

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 coloration of these feathers in this taxon is melanin-based¹. Breast feathers contain predominantly eumelanin, and flank feathers very high concentrations of pheomelanin. 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-coloured¹ 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, and then air-dried on a 50°C 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 using a scalpel, and discarded. To fracture the barbs and barbules, the remainder was mounted between two glass slides and ground lightly by manually rotating one slide past the other. Samples were then mounted on a pin stub, gold-coated, and examined using SEM (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 pheomelanosomes, as previous biochemical analyses have recorded very high abundances of pheomelanin in flank feathers¹. It is likely that both pheomelanosomes and eumelanosomes occur within a single feather (eumelanin occurs in low concentrations in flank feathers¹). However, the colour of the feather clearly corresponds to the most abundant type of melanosome. There is thus a correlation between the shape of the melanosome and feather colour. This, which has been recorded previously², forms the basis for our interpretations of colour in the fossil birds and theropods.

High decay resistance of melanosomes

It is well documented^{3,4} that melanized feathers are more resistant to bacterial degradation than non-melanized feathers. It has also been shown⁵ that melanized feathers are more abrasion resistant. Liu and Simon⁶ demonstrated that eumelanin is resistant to chemical degradation. Both eumelanosomes and pheomelanosomes are routinely extracted from inside keratinous

substrates such as hair using various methods of acid/ base extraction, indicating their strong resistance to chemical degradation; alternative methods using enzymatic extraction retain not only the physical structure, but alter the chemistry less (ref. 7, and references therein). Further, recent studies on the taphonomy of hair samples in archaeological contexts⁸, show that melanin and melanosomes are resistant to decay, even more so than keratin: “Indeed, in the most severe cases of microbial destruction to the hair shaft, the complete loss of keratinaceous material was associated with concomitant survival of melanin pigment granules.”

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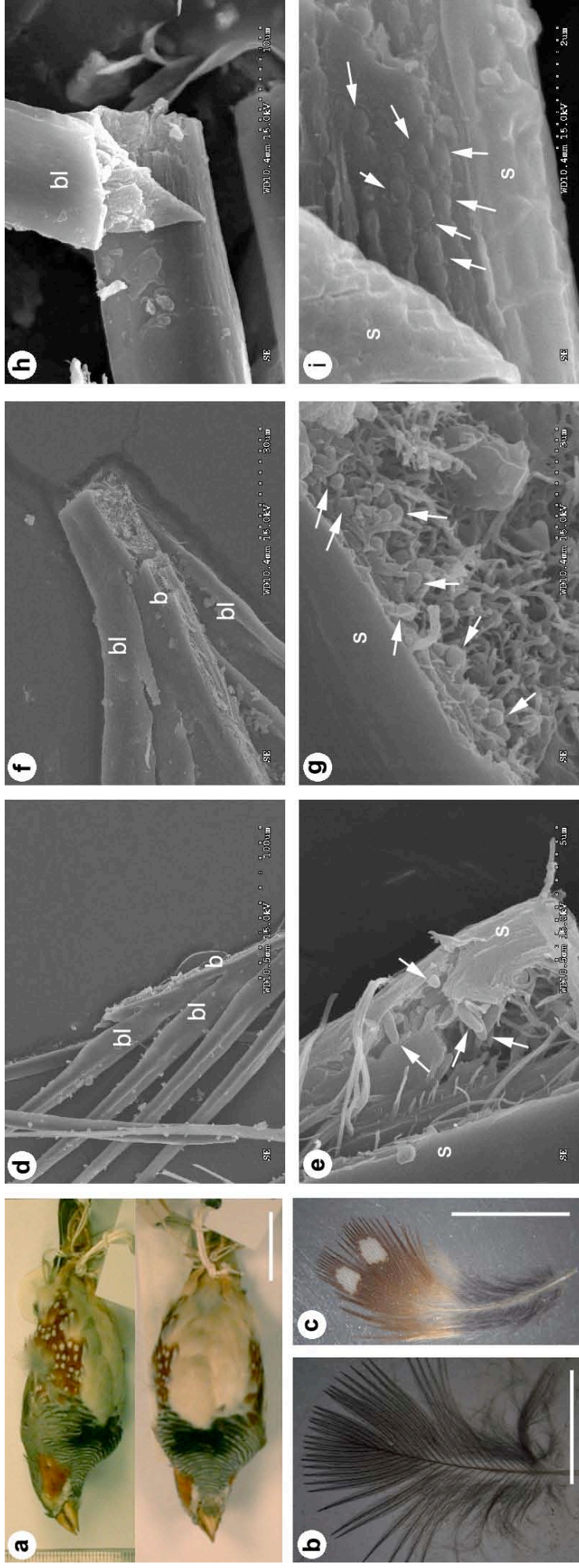


Figure S1 | Melanosomes in the feathers of the male zebra finch. (a) Optical photographs of lateral (top) and ventral views of the specimen sampled. (b, c) Optical photographs of breast feather (b) and flank feather (c). (d-i) SEM images of melanosomes inside barbs and barbules. (d-e) Eumelanosomes (at 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 been 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; d, 100 μm ; e, 5 μm ; f, 30 μm ; g, 3 μm ; h, 10 μm ; i, 2 μm .