Fossilised melanosomes and the colour of Cretaceous dinosaurs and birds

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Spectacular fossil remains from the Early Cretaceous Jehol Group[1,2] of northeastern China have greatly expanded our knowledge of the diversity and palaeobiology of early birds and dinosaurs, and contributed to 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[6-13], 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 filamentous integuments of non-avian dinosaurs, thus refuting recent claims[14-17] that the filaments are partially decayed dermal collagen fibres. Examples of both eumelanosomes and phaeomelanosomes have been identified, often preserved in life position within the partially degraded fossils of feathers and filaments. Furthermore, the data here provide the first empirical evidence for reconstructing the colours and colour patterning of these extinct birds and theropod dinosaurs: for example, the stripes on the tail of the theropod dinosaur *Sinosauropteryx* can reasonably be inferred to have exhibited chestnut to rufous tones.

Ever since their first announcement[6,7], the 'feathered' dinosaurs from the lacustrine sediments of the Jehol Group (Early Cretaceous, 131–120 Ma[1]) 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

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dinosaurs, primarily Maniraptora such as the oviraptorosaur *Caudipteryx*[7], the dromaeosaurid *Microraptor gui*[9], and unclassified maniraptorans *Protarchaeopteryx*[7], *Pedopenna*[11], and *Yixianosaurus*[10].

Integumentary filaments occur both in non-avian theropods that possessed true pennaceous feathers (e.g. *Caudipteryx*) and in those, for example *Sinosauropteryx*[6] and *Beipiaosaurus*[12], in which the latter are absent. The report of superficially similar unbranched filaments in the ornithischian dinosaurs *Psittacosaurus* and *Tianyulong*[13] 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[7-13,18]; others[14-17] 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 skin[17]. 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[18-20], and the timing of the origin of this evolutionary novelty.

Here we demonstrate, using scanning electron microscopy, that both the integumentary filaments of *Sinosauropteryx* and *Sinornithosaurus*[8] and the pennaceous feathers of the Jehol birds contain sub-micron-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 fossilised bacteria or diagenetic minerals, and interpret them as fossilised melanosomes. This 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 large part, for the colours exhibited by the feathers of modern birds. The two most common types of melanin are the yellow-reddish-brown pigment phaeomelanin, and eumelanin, responsible for black-grey colours[21]. 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), the bird *Confuciusornis* (Fig. 2), and integumentary filaments of the theropods *Sinosauropteryx* (Fig. 3) and *Sinornithosaurus* (Fig. 4).

These fossil bodies could be interpreted as bacteria, but our evidence suggests they are melanosomes. The outline of fossil feathers is often defined by layers of closely spaced, aligned, micron-sized, oblate, bodies that have been interpreted either as a film of keratinophilic bacteria that coated the surface of the feather during early diagenesis[22], or, more recently, as melanosomes[23,24]. Both melanosomes and bacteria are generally similar in size (one micron or less) and shape (spherical, oblate or elongate), so it is essential to distinguish the two.

There are two 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 arrays[25]. The typical configuration is one or more layers of regularly oriented melanosomes suspended in a beta-keratin matrix below a superficial layer of beta-keratin; melanosomes can also occur, usually arranged less regularly, medial to such layers[25]. Preservation (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 (Fig. 3c, 4b-d). Second, Vinther and colleagues[23] showed that purported eumelanosomes occur only in dark bands of banded feathers, and not in light bands: a fossilised biofilm of keratinophilic bacteria would likely occur throughout a uniformly preserved structure. Notably, those parts of a feather that lack melanosomes, the calamus and proximal part of the rachis, are repeatedly absent in

Jehol materials unless preserved in calcium phosphate[2]. 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.

The chemistry of the fossils confirms that the purported melanosomes are not diagenetic minerals. They are primarily preserved as carbon, i.e. they are organically-preserved, consistent with their being melanosomes[21]. Framboids and microcrysts of pyrite occur associated with Jehol fossils. The more spherical set of fossil bodies superficially resemble framboidal pyrite in shape, but are more than an order of magnitude smaller. Pyrite microcrystals are typically well faceted, not well-rounded; their diameters range from about 1 to 5 µm, always greater than that of the fossil bodies (about 500 nm). EDX analyses 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 'rodlike' and 'globular' geometries, respectively, in the feathers of modern birds[21, 23-24] (see also Supplementary Information). The fossilised eumelanosomes are elongate (c. 0.8 - 1 µm, long, and 200 - 400 nm wide) 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 barbule (Fig. S1f-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 birds[25]. 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 c. 20 µm long and 6-8 µm wide) that are separated by narrow (c. 2 µm 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 clearly in life position. The melanosomes are 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 keratinous matrix has decayed completely are characterised 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 synthesised endogenously in specialised pigment-producing cells, melanocytes, that occur primarily within the epidermis[21]; the melanosomes generated migrate into the dermal pulp of the developing feather germ[26]. In various avian species melanin granules also form, and are apparently retained, in dermal melanocytes[27]; melanin granules can form a discrete layer in the dermis, but below, and not as part of, the collagen layer[28]. 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[14-17]. More detailed study may resolve whether these filaments are the evolutionary precursors of true feathers[18-20], other epidermal outgrowths[29], or possibly include examples of both.

The occurrence of melanosomes in fossil birds[24,25] and non-avian dinosaurs allows the first opportunity to reconstruct certain aspects of the external colouration 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 colouration. In *Sinornithosaurus* the filaments exhibit either 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 stripes along the tail, 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 on the functions of feathers in non-avian dinosaurs, whether primarily for thermoregulation, camouflage, or communication.

Material and Methods. 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* and *Yixianosaurus* also show fossilized melanosomes.

Isolated feather (Fig, 1). IVPP V15388B. Ningcheng County, Inner Mongolia, PR China.

Confuciusornis (Fig. 2)**.** IVPP V13171. Sihetun, Beipiao City, Liaoning Province. Samples, primarily from the periphery of the specimen, were removed during preparation with needles. The reverse surfaces of over 200 such samples were examined, almost all of which contained melanosomes .

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 (Fig. 3). IVPP V 14202. Dawangzhangzhi, Lingyuan City, Liaoning Province. This is a new, as yet undescribed, specimen. A large flake was removed from the dorsal side close to the base of the tail (Figure 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 V 12811. Sihetun, Beipiao City, Liaoning Province. The holotype is accessioned with several other small pieces, the remains of the counterpart. Samples were taken from the surface of those pieces that almost certainly correspond to the area shown in Fig. 4a.

Samples were mounted on pin stubs using double-sided carbon tape and examined using a Hitachi S-3500N variable pressure scanning electron microscope equipped with an EDAX Genesis energy dispersive spectrometer, Composition was determined via energy-dispersive X-ray microanalysis (EDX) on uncoated specimens. 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..

Acknowledgements We thank Maria McNamara (University College Dublin), and Richard Prum (Yale University) for comments, and Leona Leonard and Nigel Monaghan (National Museum of Ireland) for materials. Funded by the NERC (Grant NE/E011055/1), the Major Basic Research Projects (2006CB806400) of MST of China, and the National Natural Science Foundation of China (40121202, 40472018).

The authors declare no competing financial interests.

Author Contributions Z. F., S. L. K., P. J. O., M. J. B., Z. Z., and D. J. designed the research, performed the research, analysed data and wrote the paper. XW and XX provided field assistance, discussion and materials for analysis.

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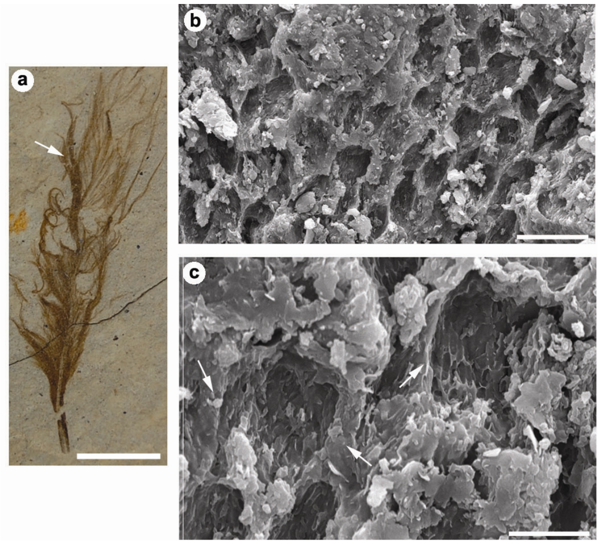

Figure 1 | Melanosomes in an isolated pennaceous feather (IVPP V15388B). a, Optical photograph; position of area analysed by SEM indicated by arrow. **b**, **c**, SEM images of eumelanosomes preserved as moulds inside small areas that are separated from each other by anastomosing ridges of degraded feather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

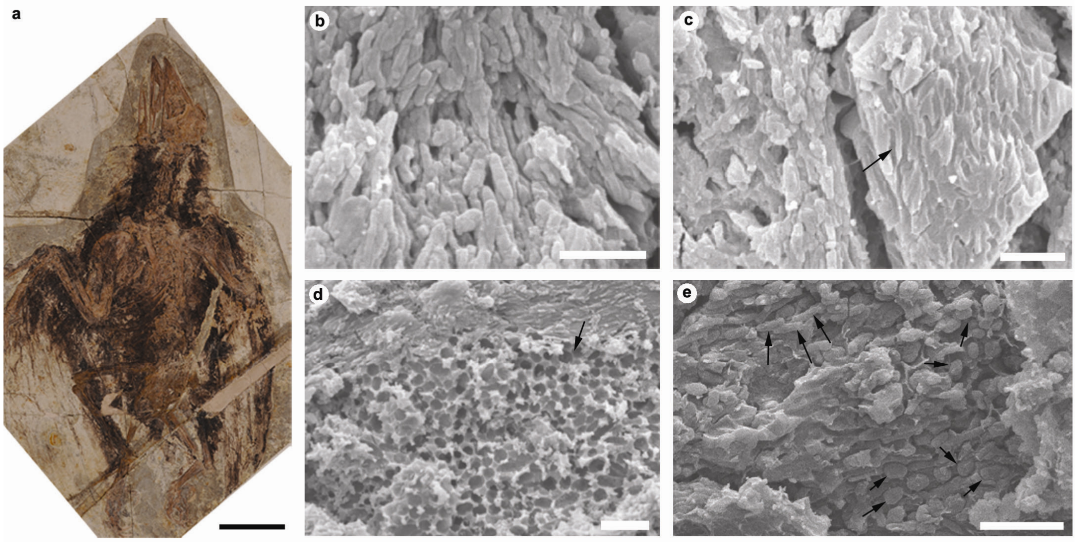

Figure 2 | Melanosomes in feathers of *Confuciusornis* (IVPP V13171). a, Optical photograph. **b**, Strongly aligned, closely spaced, eumelanosomes preserved as solid bodies. **c**, Mouldic eumelanosomes (at arrow) a short distance above a layer in which the eumelanosomes are preserved as aligned solid bodies. **d**, Strongly aligned eumelanosomes surround an area (at arrow) comprising only more widely spaced mouldic phaeomelanosomes. **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.

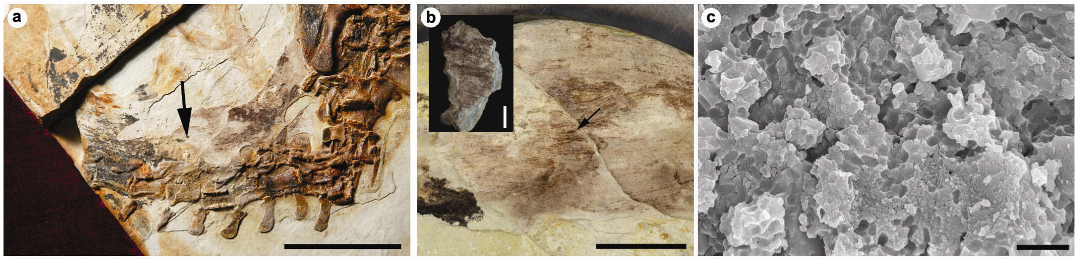

Figure 3 | Melanosomes in the integumentary filaments of the dinosaur *Sinosauropteryx* (IVPP V14202). a, Optical photograph of the proximal part of the tail. Arrow indicates position of sample removed. **b**, Optical photograph of sample of integumentary filaments; position of SEM sample (inset) indicated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

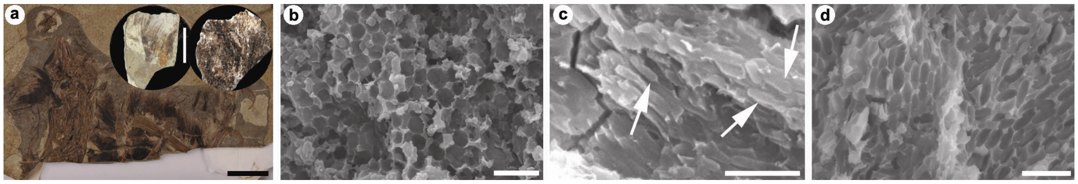

Figure 4 | Melanosomes in the integumentary filaments of the dinosaur *Sinornithosaurus* (IVPP V12811). a, Optical photographs of part of the holotype and SEM samples (insets). **b,** Mouldic phaeomelanosomes. **c,** Aligned eumelanosomes preserved as solid bodies. **d,** Strongly aligned mouldic eumelanosomes. Scale bars: a, 50 mm and, inset, 10mm; b-d, 2 µm.

Supplementary Information.

Melanosomes in an extant bird

Flank and breast feathers were plucked with tweezers from a male specimen of the zebra finch *Taeniopygia guttata castanotis* (National Museum of Ireland – Natural History Division: NMI 1909.329.1) (Fig S1a). The colouration of these feathers in this taxon is melanin-based[1]. Breast feathers contain predominantly eumelanin, and flank feathers very high concentrations of phaeomelanin. Breast feathers are either uniformly black or show black and white striping (Fig S1a); the former were used herein (Fig S1b). The distal half of flank feathers is brown-coloured with two white, unpigmented, spots; otherwise, the barbs and barbules are pigmented evenly (Fig S1c).

Method. Feathers were placed in an ultrasonic bath in distilled water for 10 minutes to remove surface detritus, then dried on a hotplate. The feathers were cut in two transversely and the proximal part discarded; the pigmented parts of samples from flank feathers thus exhibited only brown colours. The rachis was separated from the rest of the feather by a scalpel, and discarded. The remainder was mounted between two glass slides and ground lightly by manually rotating one slide past the other, then mounted on a SEM stub, gold-coated, and examined using a scanning electron microscope (see Materials and Methods).

Results. Only rod-shaped eumelanosomes were identified in breast feathers (Fig S1b, d-e). The brown-coloured distal part of the flank feathers (Fig S1c) revealed only sub-spherical melanosomes (Fig S1f-i). The sub-spherical melanosomes are interpreted as phaeomelanosomes, as previous biochemical analyses have recorded very high abundances of phaeomelanin in breast feathers[1]. It is likely that both phaeomelanosomes and eumelanosomes occur within a single barb or barbule (eumelanin occurs in low concentrations in breast feathers[1]). However, the colour of the feather clearly corresponds with which type of melanosome. There is this a correlation between the shape of the melanosome and the feather colour. This, which has been recorded previously[2], forms the basis for our interpretations of colour in the fossil theropods.

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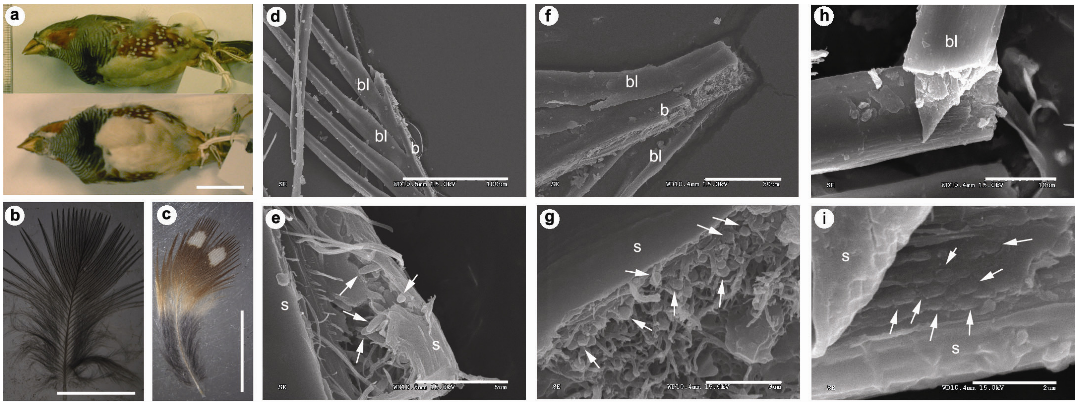

Figure S1 | Melanosomes in the feathers of the male zebra finch. (a) Lateral (top) and ventral views of the specimen sampled. (b) Breast feather. (c) Flank feather. (d-i) Scanning electron micrographs of melanosomes inside barbs and barbules. (d-e) Eumelanosomes (at arrows in e) in the barb of a breast feather. (f-g) Phaeomelanosomes (at arrows in g) in the barb of a flank feather. (h-i) Barbule of a flank feather in which the superficial layer has peeled away to expose aligned phaeomelanosomes (at arrows in i). Abbreviations: b, barb; bl, barbules; s, superficial layer. Scale bars: a, 10 mm; b, 5 mm; c, 5 mm.