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The cause of 50 million-year-old colour

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Multilayer reflectors cause structural, 'metallic' colours in a diversity of animals today, yet are unknown in extinct species. We identify a multilayer reflector, causing structural colour, in a 50-million-year-old beetle from Messel, Germany. It is proposed that the original material of this reflector has been preserved, although this is not a precondition for determining original colours from ancient multilayer reflectors. Therefore, the potential exists to reveal the original colours of other (particularly arthropod) extinct species.

Keywords: ancient colours; multilayer reflectors; beetles; Messel fossils

1. INTRODUCTION

Today, many animals, including birds, butterflies and fishes, display bright metallic-like colours that result from multilayer reflectors (Land 1972; Parker *et al.* 1998*a*). However, until now multilayer reflectors have been unknown in extinct animals. We demonstrate a case of such ancient structural colour, and reveal the potential to reconstruct the original colours of other extinct species.

Light may be strongly reflected by constructive interference between reflections from the different interfaces of a stack of thin films (of actual thickness d) of alternately high and low refractive index (n). For this to occur, reflections from successive interfaces must emerge with the same phase and this is achieved when the so-called 'Bragg condition' is fulfilled. At normal incidence, the wavelength observed under white incident light is $4(nd)$ (Land 1972). The layering within the cuticle of insects, for example, alternates between materials of relatively high and low refractive indices, and can occasionally evolve the layer thicknesses to fulfil the Bragg condition and cause iridescence (Land 1972; Parker *et al.* 1998*a*).

The Middle Eocene (50 million years ago) oil shales of Messel, Germany, are well renowned for their abundant fauna and flora preserved in exceptional detail, including both hard and soft parts (Schaal & Ziegler 1992). Messel rock contains 40% water and has a high content of original organic matter (Schaal & Ziegler 1992). Unusually, beetles have been found at Messel that have retained their original colour, although the colour disappears on drying. The cause of this colour has until now been unknown and will be explained here.

2. METHODS

A section of blue elytron (wing cover) from an unidentified beetle from Messel (figure 1*a*), collected in 1998 and stored in distilled water, was analysed immediately after collection as follows. The experimental optical reflectance was measured using a retroreflection spectrometer. Part of the section was fixed overnight in 2.5% glutaldehyde with 2% paraformaldehyde in 0.1 M sodium cacodylate buffer then washed in 0.1 M sodium cacodylate buffer. The section was subsequently post-fixed in 1% osmium tetroxide then dehydrated through an alcohol series and finally 100% acetone. After embedding in Spurr resin, 60 nm sections were cut. The sections were stained using lead citrate and uranyl acetate, then examined in a transmission electron microscope (where the high index layers of arthropod reflectors appear dark using this stain; Parker *et al.* 1998*b*).

Another part of the elytron, including the surrounding rock, was critical-point dried and coated in gold. This was examined in a scanning electron microscope. X-ray analysis was used to reveal the chemical composition. For this, part of the elytron was carbon coated and examined using an Oxford Link Isis 200 energy dispersive spectrometer (with a silicon/lithium detector and beryllium window) that detects elements with an atomic mass above that of oxygen. Results were interpreted using SPEEDMAP software (map based on counts alone, with no element corrections). X-ray analysis was also conducted on an elytron of the extant scarab beetle *Calloodes grayanus*, a structurally coloured species, for comparison and as a test of whether the original organic material had been preserved in the Messel elytron.

The ultrastructure of the cuticle was optically modelled using the programme TFCALC (employing the Matrix method of Macleod (1969)) and a predicted reflectance spectrum was obtained.

3. RESULTS

The experimental optical reflectance spectrum of the elytron revealed a peak at 480–505 nm (figure 1*b*), corresponding to human blue, with a maximum of 63% reflectivity. The exocuticle of the elytron examined contains five layers of electron-dense (high refractive index) material, each separated by a layer of low refractive index material (figure 1*c*). Each layer was flat and smooth at the nanoscale (figure 1*d*). The high and low index layers were *ca.* 80 nm and 95 nm thick (d), respectively (±5 nm). Refractive indices (n) of the two layers were taken as 1.56 and 1.33, respectively, in accordance with living beetle tissue (essentially chitin (plus other compounds) and water; Land 1972; Hariyama *et al.* 2002). The computer model of this structure predicted a peak at 490 nm with 50% reflectivity (figure 1*b*).

The high index layer of the Messel beetle reflector contained a carbon-to-oxygen ratio of 1.9 : 1, as determined from X-ray analysis. In *C. grayanus* this ratio was 2.9 : 1, whereas in the rock surrounding the Messel elytron it was 0.4 : 1.

4. DISCUSSION

The match between the experimental optical and predicted reflection spectra is excellent. Therefore, we conclude that the cause of this colour is a multilayer reflector known as a quarter-wave stack (see Land 1972; Parker *et al.* 1998*a*). The very slight discrepancy between experimental optical and predicted reflectance profiles is probably due to the larger area analysed by the spectrometer (*ca.* 1 mm^2) than the model (a 60 nm thick section) and a variation in layer quantity throughout the section. The outer surface of the elytron is broken (figure 1*d*) and it is possible that there were originally more layers in the stack, to provide a relatively brighter colour, although the hue would have remained constant (see Parker *et al.* 1998*a*).

The close match in carbon-to-oxygen ratio within the high index layer of the Messel beetle reflector and the extant beetle *C. grayanus*, compared with that of the rock surrounding the Messel elytron, suggests that the original

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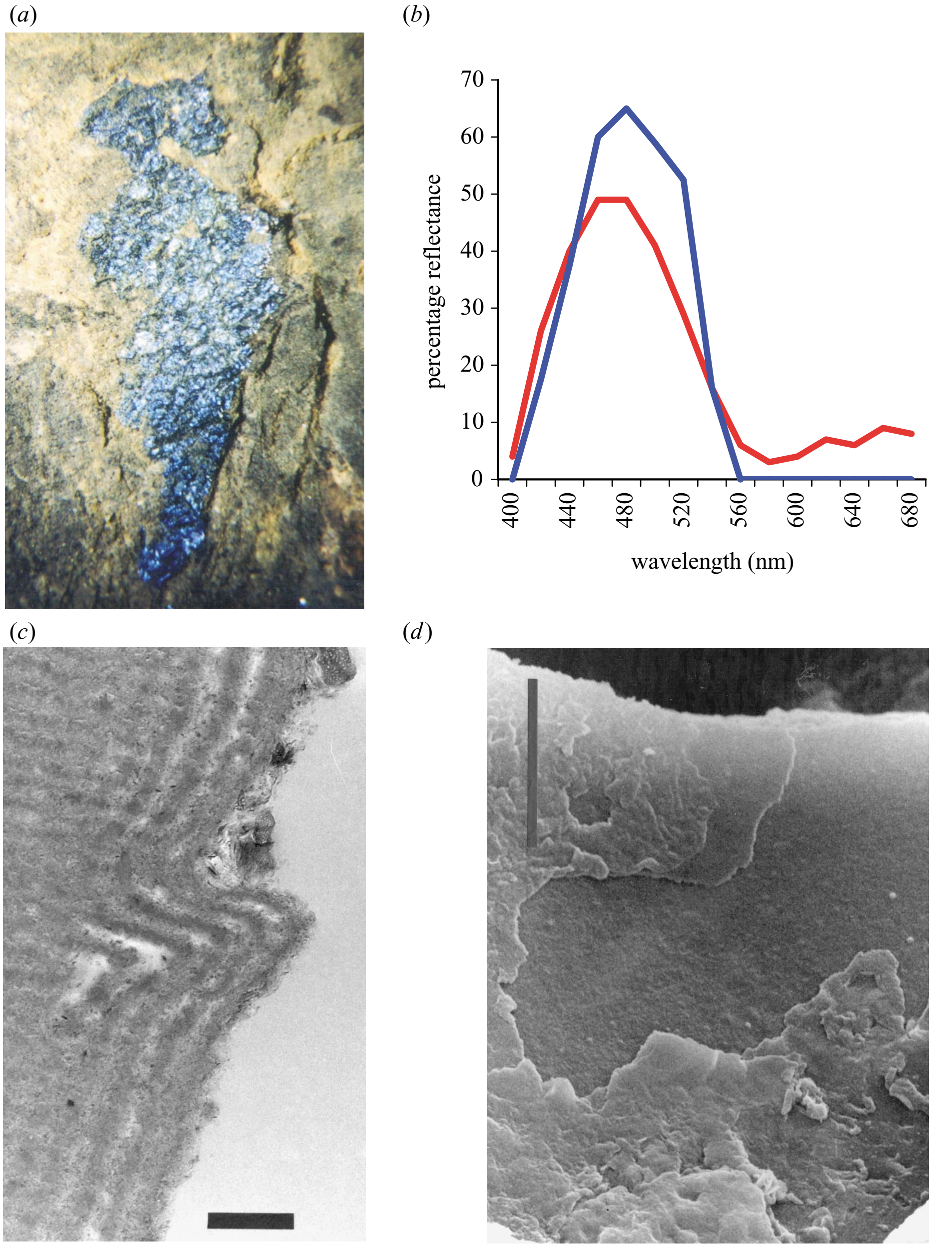

Figure 1. Elytron of an unidentified beetle from Messel (50 million years ago). (*a*) Light photograph of the specimen examined, embedded in rock. The length of the specimen is 4 mm. (*b*) Graphs showing the experimental optical (blue line) and predicted (red line) reflectance from the elytron. (*c*) Transmission electron micrograph of the outer surface of a 60 nm wide section of elytron showing the fine lamination (running vertically in the picture) in a cross-section. The surface of the elytron has become wrinkled; the bend shown here represents a wrinkle. The 'horizontal' lines are an artefact of cutting. (*d*) Scanning electron micrograph of the outer surface of the elytron, perpendicular view, showing internal fine layers where the outer layers are broken. Scale bars, (*c*) 0.5 µm; (*d*) 5 µm.

organic material has preserved in the Messel specimen examined. In addition, the (principally) water (low index) layers have been preserved. Hence, when the Messel beetles are dried the low index layers of the reflector become altered, which explains the colour disappearance. The same happens in some extant beetles on death. However, that the original colour has been preserved in this 'fossil' beetle provides an excellent test for reconstructing ancient colours based on fossilized multilayer reflectors.

Combining information on the structure of reflectors in extinct species, along with those of their living relatives, with a cladogram for that taxon (see Parker 1995), could provide information on the evolution of behaviour. Eyes were common in the fauna cohabiting with this Messel beetle, probably including conspecifics and predatory beetles, birds and amphibians. Today some beetles employ structural colour with specific characteristics for predator avoidance or mating (Schultz 1986) or camouflage (Parker *et al.* 1998*a*).

The potential for determining colour resulting from multilayer reflectors from ancient, extinct animals does not end with Messel beetles. Finely laminate structures within external parts have been preserved and illustrated in, for example, Tertiary arthropods from Riversleigh, Australia (Duncan *et al.* 1998), Cretaceous conchostracans from the Crato Formation of Brazil (Martill 1993) and Silurian trilobites from Wales (Dalingwater *et al.* 1993). No water remains in these fossils and in some cases the original materials have been replaced by opaque materials. Under these conditions, multilayer reflectors would no longer give rise to colour, but the original structure remains and this can be modelled using computer programs to provide a predicted reflectance spectrum and, consequently, original coloration. This paper shows that such a model can be accurate.

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