

Fossilized melanosomes and the colour of Cretaceous dinosaurs and birds

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Spectacular fossils from the Early Cretaceous Jehol Group^{1,2} of northeastern China have greatly expanded our knowledge of the diversity and palaeobiology of dinosaurs and early birds, and contributed to our understanding of the origin of birds, of flight, and of feathers. Pennaceous (vaned) feathers and integumentary filaments are preserved in birds^{3–5} and non-avian theropod dinosaurs^{6–12}, but little is known of their microstructure. Here we report that melanosomes (colour-bearing organelles) are not only preserved in the pennaceous feathers of early birds, but also in an identical manner in integumentary filaments of non-avian dinosaurs, thus refuting recent claims^{13–16} that the filaments are partially decayed dermal collagen fibres. Examples of both eumelanosomes and phaeomelanosomes have been identified, and they are often preserved in life position within the structure of partially degraded feathers and filaments. Furthermore, the data here provide empirical evidence for reconstructing the colours and colour patterning of these extinct birds and theropod dinosaurs: for example, the dark-coloured stripes on the tail of the theropod dinosaur *Sinosauropteryx* can reasonably be inferred to have exhibited chestnut to reddish-brown tones.

Ever since they were first announced^{6,7}, the ‘feathered’ dinosaurs from the lacustrine sediments of the Jehol Group (Early Cretaceous, 131–120 Myr ago¹) of China have been controversial. Pennaceous feathers—those with a central shaft and lateral vanes, such as the contour and flight feathers of modern birds—occur both in Jehol birds^{3–5} and in non-avian theropods, primarily Maniraptora such as the oviraptorosaur *Caudipteryx*⁷, the dromaeosaurid *Microraptor gui*⁹, and the unclassified maniraptorans *Protarchaeopteryx*⁷, *Pedopenna*¹⁰ and *Yixianosaurus*¹¹.

Integumentary filaments occur both in non-avian theropods that possessed true pennaceous feathers (for example, *Caudipteryx*) and in those in which the latter are absent, such as *Sinosauropteryx*⁶, *Sinornithosaurus*⁸ and *Beipiaosaurus*¹². The report of superficially similar unbranched filaments in the ornithischian dinosaurs *Psittacosaurus* and *Tianyulong*¹⁷ suggests that such structures might be common to all dinosaurs. Many investigators have accepted that these various filamentous to feather-like structures are epidermal in origin and represent feathers^{6–12,17,18}; others^{13–16} have disputed this view, arguing, for example, that in the theropod dinosaur *Sinosauropteryx* they represent degraded dermal collagen fibres, part of the original strengthening materials of the animal’s skin¹⁶. Resolving this fundamental difference in interpretation is important for our understanding of the biology of the taxa in which they occur, but also has wider implications; if epidermal in origin, these structures will inform models of the evolutionary origin of modern feathers^{19,20}, and the timing of steps in the acquisition of this evolutionary novelty.

Here we demonstrate, using scanning electron microscopy (SEM), that both the integumentary filaments of *Sinosauropteryx* and *Sinornithosaurus* and the pennaceous feathers of the Jehol birds contain sub-micrometre-sized bodies that are either highly elongate with rounded termini, or oblate to sub-spherical, in shape. We eliminate the possibility that these bodies represent fossilized bacteria or diagenetic minerals, and interpret them as fossilized melanosomes. Their morphology is identical to that of melanosomes in the feathers of extant birds. Melanosomes are lysosome-related organelles of pigment cells in which melanins are stored, and are responsible, in part, for the colours exhibited by the feathers of modern birds. The two most common types of melanin are the reddish-brown to yellow pigment phaeomelanin and the black-grey pigment eumelanin²¹. These melanosomes, the first examples reported from the Jehol Group, and the first fossil examples of phaeomelanosomes, are preserved in life position. Representative examples are shown from an isolated feather (Fig. 1), feathers from the bird *Confuciusornis* (Fig. 2), and integumentary filaments of the theropod dinosaurs *Sinosauropteryx* (Fig. 3) and *Sinornithosaurus* (Fig. 4).

The outline of fossil feathers is often defined by layers of closely spaced, aligned, micrometre-sized, oblate bodies that have been

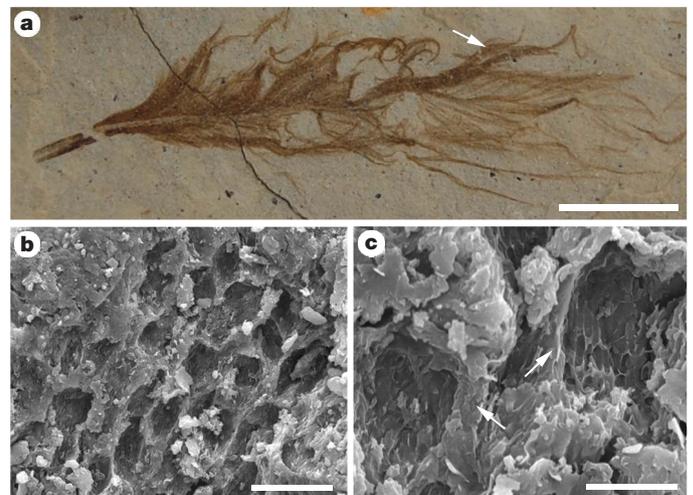


Figure 1 | Melanosomes in an isolated pennaceous feather (IVPP V15388B). **a**, Optical photograph; position of area analysed by SEM indicated by arrow. **b**, **c**, SEM images (at lower and higher magnification, respectively) of eumelanosomes preserved as moulds inside small areas that are separated from each other by anastomosing ridges of degraded feather (at arrows in **c**). Scale bars: **a**, 5 mm; **b**, 20 μm ; **c**, 5 μm .

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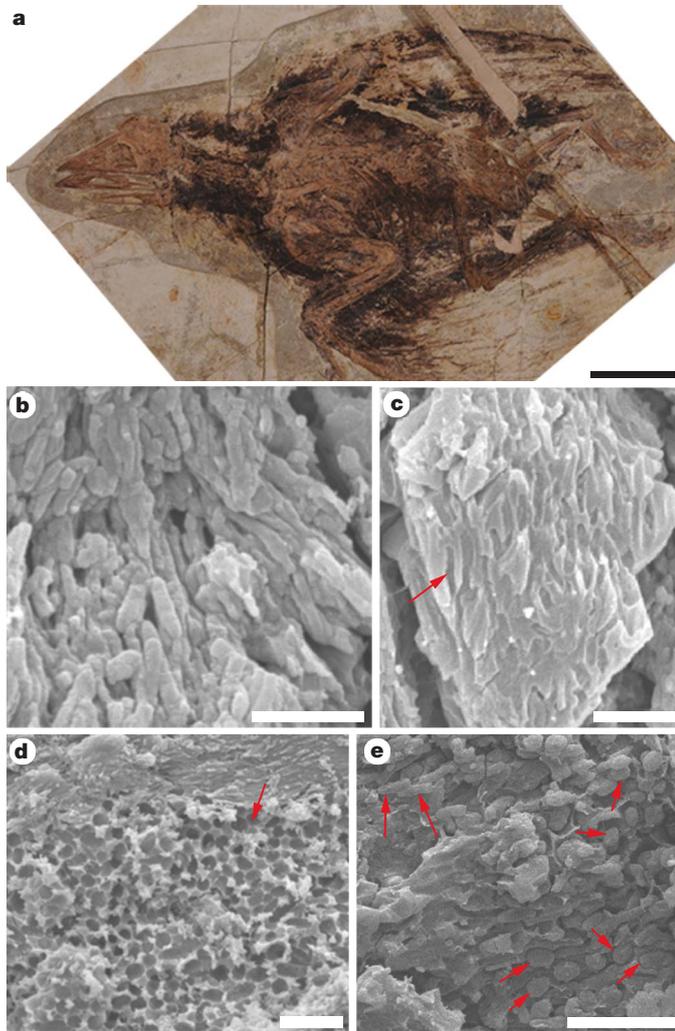


Figure 2 | Melanosomes in feathers of the bird *Confuciusornis* (IVPP V13171). **a**, Optical photograph. **b**, Strongly aligned, closely spaced, eumelanosomes preserved as solid bodies. **c**, Mouldic (that is, preserved as moulds) eumelanosomes (at arrow) a short distance above a layer in which the eumelanosomes are preserved as aligned solid bodies. **d**, Area (at arrow) comprising more widely spaced mouldic phaeomelanosomes surrounded by less distinct, aligned eumelanosomes (top of image). **e**, Gradational boundary between areas dominated by eumelanosomes (longer arrows) and phaeomelanosomes (shorter arrows), both preserved as solid bodies. Scale bars: **a**, 50 mm; **b–e**, 2 μm .

interpreted either as a film of keratinophilic bacteria that coated the surface of the feather during early diagenesis²², or, more recently, as melanosomes^{23,24}. Both melanosomes and bacteria are generally similar in size (one micrometre or less) and shape (spherical, oblate or elongate), so it is essential to distinguish the two.

One objection to our interpretation could be that melanosomes are unlikely to survive the fossilization process. However, as argued elsewhere^{23,24} there is extensive evidence that melanosomes are highly resistant to chemical and physical degradation and have higher resistance to decay than the keratin substrate of feathers and hairs in a variety of physical environments (see also Supplementary Information).

There are three pieces of evidence that confirm that the microstructures in the Jehol fossils are melanosomes, not replacement bacteria. First, the bodies occur embedded inside the feathers, and in those feather parts that exhibit melanosomes in modern birds^{21,25}. In extant birds, melanosomes in the feather barbules are arranged in complex arrays²⁵. The typical configuration is one or more layers of regularly oriented melanosomes suspended in a β -keratin matrix below a superficial layer of β -keratin; melanosomes can also occur,

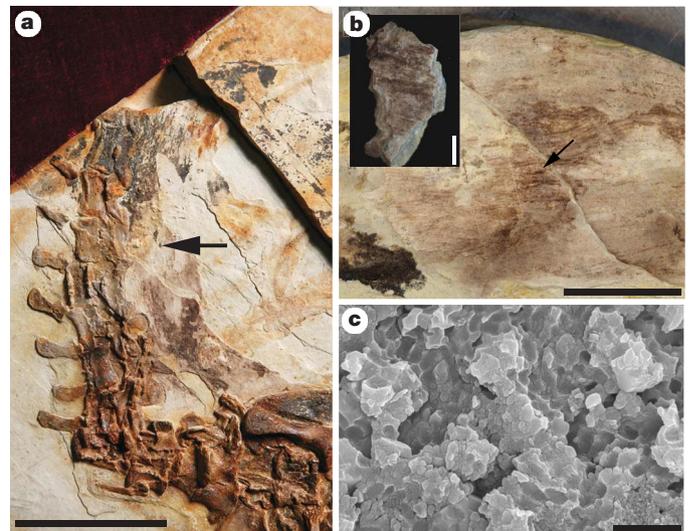


Figure 3 | Melanosomes in the integumentary filaments of the dinosaur *Sinosauropteryx* (IVPP V14202). **a**, Optical photograph of the proximal part of the tail. Arrow indicates position of sample removed previously. **b**, Optical photograph of sample of integumentary filaments; position of SEM sample (inset) indicated by arrow. **c**, Mouldic phaeomelanosomes within a filament. Scale bars: **a**, 50 mm; **b**, main panel, 20 mm, and inset, 1 mm; **c**, 2 μm .

usually arranged less regularly, medial to such layers²⁵. Preservation (presumably as primarily an organic remain) of the degraded keratinous matrix occurs locally in some of the Jehol feathers, most obviously where the fossil bodies are exposed as moulds (Fig. 2c, d); the fossil bodies are, like melanosomes, clearly embedded within this matrix (Fig. 2e), and are not a superficial coating. The integumentary filaments also exhibit this feature (Figs 3c, 4b–d). Second, it has been shown²³ that eumelanosomes occur only in dark bands of banded feathers, and not in light bands: a fossilized biofilm of keratinophilic bacteria would be likely to occur throughout a uniformly preserved structure, and not stop suddenly along an apparent feather stripe. Notably, those parts of a feather that lack melanosomes, the calamus and proximal part of the rachis, are repeatedly absent in Jehol materials (for example, Fig. 1a) unless preserved in calcium phosphate². There is no reason to suppose that a film of keratinophilic bacteria would have developed elsewhere over the surface of the feather, but not on these parts, nor could their absence imply that these portions were buried in the skin and so escaped bacterial replacement because most of the rachis would have been exposed. The third line of evidence for fossil melanosomes comes from ref. 24, which showed packing and layering of melanosomes in fossil feathers, identical to ultrastructures seen in modern feathers and in the Jehol feathers, but incompatible with a bacterial origin.

The chemistry of the fossils² confirms that the melanosomes are not diagenetic minerals, especially not inorganic pyrite framboids. The phaeomelanosomes superficially resemble framboidal pyrite in shape, but are more than an order of magnitude smaller. Further, the fossils are primarily preserved as carbon, that is, they are organically preserved, consistent with their being melanosomes²¹. Framboids and microcrysts of pyrite occur associated with Jehol fossils, but the pyrite microcrystals are typically well faceted, not well-rounded, and their diameters range from about 1 to 5 μm . Energy-dispersive X-ray (EDX) microanalyses confirm that the composition of the pyrite (now iron oxides) is different from that of the fossil bodies.

The two distinct morphologies of fossil body are interpreted as eumelanosomes and phaeomelanosomes based on their having 'rod-like' and 'globular' geometries, respectively, in the feathers of modern birds²¹ (see also Supplementary Information). The fossilized eumelanosomes are elongate (~ 0.8 –1 μm long, and 200–400 nm wide)

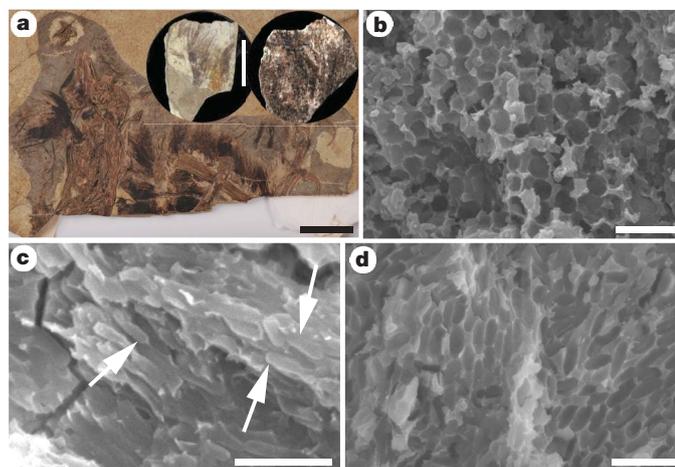


Figure 4 | Melanosomes in the integumentary filaments of the dinosaur *Sinornithosaurus* (IVPP V12811). **a**, Optical photographs of part of the holotype and SEM samples (insets). **b**, Mouldic phaeomelanosomes. **c**, Aligned eumelanosomes preserved as solid bodies (at arrows). **d**, Strongly aligned mouldic eumelanosomes. Scale bars: **a**, main panel, 50 mm and inset, 5 mm; **b–d**, 2 μ m.

with rounded termini. The phaeomelanosomes are ovoid to sub-spherical and vary more in size; most are between 500 and 700 nm long (occasionally up to 900 nm) and 300 and 600 nm wide. The variation in size falls well within that of melanosomes in modern birds, even within a single feather (Supplementary Fig. 1f–i). Eumelanosomes often occur closely packed, strongly aligned and forming a discrete layer (Fig. 2b, d), as in other fossil birds^{23,24}, and the arrays of melanosomes in extant birds²⁵. This fabric is most obvious when the remainder of the feather has decayed completely. In isolated feathers, the eumelanosomes occur in well-defined areas (each ~ 20 μ m long and 6–8 μ m wide) that are separated by narrow (~ 2 μ m wide), anastomosing, ridges of degraded feather (Fig. 1b, c). The eumelanosomes are strongly aligned, parallel to the long axis of each area (Fig. 1c). Phaeomelanosomes only may be present, as in *Sinosauropteryx* (Fig. 3c), or occur as areas surrounded by eumelanosomes, as in *Confuciusornis* (Fig. 2d); in the latter case the boundary between the two types of melanosome can be sharp (Fig. 2d) or more gradational (Fig. 2e).

The Jehol melanosomes are in life position, exposed on the plane of splitting as both solid bodies and moulds. Solid bodies can occur embedded inside, and moulds are always defined by, the variably degraded, organically preserved, feather matrix, never the host sediment. Areas where the degraded, originally keratinous, matrix has decayed completely are characterized by a fabric of densely packed, strongly aligned, eumelanosomes preserved as solid bodies; the regularity shows that this fabric could not have originated post-mortem.

Our results demonstrate conclusively that the integumentary filaments of non-avian theropod dinosaurs are epidermal structures. In birds, melanin is synthesized endogenously in specialized pigment-producing cells, melanocytes, that occur primarily in the dermis²¹; the melanocytes migrate into the dermal pulp of the developing feather germ, where the melanin is packaged into melanosomes and then those melanosomes are transferred to keratinocytes for deposition into developing feathers²⁶. In various avian species melanin granules also form, and are apparently retained, in dermal melanocytes²⁷; melanin granules can form a discrete layer in the dermis, but below, and not as part of, the collagen layer²⁸. The occurrence of melanosomes embedded inside the filaments of Jehol non-avian dinosaurs thus confirms that these structures are unequivocally epidermal structures, not the degraded remains of dermal collagen fibres, as has been argued recently^{13–16}. Our work confirms that these filaments are probably the evolutionary precursors of true feathers^{18–20}, and it will be interesting to determine whether any fossil

filaments might relate to other kinds of epidermal outgrowths in modern birds²⁹.

The occurrence of melanosomes in fossil birds^{23,24} and non-avian dinosaurs allows the first opportunity to reconstruct certain aspects of the external coloration of these organisms. The colours, and their hues and shades, of modern bird feathers derive from a variety of pigments, including melanin, as well as the microstructural arrangement of the tissues^{28,30}. Our identification of both eumelanosomes and phaeomelanosomes implies that some basal birds and non-avian theropods had black and russet coloration. In *Sinornithosaurus* the filaments are locally dominated either by eumelanosomes or phaeomelanosomes, indicating significantly different colour tones. In *Confuciusornis*, variation in colour within a single feather is indicated by changes in the relative abundance of phaeomelanosomes and eumelanosomes over short distances (Fig. 2e). Only phaeomelanosomes have been identified so far in filaments from the tail of *Sinosauropteryx*, and this suggests that the dark-coloured stripes along the tail in the fossil, and possibly also the filamentous crest along the back, exhibited chestnut to rufous (reddish-brown) tones. As the melanosomes are preserved in life position in the Jehol fossils, detailed study of differences in their spatial distribution, including orientation and density, and the relative abundance of each type, will reveal greater detail regarding both colour and colour patterning. Reconstruction of colour patterns will also inform debates on the functions of feathers in non-avian dinosaurs, whether primarily for thermoregulation, camouflage or communication.

METHODS SUMMARY

All materials used in this study are in the collections of the Institute of Vertebrate Paleontology and Paleoanthropology, Chinese Academy of Sciences, Beijing, China (IVPP). As well as material illustrated herein (Figs 1–4), samples from *Beipiaosaurus*, *Pedopenna* and *Yixianosaurus* also show fossilized melanosomes. **Isolated feather.** IVPP V15388B (Fig. 1); Ningcheng County, Inner Mongolia, China.

Confuciusornis. IVPP V13171 (Fig. 2); Sihetun, Beipiao City, Liaoning Province, China. About 300 samples, primarily from the periphery of the specimen, were removed during preparation with needles. Sizes range from 3 to 300 mm².

The relative rarity in the Jehol biota of examples of the non-avian theropod dinosaurs precluded extensive destructive sampling of existing specimens; for each taxon, samples were removed from the surface of unlacquered specimens that had been prepared previously.

Sinosauropteryx. IVPP V14202 (Fig. 3); Dawangzhangzhi, Lingyuan City, Liaoning Province, China. This is a new, as yet undescribed, specimen. A large flake was removed from the dorsal side close to the base of the tail (Fig. 3a). The integumentary filaments were better preserved on the reverse side of this flake; small samples of these were picked off with a scalpel (Fig. 3b).

Sinornithosaurus. IVPP V12811 (Fig. 4); Sihetun, Beipiao City, Liaoning Province, China. The holotype is accessioned with several other small pieces, the remains of the counterpart. Samples were taken from the surface of those pieces that correspond to parts of the area shown in Fig. 4a.

SEM. Samples were mounted on pin stubs using double-sided carbon tape and examined using a Hitachi S-3500N variable pressure SEM equipped with an EDAX Genesis energy dispersive spectrometer, at the University of Bristol. Initial screening was performed on uncoated specimens, and composition was determined via energy-dispersive X-ray microanalysis (EDX). All images herein are of specimens that were gold-coated subsequently.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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