Palaeocolour: A History and State of the Art

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Alongside being the most diverse and widespread of all tetrapod clades, birds show the most remarkable range of colours in any group of terrestrial vertebrates (Baker and Parker 1979; Hill and McGraw 2006a). From the cryptic browns and greys of dunnocks and sparrows to the glorious rich palette of colours in the birds of paradise, parrots and hummingbirds, almost every colour imaginable to the human observer appears to be present in extant birds (Hill and McGraw 2006a, b). Thanks to features such as tetrachromacy and ultraviolet (UV) vision, many birds can also see colours invisible to di- or trichromatic mammals (Vorobyev et al. 1998). Colour plays a key role in ecology. Be it for camouflage from predators or prey, sexual display, species recognition, or as warning signalling (aposematism), colour has doubtlessly been a key driver in the evolution of bird plumage (Baker and Parker 1979; Hill and McGraw 2006a; Vinther 2015a). The range of colours exhibited by birds can likely be explained, at least in part, by the importance of visual cues in avian signalling due to their excellent tetrachromatic visual capabilities (Vorobyev et al. 1998; Koschowitz et al. 2014). These are only rivalled in vertebrates by the equally colourful teleost fishes (Cuthill 2006). Bird colouration has fascinated naturalists and scientists for centuries and helped to galvanise the theories of evolution by both natural and sexual selection (Baker and Parker 1979; Darwin 1859, 1871).

The dazzling array of colours seen in birds has traditionally been attributed to two mechanisms of colour production: the utilisation of pigments, biopolymers that differentially absorb and reflect specific wavelengths of light, and nanostructural arrays within feathers (McGraw et al. 2005; McGraw 2006a, b, c; Prum 2006). Structural colours are produced in two primary ways in bird plumage. Iridescence is angle-dependent refraction, most often associated with pigment layers and keratin interacting with incident light to modulate it resulting in the reflection of specific wave lengths of light (McGraw 2006b; Prum 2006; Igic et al. 2016). Non-iridescent structural colour is produced by a complex network of quasi-ordered air bubbles inside the keratin matrix (Babarović et al. 2019). This serves to scatter certain light waves, while an underlying melanin layer usually serves to absorb the remaining unscattered light (Prum 2006).

The hues, saturation and brightness of pigmentary colours are also controlled in part by their arrangement within the keratin matrix. Thus, a likely continuum in colour production involving both structural and pigmentary mechanisms, rather than a strictly dichotomous division of pigmentary and structural production, exists (McGraw et al. 2005; McGraw 2006b; Prum 2006; Galván and Solano 2016). Novel nuances of bird colour production are still being

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recognised, and it is likely that we are far from fully appreciating the variations that exist in how certain hues are produced in extant bird plumages (Igic et al. 2016).

Pigmentary colour production involves the use of multiple different pigment types in birds. These include melanins, carotenoids, porphyrins, flavins, psittacofulvins, pterins and purines among others (McGraw and Nogare 2004; McGraw 2006a, b, c; Galván and Solano 2016). The most ubiquitous and likely ancient of these are the melanins (McGraw 2006b; Galván and Solano 2016; D'Alba and Shawkey 2018). Melanins are synthesised within the animal, whereas numerous other pigment classes including carotenoids, porphyrins and pterins are obtained directly from the diet. These diet-derived pigments may however be altered in their molecular structure after ingestion (McGraw 2006a, c). These less common pigment classes usually confer different hues to melanins, such as the reds, oranges and yellows of carotenoids and psittacofulvins. They are presumed to often be honest indicators of quality due to the need to obtain them through the diet (Olson and Owens 1998; McGraw 2006a; LaFountain et al. 2015).

Melanin is thought to be highly conserved throughout vertebrates, which synthesise the pigment within organelles called melanosomes (McGraw 2006b; Vinther 2015a; Clements et al. 2016). Melanosomes are found in multiple tissue types in vertebrates, including the eyes, internal organs and integument (Vinther 2015a; Clements et al. 2016; McNamara et al. 2018). It is those of the integument that are involved in the key role of colour production, although they serve multiple other functions alongside this including thermoregulation, as antimicrobial barriers and protection against UV radiation (McGraw 2006b; Margalida et al. 2008; Vinther 2015a; Galván and Solano 2016). In birds, most melanin involved in colour production is found in the feathers, where it is deposited by keratinocytes after being synthesised in melanocytes (McGraw 2006b; Galván and Solano 2016).

Two distinct types of melanin are known in vertebrates, including birds (McGraw 2006b; Galván and Solano 2016). Eumelanin, which is the most common of the two, imparts black, dark grey and dark brown hues (Fig. 11.1a–d) (McGraw 2006b; Vinther 2015a). Phaeomelanin produces lighter brown, yellow and reddish hues (Fig. 11.1e, f) (McGraw 2006b; Vinther 2015a). The two melanin types have distinct chemistries and are synthesised through different pathways (see below) (McGraw 2006b; Galván and Solano 2016). The physical structures of the two melanosome types are also distinct. Eumelanosomes exhibit a 'sausage-shaped' oblong morphology, while phaeomelanosomes show a spherical to ovoid morphology (Fig. 11.1; Vinther 2015a). The evolutionary origins of melanin are also

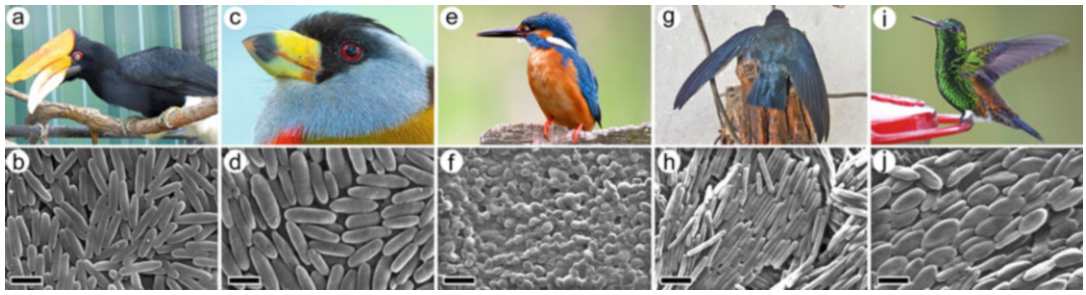

Fig. 11.1 Example of melanised colours in birds and the associated melanosome morphologies. (**a**) Black—*Buceros rhinoceros*. (**b**) Ellipsoidal eumelanosomes extracted from a black feather of *B. rhinoceros*. (**c**) Grey—*Semnornis ramphastinus*. (**d**) Large ellipsoidal eumelanosomes extracted from a grey feather of *S. ramphastinus*. (**e**) Rufous—*Alcedo atthis*. (**f**) Spherical phaeomelanosomes extracted from a rufous feather of *A. atthis*. (**g**) Glossy iridescent—*Collocalia isonota*. (**h**) Long, thin melanosomes extracted from *C. isonota*. (**i**) Bright iridescent—*Amazilia tobaci*. (**j**) Flattened plate-like melanosomes extracted from a green feather of *A. tobaci*. Scale bars represent 1 μm. Modern bird images from Pixabay, Flickr and Wikipedia: Perry Quan (**a**), Luis Fernando Serna Agudelo (**c**), Obsidian Soul (**g**) and Dominic Sherony (**i**)

currently unclear. Due to its ubiquity throughout vertebrates, the occurrence in fossils as far back as at least the Carboniferous and the presence of eumelanin in invertebrate clades as well as fungi, the pigment and its synthesis pathway likely have ancient origins (Glass et al. 2012; Clements et al. 2016; Galván and Solano 2016; D'Alba and Shawkey 2018). Cambrian vertebrate fossils, like *Haikouichthys* and *Metaspriggina* (Shu et al. 2003, Morris and Caron 2014), preserve their eyes and liver as an organic stain, which has been shown to be due to preserved melanin in younger occurrences. It has been suggested that due to melanin's ability to protect cells from UV radiation, owing to its broadband absorbance spectrum, it may have been essential for the evolution of life on Earth (Galván and Solano 2016). This may have been particularly the case when microorganisms began to inhabit environments exposed to the harmful effects of the sun. While these concepts relate to the darker melanin form, eumelanin, the origin and original functional role of phaeomelanin is much less clear (Galván and Solano 2016). Although the origins of phaeomelanin are uncertain, its importance in colouring the vertebrate integument and, in particular, that of birds and mammals is undoubted.

Much work has been performed in recent decades to better characterise the way in which melanosomes contribute to colour production in bird feathers (McGraw et al. 2005; McGraw 2006b, 2008; Galván and Solano 2016; Igic et al. 2016). A basic and longstanding assumption in bird colouration and, in particular, in the portrayal of palaeocolour has been that melanin-based colouration boils down to a dichotomy: black melanised colours produced by eumelanin and reddish to brown melanised colours produced by phaeomelanin (McGraw et al. 2005; McGraw 2006b). To date, most studies that have looked in detail at the melanin composition of extant bird feathers have concluded that both melanin types are present in almost every instance of melanised colouration. It is likely that the relative concentrations of each dictate the hue produced (McGraw et al. 2005; McGraw 2006b; Galván and Wakamatsu 2016).

Alongside work looking at the way melanins colour bird feathers, the discovery that melanin can preserve in the fossil record has helped achieve what was once thought an impossible endeavour: reconstructing colour in extinct animals (Vinther 2015a). This new field, known as palaeocolour, is helping us to uncover features of ancient animals hitherto thought intractable. It is also helping us understand new aspects of ancient ecologies like how colours reflect evolutionary changes in the dynamics between predators and their prey as well as intraspecific display (Vinther 2015a; Vinther et al. 2016; Brown et al. 2017; Smithwick et al. 2017a). It is obvious that the importance of colour in organismal evolution was as important in the past as it is in the present. This has likely been the case for as long as vision has existed—extending back to the Cambrian Explosion. This is particularly true for birds and their theropodan ancestors. Birds are important ecosystem engineers in most extant terrestrial and indeed aquatic environments. Dinosaurs would have been similarly important, also filling several niches comparable to modern terrestrial mammals. This invites several questions about how comparable the dynamics among predators and prey was in the Mesozoic to extant analogues and also in what respects it was different (Brown et al. 2017).

11.1 Overturning the Paradigm: From Bacteria to Coloured Dinosaurs

Soft tissues, that is, those that are not mineralised in life, are generally rare in the fossil record (Allison and Briggs 1993; Parry et al. 2017). When found however, they can offer unique additional insights (Allison and Briggs 1993; Briggs and Kear 1993; Wilby and Briggs 1997; Parry et al. 2017). Integumentary preservation in vertebrates is known from several exceptional fossil localities and includes scales, skin, hair and, importantly for the understanding of avian evolution, feathers (Davis and Briggs 1995; Vinther 2015a). The first fossil feather was

found in the lithographic limestone of Solnhofen, in around 1860 (von Meyer 1861a, b, 1862; Griffiths 1996; Carney et al. 2012). This well-preserved and very detailed feather is preserved as a dark organic film in a buff coloured limestone and was used to erect a new taxon: *Archaeopteryx lithographica* (Carney et al. 2012; Fig. 11.2a). The discovery of this single isolated feather was followed just a year later by that of a remarkably complete animal from which it was assumed the isolated feather came (Fig. 11.2b) (von Meyer 1861a, b, 1862; Griffiths 1996). *Archaeopteryx* displayed a mixture of bird-like and reptile-like features. Importantly, this included a full covering of pennaceous feathers that helped to spark the now near universally accepted idea that birds are directly descended from, and for all intents and purposes are living representatives of, dinosaurs (Ostrom 1974; Carney et al. 2012; Foth et al. 2014; Brusatte et al. 2015). The feathers of the first complete *Archaeopteryx* specimen, the London specimen, as well as the later Berlin specimen (Fig. 11.2b) were preserved as inorganic impressions just like all subsequent finds.

In the 160 years since the initial iconic discovery of *Archaeopteryx*, numerous fossil localities have been uncovered globally where feathers have been found to preserve (Vinther 2015a). Among the most important of these include the Early Eocene formations of Messel (near Darmstadt, Germany), Fur (Denmark) and Green River (Wyoming, USA), exceptional Early Cretaceous deposits such as the Jehol Biota of Liaoning Province (China) and the Santana Formation (Brazil) and the Mid-Cretaceous Burmese amber deposits of Myanmar (Davis and Briggs 1995; Kellner 2002; Chang 2003; Vinther et al. 2008; Prado et al. 2016; Xing et al. 2016).

Despite fossil feathers being well known for over 150 years, in-depth questioning of the nature

Fig. 11.2 The iconic Late Jurassic paravian theropod *Archaeopteryx* from Solnhofen, Germany. (**a**) The first fossil feather to be discovered in 1860—an organically preserved isolated wing feather assigned to *Archaeopteryx* and recently shown to contain abundant melanosomes (Carney et al. 2012). (**b**) The 'Berlin specimen' (HMN 1880) showing the complete animal with feathered wings and tail displayed. Scale bars represent 5 mm in (**a**) and 50 mm in (**b**). Reproduced and modified with permission from Nature Publishing

of their preservation did not start until electron microscopic investigations became a commonplace in geology and palaeontology in the late twentieth century (Wuttke 1983; Davis and Briggs 1995; Vinther 2015a). The ultrastructure of fossil feathers was first examined alongside fossil hair and frog integument from the extraordinary Messel Formation using electron microscopy in the early 1980s (Wuttke 1983; Vinther 2015a). All integumentary structures were shown to be preserved due to the presence of abundant tiny sausage-shaped microbodies, around a micron each in length that were only found within the dark patches of the integumentary structures. In feathers, they were aligned together along the axis of the barbs and barbules (shown in our Fig. 11.3 of an undescribed isolated Messel feather). It was argued that these microbodies represented bacterial colonies and bacterial glycocalyx, which were preserving an outline of the features on which they grew (Wuttke 1983). Several mechanisms through which feathers could become preserved were subsequently postulated by Davis and Briggs (1995). These included preservation as carbonised mats, bacterial autolithification and imprintation, all of which showed similar microstructures attributed to preserved bacteria. This paradigm, that feathers (and other integumentary structures) were preserved due to the presence of the bacteria that were involved in their decay, pervaded for another decade until a novel realisation came about through an investigation of exceptional preservation in an invertebrate.

Coleoid cephalopods have been known to preserve soft tissue for around 170 years (Owen 1863). An unusual feature of their preservation often observed is that the ink sacs are usually three-dimensionally preserved due to preservation of the ink melanin pigment inside the organ. The rest of the soft tissues (including the muscular mantle) are most often preserved two-dimensionally compressed (Fig. 11.4a) (Vinther 2015a). It has long been recognised that fossil coleoid ink reveals an infrared spectroscopy chemical signature consistent with

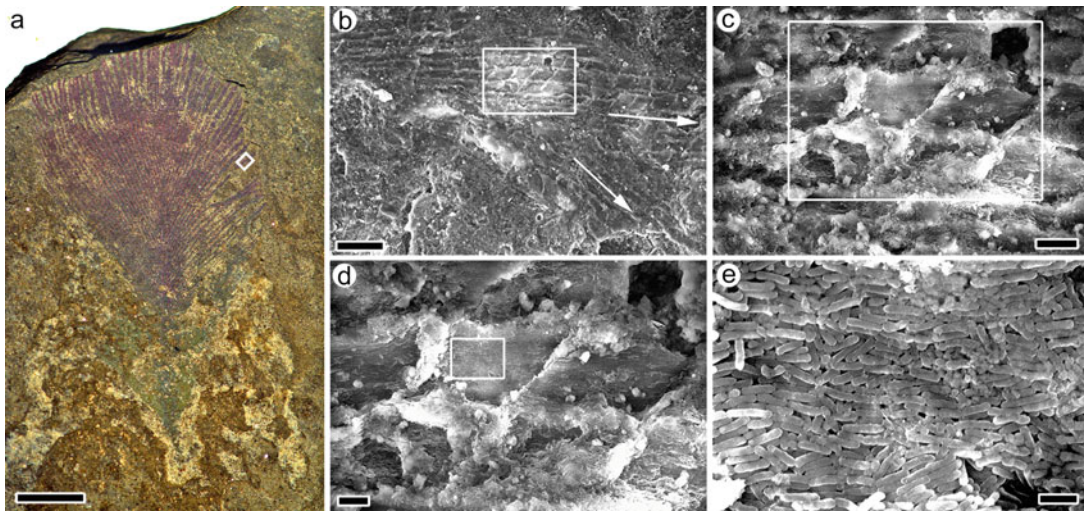

Fig. 11.3 The preservation and arrangement of melanosomes (previously identified as lithified bacteria) in an exceptional fossil feather from the Eocene Messel Formation, Germany. White boxes indicate the next SEM image in the series. (**a**) An isolated feather from an unknown bird (SMF-ME 3937) showing exceptional preservation of barbs and barbules. (**b**) SEM image of a barb showing preservation of individual barbules arranged as in a modern pennaceous feather. White arrows show the orientation of the barbules. (**c**) SEM image of the barbules showing apparent cell-like structure with each cell measuring around 10 μm in length. (**d**) Close-up SEM image of four individual cell-like structures. (**e**) SEM image of the melanosomes that explain the preservation of the barb and barbule structures, with no other original feather features (including keratin) present. The melanosomes are in their original arrangement sitting on the matrix, and it is clear that all keratin has degraded away. Scale bars represent 5 mm in (**a**), 50 μm in (**b**), 10 μm in (**c**), 5 μm in (**d**) and 1 μm in (**e**)

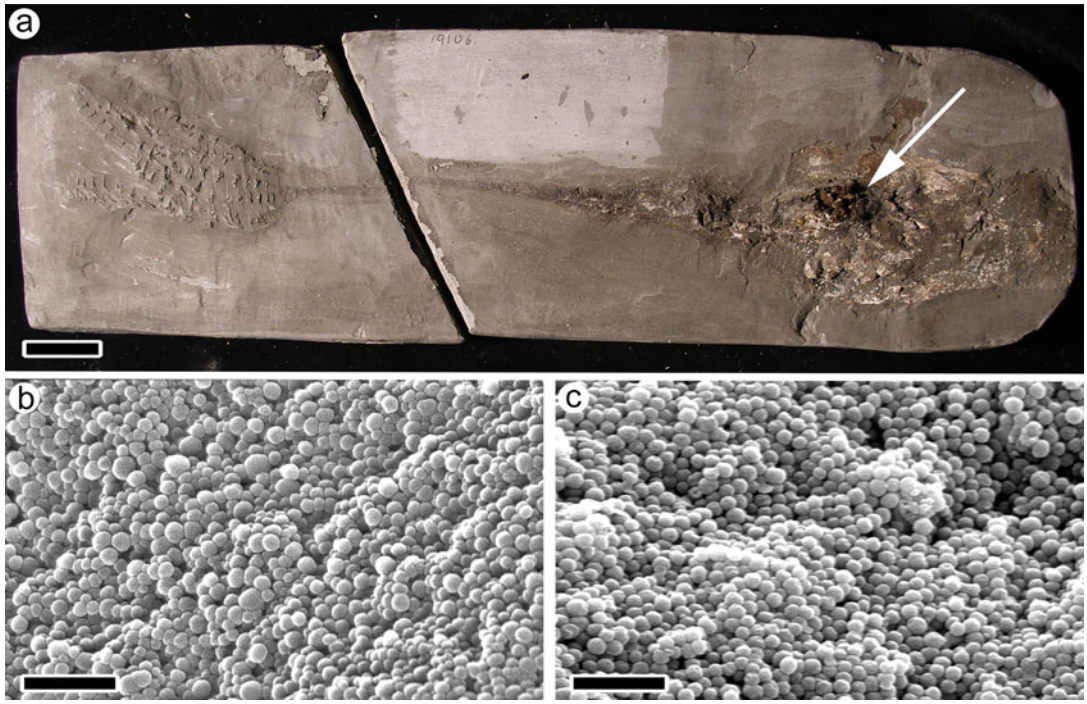

Fig. 11.4 Preserved melanin granules in fossil coleoid cephalopod ink demonstrating the remarkable recalcitrancy of the pigment. (**a**) A complete coleoid fossil of the genus *Geoteuthis* from the Early Jurassic of Lyme Regis, UK (YPM 19106), showing compressed soft tissue preservation apart from the 3D black ink sac (white arrow), uncompressed due to its constituent melanin. (**b**) SEM image of modern coleoid cephalopod (*Sepia*) ink showing the morphology of the melanin granules. (**c**) SEM image of the preserved ink from YPM 19106 showing identical melanin granule morphology to the extant *Sepia* ink. Scale bars represent 2 cm in (**a**) and 1 μm in (**b**, **c**)

melanin (Beyermann and Hasenmaier 1973). In the late 2000s, researchers examined the microstructure of Jurassic coleoid ink under an electron microscope. The preserved ink was composed of tightly packed spherical granules (Fig. 11.4c) (Vinther 2015a) identical in size and shape to modern cephalopod ink granules, which are composed of pure eumelanin (Doguzhaeva et al. 2004). The fossil ink therefore appears to be little changed over 196 million years (Fig. 11.4b) (Vinther 2015a). That the fossilised cephalopod ink sacs often showed three-dimensional preservation, despite compression of the rest of the animal, highlighted a key feature of melanin, its high recalcitrancy and resistance to degradation (Vinther 2015a, b). Subsequent work found that the precise chemical nature of the fossil ink was similar to that of modern melanin from the cuttlefish *Sepia officinalis* (Glass et al. 2012, 2013).

As modern bird feathers have been long known to be predominantly coloured by melanin, fossil feathers were an obvious next step in looking for melanin in the geological record. In 2008, Vinther et al. analysed a fossil feather from the Early Cretaceous Crato Formation of Brazil which showed distinct dark and light transverse bands (Fig. 11.5a). Within the dark bands of the feather, they found abundant oblong structures around 1–2 μm in length aligned along the barbs and barbules, identical to those previously identified as lithified bacteria in feathers from Messel (Fig. 11.5b; Vinther et al. 2008). In contrast, the light bands showed no microstructures other than the matrix itself (Fig. 11.5c). Vinther et al. (2008) noted that the oblong structures were identical to eumelanosomes found in modern bird feathers (Fig. 11.1). Due to the newly appreciated preservation potential of melanin together with

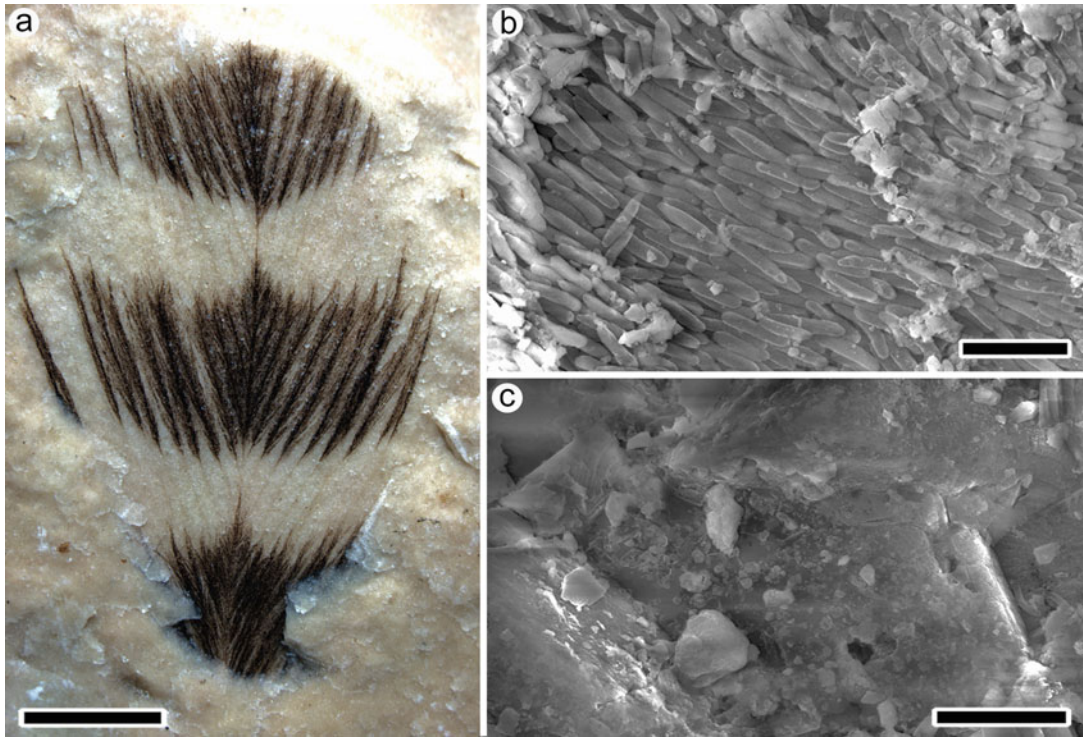

Fig. 11.5 A striped fossil feather from the Early Cretaceous Crato Formation, Brazil, the first to be unequivocally shown to preserve due to the presence of melanin (Vinther et al. 2008). (**a**) The isolated feather (LEIUG 115562) showing distinct dark and light banding. (**b**) SEM image of a sample taken from a dark band showing abundant aligned melanosomes. (**c**) SEM image of a sample taken from a light band showing no melanosomes, only the rock matrix. This shows that the striping is due to preservation of original colour patterning and the presence and absence of melanin in the dark and light bands, respectively. Scale bar represents 3 mm in (**a**), 2 μm in (**b**) and 5 μm in (**c**)

their unique arrangement and dimensions, it was considered more parsimonious that these micrometre-sized structures were in fact exceptionally preserved melanosomes rather than lithified bacteria. Furthermore, they were still arranged roughly as they would have been in the feather in life, although all remnants of the keratin matrix in which they sat had been lost. They suggested therefore that the feather was showing its original colour pattern of pigmented and unpigmented banding, i.e. the presence and absence of melanin. Based on their shape, it was surmised that the feather would have been striped black and white since it was known that spherical/oblate melanosomes contain reddish-brown phaeomelanin (Vinther et al. 2008). The bacterial explanation for this preservation was harder to justify, given that bacteria are associated with all parts of an animal during decay and thus would be highly unlikely to form distinct bands within individual structures or precisely mirror the morphology and arrangement of feather melanosomes (Vinther 2015a). Energy-dispersive X-ray spectroscopy (EDX) of the dark bands backed this up, showing high concentrations of carbon, whereas no carbon was present in the light bands. Alongside this isolated feather, a complete bird skull from the Fur Formation was also analysed in the study, which showed the same oblong-shaped structures that were aligned along the barbs and barbules in the preserved plumage forming a halo around the skull (Vinther et al. 2008). Furthermore, a dark patch within the eye orbit of the bird showed both oblong and subspherical microstructures which resembled melanosomes found within the retinas of modern birds (Vinther et al. 2008; Vinther 2015b).

This study overturned the orthodoxy that lithified bacteria were the chief explanation for integumentary preservation in vertebrate fossils

and proposed that, instead, exceptionally preserved melanosomes were a more likely scenario (Vinther 2015a). The palaeocolour literature has subsequently adopted the term melanosome for their occurrence in fossils; however, some have advocated the use of the term melanin granules instead (Galván and Solano 2016). The term melanosome refers to the living organelle within a cell, while palaeocolour most often deals with melanin deposited into inert keratin. However, the morphology of the two is identical as the melanin granules are formed through dense packing of melanin inside the organelle's membrane, which is then lost as they are transported to the keratin. For consistency with the literature, we refer to both the living organelle and the fossil occurrences as melanosomes. More recently, the bacterial hypothesis has been revisited, with suggestions that bacteria fossilise easily and are indistinguishable from melanosomes (Moyer et al. 2014; Lindgren et al. 2015; Schweitzer et al. 2015). However, these criticisms fall short in their consideration of their relative preservation potential, the actual dimensions that bacteria exhibit and the unique fashion in which melanosomes localise themselves in fossil tissues (Vinther 2015a, b). Bacteria are certainly key to inducing conditions that make soft tissue fossilisation possible and promoting many of the processes that lead to exceptional preservation (Briggs and Kear 1993; Parry et al. 2017 and references therein). As yet however, they have not been conclusively demonstrated to be organically preserved in any fossil vertebrate. Furthermore, the bacterial model does not explain why only tissues that are known to contain melanin preserve as organic stains and not other tissues that would be likely to host thriving microbiomes, such as muscles and the intestinal tract.

11.2 Mechanism of Melanin Preservation

The ubiquity and varied functions of modern melanins have made them a focus of scientific study since the nineteenth century (Wolfenden 1884; Chittenden and Albro 1899; Galván and Solano 2016). Despite such a long history of research, certain aspects of the structure and synthesis of melanin are still incompletely understood in living animals (Galván and Solano 2016). Melanins are complex biopolymers thought to be formed through subtly different pathways depending on the exact melanin type. In modern birds, melanins are synthesised in melanocytes through a process known as melanogenesis (Galván and Solano 2016). The current state of knowledge of the full process is described in detail elsewhere (Galván and Solano 2016), but in brief melanogenesis involves a number of steps, which differ between eumelanin and phaeomelanin. The initial step common to both melanin types is the oxidation of L-tyrosine to L-dopaquinone by oxygen, catalysed by the enzyme tyrosinase (Galván and Solano 2016). After this step, the chemical composition of the solution appears to determine whether eumelanogenesis or phaeomelanogenesis follows through different polymerisation reactions resulting in the final large and complexly cross-linked pigment molecules (Galván and Solano 2016). The chemical structures of eumelanin and phaeomelanin differ in key aspects that have significant implication for fossil melanin. Eumelanin comprises cross-linked 5,6 dihydroxyindole (DHI) and 5,6 dihydroxiindole-2-carboxylic acid (DHICA) polymers. Phaeomelanin incorporates monomers of benzothiazines and benzothiazoles rather than DHI and DHICA. The presence of these distinctive sulphur compounds distinguishes phaeomelanin and also provides key signatures that can be looked for in the fossil record (Glass et al. 2012; Brown et al. 2017). The complex cross-linking of melanin may help to explain its remarkable recalcitrance and ability to survive diagenetic processes.

Despite numerous studies into the preservation of melanin in fossils, its precise taphonomy and preservation are at present still to be fully understood. This is in part due to the lack of complete clarity on the molecular structure of modern melanin, but also due to the relative novelty of the field. Although a small number of references to

melanin being inferred to be present in fossils were made throughout the twentieth century (e.g., Voigt 1936, 1988; Whitear 1956; Beyermann and Hasenmaier 1973; Gottfried 1989; Mapes and Davis 1996), it has only been the past decade that has seen any serious advances made in unravelling the intricacies of the pigment's preservation in deep time (Vinther 2015a). Since the initial confirmation that melanin survives in fossils, it has become apparent that the pigment may be relatively common in the fossil record where soft tissues are present (Glass et al. 2012; Vinther 2015a, b; Clements et al. 2016). While it is generally accepted that melanin can survive taphonomic and diagenetic processes, work to better elucidate the mechanisms by which the pigment can become preserved has thus far been comparatively limited, and the subject is still in its infancy. A small number of important studies over the past decade have however attempted to further our understanding of the nature of melanin preservation and have provided key information.

Identifying chemical signatures for melanin and its diagenetic products has been attempted using a number of different techniques (e.g. Vinther et al. 2009; Barden et al. 2011; Wogelius et al. 2011; Glass et al. 2012, 2013; Lindgren et al. 2012; Colleary et al. 2015; Pan et al. 2016; Gren et al. 2017). Maturation experiments have also been performed to investigate how the effects of heat and pressure (both key factors in diagenesis) affect the morphology and chemical structure of melanin and melanosomes (McNamara et al. 2013; Colleary et al. 2015; Saitta et al. 2017).

While preliminary work in the 1970s studied fossil melanin using infrared spectrometry in order to understand melanin preservation (Beyermann and Hasenmaier 1973), only more recently after the discovery of widespread melanin preservation have scientists attempted to understand the chemical makeup of fossil melanins. Some studies were able to identify chelating metals associated with melanin using synchrotron X-ray fluorescence (XRF) (Wogelius et al. 2011). These could be mapped across a whole specimen. Similar elemental mapping confirmed the carbonaceous nature of melanosomes, using scanning electron microscopy (SEM) and EDX (Vinther et al. 2008). While melanin is unique for its ability to chelate an assortment of metals (Liu and Simon 2005), the use of these techniques in identifying fossil melanins is limited because other organic fossils will bind metals in life and after death (Vinther 2015a). Furthermore, the elemental mapping does not identify areas in which melanosomes have dissolved due to oxidation, but can be identified as impressions, giving a false appearance of colour pattern (Vinther 2015a).

Studies into the chemical nature of melanin in fossils using techniques other than whole-specimen elemental surface mapping have provided more insight into the nature of its preservation. Barden et al. (2011) performed Fourier transform infrared spectroscopy (FTIR), electron paramagnetic resonance (EPR) and pyrolysis gas chromatography mass spectrometry (py-GCMS) alongside SEM and EDS on isolated fossil feathers from the Early Cretaceous Xiagou Formation of China attributed to the amphibious bird *Gansus yumenensis*. Imaging demonstrated the presence of melanosomes, and the infrared spectra and chromatograms of the organics were very similar to a modern eumelanin standard. FTIR allows for the identification and quantification of functional groups, such as hydroxyls, ketones and carboxyls. Hydroxyls and carboxyls tend to be lost during maturation and hence allow for understanding the alterations of melanins. While the py-GCMS did not serve to identify melanin markers in this study, it importantly showed no significant contribution of bacterial hopanoid biomarkers, which helped rule out the possibility of a bacterial origin of the microbodies.

In 2012, Glass et al. performed what is still one of the most comprehensive chemical studies into fossil melanin preservation to date. The study focussed on one of the least controversial and likely purest sources of eumelanin in the fossil record: that of coleoid cephalopod ink sacs. Using a suite of analytical techniques, including py-GCMS, alkaline hydrogen peroxide oxidation, FTIR and X-ray photoelectron spectroscopy (XPS), Glass et al. determined that the fossil ink of coleoids from the Middle Jurassic of Christian Malford (Wiltshire, UK) and Early Jurassic of

Lyme Regis (Dorset, UK) was unequivocally preserved as eumelanin and its breakdown products. In fact, around 10–15% of the preserved ink was still composed of intact eumelanin with carboxyl and hydroxyl groups intact. This further demonstrated the incredible recalcitrance of the pigment and its ability to survive relatively unchanged for over 190 million years. Numerous melanin-derived products were found in the Jurassic ink as well as multiple diagenetic components using py-GCMS. Some had been secondarily sulphurised, that is, they had reacted with sulphur to form various thiophenes (Glass et al. 2012; Vinther 2015a).

A follow-up study by Glass et al. (2013) examined ink from coleoids found at the late Early Jurassic locality of Holzmaden (Germany). Although these were of an age intermediate to the two English coleoids studied previously, the melanin content was found to be just 1% that of those specimens and just 0.1% that of a modern eumelanin extract. The only major difference between the fossils was the burial and thus diagenetic history of the formations. The Posidonia Shale of Holzmaden was buried deeper than the other two Jurassic localities. This meant it had entered the oil window (the point at which insoluble organic matter, kerogen, thermally matures into oil, which is dependent on burial depth-controlled temperature). The level of organic maturation was therefore significantly higher, resulting in the alteration and enhanced breakdown of the original melanin. Despite this, numerous pyrolysate compounds were found in the Holzmaden specimens that were similar to the other less-matured fossils. This showed that py-GCMS is capable of detecting melanin-derived breakdown products even in heavily matured specimens (Glass et al. 2013; Vinther 2015a). Py-GCMS is a destructive technique however (although modern GCMS machines can analyse down to 50 μg), meaning that samples are destroyed in the analysis. This destructive technique may therefore prove unsuitable for particularly rare specimens, as is often the case with exceptionally preserved fossils.

A recent development for exploring fossil melanins is the relatively nondestructive time-of-flight secondary ion mass spectrometry (ToF-SIMS). This sensitive technique involves firing a pulsed ion beam at small areas of the surface of samples and determining the mass of secondary ions removed from the outermost surface (Vickerman and Briggs 2001). Both positively and negatively charged ions can be detected but have to be collected separately in successive runs. These secondary ions can give information about the elemental and molecular makeup of the sample surfaces (Vickerman and Briggs 2001; Lindgren et al. 2012; Colleary et al. 2015). For melanins, the technique is best suited to characterise low-molecular-weight secondary ions—fragments of the larger and poorly understood molecule. Spectra can be generated and compared to those of other samples with known compositions (Colleary et al. 2015). Importantly, each individual peak, composed of a single or a couple of secondary ions, is not diagnostic of melanin, but the relative intensity creates a spectral fingerprint that can be compared between samples.

Lindgren et al. (2012) used ToF-SIMS to probe the preserved eye of a fish from the early Eocene Fur Formation and compared the resulting spectra to the surrounding sediment, other body regions and, importantly, a modern melanin standard. The results from this showed that the mass spectra derived from the eye were very similar to that of the modern melanin standard and dissimilar to that of the surrounding sediment (Lindgren et al. 2012). ToF-SIMS has its limitations however, as it only provides fragments of the molecular makeup from a sample surface. The fragmentation of the molecules in situ leads to complex arrays of secondary ion fragments that could be derived from several sources in a heterogenous sample. Furthermore, the mass of the secondary ions is obtained from the time-of-flight from the sample to the detector, which gives poor mass resolution, further conflating the ability to characterise distinct ions of similar relative mass.

In spite of its limitations, ToF-SIMS has now become something of a standard in analysing melanin in the fossil record. It is currently considered to be one of the most suitable and easily

applicable techniques to probe the molecular makeup of fossil samples without excessive damage to the specimen (Colleary et al. 2015; Clements et al. 2016; Gabbott et al. 2016; Gren et al. 2017). The use of multivariate statistics, such as principal component analysis (PCA), also allows more objective comparison of the appearance of the ToF-SIMS spectra. Incorporating PCA provides both the ability to distinguish melanin from negative non-melanin samples in an objective fashion (Fig. 11.6c) and the possibility of identifying alterations to melanin that occurred during maturation (Colleary et al. 2015).

11.2.1 Maturation Experiments

It has become apparent that melanin, while recalcitrant, does not survive molecularly intact. Instead, it must undergo alterations similar to other fossil organic materials (Eglinton and Logan 1991). Hence, researchers have begun to experimentally understand the nature of alterations to melanins during fossilisation, driven by maturation under elevated heat and pressure (McNamara et al. 2013; Colleary et al. 2015). Artificial maturation experiments have been carried out on both feathers and pure melanin samples under experimental conditions to elucidate how the pigment may alter during diagenesis (McNamara et al. 2013; Colleary et al. 2015; Saitta et al. 2017). As the geologic processes of organic alteration are contingent on time and the levels of temperature and pressure they have experienced, experimentalists resort to using more elevated P/T conditions in a shorter time window to speed the process up.

It has been observed that impressions of fossil melanosomes are often larger than the actual preserved ones (Clarke et al. 2010; Carney et al. 2012). Maturation experiments have demonstrated that this difference can be explained by dehydrating alterations taking place during maturation causing condensation reactions (Eglinton and Logan 1991) and hence shrinkage without affecting the preservation and overall morphology of the melanosome organelle (McNamara et al. 2013). Melanosome geometry has been shown to change between 7.6 and 20% depending on the conditions to which they were exposed (lower temperature regimes result in less shrinkage). Although the potential shrinkage of melanosomes had been highlighted previously (Clarke et al. 2010; Carney et al. 2012), this study (McNamara et al. 2013) highlighted how the effect of thermal maturation can induce the phenomenon. As the temperature controlled the degree of shrinkage, burial history and, in particular, the level of thermal maturation should therefore ideally be taken into account when melanosomes are analysed for palaeocolour reconstructions by their morphology. However, as the shrinkage appears to be isometric (i.e. the aspect ratio of melanosomes remains the same), it is observed to have a negligible effect. This is because it has been shown that aspect ratio is one of the most important variables in statistical comparisons for colour prediction by melanosome morphology (see below). Shrinkage can in theory be mitigated by scaling up fossil melanosome measurements by 10–20% in statistical analyses. Additionally, measuring both mouldic impressions and actual 3D preserved melanosomes (if present within a single sample) could allow the potential degree of shrinkage to be examined.

While melanosomes may shrink during diagenesis, the presence and absence of the pigment should not change; therefore, original colour patterns are likely to be visible even in highly matured deposits provided that soft tissue preservation occurs (Vinther et al. 2008; Vitek et al. 2013; Colleary et al. 2015; Vinther 2015a). This is highlighted by deposits such as those of the Jehol Biota, which are thought to have undergone a deeper burial history than many other feather-bearing sites, yet still show remarkable integumentary structure preservation including original colour patterning (Vinther 2015a, Vinther et al. 2016, Smithwick et al. 2017a, b). Younger deposits that have undergone less harsh burial conditions, such as Messel, can also preserve colour patterns in exquisite detail, suggesting minimal alteration (Fig. 11.7).

The most comprehensive maturation study looked at the effects of elevated temperatures

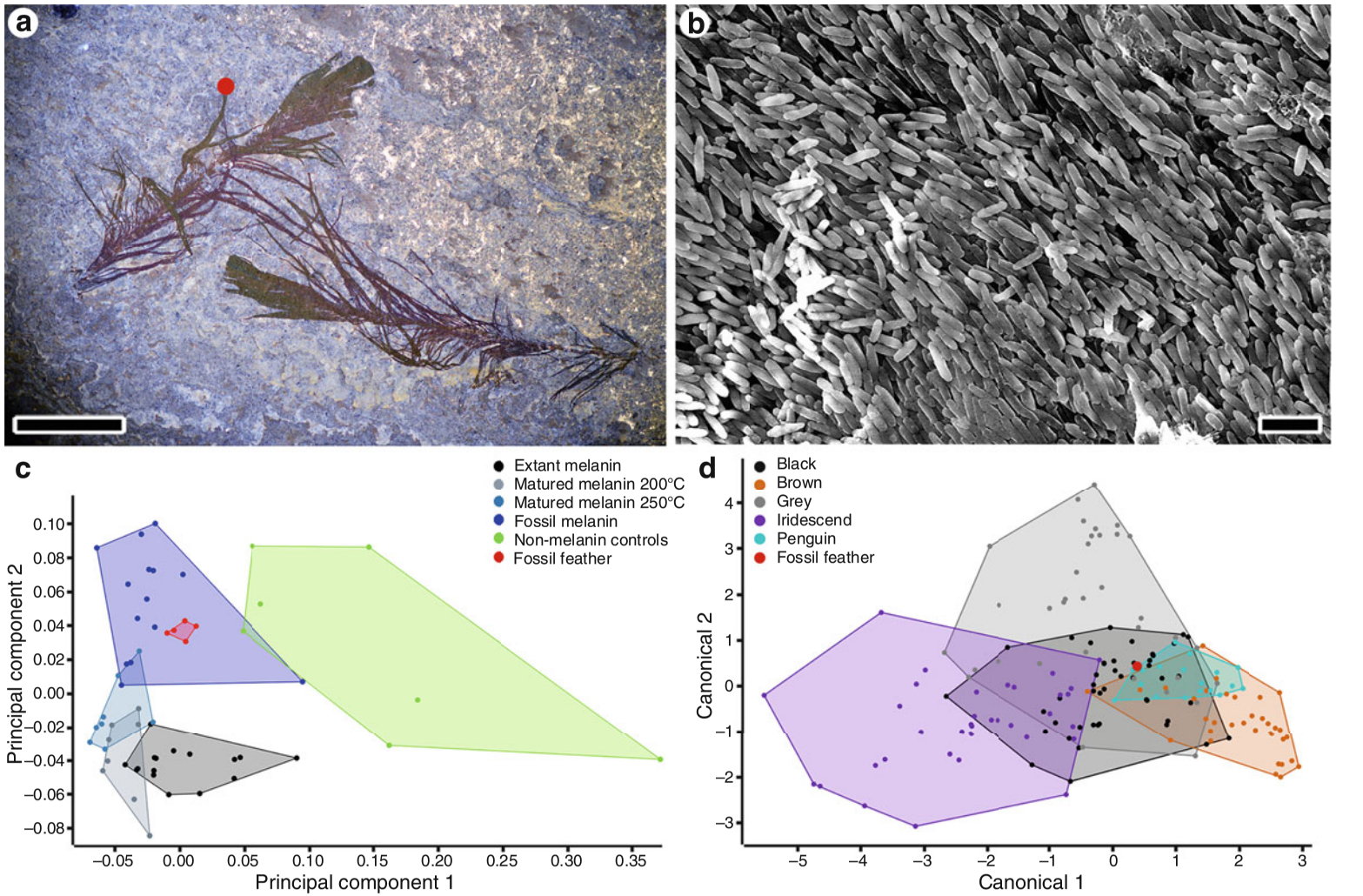

Fig. 11.6 Melanin preservation in Eocene feathers and palaeocolour inferences possible from minimal sampling. (**a**) A pair of feathers (SMF-ME 3855) from an unknown bird from the Eocene Messel Formation, Germany. The red dot indicates the point at which a sample was removed for analysis. (**b**) SEM image of melanosomes that mark

and pressures on melanins in conjunction with ToF-SIMS (Colleary et al. 2015). In addition to effects on the morphology of melanosomes, Colleary et al. (2015) also investigated how the chemical structure of melanin was altered. They analysed a range of fossil taxa, including bird feathers, mammal hair, fish eyes, amphibian skin and coleoid cephalopod ink. This provided for a broad sample straddling almost 300 million years of the fossil record, bracketing bilaterian metazoans and including melanosomes of varying morphology and hence likely different eu- and phaeomelanin composition. Maturation experiments were also run on extracts of pure melanin from modern bird feathers. As with previous experiments, samples were subjected to elevated temperatures and pressures (200 and 250 °C and 250 bar) for 24 h sealed in gold tubes. To compare the more than 55 ToF-SIMS spectra, PCA was used. Most significantly, the ToF-SIMS spectra could show that fresh and fossil melanins were distinct from negative samples and that they in turn showed subtle differences with matured melanins being intermediate in spectral composition (reproduced in our Fig. 11.6c). Furthermore, the spectra showed differences correlating the morphology of fossil melanosomes, suggesting that ToF-SIMS is also able to characterise different compositions of eu- and phaeomelanin. These results also show that the fossil samples did not cluster according to lithology, age or locality, showing that the framework mainly allows for characterising differences in original melanin chemistry. Some differences in how melanin chemistry spread in extant and fossil samples in PCA need further scrutiny and are the focus of current research (Colleary et al. 2015).

11.2.2 Melanin and Sulphurisation

In the comprehensive chemical characterisation of cephalopod ink by Glass et al. (2012), it was shown that the eumelanin had reacted with sulphur to form a host of thiophenes among the pyrolysates in the py-GCMS spectrum. Sulphurisation is a well-known phenomenon in organic geochemistry (Sinninghe Damsté et al. 1989) and is noted to likely be an important pathway for preserving both bone marrow and muscle tissue in a particularly sulphuric Miocene fossil deposit (McNamara et al. 2006, 2010). Since phaeomelanin is composed of monomers of benzothiazines and benzothiazoles, which contain sulphur, concerns had been raised about whether secondary sulphurisation could lead to unwanted conflation between original pheomelanin and secondarily sulphurised eumelanin (McNamara et al. 2016a). However, thiophenes and thiazines/thiazoles are molecularly distinct due to the presence of nitrogen in the latter. Hence, the secondary ions chosen by Lindgren et al. (2014) to characterise pheomelanin in ToF-SIMS analyses (of which many combine C, N and S) would be distinct from the thiophenes (which would not contain N). It is therefore observed that the sulphurised fossil coleoid ink analysed by Colleary et al. (2015) is not conflated with both modern and fossil melanins that contain benzothiazoles (Brown et al. 2017) or modern and fossil melanosomes that possess the distinct small and ovoid morphology characteristic of phaeomelanin-rich compositions (Colleary et al. 2015).

Fig. 11.6 (continued) out the barbules of the fossil feather. (**c**) Time-of-flight secondary ion mass spectrometry (ToF-SIMS) principal component analysis (PCA) plot showing the distribution of extant, matured and fossil melanin samples based on the first two PC axes derived from 54 mass peaks known to be associated with melanin (adapted using data from Colleary et al. 2015). The isolated fossil feather plots in the centre of the range of other fossil melanin samples, separate from the non-melanin controls, confirming a likely melanin affinity. (**d**) Canonical discriminant analysis results showing the relationship between melanosome morphology and colours produced. Modern feather melanosomes with known associated colours were categorised by colour and used to predict the likely colour of the fossil feather, shown here to fall within the range of black and 'penguin' feather melanosomes. Data adapted from Li et al. (2012). Scale bars represent 1 cm in (**a**) and 2 μm in (**b**)

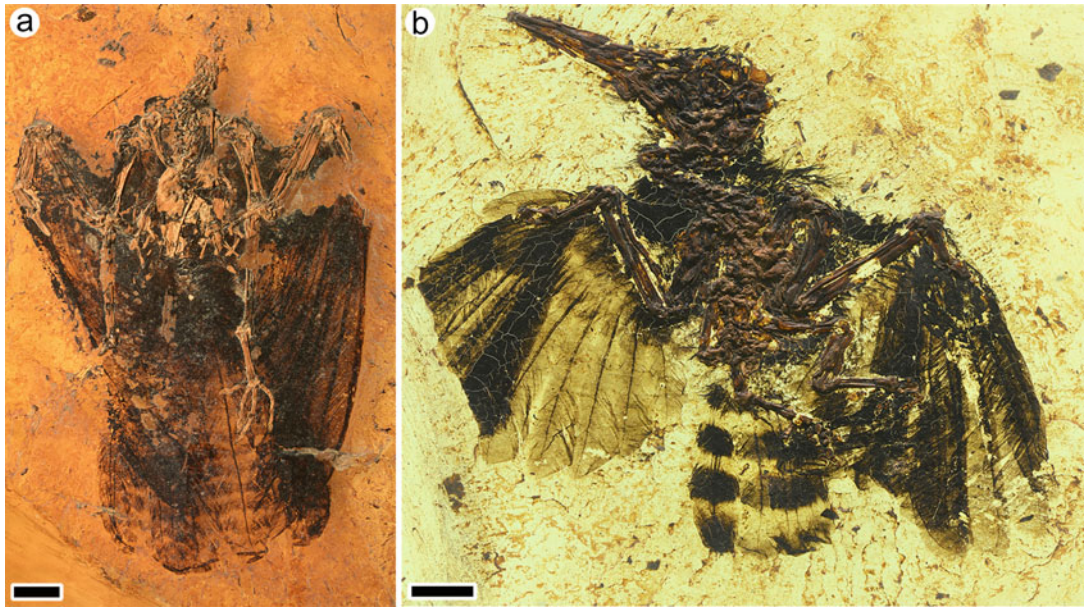

Fig. 11.7 Examples of preserved original colour patterns in fossil birds from the Eocene Messel Formation, Germany. (**a**) A near-complete specimen of the genus *Hassiavis* (SMF-ME 9047a) missing the head but showing exceptional feather preservation including dark pigmented wings and a finely banded tail. (**b**) A complete specimen of the species *Messelirrisor grandis* (HLMD-Be 178) showing a striking thickly banded tail. These colour patterns are retained due to the presence of melanosomes. Scale bars represent 10 mm

11.3 Preservation of Non-melanin Feather Components

A feature that has been frequently noted in studies on fossil feathers is the absence of any feather ultrastructure apart from the preserved melanosomes (Vinther et al. 2008, 2009; Carney et al. 2012; Li et al. 2012; Field et al. 2013; Vitek et al. 2013; Vinther 2015a). Most SEM analyses of fossil feathers to date have shown that when feathers are preserved, melanosomes mark out the structure of the barbs and barbules, but lie freely in the matrix with little morphological evidence of the other key component of feathers, keratin, present (highlighted in Fig. 11.3, also clear in Figs. 11.5, 11.6 and 11.8) (Vinther et al. 2008, 2009; Carney et al. 2012; Li et al. 2012; Field et al. 2013; Vitek et al. 2013; Vinther 2015a, b). Maturation experiments have shown that keratin, like other proteins, does not survive in diagenetic environments and becomes fluid and water soluble readily when temperatures and pressures are elevated (Saitta et al. 2017), leaving only melanosomes (Colleary et al. 2015). McNamara et al. reported on experiments in which feather keratin appeared to survive (McNamara et al. 2013), but it turned out that there was a mix up in the presentation of their experimental protocol and only experiments that had been performed under brief intervals (1 h vs. 24 h) were reported (McNamara et al. 2017, recent correction), which is not a standard protocol.

Some have however claimed that keratin is preserved organically in fossil feathers and that molecular signatures remain (Schweitzer et al. 1999; Moyer et al. 2016; Pan et al. 2016). A recent study of the Early Cretaceous bird *Eoconfuciusornis zhengi,* from the Jehol Biota of China, looked at the possibility of keratin being preserved using immunohistochemistry techniques alongside SEM imaging, transition electron microscopy (TEM) and ChemiSTEM techniques (Pan et al. 2016). Antibody antigen binding suggested that original components of the feather beta keratin were preserved and

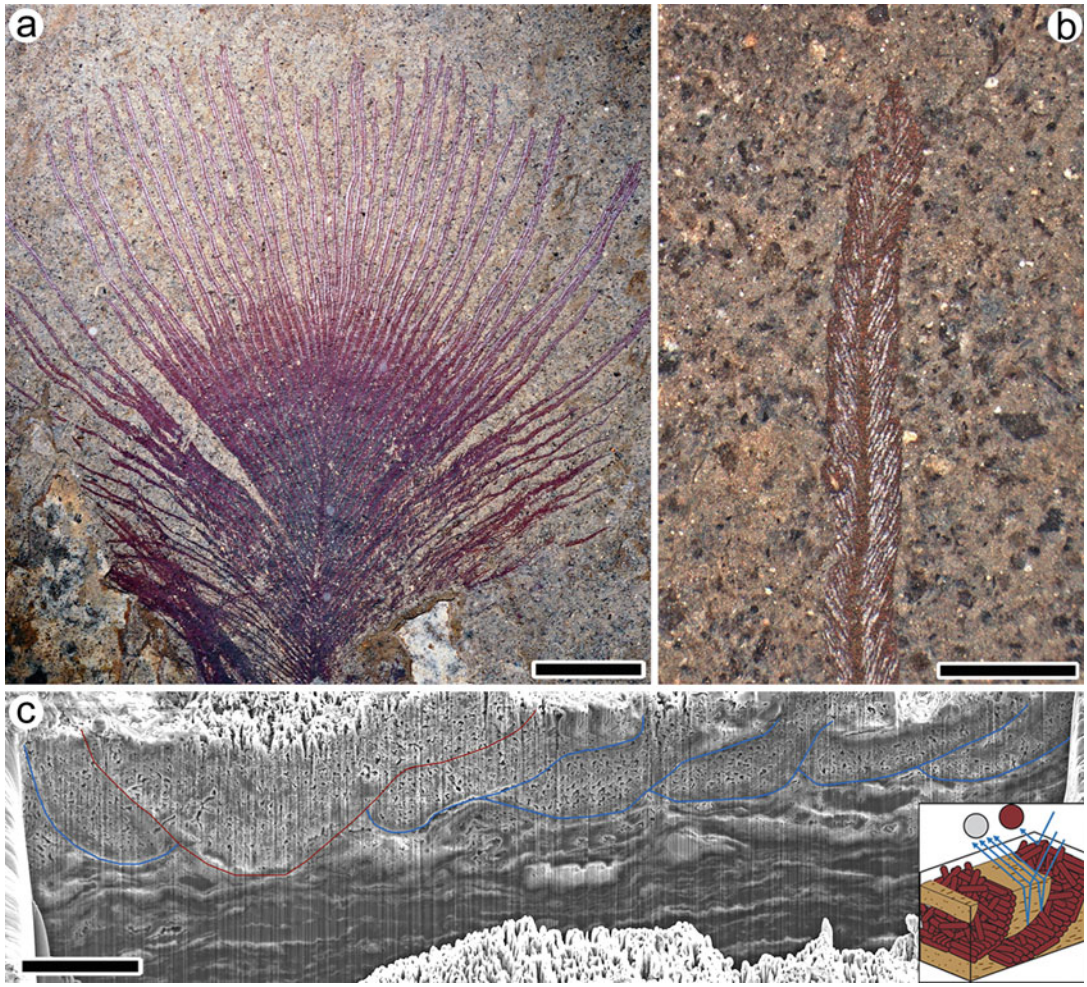

Fig. 11.8 Three-dimensional preservation in a fossil feather from the Eocene Messel Formation, Germany, shown to be originally iridescent (from Vitek et al. 2013). (**a**) Overview of the pennaceous portion of an isolated feather (SMF Me 3850) from an unknown bird. (**b**) Close-up of an individual barb showing preserved barbules and a silver sheen. (**c**) SEM image of a cross section of a barb and associated barbules cut using a focused ion beam (FIB). The barb is outlined in red, and the individual barbules are marked in blue. The structures can be seen to be three-dimensionally preserved, thanks to the presence of melanosomes. All keratin has degraded away, but the melanosomes sit in their original arrangement on the rock matrix and show distinct similarity in their arrangement to modern iridescent feathers. The silver sheen visible to the naked eye is caused by the three-dimensional arrangement of melanosome layers and a thin layer of sediment (inset diagram). Scale bars represent 5 mm in (**a**), 1 mm in (**b**) and 10 μm in (**c**)

melanosomes were present within a presumed filamentous keratin matrix. Issues surrounding the immunohistochemistry techniques have however been raised, including their propensity to provide false positives (e.g. from consolidants used to conserve fossils) and statistical artefacts (Saitta et al. 2017, 2018). Other studies have maintained the possibility of keratin protein preservation using antibody experiments (Lindgren et al. 2017). However, the observation that these fossils preserve melanosomes liberated from their keratin matrix and that unmelanised keratinous tissues preserve no organic residues (Vinther et al. 2008) in addition to previous knowledge on protein stability (Eglinton and Logan 1991; Demarchi et al. 2016; Saitta et al.

2017) goes against the claimed presence of these highly unstable molecules in such ancient samples. Additionally, the mass spectroscopic methods employed fail to recover a protein signal. Antibody experiments are therefore demonstrably unsuitable for analysing fossil samples due to the well-known problems with frequent unspecific binding of antibodies (Saitta et al. 2017, 2018).

While organically preserved keratin is controversial, some originally keratinous structures can survive in the fossil record under certain circumstances through authigenic mineralisation. Claw sheaths, for example, are relatively common in deposits such as Jehol (e.g. Gong et al. 2012; Smithwick et al. 2017a, b) and Solnhofen (e.g. Frey et al. 2003; Fig. 11.2b), and fossil baleen has been shown to be prevalent in whales from the Neogene Pisco Formation of Peru (Esperante et al. 2008; Gioncada et al. 2016). The preservation of these features is likely due to the presence of hardening calcium phosphate salts (apatite) (Saitta et al. 2017). Hard keratinous tissues in living animals can contain up to 15% calcium phosphate by dry weight (O'Connor et al. 2015; Gioncada et al. 2016). Apatite is one of the most important minerals in both hard and soft part preservation in vertebrate fossils (Briggs and Kear 1993; Briggs et al. 1993; Briggs and Wilby 1996; Parry et al. 2017 and references therein), and its presence in keratinous tissues likely aids its mineralisation and preservation in fossils. These mineralised keratinous tissues can retain the structure's original morphology but are highly unlikely to preserve any organic traces of the original decay-prone and labile proteins (Saitta et al. 2017). The only part of feathers that has been shown to be hardened in this manner is the rachis (Blakey et al. 1963; Saitta et al. 2017), but none have been found preserved in fossils with intact keratinous ultrastructure. While it is plausible that apatite is present in other parts of feathers (Blakey et al. 1963), it seems that levels are not sufficient to allow for authigenic mineralisation of feather keratin in most circumstances. An alternative proposed pathway for mineralisation of organic features (mineral preserved organics—MPO) such as keratin has been proposed based on close association with metals that could coat and/or invade the tissues, promoting preservation (O'Connor et al. 2015). As yet however, this has not been identified in fossil feathers and is more relevant to much younger archaeological remains.

11.4 Bringing the Past to Life: Palaeocolour Reconstructions of Extinct Dinosaurs

Alongside research into how and why melanin may preserve, much work has been carried out since the initial studies of fossil melanosomes (Vinther et al. 2008, 2009) to better understand what the preservation of melanin and other pigments can tell us about the colouration of extinct animals, particularly birds and other dinosaurs, and how we can use that information to better inform understanding of past ecologies.

Once it had been established that melanosomes could be found preserved with high fidelity in fossil feathers, attention turned to whether the original arrangement of the melanosomes within a fossil feather could be found and thus provide information about potential structural colouration in extinct taxa. In 2009, Vinther et al. looked at isolated fossil feathers from Messel, some of which showed a silvery sheen in the barbules visible to the naked eye (Fig. 11.8a, b). The aim was to determine whether this was a preservational artefact or remnants of original structural arrangements of melanosomes. In one particular contour feather, which became the focus of the study, the arrangement of the melanosomes distinctly varied with a contrast being apparent between the proximal and distal portions. This variation matched the visible differences in the feather. Proximally, melanosomes were arranged in an aligned but loosely packed configuration in the barbs and barbules as well as the rachis. In the distal portion however, the barbs formed an open and pennaceous arrangement with the barbule melanosomes forming solid, smooth and continuous dense external layers. Underlying these layers, further melanosomes were more loosely packed and less densely arranged. This arrangement is similar to the single thin-film nanostructural array seen in many modern bird

feathers exhibiting iridescence (Prum 2006; Vinther et al. 2009; Vitek et al. 2013).

Subsequent work on the same originally iridescent feathers from Messel revealed that the silvery sheen was related to the original structural arrangement of melanosomes in the feather (Fig. 11.8c). Focused ion beam scanning electron microscopy (FIB-SEM), a technique that cuts a micrometre-scale trench in a sample allowing three-dimensional structures to be observed, was used to examine this. Thin wedges of sediment were found to be acting in place of the original keratin as a material with a different refractive index than the underlying melanosomes (Vitek et al. 2013). Due to the variable thickness of the wedge of sediment, all light waves were scattered, like in a chirped mirror, hence creating the observed silvery sheen. This showed that the feather was most likely strongly iridescent in life. The presence of structural colour in an Eocene bird feather provided novel information about the Messel ecosystem.

Messel has the richest avifauna of any known fossil location, with a level of diversity and ecological disparity rivalling modern forested ecosystems (Mayr 2017). While skeletal remains of these birds can inform us about their potential habits, such as feeding strategies and perching ability (Mayr 2017), being able to infer plumage colouration has the potential to deepen our understanding of social and behavioural interactions of this extinct ecosystem. In modern birds, iridescence is often associated with social signalling (Cuthill et al. 1999; Prum 2006; Doucet and Meadows 2009; Stavenga et al. 2010; Vukusic 2011). Due to the exceptional preservation of original structural colours in Messel feathers, we can infer that such interactions were likely occurring in the Early Eocene bird assemblages (and indeed earlier).

Most fossil feathers do not show evidence of original structural colours. Instead, we must rely on the morphology, density and chemistry of the melanosomes to infer original colouration. Since melanosome morphology provides the ability to distinguish pigmentary colours in extant taxa (Fig. 11.1), methods for predicting colour from melanosome shape in fossils have been investigated. In 2010, two research groups independently analysed the integument of Mesozoic feathered dinosaurs. Zhang et al. (2010) were able to demonstrate the preservation of both elongate and smaller ovoid melanosomes in theropods and pygostylians from the Jehol Biota. Li et al. (2010) described melanosomes from the Late Jurassic paravian *Anchiornis huxleyi* from the Tiaojishan Formation of China. SEM imaging of 29 samples from feathers across the body of *Anchiornis* revealed abundant impressions of oblong structures again identical to modern melanosomes. These melanosomes preserved the original alignment along feather structures in most places. Some light-coloured feather impression samples were barren of melanosomes and organic remains and hence were considered unpigmented. From the preserved melanosomes and impressions in the rock matrix, measurements were taken of the long and short axis of each structure and added to a database of measurements from modern bird feathers with known associated colours (10 black, 10 brown and 10 grey samples). A canonical discriminant analysis was performed on this database which predicted the likely colour of *Anchiornis*. An example of how this method can be done is presented for a Messel feather in Fig. 11.6. Samples from different areas of *Anchiornis* were predicted as black, brown and grey with different but generally high P-values. This was used to create a complete reconstruction of the plumage of this dinosaur (Fig. 11.9a, b). The body of the animal was predicted as a mixture of grey and black with unpigmented white bands on the fore and hind wings with spangled, black tips. Melanosomes taken from the feathers on the distal crown feathers were particularly small, clustering with rufous-red feathers in the brown category in the canonical analysis. This contrast to the body melanosomes suggests a display function. This method of statistically comparing fossil melanosome morphologies to extant feather melanosomes with known colours has become the standard for many palaeocolour predictions (Fig. 11.6).

Further studies of dinosaur integumentary structures expanded the extant colour database to include iridescence as a category. Generally,

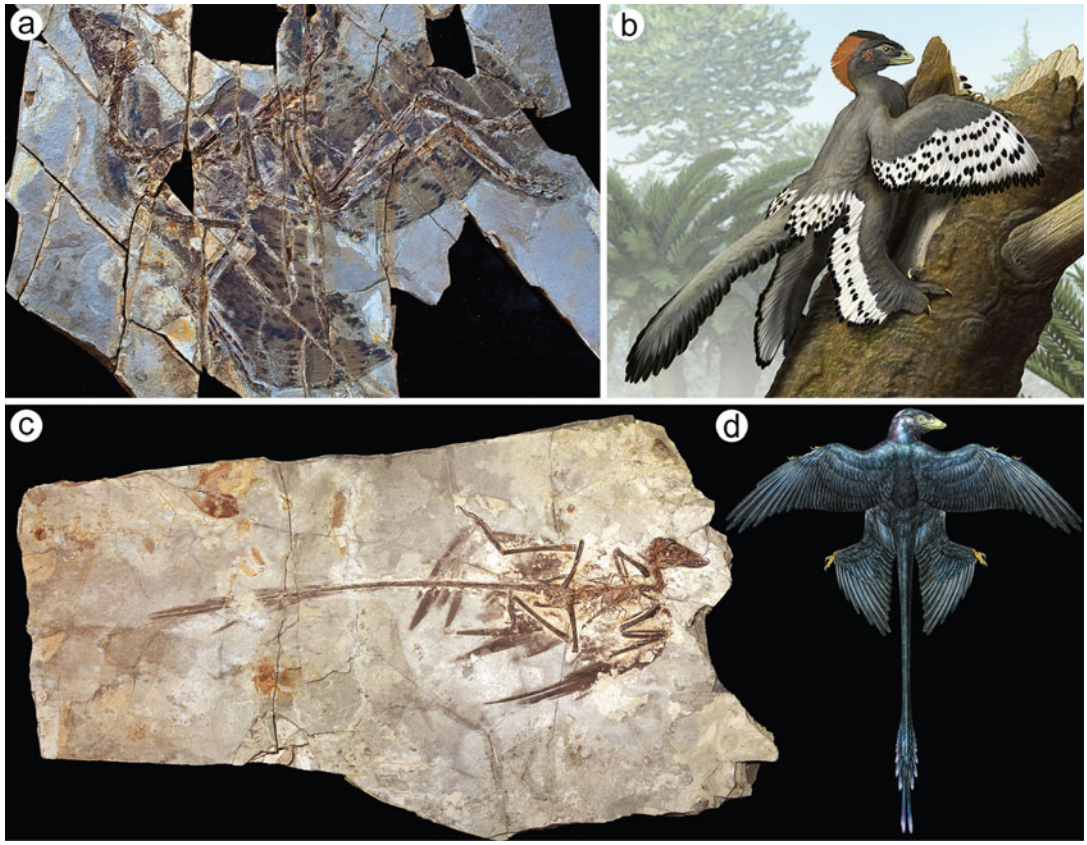

Fig. 11.9 Palaeocolour reconstructions of paravian dinosaurs. (**a**) *Anchiornis* from the Middle Jurassic of Liaoning, China. (**b**) Palaeocolour reconstruction of *Anchiornis* based on melanosome sampling showing a black and grey body with mottled wings and a rufous head crest. (**c**) *Microraptor*, a four-winged dinosaur from the Early Cretaceous of Liaoning, China. (**d**) Palaeocolour reconstruction of *Microraptor* showing subtle corvid-like iridescence based on melanosome sampling. Reconstructions courtesy of Robert Clark (**a**), Carl Zimmer (Commissioner) and Carl Buell (Illustrator), from 'Evolution—making sense of life' (Zimmer and Emlen, Roberts and Co. Publishers) (**b**) and Mick Ellison (**c**, **d**)

iridescent melanosomes are consistently longer and more skinny than black melanosomes apart from in certain clades such as hummingbirds (Fig. 11.1h, j). These works have shown that some dinosaurs likely exhibited iridescence in a similar fashion to modern birds. In 2012, Li et al. looked at the paravian *Microraptor gui*, an unusual 'four-winged' dinosaur from the Jehol Biota. The morphology of the melanosomes in *Microraptor*, being relatively longer and thinner and preserved as aligned impressions, predicted them as iridescent. The limited evidence of the original keratin nanostructure, and thus structural colouration, but with the dense end-to-end arrangement of the melanosomes in *Microraptor* allowed for a conservative interpretation that it would have most likely exhibited thin-film iridescence. This type of iridescence is common to some extant birds such as members of the family Corvidae (Li et al. 2012; Lee et al. 2016), which only show weak iridescence (Fig. 11.9c, d). As aforementioned, since the overlying keratin layer which is integral in determining exact iridescent hues does not fossilise, this cannot be inferred from the fossils. From this reconstruction, inferences were also made about the potential ecology of *Microraptor*. Previous analysis of the scleral ring morphology of the dinosaur indicated

a nocturnal lifestyle; however, dark glossy plumage is more common in diurnal modern birds (Li et al. 2012). More recently, another paravian, *Caihong juji* (Hu et al. 2018), was described with solid platelet-shaped melanosomes, which is a feature only known from brightly iridescent extant birds such as hummingbirds and tree swifts (Fig. 11.1j), thus extending this colour-producing feature back into the Jurassic.

More recent studies utilising palaeocolour have started to further explore the intricacies of behaviour and ecology in dinosaurs outside of Maniraptora. Integumentary structures suggested as potentially homologous to feathers are present in certain ornithischian taxa (Zheng et al. 2009; Godefroit et al. 2014) as well as the so-called protofeathers present in basal theropods (Chen et al. 1998; Rauhut et al. 2012). These structures have also been contended as possible decayed scales or collagen fibres rather than feathers of feather antecedents (Lingham-Soliar et al. 2007). The presence of melanosomes preserved in these features (Zhang et al. 2010; Godefroit et al. 2014) however makes it possible to characterise them as genuine integumentary appendages rather than dermal collagen (which does not contain melanosomes). Further, significant issues surrounding the identification of the structures as dermal in origin have been highlighted (Smithwick et al. 2017b). Along with likely feathers (or at least feather homologues) in some early theropod dinosaurs, certain cases of exceptional preservation of genuine scales with original melanised colour patterns have been described.

Fossil colour patterns in these ancient and ground-dwelling dinosaurs are able to provide important clues to the nature of the terrestrial predator-prey landscape and how these adapted to it. A well-preserved specimen of the small ceratopsian *Psittacosaurus* sp. from the Jehol Biota preserves visible colour patterns (Lingham-Soliar and Plodowski 2010; Mayr et al. 2016; Vinther et al. 2016). SEM imaging of samples of dark patches on the externally scaled integument of *Psittacosaurus* revealed abundant melanosome impressions which resembled phaeomelanosomes and were predicted as being brown in quadratic discriminant analysis (Vinther et al. 2016). The distribution of the melanosome-containing organic material exhibits distinct patterns that resemble those seen in extant animals, such as stripes, spots and countershading (Fig. 11.10a). Countershading is a common form of camouflage in modern animals which acts to reduce the three-dimensionality of an object by optically flattening the appearance of the body by reducing self-shadowing (Thayer 1896; Rowland 2009; Allen et al. 2012; Vinther et al. 2016). Self-shadowing is an important visual cue (shape from shading) in practically all vertebrate visual systems (Allen et al. 2012). Unpigmented scales are also discernible across the whole body of *Psittacosaurus* by their modest relief, but they preserve as films of calcium phosphate (see above) that fluoresce in UV- and laser-stimulated fluorescence (LSF) imaging (Vinther et al. 2016). To understand the distribution of the observed colour patterns, an anatomically accurate 3D model was made of dinosaur which had the pigment distribution carefully projected onto it (Fig. 11.10b). This allowed further investigation of the countershading pattern and in turn the likely habitat that the animal live in.

Studies have shown that there is distinct relationship between extant animals living in closed versus open habitats and the transition in countershading, being sharper and higher on the body when directly illuminated versus more gradual and further down the body in closed habitats (Allen et al. 2012). To understand the light environment that the countershading gradient of *Psittacosaurus* would have been best adapted for, a further 3D model (painted grey) was imaged under different lighting conditions found in open and closed habitats. By comparing the shadows produced under each lighting condition with the actual preserved colour patterns, it was shown that *Psittacosaurus* would have been best adapted to living in a closed forested habitat.

The principals behind this study were extended to a ground-dwelling feathered non-avian theropod dinosaur from the same deposits by Smithwick et al. (2017a). *Sinosauropteryx prima*, one of the first dinosaurs to have its colour deduced from melanosome imaging (Zhang et al. 2010), also shows a darker dorsum and lighter (likely unpigmented) ventrum indicating a countershaded pattern (Fig. 11.10c). Reconstructions of the colour pattern across the

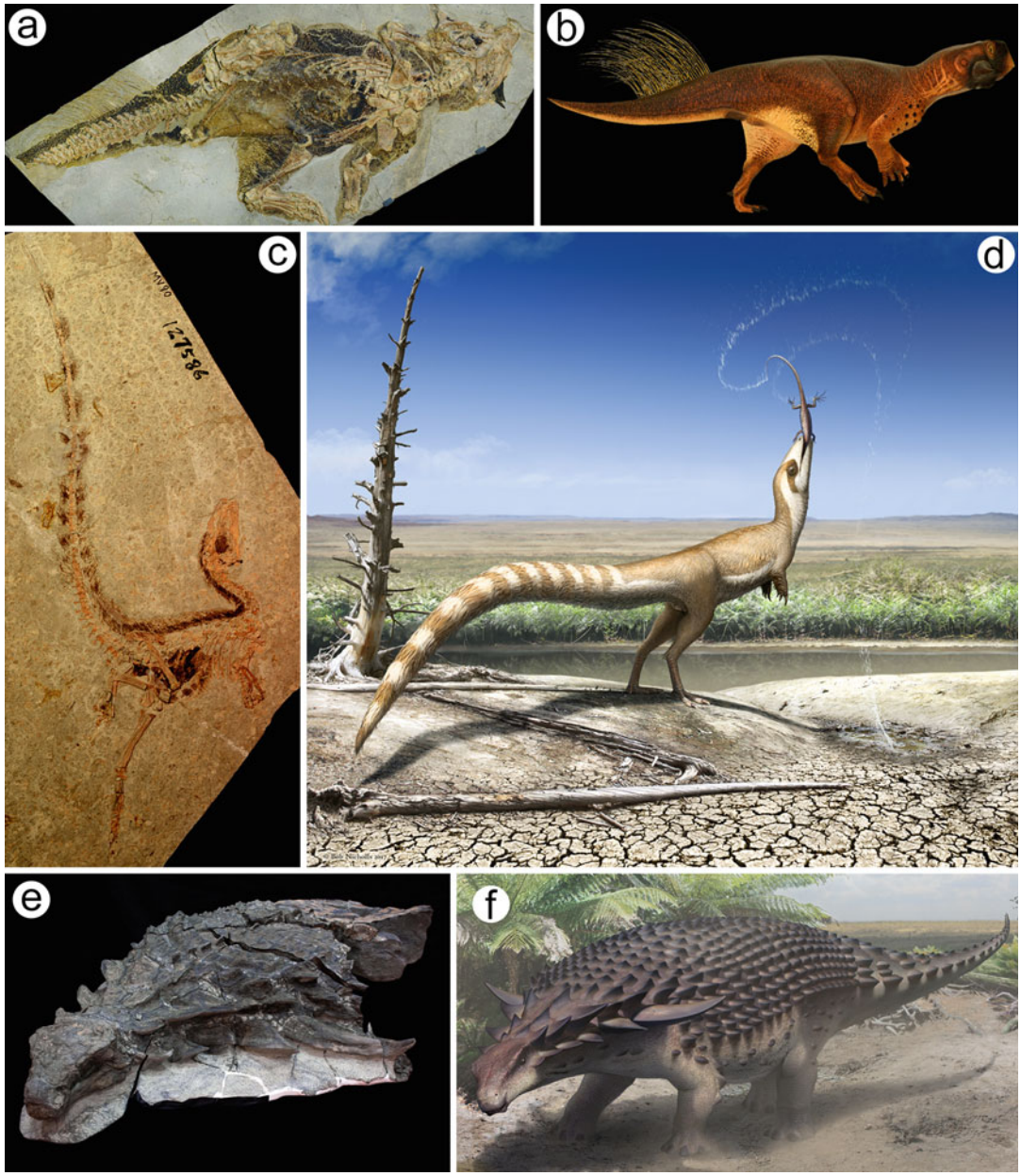

Fig. 11.10 Palaeocolour reconstructions of non-maniraptoran dinosaurs. (**a**) *Psittacosaurus*, a ceratopsian from the Early Cretaceous of Liaoning, China. (**b**) 3D palaeocolour model of *Psittacosaurus* based on melanosome sampling and pigment distributions showing a low countershaded pattern and dark brown hue. (**c**) *Sinosauropteryx*, a non-maniraptoran theropod from the Early Cretaceous of Liaoning, China. (**d**) Palaeocolour reconstruction of *Sinosauropteryx* showing countershading, a striped tail and a 'bandit' eye mask, based on the distribution of pigmented feathers across the body. (**e**) *Borealopelta*, a large armoured ankylosaur from the Early Cretaceous of Alberta, Canada. (**f**) Palaeocolour reconstruction of *Borealopelta* showing a phaeomelanised countershaded pattern, based on the distribution of pigment across the body and the chemistry of the preserved melanin. Reconstructions courtesy of Robert Nicholls

dinosaur (Fig. 11.10d) were again compared to predicted optimal countershading patterns based on 3D models imaged under different conditions. This time however, a countershading pattern more closely matching that predicted for an animal living in an open habitat was seen, despite the fact that both *Sinosauropteryx* and *Psittacosaurus* came from the same deposits. This suggests that the Jehol Biota was made up of a range of different habitat types with different animals adapted to different conditions. Other important features of the plumage of *Sinosauropteryx* were also described in detail in this work. These included a previously noted banded tail (Zhang et al. 2010), which is a very common plumage pattern seen in modern birds and is associated with both social signalling and camouflage (Marques et al. 2016), and an eye stipe across the face (Fig. 11.10c, d). This feature is informative with regard to bird evolution, as it is a common feature seen in many modern bird taxa and is known to be associated with both camouflage (hiding the presence of the eye) and as an anti-glare device to protect the eye from the sun (Ficken and Wilmot 1968; Bortolotti et al. 2006).

Countershading has also recently been observed in a large Cretaceous nodosaurid ankylosaur *Borealopelta markmitchelli* (Brown et al. 2017). This provided an opportunity for understanding the non-actualistic nature of Mesozoic predator-prey landscapes. The frequency of countershading occurring in living terrestrial mammals drops with increasing body size and is lost above 1000 kg. This is due to the safe haven provided by gigantism at this threshold. Showing that a heavily armoured ornithischian dinosaur, estimated to have weighed more than 1300 kg, was countershaded (Fig. 11.10e, f) demonstrates the difference in the nature of the predator-prey balance in the Mesozoic to today. This was likely due to the presence of large theropodan predators that meant the safety of large body sizes was only effective at even greater magnitudes than would be necessary today, as exemplified by the giant sauropods (Brown et al. 2017).

Fossil colour patterns therefore provide important insights to extinct ecologies, which would be limited from conventional lines of evidence, such as osteology, trace fossils and stable isotopes. As our ability to reconstruct colour in extinct taxa improves, a more comprehensive picture of the past landscape is becoming clearer.

11.5 Limitations

While a wealth of information on past animal colour has been revealed since the discovery of melanin preservation in birds and extinct dinosaurs, there are currently limitations. These include inferences of specific hues, detection of other pigments and some taphonomic considerations.

Although melanin is the most common pigment utilised by vertebrates for colouration, the myriad of less common pigments contributes a significant extra gamut of possible colours (McGraw 2006a, c). By contrast, melanised colours are limited to black, browns, rufous reds and greys (McGraw 2006b).

Carotenoids are the most widespread pigment in extant avian clades after melanin. This pigment is taken up through the diet and appears with little phylogenetic constraint in different groups. Passerines most commonly exhibit carotenoid-based patches of plumage in about 40% of taxa (Thomas et al. 2014b), while in non-passerines it is much more restricted to only 13% (Thomas et al. 2014b). It is likely that dinosaurs could have exhibited carotenoid-based plumage and integument. It is a common feature in other diapsids. The pigment does have a preservation potential (Damsté and Koopmans 1997; Summons 2014), but is not hosted inside organelles with a preservation potential as the pigment does not form polymerised macromolecules like melanin does. Carotenoid preservation, or the vesicles containing them, has been proposed in a Late Miocene snake (McNamara et al. 2016b), but no evidence of the pigment has been found in any fossil feather (Thomas et al. 2014a; Vinther 2015a), which may be due to preservation and its utmost rarity. A number of other non-melanin pigments found in modern birds are clade specific

(e.g. psittacofulvins in the Psittaciformes and green turacoverdin in turacos) (McGraw 2006c) and are therefore unlikely to have been present in any birds outside of these groups. Whether any extinct birds or other dinosaurs had their own unique class of pigments is an open question, but given the rarity in modern birds, it is unlikely.

In order to detect non-melanin pigments, mass spectroscopic or other chemical methods would have to be employed, and most of these are highly destructive. Porphyrins have been characterised compellingly with the otherwise less diagnostic ToF-SIMS (Greenwalt et al. 2013). How much of a concern is this when proposing broad colour patterns from a non-avian dinosaur?

First, these pigments are very rare as previously mentioned. Hence, the likelihood of having to entertain non-melanin pigments outside of passerines, turacos, owls and parrots is small. Second, co-occurring melanin pigments mask the colour of these pigments (McGraw 2006a, b, c; Vinther 2015a). Hence, only feathers lacking melanosomes are likely to have been either white or patterned with alternative pigments. It is possible that labile non-melanin pigments could have been present in these presumed unpigmented areas and have since been lost through diagenesis. However, the most parsimonious interpretation would be that these regions could have been white, given its higher abundance than these pigments. Alternatively, one can entertain exploring for these pigments in these particular regions of the body. However, if the white region forms a dorsoventral gradient, it is most likely that it represents countershading transition as this is one of the commonest colour patterns in modern animals (Rowland 2009).

While pigments other than melanin have been found in certain fossils and sediments [e.g. flavonoids in leaves (Rieseberg and Soltis 1987) and geoporphyrins, derived from haem, in a mosquito (Greenwalt et al. 2013)], assigning them as endogenous to a specific fossil is often problematic due to the propensity of the pigments to remobilise during decay and diagenesis (Vinther 2015a). While it may be possible to find other pigments in fossil birds and dinosaurs, ruling out contamination from remobilisation (e.g. from decaying algae) would require careful comparison of integumentary features to surrounding sediments (Vinther 2015a).

Another limitation of palaeocolour reconstructions is the preservation potential of keratin. As keratin is lost early on in diagenesis [e.g. within decades to millennia in archaeological sites of otherwise promising preservation potential (O'Connor et al. 2015)], original non-iridescent structural colouration that is formed via light-scattering air bubbles inside the keratin is also lost (Saitta et al. 2017). As outlined previously, iridescence, which is generated by organised melanosomes, can be identified, however, through either the preserved arrangement of the melanosomes (Vitek et al. 2013) or through their characteristic shape (Li et al. 2012; Hu et al. 2018).

As it stands, palaeocolour can only provide information on broad hues and iridescence. Non-iridescent structural colour cannot be identified through melanosome preservation (Babarović et al. 2019), and detecting albeit rare non-melanin pigments is complicated. However, inferences about distinct colouration strategies have been performed from fossils such as display (Li et al. 2010, 2012) and camouflage (Vinther et al. 2016; Brown et al. 2017; Smithwick et al. 2017a). In addition, broad-scale colour patterns such as countershading, stripes and spots can be highly informative as to an animal's ecology and behaviour irrespective of the precise hues.

11.6 Conclusions

Over the last decade, palaeocolour has evolved significantly as a discipline. The preservation potential of melanin and other pigments under exceptional circumstances has allowed for inferring aspects of dinosaurian appearance and ecology that was thought to be impossible. While palaeocolour is limited to a few fossils from few localities, it has shown its potency for contributing crucial input to evolutionary and ecological studies of extinct ecosystems.

Palaeocolour has contributed significantly to our understanding of the evolution and origin of avian plumage, and its colour gamut and many discoveries are still to be made. Colour

reconstructions have helped to advance our knowledge of the predator-prey landscape in the Mesozoic, further highlighting major differences to today, but also some important similarities. Understanding which types of camouflage were present and in which groups helps to elucidate how Jurassic Park would have played out, if the book were written today.

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