The aim of palaeontological research is to decipher the evolution, biology and function of life on Earth by studying the fossil remains of organisms preserved in the geological record. Most often the material that survives over tens of millions of years is typically hard, biomineralized tissue such as bone and shell. This is mostly due to the inherent robust physical and chemical properties of such hard biomaterials. ‘Soft tissues’ including hair, feathers, skin, internal organs, cuticles and plant matter rapidly degrade in most environments, but in exceptional and isolated circumstances, these less durable biomaterials can be preserved over millions of years. The morphological details revealed by such preservation have not only allowed palaeontologists to interpret and reconstruct how many ancient and now long-extinct organisms may have appeared, but have also provided insight to their biology, ecology, behaviour, physiology and possible interactions with their environment.

However, one extremely important characteristic of ancient organisms has until recently been conspicuously elusive: colour. Colour is known to play a vital role in many facets of the behaviour of living organisms, including adaptations to specific environments such as camouflage, and also has direct impacts upon ecology, such as communication and sexual selection. Indications of colour in fossilized organisms may help to resolve these characters in fossil remains and how they may have influenced the evolution of a species.

In some very rare cases, fossils may show a visible indication of pigment patterns such as spots or stripes, but positive identification of specific colour or pigment mechanisms has so far remained extremely difficult. This is in part due to the limitations, such as detection limits, of analytical techniques that may be used to search for pigments, and partly due to the entrenched belief that the chemistry and micromorphology of a living organism is altered or replaced by a number of fossilization processes to such a degree that all useful information has been lost.

In recent years, a growing number of studies have investigated the potential preservation of pigments within fossil tissues, with particular emphasis falling upon melanin. The focus on melanin is a result of the fact that melanin is almost ubiquitous in life on Earth, having been identified in a wide variety of living organisms including most chordates (Figure 1), many groups of invertebrates including echinoderms, sponges, molluscs and arthropods, and is also observed in plants, fungi and bacteria. Its presence in both highly derived and more primitive species indicates that melanogenesis represents a very ancient biosynthetic pathway utilized by the earliest life on the planet. Indeed, the tyrosinase enzyme, a key copper-bearing participant in melanogenesis, may have evolved from an extremely primitive biochemical function going back over one billion years (Van Holde et al., 2001). Additionally, the focus on melanin is also a result of the fact that unlike some of the other forms of pigmentation, melanin is stored in organelles – melanosomes – that form diagnostic shapes indicative of the type of melanin pigment contained within. This structural component, albeit microscopic in scale, has the potential to be preserved over millions of years and may be detectable by standard imaging techniques such as scanning electron microscopy (SEM).

Zhang et al. (2010) was one of the first of several studies by different groups to identify melanosomes preserved in fossil tissues. Using SEM imaging, they provided evidence of melanosome like structures, rod-shaped eumelanosomes (~2 microns in length) and spherical phaeomelanosomes (~1 micron in diameter) preserved in the feathers of derived theropod (predatory) dinosaurs and birds from the Cretaceous Period (~120 million years old, China). This and several subsequent studies proceeded to present whole-organism pigment reconstruc-

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**Figure 1.** Basic phylogeny of the chordates, with groups in which melanin has been identified, shown in bold. A version of this figure with full references is available at www.chemicalghosts.org. † = Extinct.
fossil structures as melanosomes is still under active debate with a study by Moyer et al. (2014), published soon after Lindgren et al., convincingly arguing a bacterial origin for structures which had been interpreted by others as melanosomes. Despite the melanosome/bacterial debate continuing (Moyer et al., 2014), newly published studies are starting to assign these structures as melanosomes by default (Li et al., 2014) without presenting corroborating data for the SEM imaging from each specimen studied. For these reasons, including chemical analysis as Lindgren et al. have done is tremendously important, as relying on structure alone is fraught with peril.

Another important limitation of studies that utilize microstructural imaging is that only a small fraction of a specimen can be analysed. Every study published so far using SEM images, including Lindgren et al., has examined <1% of the remnant soft tissue and then extrapolated pigment patterns over large areas. By analogy, examination of <1% of a zebra could lead one to seriously misinterpret pigment distribution in that species. Additionally, it is likely that in many fossil samples, structural preservation will not be uniform, thus creating further uncertainty in reconstructions of pigment patterns derived from melanosomes.

While structural information is important, given the technical limitations of the SEM (sample size, high vacuum, slow data acquisition rates), the interpretations that can be derived will therefore be equally limited. The technology exists to be able to perform elemental analysis of 100% of a fossil specimen (Wogelius et al., 2011), which can help avoid the problems of positively discriminating microbial structures from melanosomes by correlating structure with the remnant chemistry of an entire specimen. However, chemical approaches are also not without their obstacles. Differentiating between geologically and biologically sourced chemical signals is challenging, but so far the data show that original chemical retention may be more common than previously believed.

Overall, the various studies and approaches strongly indicate that remnants of pigment, such as eumelanosome, phaeomelanosome and the chemical residue of eumelanin, can indeed be preserved for over a 100 million years in exceptionally preserved fossils. But additionally, the current body of work suggests that pigment studies must make use of a suite of techniques to draw supportable conclusions and that colour reconstructions of entire organisms based on extrapolation from limited sampling may not be reliable.

The study of pigments through time is an exciting area of research that has garnered widespread attention, both by academics and the general public, but is still in its relative infancy.

Results presented by Lindgren et al. give us confidence that an integrated chemical and structural approach to analysing soft tissue fossils can indeed resolve pigment residue. Due to the fact that eumelanin residue is resolved in three fossil marine reptiles, the authors postulate that melanization in this group may provide a clue to the selective pressures acting on these organisms including homeothermy. This demonstrates how resolving pigmentation may provide critical information regarding evolution which has thus far been beyond the reach of palaeontology. However, because pigmentation is such an important aspect of many forms of ancient life and because of the serious difficulties involved in analysing specimens that have endured aging over deep geological time, research in this area will continue to fascinate and frustrate for years to come.

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