The colour of fossil feathers

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Feathers are complex integumentary appendages of birds and some other theropod dinosaurs. They are frequently coloured and function in camouflage and display. Previous investigations have concluded that fossil feathers are preserved as carbonized traces composed of feather-degrading bacteria. Here, an investigation of a colour-banded feather from the Lower Cretaceous Crato Formation of Brazil revealed that the dark bands are preserved as elongate, oblate carbonaceous bodies 1–2 μm long, whereas the light bands retain only relief traces on the rock matrix. Energy dispersive X-ray analysis showed that the dark bands preserve a substantial amount of carbon, whereas the light bands show no carbon residue. Comparison of these oblate fossil bodies with the structure of black feathers from a living bird indicates that they are the eumelanin-containing melanosomes. We conclude that most fossil feathers are preserved as melanosomes, and that the distribution of these structures in fossil feathers can preserve the colour pattern in the original feather. The discovery of preserved melanosomes opens up the possibility of interpreting the colour of extinct birds and other dinosaurs.

Keywords: fossil preservation; feather colour; bird; dinosaur

1. INTRODUCTION
In contrast to most mammals, birds are able to see a broad range of colours. This colour vision complexity has led to the evolution of coloured plumage for sexual display and camouflage. A number of pigments and nanostructures produce plumage colours. Black and brown feather colours are generated by eumelanins and phaeomelans, respectively. Melansins are complex cross-linked oligomeric to polymeric structures composed of units of dihydroxyindole and dihydroxyindole carboxylic acid (Liu & Simon 2003). Melansins are the most common and broadly distributed pigments in feathers (McGraw et al. 2005; McGraw 2006) as well as in the rest of the animal kingdom (Liu & Simon 2003; Liu et al. 2005). Melanosins are synthesized within membrane-bound, lysosome-like organelles called melanosomes inside pigment cells called melanocytes (Marks & Seabra 2001). The melanosomes are then transferred to keratinocytes during feather morphogenesis to create pigmentation patterning (Durrer 1986; Prum & Williamson 2001, 2002). Laminar nanoscale organization of melanosomes in feather barbules produces iridescent structural coloration in many birds (Prum 2006).

Fossil feathers are found from approximately 50 deposits ranging in age from Jurassic to latest Tertiary (Davis & Briggs 1995). They are preserved as carbonaceous residues in the majority of localities (Davis & Briggs 1995). Previous investigations of samples from a number of different deposits using scanning electron microscopy (SEM) revealed masses of small oblate bodies approximately 2 μm in length. These bodies, which delineate the feather outline, were interpreted as fossilized feather-degrading bacteria (Wuttke 1983). We decided to reinvestigate these structures to determine whether they might represent melanosomes rather than bacteria.

2. MATERIAL AND METHODS
A fossil feather with colour bands and a fossil bird skull with preserved feathers and an eye were studied together with modern material for comparison: black melanin-pigmented feathers from a Red-winged Blackbird (Agelaius phoeniceus, Icteridae) and the retina of a Whip-poor-will (Caprimulgus vociferus, Caprimulgidae). The fossil feather is from the Lower Cretaceous Crato Formation, Brazil (Leicester University, UK, Geology Department, LEIUG 115562) and was originally described by Martill & Frey (1995). The bird skull is from the Early Eocene Fur Formation, Denmark (Danevæ 2004; Geological Museum of Copenhagen, MGUTH 28 57). Details of the fossils were photographed using a Leica MZ16 dissecting microscope with Optronics camera. The Fur specimen was photographed using a Fujifilm Finepix s9100 with a 28–300 mm objective. The modern feathers were prepared by grinding, following freezing in liquid nitrogen. The ultrastructure of the fossils and living counterpart was studied uncoated in a Philips XL 30 environmental scanning electron microscope (ESEM). The elemental composition of the fossil feathers was analysed using the energy dispersive X-ray analysis in the ESEM with a low accelerating voltage. The retina of a Whip-poor-will was investigated fixed in 1.25% glutaraldehyde for 1.4 hours and then transferred to and stored in 0.2 mol l−1 cacodylate buffer (0.2 mol l−1 sodium cacodylate, 1.5 mmol l−1 calcium chloride, 2% sucrose). Retinal samples were postfixed in 2.4% osmium tetroxide for 1.5 hours. They were then stained with 2% aqueous uranyl acetate for 1 hour. Tissue pieces were then dehydrated through an ethanol series, embedded in Eponate 12, and sectioned with a diamond knife to approximately 100 nm thickness. Specimens were viewed with a JEOL EXII transmission electron microscope (TEM), and images using a Soft-Imaging Megaview II CCD camera.

3. RESULTS
The pennaceous contour feather from the Crato Formation of Brazil (figure 1a) shows striking black and white bands. The margins of the bands match isochronic sections in melanin pigment patterning in modern feathers (Prum & Williamson 2001, 2002), indicating that these colour bands are not preservation artefacts. Relief on the fossil defines the rachis, barbs and rarely barbules, and reveals places where the barbules were ‘unzipped’. Under the ESEM, the dark bands of the Crato feather showed masses of elongate, oblate bodies 1–2 μm long, aligned along the barbs and barbules (figure 1b). Energy dispersive analysis (EDS) of the dark bands showed that these structures are composed mainly of carbon, as are most fossil feathers (Davis & Briggs 1995). By contrast, in the light bands, the relief on the specimen is less pronounced. Furthermore, no oblate structures were evident under the ESEM, which revealed only the texture of the rock matrix (figure 1c). EDS of the light bands detected no carbon residue.
Figure 1. Cretaceous feather ultrastructure compared with that in a living bird. (a) Feather from the Crato Formation, Early Cretaceous, Brazil (Leicester University, UK, Geology Department, LEIUG 115562) showing colour bands; margins of colour bands are similar to those found in living birds and barbules are clearly preserved. (b) Dark bands, composed of aligned eumelanosomes, contrast with (c) light areas that reveal only the rock matrix. (d) A broken barbule from a modern Red-winged Blackbird (*Agelaius phoeniceus*, Aves: Icteridae, Yale Peabody Museum 1047) reveals eumelanosomes aligned along the barbule enclosed in a keratin matrix. Scale bars, (a) 3 mm, insert 1 mm; (b) $1 \mu m$; (c) $10 \mu m$; (d) $1 \mu m$.

Figure 2. (a) Skull of undescribed bird from the Fur Formation, Early Eocene, Denmark (Danekræ 200, MGUH 28.929), preserving feathers and the eye as an organic film. (b,c) Details of the feather region showing aligned eumelanosomes. (d) Detail of the eye showing elongate and oblate eumelanosomes. (e) TEM of a section through the retina of a Whip-poor-will (*Caprimulgus vociferus*, Caprimulgidae). Scale bars, (a) $10 \mu m$; (b) $1 \mu m$; (c) $3 \mu m$; (d) $1 \mu m$; (e) $5 \mu m$. 

The feathers associated with the skull of the Early Eocene bird (figure 2a) are preserved as aligned oblate bodies in the same size range as those in the Crato fossil (figure 2b,c). The organic material of the feathers in both specimens consists entirely of aligned oblate bodies.

The oblate structures from the dark bands in the fossil Crato feather are strikingly similar in size, shape and orientation to eumelanosomes from the barbules of a black-pigmented contour feather from a Red-winged Blackbird (figure 1d), and to eumelanosomes from other modern bird feathers (Durrer 1986; Zi et al. 2003).

4. DISCUSSION
We interpret the oblate structures in the fossil feathers as fossilized eumelanosomes based primarily on their similarity to these structures in modern feathers. There is no reason for bacteria to be preserved on the dark bands alone and not on the light bands of the fossil feather. Rather, eumelanin is resistant to chemical degradation (Liu & Simon 2003), and the presence of eumelanin in dark feathers makes them less prone to bacterial decay than white feathers (Goldstein et al. 2004). This supports the interpretation of the carbon residue found in the Crato feather and other fossil feathers (Davis & Briggs 1995) as a result of the preservation of eumelanin-containing melanosomes.

Similar oblate structures, previously interpreted as feather-degrading bacteria, have been revealed by SEM of feathers from the Cretaceous of Alabama (Bingham et al. 2008) and the Eocene of Grube Messel, Germany (Wuttke 1983). As in the feather analysed here, it is clear that these structures are fossil melanosomes. Likewise structures in a feather from the Oligocene of Cereste interpreted by Davis & Briggs (1995, fig. 1b) as bacteria surrounded by glycocalyx can now be identified as melanosomes surrounded by the remains of β-keratin fibres. The structures in a decaying osprey feather illustrated by Davis & Briggs (1995, fig. 1a) for comparison are also presumably melanosomes. Feathers in dinosaurs may also preserve melanosomes, although they have yet to be investigated; colour banding has been reported in feathers of the Cretaceous theropod dinosaur Caudipteryx zoui (Ji et al. 1998).

Phaeomelanosomes are distinct morphologically from eumelanin-containing melanosomes in both birds (McGraw 2006) and mammals (Liu et al. 2005) and are generally more globular in shape. Their chemical composition, however, is somewhat different (Liu et al. 2005) and their preservation potential remains to be assessed. Their preservation in fossil feathers would also be of interest as they produce rusty red to buff yellow colours.

Many melanin-bearing structures are preserved in fossils. The eye of the Eocene bird (figure 2d) appears to preserve retinal eumelanosomes based on a comparison with a modern bird (figure 2e). Similar structures have been reported in the eyes of fossil fish from the Cretaceous of Spain (Gupta et al. 2008, fig. 3c) and from the Eocene of Grube Messel (Liebig 1998, Pl. 5, fig. 6). Structures preserving the fur of mammals from Messel are strikingly similar to melanosomes (e.g. in the bat Palaeochiropteryx: Wuttke 1983, Pl. 2, fig. 4). It has also been suggested that the organic imprint of the integument of ichthyosaurs could be composed of melanocytes (Whitear 1956). Thus, the preservation of eumelanin may be important in the preservation of soft tissue outlines in animal fossils from a number of localities. Fossil squids preserve the ink sac as an organic mass composed of fossilized eumelanin granules (Doguzhaeva et al. 2004). The colour bands in some fossil insects presumably also reflect the distribution of eumelanin, but as insects do not generate melanosomes, such structures are unlikely to be evident under the ESEM.

Our observations indicate that the structures on fossil feathers previously reported as bacteria are melanosomes, and that the Cretaceous feather described here (figure 1) was originally banded with a black and white eumelanin pattern. It is likely that melanosomes are the source of the carbon in fossil feathers, as the eumelanin inside the melanosomes remains even after the keratin that encloses them has degraded. The absence of carbon in other feathers, such as those of the famous Archaeopteryx specimens from the Jurassic Solnhofen Limestone of Bavaria, does not necessarily mean that they were white. Textures in the imprints of these feathers suggest that oblate structures were originally present (Davis & Briggs 1995, figure 2b), and the carbon coating has presumably been lost due to oxidation, except in the single isolated feather, which is still carbonaceous. Different shapes and arrangements of melanosomes in bird feathers are associated with different colours, including black, brown, red, buff and even iridescent structural colours (Prum & Williamson 2002; McGraw 2006; Prum 2006). Our discovery of melanosomes in a fossil feather therefore opens up the possibility of predicting feather colour in ancient birds and perhaps in other theropod dinosaurs, with obvious implications for understanding their ecology and behaviour.

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