



Research

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Palaeontology

Experimental maturation of feathers: implications for reconstructions of fossil feather colour

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Fossil feathers often preserve evidence of melanosomes—micrometre-scale melanin-bearing organelles that have been used to infer original colours and patterns of the plumage of dinosaurs. Such reconstructions acknowledge that evidence from other colour-producing mechanisms is presently elusive and assume that melanosome geometry is not altered during fossilization. Here, we provide the first test of this assumption, using high pressure–high temperature autoclave experiments on modern feathers to simulate the effects of burial on feather colour. Our experiments show that melanosomes are retained despite loss of visual evidence of colour and complete degradation of other colour-producing structures (e.g. quasi-ordered arrays in barbs and the keratin cortex in barbules). Significantly, however, melanosome geometry and spatial distribution are altered by the effects of pressure and temperature. These results demonstrate that reconstructions of original plumage coloration in fossils where preserved features of melanosomes are affected by diagenesis should be treated with caution. Reconstructions of fossil feather colour require assessment of the extent of preservation of various colour-producing mechanisms, and, critically, the extent of alteration of melanosome geometry.

1. Introduction

Coloration is one of the most elusive aspects of the biology of ancient organisms. Recent discoveries of melanosomes preserved in fossil feathers [1] and biophotonic nanostructures in the cuticle of fossil insects [2], however, confirm the potential of the fossil record to illuminate the evolution of colour and visual signalling strategies. Previous studies of the coloration of fossil birds and non-avian dinosaurs were based on morphological and/or chemical evidence for the presence of melanin [3–8]. Other pigments and colour-producing nanostructures generate colour in the feathers of many extant birds and can modify colour generated by melanosomes [9], but fossil evidence of these mechanisms is elusive; the relative preservation potential of the various colour-producing mechanisms in feathers is unknown. Several studies have inferred the original coloration of fossil feathers by comparing the preserved geometry and/or spatial arrangement of melanosomes to those in extant analogues [4–6,8,10,11]. These reconstructions assume that data from fossil and modern feathers can be compared directly, but the fidelity with which melanosomes are preserved is unknown. Critically, the impact of burial on melanosome geometry has not been investigated. These issues have significant implications for reconstructions of original plumage coloration in fossils: sediments hosting melanosome-bearing fossil feathers vary in their burial history (see the electronic supplementary material, table S1).

The morphology and chemistry of fossil tissues are subject to taphonomic overprints involving the effects of decay, burial and weathering; many of these processes are amenable to experimental testing. Artificial maturation techniques can simulate the chemical and morphological effects of burial on integumentary tissues [12,13], including how colour-generating ultrastructures are altered [14]; elevated pressures and temperatures in experiments accelerate geochemical reactions that occur at lower pressures and temperatures over longer geological timescales [15] (see the electronic supplementary material). We employed high pressure–high temperature autoclave experiments to test, for the first time, the effect of burial on feather ultrastructure and to identify constraints on melanosome-based reconstructions of fossil feather colour. We focused on changes in visual colour and morphology and, in particular, on changes in melanosome geometry as this is an important contributor to colour in fossil feathers [6,11].

2. Material and methods

Melanosome-bearing feathers were selected to represent diverse hues, colour-producing mechanisms and taxa within Neornithes; melanosomes do not produce the hue observed in the feathers with biophotonic nanostructures or non-melanin pigments (figure 1 and table 1; electronic supplementary material, S1). Contour feathers were dissected from dried specimens held by the Yale Peabody Museum of Natural History. Table 1 summarizes details of the taxa examined, feather location on the body and the colour-generating mechanism.

Feathers were wrapped in aluminium foil and inserted into an autoclave pressurized using Ar gas at 200°C, 250 bar and 250°C, 250 bar; each experiment lasted 24 h (see the electronic supplementary material). Fresh and experimentally matured feathers were examined using a FEI XL-30 ESEM-FEG SEM (see the electronic supplementary material). Changes in hue were assessed visually. Long and short axes of between 20 and 40 melanosomes were measured from each specimen. Differences in the dimensions of fresh and experimentally matured feather samples from each taxon were tested using one-way analysis of variance (ANOVA).

3. Results

During the 250°C, 250 bar treatment, the hue of all feathers changed to black regardless of the colour-generating mechanism (figure 1*a,d,g,j,m,p*; electronic supplementary material, figure S1); the black hue occluded original patterning (figure 2). This alteration was accompanied by complete degradation of the barb cortex and quasi-ordered biophotonic nanostructures (figure 1*b,c,e,f*; electronic supplementary material, figure S1) and reduction in the dimensions of barbs, barbules and barb cortices (see the electronic supplementary material, table S2). Eumelanosomes (figure 1*c,f*; electronic supplementary material, figure S1) and phaeomelanosomes (figure 1*i,l,o,r*) survived the experiments; they were usually visible on transverse fractured sections of barbs and barbules but rarely on the feather surface. Importantly, the geometry of the melanosomes changed markedly during the experiments. Long and short axes decreased in length by $18.5 \pm 9.2\%$ and $20 \pm 7.9\%$, respectively (table 2); differences between fresh and experimentally treated samples are statistically significant for all taxa (table 2).

During the 200°C, 250 bar treatment, some feathers retained visual evidence of the original hues and quasi-ordered nanostructures were incompletely degraded (see the electronic supplementary material, figure S2). The geometry of melanosomes was altered less than during the 250°C, 250 bar treatment (see the electronic supplementary material, table S3); long and short axes decreased in length by $7.6 \pm 4.6\%$ and $12.5 \pm 7.9\%$, respectively.

4. Discussion

Our experiments demonstrate that modern feathers characterized by different hues and colour-generating mechanisms follow a convergent degradation pathway under the combined effects of elevated pressure and temperature. Melanosomes are the only colour-producing features that resist degradation; as with other anatomical features of feathers, however, their geometry is altered. This alteration characterizes melanosomes from all feathers investigated herein regardless of taxonomy and melanosome type. These results—preferential preservation of melanosomes and alteration of their original geometry—have significant implications for studies of fossil feather colour. The extent to which melanosome geometry is altered varies under different temperature regimes: diagenetic contraction is greater at higher temperature. This may reflect dehydration during condensation/polymerization reactions [12]. Combining such experimental data with information on the burial history of feather-bearing biotas will allow the extent of diagenetic alteration of melanosomes to be predicted; a similar approach has been used in investigations of structural colour in fossil insects [14]. In general, melanosomes in fossil feathers from sediments characterized by deep burial (and/or hydrothermal alteration) should be more degraded than those buried to shallow depths. The burial history of the Yixian Formation (Cretaceous, China) indicates that the Jehol biota experienced elevated temperatures relative to other important feather-bearing biotas, e.g. Messel, Florissant and the Fur Formation (see the electronic supplementary material, table S2). Notably, our results show that individual melanosomes from a monochromatic feather region vary in the extent to which their geometry is altered (table 2). Future studies are needed to constrain the extent of this variation under different burial regimes and for different taxa.

Reconstructions of original plumage colour will be most accurate in cases where fossil feathers lack evidence of thermal maturation. Our experiments reveal that alteration of keratinous feather structures and melanosomes occurs in tandem, but to different extents. This difference explains certain distinctive features of fossil feathers as taphonomic artefacts that may serve as indicators of alteration. Many fossil melanosomes are preserved as external moulds within an amorphous organic matrix (which presumably represents the degraded remains of the keratinous feather medulla and cortex (e.g. fig. 1*h–f* in [6]), whereas others are preserved as discrete, three-dimensional bodies (e.g. fig. 1*b* in [1]). These different preservational modes can yield disparate colour predictions for the same feather region [5], possibly because the organic matrix containing the melanosome moulds has contracted to a greater extent than the melanosomes. Even where preserved as three-dimensional bodies, melanosomes can exhibit microfractures (e.g. fig. 1*d* in [6]) that may indicate diagenetic contraction.

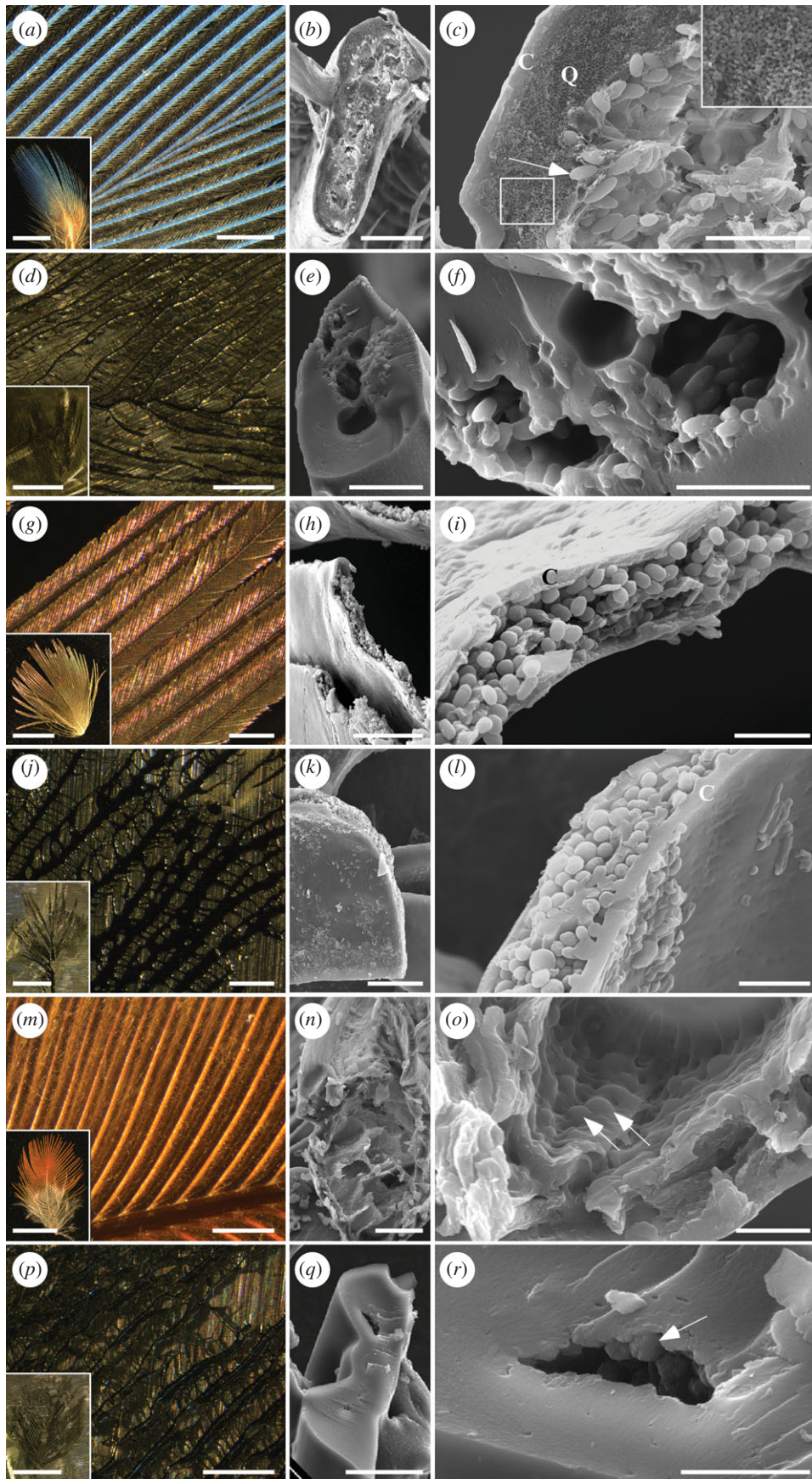
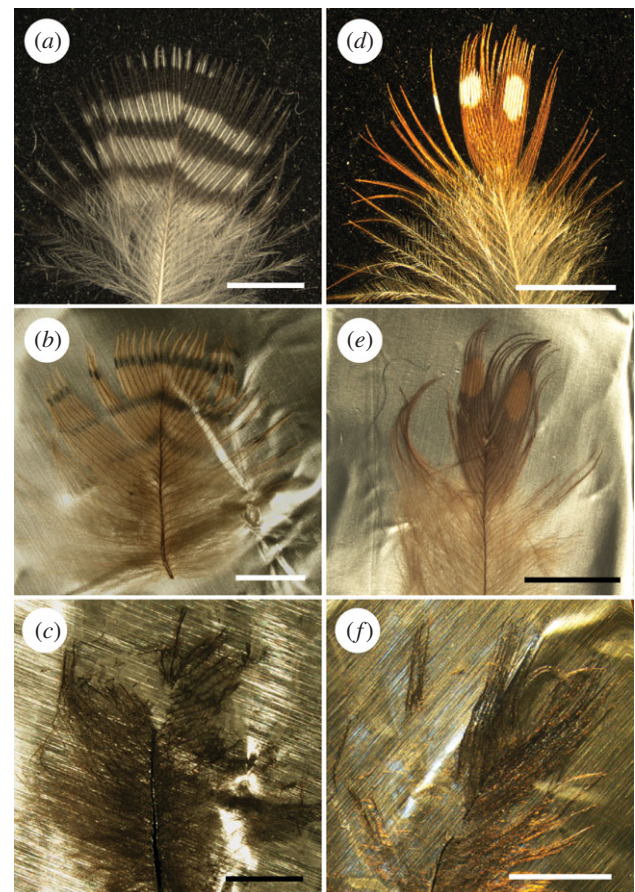


Figure 1. Effect of temperature (250°C) and pressure (250 bar) on feather colour. (a–c) Fresh and (d–f) experimentally treated *Sialia sialis* feathers. Melanosomes (arrow, (a)) survive in rami of treated feathers (f) but the keratin cortex (C) and quasi-ordered nanostructure (Q) are degraded. (g–i) Fresh and (j–l) experimentally treated *Columba livia* feathers. Note melanosomes within barbules of treated feathers (l). (m–o) Fresh and (p–r) experimentally treated *Carduelis carduelis* feathers. Melanosomes (arrows in (o,r)) line ramus void space. Scale bars, (a,d,g,j,m,p) 500 μm , (b) 20 μm , (c,f,i,l,o,r) 5 μm , (e,h,k,n,q) 10 μm , inset in (a,d,g,j,m,p) 5 mm.

Table 1. Details of experimentally treated feathers. Numerals in brackets refer to studies listed in the electronic supplementary material.

taxon	family	specimen accession number	location on body	colour-producing mechanism
<i>Ara ararauna</i>	Psittacidae	YPM 81424	nape	quasi-ordered nanostructure in barbs (1)
<i>Cardinalis cardinalis</i>	Cardinalidae	YPM 5677	secondary remiges	carotenoids plus eumelanosomes (2)
<i>Caruelis chloris</i>	Fringillidae	YPM 24374	rump	carotenoids plus eumelanosomes (2)
<i>Columba livia domestica</i>	Columbidae	YPM 97580	breast	carotenoids plus eumelanosomes (3)
<i>Cyanocitta stelleri</i>	Corvidae	YPM 98708	neck	barbule cortex thin film (4)
<i>Irena puella</i>	Irenidae	YPM 64175	secondary retrices	quasi-ordered nanostructure in barbs (5)
<i>Meleagris gallopavo</i>	Phasianidae	YPM 70208	back	quasi-ordered nanostructure in barbs (6)
<i>Ptilonorhynchus violaceus</i>	Ptilonorhynchidae	YPM 41747	breast	barbule cortex thin film (7)
<i>Sialia sialis</i>	Sialidae	YM 127699	rump	barbule cortex thin film (8)
<i>Taeniopygia guttata</i>	Estrinidae	YPM 91410	rump	quasi-ordered nanostructure in barbs (9)
			nape	eumelanosomes (10)
			flank	phaeomelanosomes (10)

**Figure 2.** Degradation of melanosome-based colour patterning in *Taeniopygia guttata* (a–c) nape and (d–f) flank feathers. (a,d) Fresh, (b,c,e,f) feathers treated to (b,e) 200°C 250 bar and (c,f) 250°C 250 bar. Scale bars, 2 mm.

Our experiments highlight additional issues relating to study of fossil feather colour. Experimentally treated feathers can exhibit melanosomes on transverse fractured sections despite amorphous surface textures, supporting the hypothesis [11] that the absence of visible melanosomes in fossil feathers exhibiting an amorphous organic layer may be a taphonomic artefact and not indicative of light-toned coloration. Uniform tones in some fossil feathers may also be artefacts; original patterning is obliterated by elevated pressure and temperature. Survival of patterning in fossil feathers indicates relatively mild burial conditions and/or complete degradation of the keratin feather matrix, in which cases the presence/absence of melanosomes generates differences in tone. Except for ordered melanosome arrays [10,11], biophotonic nanostructures are unknown in fossil feathers. This may reflect a real absence in the living organism, a taphonomic artefact or an inability to recognize partially degraded examples in fossils. Our results document intermediate stages in the degradation of biophotonic nanostructures (e.g. electronic supplementary material, figure S1). Identification of similar features in fossil feathers will yield more accurate predictions of original colour.

Reconstructions of original plumage colour in fossils to date have relied on the assumption that the original geometry and density of melanosomes is preserved [5,6,15]. Future modelling of the relationship between melanosome geometry and feather colour [5,6,11] will inform on how much deviation from a particular geometry is required to produce a different colour. Incorporating evidence of other

Table 2. Melanosome long and short axis length (mean \pm 1 s.d.) for fresh and experimentally treated (200°C 250 bar) feathers from each taxon. One-way analysis of variance (ANOVA) results for each taxon show the *F*-test result, the degree of freedom (d.f.) and a probability value (*p*-value).

taxon	long axis (μm ; fresh)	long axis (μm ; 250°C 250 bar)	ANOVA <i>F</i> (d.f.), <i>p</i> -value	short axis (μm ; fresh)	short axis (μm ; 250°C 250 bar)	ANOVA <i>F</i> (d.f.), <i>p</i> -value
<i>Ara ararauna</i>	1.27 \pm 0.11	1.04 \pm 0.11	31.44 (1,41), 1.563 \times 10 ⁻⁶	0.72 \pm 0.06	0.56 \pm 0.05	31.44 (1,41), 1.563e-6
<i>Cardinalis cardinalis</i> (retrices)	0.75 \pm 0.2	0.53 \pm 0.12	7.732 (1,38), 0.008	0.66 \pm 0.12	0.5 \pm 0.13	7.732 (1,38), 0.008
<i>Carduelis chloris</i>	0.94 \pm 0.23	0.6 \pm 0.11	15.97 (1,39), 0.006	0.75 \pm 0.14	0.51 \pm 0.12	15.97 (1,39), 0.006
<i>Columba livia domestica</i>	0.53 \pm 0.07	0.48 \pm 0.06	4.066 (1,57), 0.049	0.46 \pm 0.08	0.41 \pm 0.07	4.066 (1,57), 0.049
<i>Cyanocitta stelleri</i>	1.37 \pm 0.2	1.18 \pm 0.06	5.558 (1,46), 0.032	0.45 \pm 0.07	0.36 \pm 0.06	5.558 (1,46), 0.032
<i>Irena puella</i>	0.91 \pm 0.13	0.79 \pm 0.33	4.368 (1,57), 0.007	0.29 \pm 0.03	0.22 \pm 0.05	4.368 (1,57), 0.007
<i>Meleagris gallopavo</i>	—	—	—	0.38 \pm 0.04	0.35 \pm 0.03	—
<i>Ptilonorhynchus violaceus</i>	1.59 \pm 0.3	1.33 \pm 0.3	9.402 (1,52), 0.003	0.4 \pm 0.03	0.28 \pm 0.05	9.402 (1,52), 0.003
<i>Sialia sialis</i>	1.63 \pm 0.15	1.45 \pm 0.25	7.732 (1,38), 0.008	0.64 \pm 0.1	0.55 \pm 0.08	7.732 (1,38), 0.008
<i>Taeniopygia guttata</i> (flank)	0.57 \pm 0.08	0.51 \pm 0.04	3.52 (1,64), 0.012	0.51 \pm 0.06	0.45 \pm 0.05	3.52 (1,64), 0.012
<i>Taeniopygia guttata</i> (nape)	1.08 \pm 0.2	0.79 \pm 0.1	8.54 (1,46), 0.006	0.31 \pm 0.03	0.24 \pm 0.09	8.54 (1,46), 0.006
percentage change		18.5 \pm 9.2			20 \pm 7.9	

colour-producing mechanisms into plumage colour reconstructions requires understanding of their chemical and anatomical degradation. Even so, the presence/absence and type (i.e. eu-/phaeomelanosome) of fossilized melanosomes is sufficient to allow general inferences regarding original hue and, more importantly, colour patterning in fossil specimens, providing evidence of the functional evolution of feathers and communication strategies among birds and non-avian dinosaurs. Future targets for reconstruction include different growth stages and sexes of the same fossil taxon, and representatives of different ecologies from the same biota. Such investigations will yield critical data on

the evolution of feathers as media for visual signalling in the context of ontogenesis, sexual selection and ecosystem function. An improved understanding of the taphonomy of melanosomes, plus that of other colour-producing mechanisms in feathers, is critical to test pioneering reconstructions of fossil feather colour and to facilitate future interpretations.

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