# THE WAYS IN WHICH FEATHER COLOURS ARE PRODUCED AND THEIR POTENTIAL FOR IDENTIFICATION OF FEATHER REMAINS

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### Summary.

Feather colours can be produced in principally two ways.

1. By the deposition of pigments (dyestuffs) in the feathers.

2. By the formation of light-reflecting structures in the feathers.

Pigment colours are the most frequent, giving rise to black, brown, grey, yellow and red colours. Structural colours may have any hue, but are particularly important with green, blue and violets.

There is great variation between species in the appearance of sections of coloured feathers under the electron microscope. Examples of this variation will be given and in relation hereto the potentials of feather colours for the identification of feather remains will be discussed.

# 1. INTRODUCTION

Ornithologists rely to a very large extent on the great variety of colours and colour patterns when they identify bird species.

It is therefore natural to ask whether we can use the ways in which feather colours are produced when we are to identify tiny feather fragments to species.

The method which gives most information probably is to study ultrathin sections of feather parts with the transmission electron microscope. A number of such studies has appeared since the early 1960'ies, and there now exists a general knowledge of the appearance of the various types of feather colours under the transmission electron microscope.

Neither time for preparation nor space for printing permits a rather complete review. Another relevant fact is that most studies have been performed on bright-coloured feathers of exotic species, the results of which are of little use in practical identification work with European species.

So I shall restrict myself to a very brief review with references to some of the existing literature. Durrer (1986) provides a recent review.

# 2. APPEARANCE OF COLOURED FEATHER PARTS UNDER THE TRANSMISSION ELCTRON MICROSCOPE.

Feather colours can be produced in principally two different ways. (1) By the deposition of coloured substances, pigments, within the feather keratin, and (2) by the enhancement of reflection of light of a certain portion of the visible spectrum, due to the presence of structures of a particular size, shape and refractive index within the feather keratin.

# 2.1 Pigmentary colours

By far the two most widespread feather pigments are the melanins and carotenoids.

Melanins. These give rise to the very frequent black, brown, redbrown and yellowish-brown feather colours. They occur as granules which are easily observed with the electron microscope.

In the black feathers of the Blackbird (Turdus merula) the granules are short rods with rounded ends oriented lengthwise in barbules and rami. In transverse sections of barbules the granules appear with a circular outline (diameter approx. 0.2 µm (E. Sønder, unpubl. study).

Melanins of black and dark brown feathers (eumelanins) vary both with respect to shape and size, but my knowledge of this variation is based only on scattered observations. No systematic survey of these melanins has been carried out to my knowledge.

Granules may be rod-shaped, ellipsoid and (less frequently?) globular. Large variation in size (0.2 - 1  $\mu m)$  has been observed in the Columbiformes. In species belonging to different passerim families moderate variation in transverse diameter has been noted.

References: Dyck (1971a), Hürter (1980).

Lighter brown melanin granules (phaeomelanins) generally seem to be much more irregular in shape than eumelanins, but my observations are very few. Hübel (1975) has studied them in Coturnix.

Carotenoids. These give rise to, often intense, yellow, orange and red feather colours. Olive green colours arise where a feather is partly yellow, partly black pigmented.

In contrast to melanins carotenoids are diffusely distributed in the feather keratin and do not show up under the electron microscope, at least not with the stains usually applied. However, two other types of structures associated with carotenoids are frequently present.

Aggregations of membranes and tubules may be present in the carotenoid-containing cells (Dyck 1973). I have observed them in woodpeckers and barbets and some passeriform families, while other passeriform families lack them. Variation in this type of structure occurs; thus in woodpeckers aggregations of membranes dominate.

The air-filled medullary cells in the interior of yellow pigmented rami contain a well-developed keratin rod network not present in black and white rami. There is some inter-specific variation in this structure, as can be observed both with the transmission and scanning electron microscope (Dyck 1978).

# 2.2. Structural colours

Two main types can be distinguished according to whether the colour-producing structures are present in the barbules or in the rami.

In barbules. The shining, metallic, iridescent plumage colours so widespread among birds represent this main type. Physically it is dependent on the interference of light in thin films.

Usually a thin film is represented by a layer of melanin granules parallel to the outer surface of the barbule cell. Important for the production of colour is the very high refractive index of melanin; the hue of the colour is determined primarily by the thickness of the melanin layer, not by the colour of the melanin pigment.

Great variation occurs in the building and arrangement of the layers. Durrer, who has studied these structures extensively, in an overview (1977) distinguishes 23 different types. The melanin granules may be ellipsoid, thin or thick compact rods, rods with the interior hollow, long plates or large discs with hollow interior. There may be one or several layers; they may be close together or separated by keratin layers, etc. To this comes variation in colour, which results from fine adjustments of melanin granule size and spacing of layers (Dyck 1987). Also the modifications of the barbules (twisting etc.) necessary to expose a flattened surface vary between groups of birds (Lucas & Stettenheim 1972) and can conveniently be studied with the scanning electron microscope.

In rami. Non-metallic blue colours as seen f.i. in a Blue Tit (Parus caerulus) belong to this main type. In combination with yellow pigments they produce pure, green colours. Both the violet, blue and green colours of parrots are due to this type of structural colour (Dyck 1976).

Colour production is in the air-filled medullary cells, where a spongy structure consisting of keratin penetrated by air-spaces is present (Dyck 1971a). For the colour to appear vivid a dark background is necessary. This is usually accomplished by part of the ramus containing dark melanin.

Variation in colour results from variation in dimensions of spongy structure and type and distribution of yellow pigments (Dyck 1971a). Variation of structurally coloured rami between different groups of birds is mainly in (1) shape of rami in transverse section (Auber 1957), (2) distribution of dark melanin within rami (Auber 1957) and (3) shape of air-spaces in spongy structure (Dyck 1971b).

#### 2.3 White

Since gulls very frequently are involved in bird-strikes, a few words may be added on white feathers.

A white feather typically results when no pigments or colourproducing structures are present. Reflection of light occurs from the numerous keratin-air interfaces present, both in the medulla of the rami and on the outer surface.

I have found no specializations in the white feathers of the Black-headed Gull (<u>Larus ridibundus</u>) (Dyck 1978) and therefore consider it rather unlikely that structures associated with whiteness can be found which make differentiation of white gull feathers possible. Specializations of wing feathers producing a silvery gloss in terns have, however, been described (Rutschke 1965). The body feathers of the white winter plumage of the Rock Ptarmigan (<u>Lagopus mutus</u>) are modified to increase whiteness (Dyck 1979).

# 3. DISCUSSION

From the foregoing it will be clear that there is considerable variation in the appearance of coloured feather parts under the transmission electron microscope, and I consider it probable that in many cases it is possible to identify feather fragments as originating from a certain species or a group of closely related species.

It will likewise be clear from the foregoing that the possibilities are different for pigmentary and structural colours. Evidently variation, and thereby potentials for identification, are much greater among the latter. This is unfortunate because structurally coloured feathers are much less frequent among European species than are feathers coloured by pigment. I guess that in the great majority of cases it will be possible to identify a structurally coloured feather fragment of a European species correctly, while for a fragment coloured by pigment the likelihood is considerably less. Some improvement of the situation is provided by the fact that frequently several ways of producing colour are present in a feather, e.g. one type of pigment in the

terminal parts of the barbules, another type basally in the barbules and in the rami, and furthermore because frequently feather fragments of different colours are present in a sample.

It is necessary to emphasize strongly, however, that this identification method requires knowledge of the electron microscopical appearance of the feathers of European species, and that this knowledge is generally lacking.

During the past 15-20 years I have made a number of identifications of bird-strike remains, and have never used transmission electron microscopy. In a few cases it would perhaps have been appropriate to do so, but usually I have succeeded to identify the fragments to at least a group of closely related species by using the characteristics of downy barbules according to Brom (1980) and examining skins. When feather fragments of natural elasticity and colour are not present because the sample is sticky, containing blood and remnants of soft tissue, I place the sample in a solution of soap powder with enzymes at room temperature for some days. Then it is usually possible to isolate feather fragments and after cleaning them by rubbing and several ultrasonic treatments and drying, they have normally regained their elasticity and colour.

I suspect that in the major part of difficult cases, where the above 'standard method' does not suffice, other methods will be less time-consuming and perhaps more diagnostic than transmission electron microscopy of colour-related elements. These other methods are e.g. scanning electron microscopy of external surface structures, electrophoresis of feather keratins, and last, but not least, sequence analysis of DNA. The latter method requires 'soft material', e.g. blood or connective tissue. Very little material is required, and f.i. a little feather pulp from a growing feather will do. Whether it can also be applied to dry feathers without living tissue is unknown (P. Arctander, pers. comm.).

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