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Iridescent colour production in hairs of blind golden moles (*Chrysochloridae*)

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Relative to other metazoans, the mammalian integument is thought to be limited in colour. In particular, while iridescence is widespread among birds and arthropods, it has only rarely been reported in mammals. Here, we examine the colour, morphology and optical mechanisms in hairs from four species of golden mole (Mammalia: Chrysochloridae) that are characterized by sheens ranging from purple to green. Microspectrophotometry reveals that this colour is weak and variable. Iridescent hairs are flattened and have highly reduced cuticular scales, providing a broad and smooth surface for light reflection. These scales form multiple layers of light and dark materials of consistent thickness, strikingly similar to those in the elytra of iridescent beetles. Optical modelling suggests that the multi-layers produce colour through thin-film interference, and that the sensitivity of this mechanism to slight changes in layer thickness and number explains colour variability. While coloured integumentary structures are typically thought to evolve as sexual ornaments, the blindness of golden moles suggests that the colour may be an epiphenomenon resulting from evolution via other selective factors, including the ability to move and keep clean in dirt and sand.

Keywords: structural colour; biophotonics; hair

1. INTRODUCTION

Iridescent coloration, which changes hue with the angle of observation or light incidence, is widespread in metazoans [1]. In contrast to pigment-based colours, iridescent colours are produced by coherent scattering of light from nanostructures that are highly ordered at the nanometre scale in one, two or three dimensions [1]. The simplest nanostructures are single or multiple layers of materials with different refractive indices (RI) that act as thin-film reflectors in clades as diverse as beetles and spikemosses [1]. While several different

functions have been hypothesized for these flashy colours, most have focused on sexual display [2].

The colours of Mammalia are largely thought to be relatively drab [3], although non-iridescent blue skin colours produced by organized collagen fibres have been described [4]. Golden moles (Mammalia: Afrosoricida: Chrysochloridae) are blind insectivorous burrowing animals endemic to sub-Saharan Africa [5]. Their thick fur has a unique sheen that can feature green, blue and violet highlights [5]. However, the morphology and mechanism(s) producing colour in these hairs has not been described. Thus, we examined the colour and its physical basis in four golden mole species.

2. MATERIAL AND METHODS

We pulled iridescent guard hairs and non-iridescent down hairs from specimens of four golden mole species (*Amblysomus hottentotus*, *Amblysomus septentrionalis*, *Chrysochloris asiatica* and *Eremitalpa granti*; table 1) from the Museum of Vertebrate Zoology at the University of California, Berkeley. We measured reflectance of iridescent hairs using a microspectrophotometer (range 400–1000 nm; MSP300, Angstrom Sun, Acton, MA, USA) at coincident normal (90° incident light/90° measurement) with a 1 µm spot size. All measurements were averaged from 10 spectra (100 ms integration time), relative to a diffuse white standard (WS-2, Avantes Inc., Boulder, CO, USA) with dark standard subtracted. At least five measurements were collected for each species, each from a different location along the shaft.

To examine the hairs' external morphology, we used scanning electron microscopy (SEM). We mounted whole hairs with the dorsal side up on stubs with carbon tape, sputter-coated them with silver and viewed them on a JEOL SEM (JSM7401F, JEOL Japan).

To examine the hairs' ultrastructure, we used transmission electron microscopy (TEM). We prepared hairs for microscopy following standard methods [4]. We cut thick (1 µm) and thin (70 nm) cross sections using a Leica UC-6 ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany). Thick (1 µm) sections were placed on slides, stained in 5% toluidine blue and viewed on a Leica DM2500 light microscope at 100× magnification using transmitted light. Thin sections were placed on a 200 mesh formvar copper grid and viewed on a Tecnai 12 (Tecnai Company, OR, USA) TEM operating at 120 kV.

Using Image J [6], we measured the thickness of all light and dark layers in the outer cortex from three TEM micrographs for each species at five evenly spaced points. We also counted the number of layers per image, and calculated the mean thickness and number of light and dark layers.

To identify the optical basis of iridescent colour production, we used standard multi-layer thin-film optical modelling [7]. We simulated light interacting with stacks of sequential light and dark layers, with the number of layers and their thicknesses equal to the average values measured from the electron micrographs, to predict the theoretical reflective curves produced by the structures. We used the transfer matrix method as implemented in the *multi-layer* function [7] in R [8]. Hair keratin has an estimated RI of ~1.55 [9], which was used as a first approximation in all models. However, because the material constituting the dark layers and the extinction coefficient (k ; a measure of how much light a material absorbs) of hair keratin is unknown, several parameters of the model (RI of dark material; k of dark and light materials) were allowed to vary under an optimization procedure (see electronic supplementary material). Optimization was performed by minimizing the sum of squared differences between normalized values of measured and estimated reflectance at each wavelength (modified from Stavenga *et al.* [10]). We compared measured and predicted reflectance curves to determine the accuracy of modelling.

3. RESULTS

Iridescent golden mole hairs are straight and broad in their distal portion and come to a point at their distal tip. At their proximal end, they are considerably thinner, more tubular and curled. The distal ends have sheens with faintly visible colours that vary along the length of the hair (figure 1*a*). The surfaces of these distal ends of the hairs are flattened and have numerous small, compressed and irregularly arranged cuticular scales, producing a somewhat smooth surface (figure 1*b*). They are highly flattened dorsoventrally

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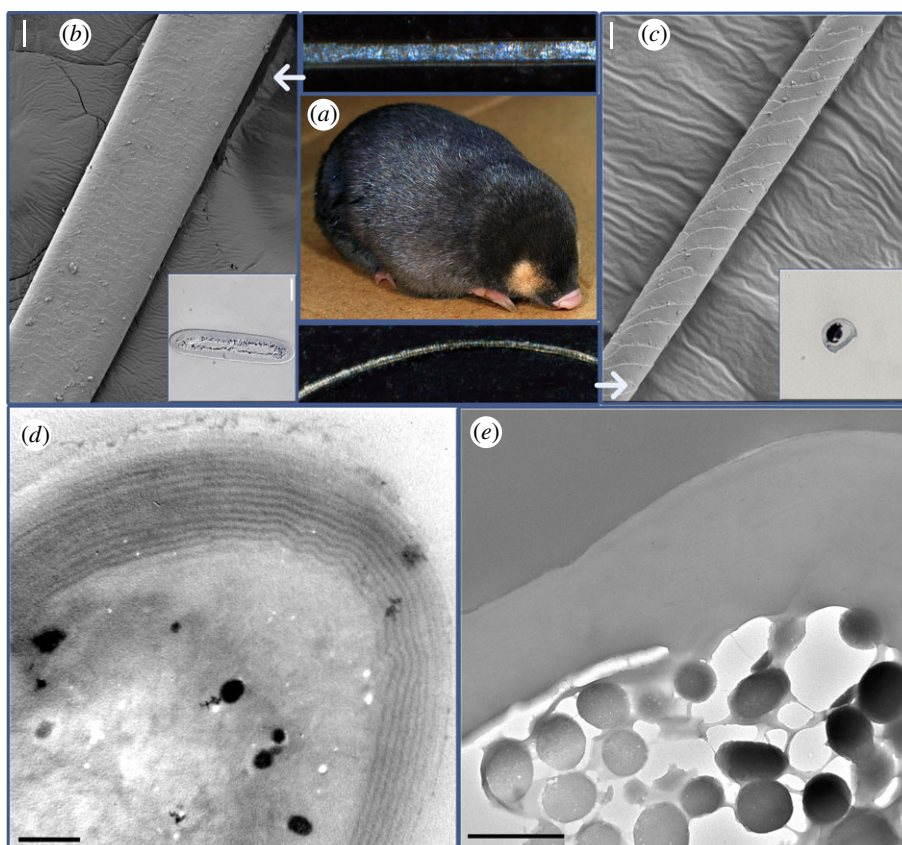


Figure 1. Structure of iridescent hairs from the golden mole species *Chrysochloris asiatica* (Photo Hamish Robertson, Iziko, South Africa, used by permission). (a) Optical microscope image of the distal end of a single iridescent (top) and non-iridescent (bottom) hair. (b,c) SEM image of the outer surface of the (b) iridescent or (c) non-iridescent hair. (b) Shows flattening and multiple small, compressed cuticular scales, while (c) shows tubular shape and large, protruding scales. Insets: Optical microscope image of a cross section of each hair, showing clear shape differences. (d,e) TEM images of a cross section of each hair, showing multiple alternating dark and light layers in the cuticle of the (d) iridescent, but not (e) non-iridescent hair over the cortex containing melanosomes. Scale bars, (b,c) 10 μm ; insets, 20 μm ; (d,e) 1 μm .

Table 1. Mean (± 1 s.d.) values of layer thickness from measurements of TEM images of each species.

species	MVZ catalogue no.	light layer (nm)	dark layer (nm)	number of layers
<i>Amblysomus hottentotus</i>	183 373	121.7 (15.3)	27.8 (6.7)	08 (0.7)
<i>Amblysomus septentrionalis</i>	81 568	237.4 (31.7)	34.3 (8.7)	03 (0.5)
<i>Chrysochloris asiatica</i>	117 920	108.6 (15.2)	30.7 (2.4)	19 (3.4)
<i>Eremitalpa granti</i>	117 919	145.8 (21.5)	20.9 (3.9)	06 (1.1)

(figure 1b) and composed, as in most hairs, of three distinctive layers—moving inward from the outer surface: a darkly stained cuticle, a more lightly stained cortex containing low densities of melanosomes, and an air-filled medulla (figure 1b,c). The cuticle contains discrete, alternating thin layers of dark (electron-dense) and light (electron-lucent) materials (figure 1c). The light layers were several times thicker than the dark layers and the thickness and numbers of layers varied between species (table 1). By contrast, non-iridescent golden mole hairs were uniformly thin, tubular, had large protruding cuticular scales (figure 1d) and did not contain multi-layers (figure 1e).

Reflectance curves of hairs were uniformly low, but considerably variable between and within species (electronic supplementary material, figure S1). The optimization procedure estimated the RI of the dark layer to be between 1.51 and 1.54, and thus a value of

1.53 was used in all models. Both materials were also estimated to have low absorbance, with values between 0.02 and 0.05 for light layers and 0.01 and 0.03 for dark layers. Low extinction coefficients are predicted because multiple oscillating secondary peaks observed in the measured spectra (electronic supplementary material, figure S1) result from light resonating within the interfaces of the layers (i.e. Fabry–Pérot interference; [1]). We therefore used values of 0.04 and 0.02 for the light and dark layers, respectively. Variation in measured spectral curves (electronic supplementary material, figure S1) complicated comparison (as in [10]), and is consistent with slight variations in the thickness (position of the main peak) or number (position and number of secondary resonating peaks) of layers (table 1). Nonetheless, in all cases, more than half matched reasonably well in shape and peak reflectance with the predicted curves (figure 2).

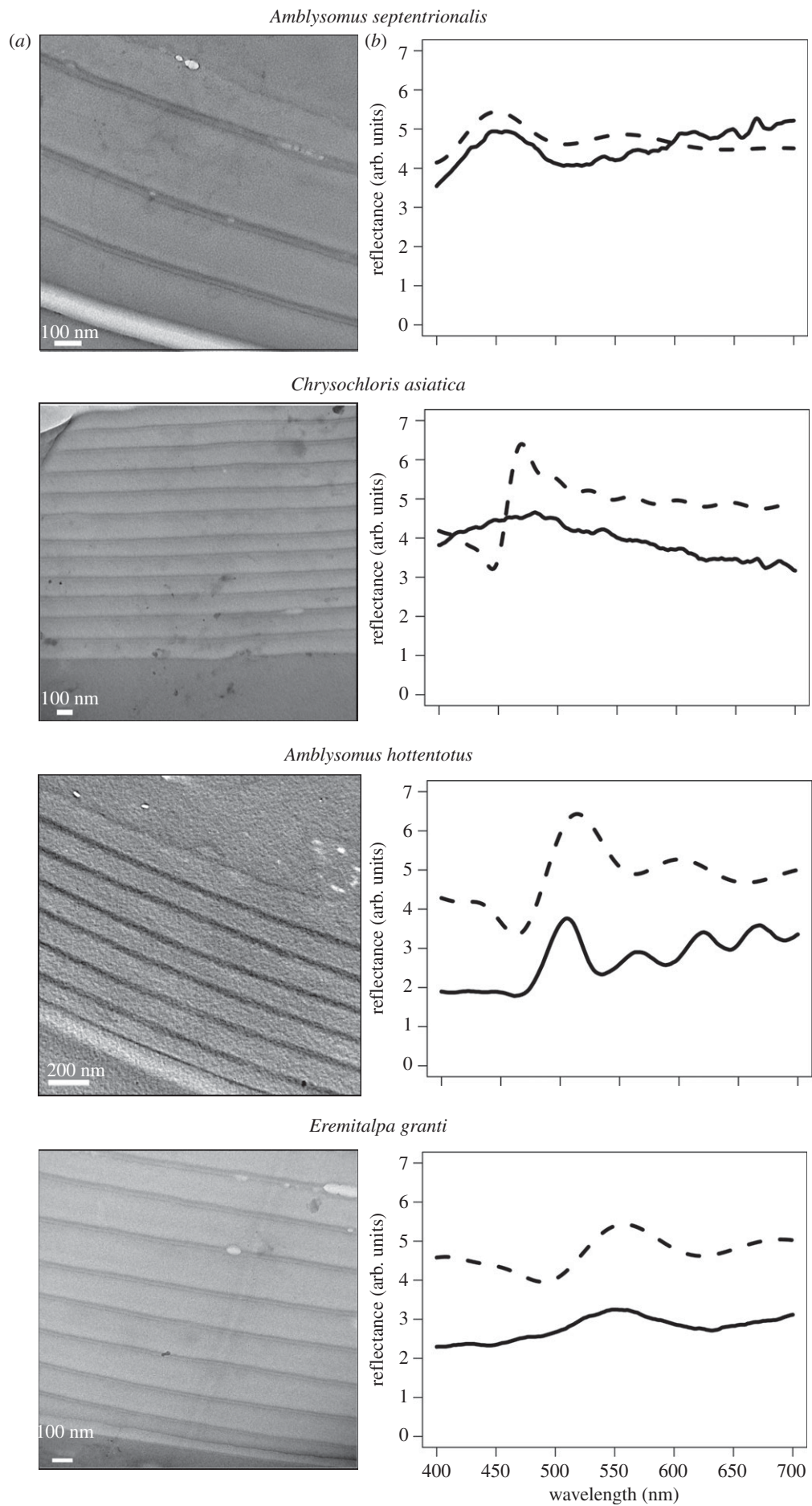


Figure 2. (a) TEM images, (b) best-fitting measured (solid line) and predicted (using optical modelling; dashed line) reflectance curves for hairs of four species of golden moles. The TEM images show dark and light layers in the cuticle of each species.

4. DISCUSSION

To our knowledge, this is the first report of the nano-structural basis of iridescent colours produced in mammal hairs. Several key morphological features contribute to this coloration. First, their flattened paddle-like shape increases the surface area available for reflection. Second, the compressed cuticular scales provide a smooth reflective surface that enhances specular reflectance. Third, the layers of light and dark materials in the cuticle act as multi-layer reflectors that produce colour through thin-film interference. Finally, the low estimated contrast in RI between these layers explains why the observed large number of repeating layers does not produce bright colours, as similar structures do in other cases [1,10]. Typical non-iridescent hairs are circular to ovoid in cross section and have large and protruding cuticular scales (figure 1; electronic supplementary material, figure S2), but may have multi-layered cuticles [11]. Those in human hairs, the most well-studied example, are larger but of the correct thickness and regularity [11] to produce iridescent colours as second-order peaks [12], suggesting that their presence is a necessary, but not sufficient condition for iridescent colour. As in barbules from iridescent feathers [1], the additional surface area provided by flattening probably contributes to the enhanced reflection from iridescent hairs.

Smooth surfaces are more conducive to iridescent colour production than are rough surfaces [13]. The enhancement of hair gloss through smoothing of scales is a well-studied phenomenon and is one of the primary mechanisms of many hair care products [13]. High specular reflectance relative to diffuse reflectance occurs when light hits a flat and smooth surface. Thus, the small size and flattening of scales on golden mole hairs enhances, while the large protruding scales on non-iridescent hairs ([11]; figure 1*d*), decreases, specular reflectance and thereby the expression of colour from the multi-layers.

These scales form the layers of the multi-layer structure, and thus their morphology is critical to colour production in golden moles in two ways. First, their thickness produces the observed iridescent colours through thin-film interference. Second, their irregular longitudinal shape likely leads to high local variability in the number of layers at different points on the hair. Changes in the numbers and/or thickness of layers can result in remarkable variation in local reflectance and consequently to the perceived colour [1]. Thus, local variation in thickness and number of scales may explain the high variability of colour and mismatches of measured and predicted reflectance within hairs.

While iridescent and other bright colours are typically thought to evolve through sexual selection, the blindness of golden moles is not consistent with this hypothesis. Furthermore, iridescent hair is not likely to function as camouflage, and it is unlikely that such conspicuous pelage would be neutrally maintained across a deeply divergent lineage [14]. Hypotheses on the evolution of iridescent golden mole hairs should explain both the presence of the multi-layer and the flattening of the hairs and scales. The colour-producing multi-layer itself may have arisen through selection for larger numbers of scales to prevent breakage through wear from

friction, and these scales may simply happen to be in the correct size range to produce colour. Flattening of the scales and hairs may streamline the profile and create a less turbulent flow, thereby easing movement through a highly viscous medium (in this case, dirt and sand). These hypotheses should be tested through future comparative and experimental work. While typically considered as functional in their own right, iridescent colours may also be byproducts of selection on mechanical or other functions.

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