) also influenced significantly the spectral curves (Table 2).

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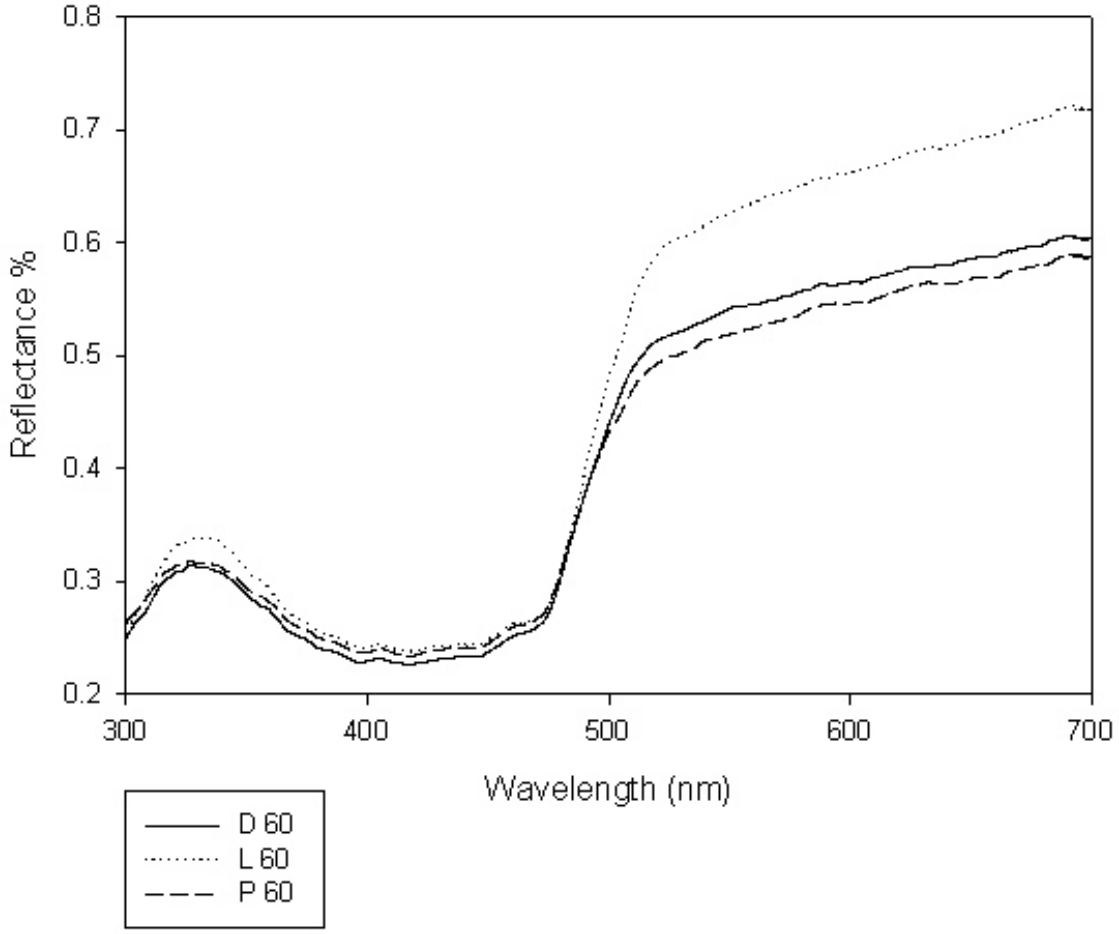
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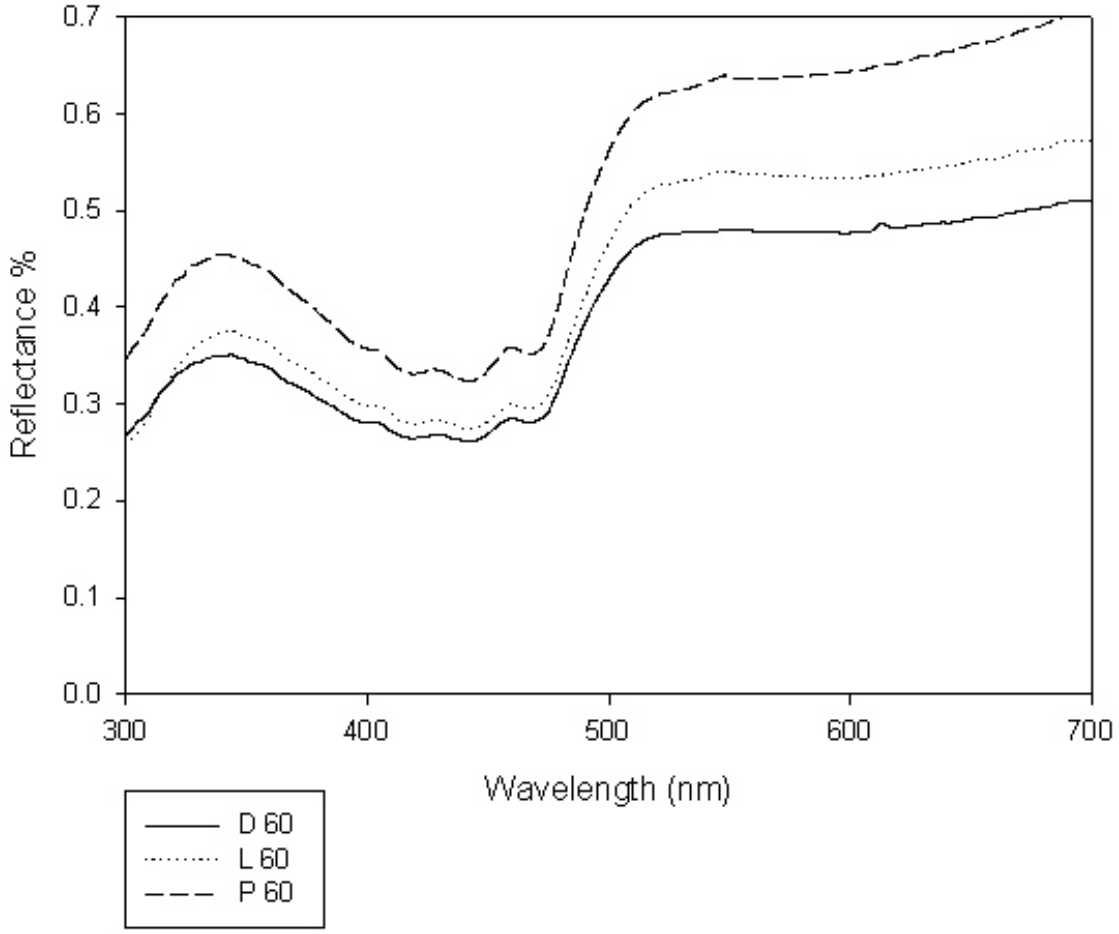


Figure 18. Variation of the mean reflectance spectra of different contour feathers from the Budgerigar (*Melopsittacus undulatus*) with different feather barb orientation. D, L and P stand for diagonal, longitudinal and perpendicular, respectively. The spectra were measured with the observation angle at 60° and incident light at 0º, both relative to normal. A – structural light blue feather; B – structural white contour feather; C – pigment yellow feather; D – combination of pigment and structural features: green feather. Each line represents the average of nine measurements.

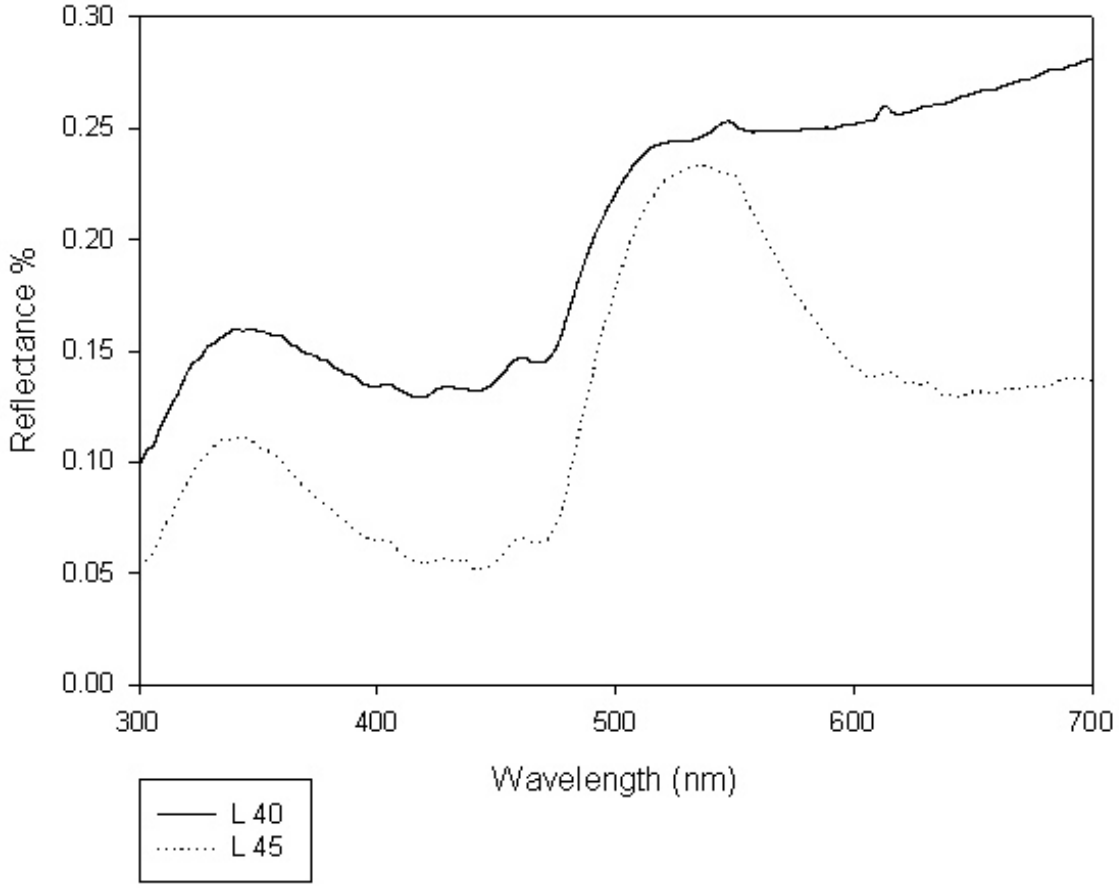


Figure 19. Variation of the mean reflectance spectra from a green contour feather of a Budgerigar (*Melopsittacus undulatus*) measured at two different observation angles (40º and 45º) with the same longitudinal barb orientation and light at 0º. The solid line corresponds to 40º while the dotted line corresponds to 45º relative to normal. Each line represents the average of nine measurements.

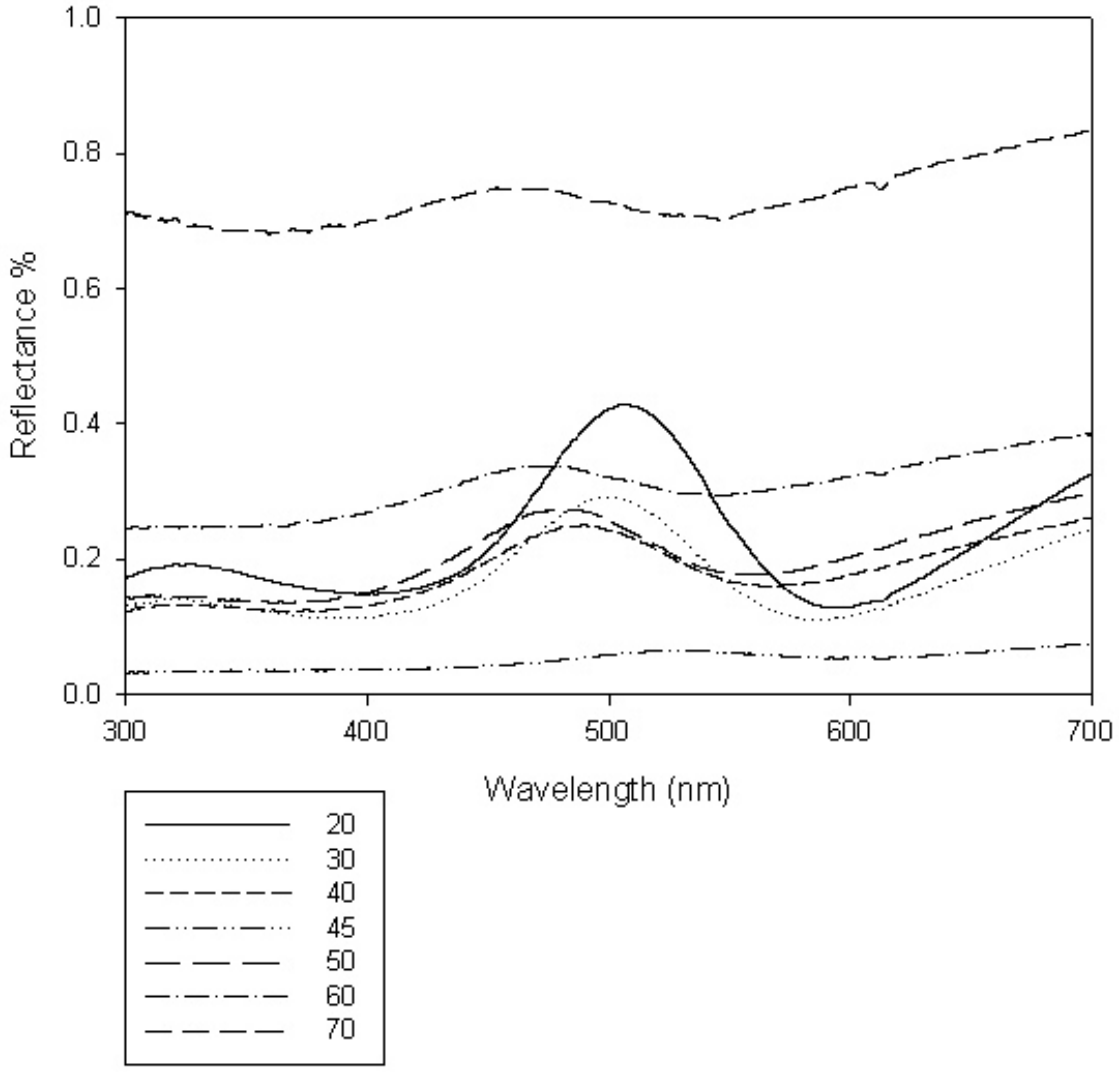
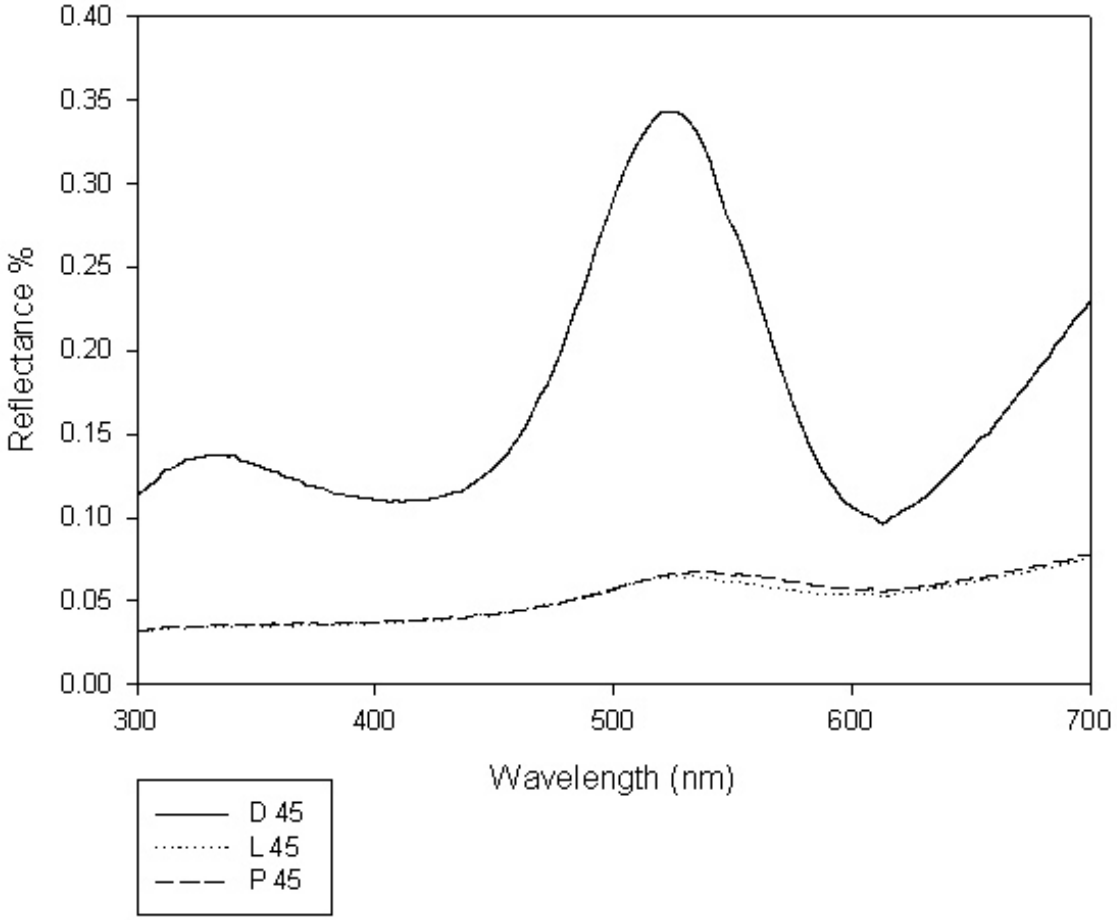


Figure 20. Mean reflectance spectra from an iridescent contour feather of a Mallard (*Anas platyrhynchos*). The feather was set in a fixed position and the angle of observation varied from 20º to 70º, illumination was kept at 0º, all relative to normal. The measurements at 45º show the lowest reflection, both in the UV and in the visible wavelengths. It is noticeable that there is a tendency for the spectral peak to shift towards longer wavelengths while a decrease in the observation angle occurs. Each line represents the average of nine measurements.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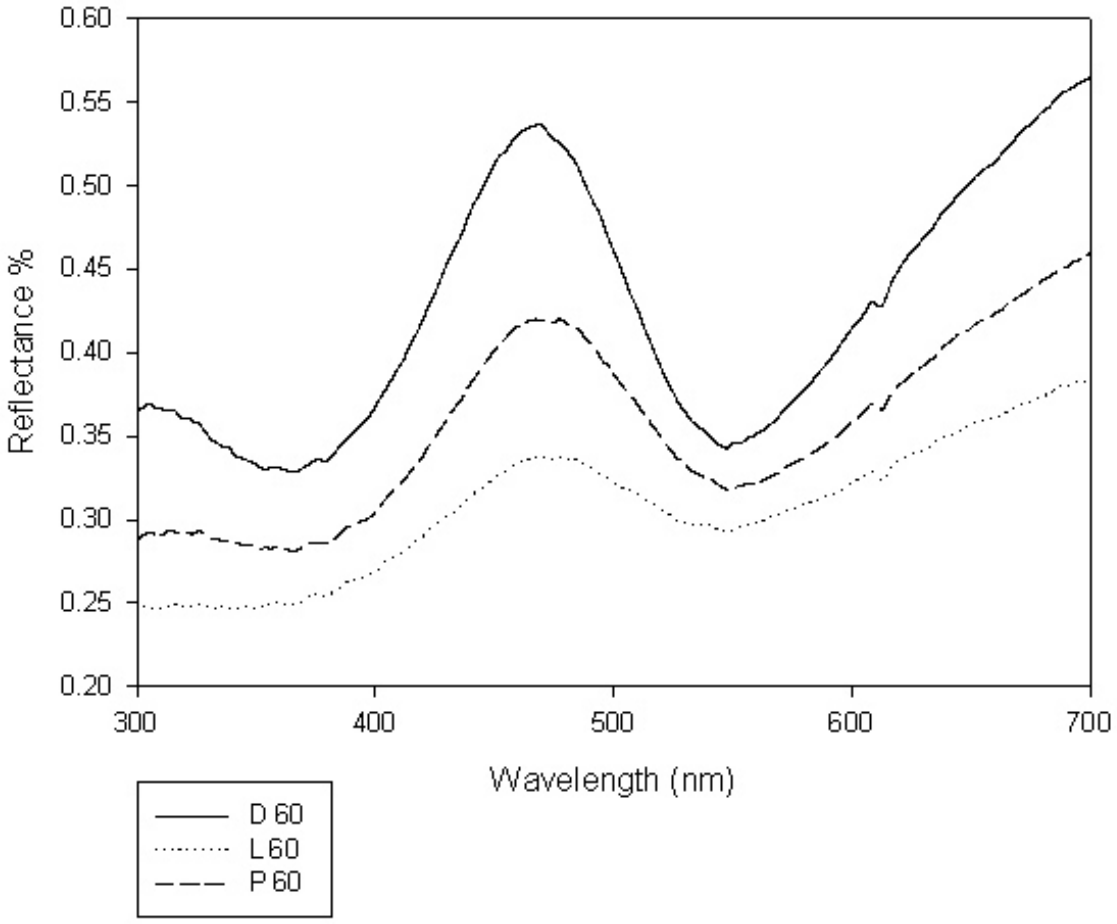

Figure 21. Variation of the mean reflectance spectra with angle and orientation from a structural feather color (iridescent) from a contour feather of a Mallard duck (*Anas platyrhynchos*) feather. The influence of the feather barb orientation in the reflectance spectra is shown. In (A) the observation angle was 45º and in (B) the observation angle was 60º. D, L and P correspond to the diagonal, longitudinal and perpendicular barb orientation, respectively. Each line represents the average of nine measurements.

		Raw data (compressed)		Standardized data (mean value subtracted)	
Feather color	Principal component	Fraction of the variance explained	Variance ANOVA *p*	Fraction of the variance explained	Variance ANOVA *p*
Green	PC1	0.995	0.000	0.879	0.000
	PC2	0.004	0.018	0.089	0.000
	PC3	0.001	0.000	0.020	0.000
Yellow	PC1	0.993	0.000	0.987	0.000
	PC2	0.006	0.140	0.009	0.000

	PC3	0.000	0.000	0.003	0.000
Blue	PC1	0.994	0.000	0.520	0.000
	PC2	0.004	0.000	0.217	0.000
	PC3	0.002	0.134	0.192	0.000
White	PC1	0.998	0.000	0.894	0.000
	PC2	0.003	0.001	0.096	0.000
	PC3	0.000	0.000	0.007	0.000
Iridescent	PC1	0.977	0.000	0.545	0.000
	PC2	0.020	0.000	0.372	0.000
	PC3	0.003	0.000	0.046	0.000

Table 1: Effect of observation angle on feathers with different colour origins.

Results from the principal component analysis (PCA), using the correlation matrix on raw data (no transformation) and standardized data (mean value subtracted), and ANOVA of the resulting PC scores. The illumination angle was 0º and the observation angles were 20º, 30º, 40º, 45º, 50º, 60º and 70º, longitudinal in relation to the feather barbs. The column of the fraction of the variance explained by the principal component presents the variation between spectra that is explained by each principal component.

		Raw data (compressed)		Standardized data (mean value subtracted)	
Feather color	Principal component	Fraction of the variance explained	Variance ANOVA *p*	Fraction of the variance explained	Variance ANOVA *p*
Green	PC1	0.989	0.000	0.937	0.001
	PC2	0.010	0.000	0.033	0.007
	PC3	0.001	0.038	0.024	0.001
Yellow	PC1	0.921	0.149	0.957	0.014
	PC2	0.070	0.000	0.034	0.000
	PC3	0.007	0.003	0.007	0.045
Blue	PC1	0.995	0.008	0.792	0.007
	PC2	0.004	0.000	0.162	0.617
	PC3	0.002	0.034	0.020	0.000
White	PC1	0.986	0.000	0.827	0.000
	PC2	0.013	0.000	0.163	0.000
	PC3	0.001	0.236	0.005	0.002
Iridescent	PC1	0.997	0.000	0.933	0.000
	PC2	0.002	0.000	0.046	0.002
	PC3	0.001	0.001	0.018	0.765

Table 2: Effect of feather positioning (feather barb orientation) in reflectance spectra in feathers with different colour origins.

Results from the principal component analysis (PCA), using the correlation matrix on raw data and normalized data, and ANOVA of the resulting PC scores. The illumination angle was 0º and the observation angle was 70º, the positioning of the feather barbs varied from perpendicular, longitudinal and two different diagonals. The column of the fraction of the variance explained by the principal component presents the variation between spectra that is explained by each principal component.

The results of this exploratory study provided the first experimental evidence for spectrometric differences induced by changing of feather position and/or observation angle in non-iridescent feathers. There were significant differences in spectral shape and spectral mean reflectance when the observation angle and feather orientation changed. These differences occurred both in iridescent feathers and in non-iridescent feathers.

Two factors that determine the reflection properties of materials were recognized [27]: the reflection properties of points on the surface of the structure and the microstructure of the surface. Regarding the first factor, two situations can occur: specular reflection and diffuse reflection. Due to the stated components and considering the complex feather structure, a difference on the reflectance spectra of different feathers would be expected when changing positioning and angles of measurement. The second factor implies local deviations from the normal mean surface causing an effect on both specular and diffuse components. The appearance of objects is usually modeled as a combination of scattering (for which the reemitted radiance is roughly uniform in all directions) and *Fresnel* reflection (which occurs at the material-air interface, this component is mirror like when the surface is smooth). This model suggests that the reflectance will reach its maximum intensity at roughly the specular angle [14]. This model has been the base for most spectrometric studies in plumage assessment. However, this model may fail completely in the case of structured surfaces since the feather colour, as a rough surface, depends on observation and source of direction [3]. Therefore, we cannot conclude that there is a certain appropriate angle for feather colour quantification. Possibly, in more specific studies, a specific angle can be defined to show more pronounced sex differences in a certain species (e.g. [2]) but the limitation to a particular measuring technique can lead to an oversight of an important trait.

Changes in colour with the angle geometry and /or feather orientation are to be expected since feathers are not perfect diffuse surfaces. This is common sense, however, it is only regarded in iridescent feathers and not in other coloured feather types. Pigmented coloured feathers are characterized by reflecting evenly in all directions and, therefore, their reflectance peak (hue), in theory, does not change with the illumination and observation [5] [21]. Nevertheless, in this study we proved that the influence of the angle geometry in colour characteristics can be significant. The fact that a fixed viewing geometry can be used to record spectra from pigmented colours was asserted [21], however, that this procedure does not capture the surface luster. Therefore, single spectra collected with a single angle geometry, can never quantify a rough surface like feathers and extra attention needs to be taken to feather, or plumage, structures producing coherent scattering in different angle geometries. Although this is a rather oversimplified study, using only few feather samples (one of each type) and only one spectra analysis method (the principal component analysis) which others currently use, it simply calls attention for the fact that differences in spectra reflectance, with change in angle geometry and feather barb orientation, can be significant. This is particularly important in the non-iridescent coloured feathers.

Biological implications of the misapplication of reflectance spectrometry

A study in which sexual dichromatism of the Long-tailed finch was disproved called our attention [13]. This was the only study, to date, were sexual dichromatism in the total spectrum (human visible + ultraviolet) was investigated and unproven. Multiple angle spectrometry was applied in this same species and results demonstrated that, when other angle geometries were used the sex differences could be revealed. In this particular study, the immediate implication was sex identification by colour. However, this may have implications in different biological fields. For

instance, the identification of the Long-tailed finch as a "monomorphic" species [13], led to a behavioural hypothesis of sexual concealment. The use of multiple angle spectrometry was used in three other species (Blue fronted Amazon parrot [25]; Java finch – submitted for publication; and European magpie – submitted for publication) to prove its usefulness. In all the considered species, the use of single angle reflectance spectrometry might have led to misclassification. This provided enough evidence for the possible "inadequacy" of single unidirectional reflectance spectrometry and how some plumage characteristics may be overviewed with the use of this technique. This study alerts for the imperative need to consider the bird's body shape and the physical and reflective properties of the feathers/plumage when assessing plumage coloration by reflectance spectrometry. There are some bias factors that also need to be accounted when using reflectance spectrometry as methodological approach. The repeatability of the measurements depends on the technique of the researcher. Due to the feathers smooth and fluffy surface the amount of pressure applied of the plumage brings extra variability. According to Gomez (communication at the 1st European Conference on Avian Colour Vision, Utrecht 2002) this variability is often around 25% and can go up to 50%. Therefore, all the measurements should be done by the same person. Mounting feathers in any way (either by gluing them to papers or by inserting them into slides) might give rise to considerable experimental error. These procedures alter completely the feather structure.

Plumage coloration, not only pigmentary but also structural, is affected by nutritional influences (e.g. [9]). Moreover, coloration and structure appearance change along seasons. Therefore, the time of the year in which the measurements are made needs also to be considered. As basic procedural errors to consider are also the influence of the "whiteness" of the white standard and the warming period for some light sources.

The use of BRDF for the comparison of the plumage characteristics of both males and females, would be an ideal approach for plumage surface measurement. For this approach one would need a CCD camera sensitive to the UV and a special set of quartz lenses, which are transparent to the UV. This would allow getting information in four separate channels. However, although this technique gives all the needed information for surface assessment, it is very time-consuming and meticulous and should probably be done with dead specimens.

Multispectral imaging may be an alternative approach to BRDF. In a multispectral image each pixel contains information about spectral reflectance of the image and the spectral reflectance of each pixel of the image can be then calculated by the camera signals using a non-linear iterative method. The set-up consists of a UV sensitive CCD camera, UV lens system and a set of chromatic filters mounted on a rotating filter wheel. The filters' sensitivities can be determined based on the birds' cones spectral sensitivity for better tuning of the images.

The application of multiple angle reflectance spectrometry in the poultry industry is also an interesting and appealing subject. Chick sex separation after hatching can have severe impact in profit maximisation in this industry of some unexplored breeds. Males and females have different nutritional requirements and are aimed for different industry sectors. They should be, therefore, separated to get the ideal diet for each product. The currently used sexing techniques (vent and feather sexing) are highly demanding in specific training and time, strain restricted, and with health risk to the operator. Sex differences in chicken plumage have been defined before by reflectance spectrometry in adult birds so research into its hatching "plumage" should be worth to investigate. If these sex differences apply, an automating separation process based on reflectance spectrometry could be a useful development for the poultry industry.

The results in this study focus simply on the colour characteristics of the plumage. Although we are aware of the complexity of colour study, in this study, colour was considered simply as a surface property, measured by a reflectance spectrometer and expressed as a ratio of the light reflected by the surface to the incident light on the surface. Whether the birds are able to perceive colour differences and whether they use them is beyond the scope of this study. Additional behavioural work, in order to verify this should be done.

The results of this work have exposed a possible flaw for incomplete data collection and general unawareness of the complexity of measurement of surface colours in the field of biology. This

misapplication of reflectance spectrometry may have implications not only in species classification but also in all related studies in which spectrometry is the elected method. This study highlights the oversimplification that is being made when using single angle spectrometry to characterise plumage colour. Many new questions may arise from the entire outcome here presented.

Acknowledgments

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References

1. Andersson, S., Örnborg, J., Andersson, M. (1998) Ultraviolet sexual dimorphism and assertive mating in blue tits. Proceedings of the Royal Society of London, series B, Biological Sciences, 265: 445-450.

2. Cuthill, I. C., Bennett, A. T. D., Partridge, J. C., Maier, E. J. (1999) Plumage reflectance and the objective assessment of avian sexual dichromatism. American Naturalist, 153:183-200.

3. Dana, K. J., Nayar, S. K., Ginneken, B., Koenderink, J. J. (1999) Reflectance and Texture of Real-World Surfaces. ACM Transactions on Graphics 18, 1-34.

4. Das, D., Wilkie, S. E., Hunt, D. M., Bowmaker, J. K. (1999) Visual pigments and oil droplets in the retina of a passerine bird, the canary Serinus canaria: microspectrophotometry. Vision Research, 39: 2801-2815.

5. Endler J. A. (1990) On the measurement of classification of color in studies of animal color patterns. Biological Journal of the Linnean Society, 41: 315-352.

6. Gill, F. B. (1995) Ornithology, 2nd ed. W.H. Freeman and Co., New York, NY.

7. Goldsmith, T. H., Collins, J. S., Perlman, D. L. (1981) A wavelength discrimination function for the hummingbird (Archilochus alexandri). Journal of Comparative Physiology A, 143: 103-110.

8. Hart, N. S. (2001) The visual ecology of avian photoreceptors. Progress in Retinal and Eye Research, 5: 675-703.

9. Hill, G.E., Montgomerie, R. (1994) Plumage colour signals nutritional condition in the house finch. Proceedings of the Royal Society of London, series B, Biological Sciences, 258: 47-52.

10. Huth, H. H., Burkhardt, D. (1972) Der Spektrale sehbereich eines Violetta Kolibris. Naturwissenschaften, 59: 650.

11. King, A. S., McLelland, J. (1984) Integument. Birds, Their Structure and Function. Baillière Tindall. London, Philadelphia, Toronto, Mexico City, Rio de Janeiro, Sydney, Tokyo, Hong Kong, 23-42.

12. Lambert, J. H. (1760) Photometria sive de mesura et gradibus luminis, colorum et umbrae. Eberhard Kleet, Augsburg.

13. Langmore, N. E., Bennett, A. T. D. (1999) Strategic concealment of sexual identity in an estrildid finch. Proceedings of the Royal Society of London, series B, Biological Sciences, 266: 543-550.

14. Lu R., Koenderink J. J., Kappers A. M. L. (1998) Optical properties (bi-directional reflection distribution functions) of velvet. Applied Optics, 37: 5974-5984.

15. Maier, E. J., Bowmaker, J. K. (1993) Colour vision in the passeriform bird, Leiothrix lutea: correlation of visual pigment absorbency and oil droplet transmission with spectral sensitivity. Journal of Comparative Physiology A, 172: 295-301.

16. Nicodemus, F. E., Richmond, J. C., Hsia, J. J. (1977) Geometrical considerations and nomenclature for reflectance. US Department of Commerce, National Bureau of Standards, NBS Monograph 160, Washington, DC.

17. Nishino, K., Zhang, Z., Ikeuchi, K. (2001) Determining reflectance parameters and illumination distribution from a sparse set of images for view-dependent image synthesis. Proceedings of the International Conference on Computer Vision, Vancouver, Canada.

18. Ödeen, A., Håstad, O. (2003) Complex distribution of avian color vision systems revealed by sequencing the SWS1 opsin from total DNA. Molecular biology and evolution, 20: 855-861.

19. Oren, M., Nayar, S. K. (1995) Generalization of the Lambertian model and implications for the machine vision. International Journal of Computer Vision, 14: 227-251.

20. Osorio, D., Vorobyev, M., Jones, C. D. (1999) Colour vision of domestic chicks. The Journal of Experimental Biology, 202: 2951-2959.

21. Osorio, D., Ham, A. D. (2002) Spectral reflectance and directional properties of structural coloration in bird plumage. The Journal of Experimental Biology, 205:2017-2027.

22. Perrins, C. (1992) Evolution and Classification; Anatomy, Locomotion and Behaviour. Bird Life- An introduction to the world of birds. Magna Books, 7-12; 12-48.

23. Prum, R. O. (1999) The anatomy and physics of avian structural colours. Proceedings of the 22nd International Ornithological Congress. Durban. 1633-1653.

24. Santos, S. I. C. O. (2005) Seeing the invisible. Ph.D. Thesis, Utrecht University, The Netherlands.

25. Santos, S. I. C. O., Elward, B., Lumeij, J. T. (2006) Sexual Dichromatism in the Blue-fronted Amazon Parrot (Amazona aestiva) Revealed by Multiple-angle Spectrometry. Journal of Avian Medicine and Surgery, 20: 8-14.

26. Shi, Y., Yokoyama, S. (2003) Molecular analysis of the evolutionary significance of ultraviolet vision in vertebrates. Proceedings of the National Academy of Sciences U.S.A., 100: 8308-8313.

27. Van Ginneken B., Stavridi M., Koenderink J. J. (1998) Diffuse and specular reflectance from rough surfaces. Applied Optics, 1: 130-139.

28. Vorobyev, M. (2003) Coloured oil droplets enhance colour discrimination. Proceedings of the royal Society of London series B, Biological Sciences, 270: 1255-1261.

29. Vorobyev, M., Osorio, D. (1998) Receptor noise as a determinant of colour thresholds. Proceedings of the Royal Society of London series B, Biological Sciences, 265: 315-358.

30. Vorobyev, M., Osorio, D., Bennett, A. T. D., Marshall, N. J., Cuthill, I. C. (1998) Tetrachromacy, oil droplets and bird plumage colours. Journal of Comparative Physiology A., 183: 621-63.

31. Wright A A. (1972) The influence of ultraviolet radiation on the pigeon's colour discrimination. Journal of the Experimental Analysis of Behavior, 17: 325-337.

32. Yokoyama S., Radlwimmer F. B., Blow, N.S. (2000) Ultraviolet pigments in birds evolved from violet pigments by a single amino acid change. Proceedings of the National Academy of Sciences, U.S.A. 97:7366-7371.

33. Yoshioka, S., Kinoshita, S. (2002) Effect of macroscopic structure in iridescent color of the peacock feathers. Forma, 17: 169-181.