The Iridescent Color of Golden Moles: Morphology and Potential Mechanisms

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ABSTRACT

We examined the physical mechanisms and morphology that may produce the iridescent sheen in the hair of golden moles (family Chrysochlorida). We observed these differences within four species of the golden mole using light microscopy, transmission electron microscopy, and scanning electron microscopy while comparing our observations to that of the morphology of the common mole (Scalopus aquaticus). The golden mole and the common mole are distantly related, yet sympatric. The iridescent hairs of the golden mole present a smooth surface as well as a flattened, elliptical shape cross section with a pattern very similar to a multilayer reflector. The common mole hair presents a circular morphology similar to the golden mole non-iridescent hair, and is not composed of a smooth surface, yet a rough surface. We conclude that the smooth hair surface and flattened shape contributes most to iridescence in golden moles.

INTRODUCTION

Iridescent color is distributed among many metazoans, and has been extensively studied in birds (Shawkey et al. 2006), fish (Lythgoe 1975), and arthropods (Shultz 1987). In every case, iridescent color is caused by coherent scattering of light from highly ordered nanostructures. This type of color is termed structural color and is distinctive from that produced by selective light absorption by various pigments. Iridescent color is exceptional compared to non-iridescent color because it changes based on the angle of incidence (Fox 1976). Iridescent color production has been found to play an important role in mating success of birds such as peacocks, as well as survival mechanisms such as corneas of teleost fish (Loyau 2007; Lythgoe 1975).

There are a variety of structural mechanisms that have been found to be associated with iridescent color production. For example, butterflies (Morphidae family) produce a blue iridescent color based on their arrangement of the microstructures of scales. This scale’s orientation as well as the refractive index of the material plays a significant role in the iridescent production in the male Morho menelaus (Berthier 2003). Beetles have layers that interchange, composed of alternating different refractive indexes with thicknesses that correspond to the wavelengths produced by light (Shultz 1987). A similar multilayer structure is responsible for the iridescent color in the elytral wing of C. raja (Noyes 2007). Multilayer reflectors are episodic and the refractive index has discontinuity between the alternating layers (Land 1972). In birds, the mechanisms producing iridescent color vary considerably, but are generally composed of an arrangement of layers composed of melanin, keratin, and air. Multilayer iridescence is a prevalent mechanism, such as in hummingbirds, but single layer iridescence has also been investigated as well in satin bowerbirds (Greenewalt 1960; Doucet et al. 2005). While several mammal species have non-iridescent skin color produced by organized collagen fibers (Prum et
as far as we know iridescent or other structural colors have never been described in mammal hairs.

Golden moles are small, blind insectivorous burrowing animals that reside in sub-Saharan Africa, typically South Africa (Meester 1976). There are nine genera consisting of twenty-one species including one species that has recently been discovered, Chrysochlorinae (Bronner & Jenkins 2005; Bronner 2000). These subterranean mammals are thermoregulators and have a notable ability to reduce energy expenditure through behavior and torpor (Jackson 2009). Golden moles are not closely related to other mole species, but instead are placed in a clade of animals including aardvarks, elephants, elephant shrews, manatees, and hyraxes. Their fur is composed of a thick undercoat and has a sheen that can feature green, blue, and violet highlights (Gorman 1990). Like most mammals, moles and shrews have overhairs and furhairs, but they have unique whiplike tips that distinguish them from very similar haired mammals such as mice and bats. Mole hairs tend to be at least 8mm in length and like most mammal hairs contain a medulla, cortex, pigment granules, and cuticle (Williams 1938; Hausman 1920); although the medulla is discontinuous along the hair (Williams 1938). The golden mole and common mole under hairs are composed of elongate cuticular scales (Hausman 1920).

The morphological basis of this color is completely unstudied, so our goal was to identify this basis and determine how weak iridescent color is produced in these species. To this end, we examine the macro- and nanostructure of four species of the golden mole (family Chrysochlorida) as well as the common mole (Scalopus aquaticus). We used spectrometry, light microscopy, transmission electron microscopy, scanning electron microscopy, and thin film optical modeling.

METHODS AND MATERIALS

We examined four species of the Family Chrysochloridae: Amblysomus hottentotus, Amblysomus septentrionals, Chrysochloris asiatica, and Eremitalpa granti. Hairs were obtained from study skins in the Berkeley Museum of Vertebrate Zoology at the University of California. Iridescent hair was pulled from the dorsal side of the specimen and non-iridescent hair was obtained from the ventral side of the specimen. The iridescent and non-iridescent hair morphology and iridescent color was examined using a dissecting scope (Leica S8APO) using a light angle of 45 degrees. We measured the reflectance of the hairs using a microspectrometer at normal incidence.

We prepared hairs for microscopy following the methods of Shawkey et al. (2003). Briefly, we dehydrated them twice in 100% Acetone for twenty minutes. The hairs were infiltrated with EPON in 15, 50, 75, and 100% consecutive concentrations for twenty-four hours for each step. The hairs were placed in moulds in an oven at 60°C for sixteen hours. Cross sections of the iridescent and non-iridescent hairs were cut using a diamond knife on a Lecia EM UC6 Ultra
Microtome. Sections of 1µm were cut and placed on slides to be viewed on a Leica DM2500 light microscope at 100x magnification using transmitted light. In addition, we mounted cross-sections on stubs with carbon tape, sputter-coated them with silver, and viewed them on a JEOL JSM7401F scanning electron microscope. Sections of 70nm were cut and placed on a 200 mesh formvar copper grid to be used for the Tecnai12 transmission electron microscope (120 kV, Tecnai Company, OR, USA).

Using Image J (available for download at http://rsbweb.nih.gov/ij/), we measured the thickness of light and dark layers in the outer cortex from TEM micrographs at five evenly spaced points (with exception of *Amblysomus hottentotus*, which was only measured at three points due to limited area of the TEM images (Figure 1)). We also counted the number of each layer per image, and calculated the mean thickness and number of the light and dark layers from each species.

To identify the optical basis of iridescent color production, we used standard thin-film optical modeling (R-project). These models predict how light changes as it moves through the material by generating predictive reflectance curves based on the multilayer inference. Based on the thickness of the layers, refractive index of the material, and the number of layers, the model is able to predict if the structural organization plays a role in the iridescent production by comparison to the microspectrometer data. Thus, we used the thin-film model to predict the theoretical reflective curves that are produced by the structures that influence the iridescent color. We used the “multilayer” function in the R script thinfilm.r, (Maia et al. 2009). The refractive index increases with the density of a material and determines the speed of light that passes through the object. Consructive inference is determined by the refractive index, number of layers, and thickness of the material. The extinction coefficient is the amount of intensity lost due to absorption. Estimated refractive indices (n) and extinction coefficients (k) were used from previous publications, including air (n=1.00, k=0.00), keratin (n=1.56, k=0.03), and melanin (n=2.00, k=0.6) (Land 1972; Brink & van der Berg 2004). It is essential to evaluate the stack number, which is the number of light and dark layers, because in a thin-film model, the reflectance is maximized with both an increase in the number of layers and a higher difference between refractive indices (Land 1972). The number of stacks was the mean number of rows for that species (light and dark layer equals one stack).
RESULTS

Stereo Microscope Images

Iridescent hairs are straighter and thicker relative to the non-iridescent hairs (Figure 2) and have sheens that vary in color along their entire length (Figure 2, Table 1). The non-iridescent hair structure is similar to a curly overhair which is weak and flexible in two thirds of the hair structure or a furhair that is considered weak and flexible along the whole hair. The furhairs in moles are also considerably finer than overhairs. (Williams 1938). The common mole hairs exhibit a straighter and thicker structure similar to the iridescent golden mole hairs as well as a whip-like curly end (Figure 2C).

Fig. 2. Stereomicroscopy Images. Golden Mole (GM) Iridescent hairs (*Eremitalpa granti*) are flat and have a blue/purple sheen (A). GM Non-Iridescent hairs (*Eremitalpa granti*) are curly and have no distinct sheen (B). Common Mole (CM) has flat hairs with shine (C).
Scanning Electron Microscope Images

SEM revealed clear differences in surface morphology between the hair types. The surfaces of the iridescent hairs are semi-flat and have small scales providing a smooth surface, while the non-iridescent hairs have cuticular scales (Figure 3A, B). Interestingly, both hair types (straight and curly) of the common mole hairs (*Scalopus aquaticus*) have similar cuticular scales to the non-iridescent hairs (Figure 3C, D).

![SEM Images](image)

Fig. 3. SEM Images. GM Iridescent hairs (*Chrysochloris asiatica*) have a smooth surface (A). GM Non-Iridescent hairs (*Chrysochloris asiatica*) has cuticular scales (B). CM straight hairs have visible rough scale-like structure (C). CM hairs have a straight and curly segment. The curly segment has more longitudinal scales (D).

Light Microscope Images

Light Microscopy revealed that the iridescent hairs are long, flattened, elliptical hairs and are composed of three distinctive layers (Figure 4). A dark stained structured outer layer is the first layer, the 2nd layer is an inner lighter layer, and the last layer is air. By contrast, the non-iridescent hairs are small and round to elliptical and have no structured layers (Figure 5). Interestingly, the common mole hairs, like the non-iridescent hairs, are circular and also have melanin granules in the center (Figure 6).
**Fig. 4. Light Microscope Image.** The iridescent hair *(Eremitalpa granti)* has a flat morphology composed of a dark structured layer and an inner lighter layer.

**Fig. 5. Light Microscope Images.** a) GM Iridescent *Eremitalpa granti* b) GM Iridescent *Amblysomus hottentotus* c) GM Iridescent *Amblysomus septentrionals* d) GM Iridescent *Chrysochloris asiatica* e) GM Non-Iridescent *Eremitalpa granti* f) GM Non-Iridescent *Amblysomus hottentotus* g) GM Non-Iridescent *Amblysomus septentrionals* h) GM Non-Iridescent *Chrysochloris asiatica.*
**Fig. 6. Light Microscope Image.** The common mole (*Scalopus aquaticus*) hair exhibits a similar structure to the GM non-iridescent hair. The CM hair also has melanin granules inside of the outer layer.

**Transmission Electron Microscope Images**

TEM revealed the presence of discrete, alternating thin layers of dark and light material in the outer cortex of iridescent mole hairs (Figure 7). The light layers tended to be thicker (ranging from 77.4–155.9nm) than the dark layers (ranging from 29.9-79.3nm; see Table 1). The species tend to vary among their stack size as well as in their light and dark layer means (Table 1). The biochemical makeup of these layers is unclear, but since mammal hair is composed of alpha keratin, we assume that the light layer is alpha keratin, while the darker layer is either a different conformation of alpha keratin or air. Below this structural cortical layer is a layer of unstructured alpha keratin containing melanosomes (Figure 4). By contrast, the alpha keratin of the non-iridescent hairs and common mole hairs are entirely unstructured (Figure 8E and Figure 9).

**Fig. 7.** The iridescent hairs have structured layers (*Eremitalpa granti*).
Fig. 8. TEM Images. a) GM Iridescent *Eremitalpa granti* b) GM Iridescent *Amblysomus septentrionals* c) GM Iridescent *Amblysomus hottentotus* d) GM Iridescent *Chrysochloris Asiatica* e) GM Non-Iridescent *Chrysochloris asiatica*.

Fig. 9. TEM Image. The common mole hair exhibits no structured multilayers and therefore is similar to the golden mole non-iridescent hair.
Thin Film Optical Modeling

Using the average thickness of the light layer for $d_1$ and $d_2$, the predicted curves showed a slight relation to the color seen through the dissecting microscope observations in *Amblysomus hottentotus*, *Amblysomus septentrionals*, and *Eremitalpa granti* (Table 1; Figure 11). $D_1$ is the average light layer thickness and $d_2$ is the average dark layer thickness (Figure 10). All species except *Chrysochloris asiatica* proved to show slightly similar trends between the theoretical and microspectrometry data. The best fit microspectrometry curve was utilized (Figure 11). *Chrysochlorisasiatica* may not have shown a significant trend due to error in the microspectrometry readings. Initially, we observed the results using the actual average light layer and dark layer thicknesses for $d_1$ and $d_2$, unfortunately these results did not show any correlation. We then predicted the light and dark layers theoretically should demonstrate the same thickness. We utilized the average light layer thickness for both $d_1$ and $d_2$. The elytral wing of the buprestid beetle has multilayer reflectors along its surface. Both the light and dark layers have a similar thickness and thus the predicted colors match with the color seen (Noyes 2007). The predicted color sheens in golden moles include violet, gold, and green.

![Diagram](image_url)

**Fig. 10.** Parameter model for transmission electron micrographs.
Fig. 11. Predicted curves in comparison to the microspectrometry curves. a) *Amblysomus hottentotus* b) *Amblysomus septentrionals* c) *Eremialpa granti* d) *Chrysochloris asiatica*. X axis is wavelength (nm) and Y axis is reflectance (arb. units).
<table>
<thead>
<tr>
<th>Species</th>
<th>Light Layer (nm)</th>
<th>Dark Layer (nm)</th>
<th>Stack</th>
<th>Light Layer Std Dev.</th>
<th>Dark Layer Std. Dev.</th>
<th>Stack Std. Dev.</th>
<th>Predicted Sheen</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amblysomus hottentotus</em></td>
<td>109.8</td>
<td>35.9</td>
<td>6</td>
<td>2.71</td>
<td>0.90</td>
<td>0.50</td>
<td>*Gold (550 nm)</td>
</tr>
<tr>
<td><em>Amblysomus septentrionalis</em></td>
<td>153.5</td>
<td>29.9</td>
<td>3</td>
<td>5.10</td>
<td>1.19</td>
<td>0.20</td>
<td>*Violet (400-450nm)</td>
</tr>
<tr>
<td><em>Eremitalpa granti</em></td>
<td>77.4</td>
<td>42.0</td>
<td>13</td>
<td>0.91</td>
<td>0.51</td>
<td>0.92</td>
<td>*Violet</td>
</tr>
<tr>
<td><em>Chrysochloris asiatica</em></td>
<td>155.9</td>
<td>79.3</td>
<td>15</td>
<td>5.24</td>
<td>5.53</td>
<td>0.5</td>
<td>Green (480-560 nm)</td>
</tr>
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Table 1. The average values based on measurements of TEM images of each species. The light layer value was used for the theoretical curve. One stack=one light and one dark layer.

**DISCUSSION**

The iridescent golden mole hair exhibit a flat morphology and organized layers around the circumference of the hair. There are also alternating light and dark layers present in the first layer as well as melanosomes in a second lighter layer of alpha keratin. The non-iridescent golden mole hair and common mole hairs show a completely different morphology, the hairs are circular to elliptical in shape and exhibit no organized layers. We hypothesize, the flat morphology of the iridescent hair could perhaps aid to increase the surface area exposed to light and thus increase the amount of light that can be reflected.

Through optical modeling, comparison of the microspectrometry curves and the predicted curves seem to show slight analogous trends. We were able to obtain usable data from three of the four species for the comparison of the theoretical values and microspectrometry readings. For the R program, we tried utilizing the empirical data of the light and dark layer thicknesses, but this showed absolutely no comparable trends. We then looked at an idealized situation based on previous work by using only the average thickness value of the light layers presenting the light and dark layers should hold similar thickness values (Noyes 2007). The actual microspectrometry curves do not indicate a strong color, but a faint color which is logical since the hairs have an iridescent sheen as opposed to a strong iridescent color seen in other organisms such as birds or insects. The sheen color observed coincides slightly with the predicted color of the curve and the spectrometry readings for three of the four species. Because a strong relationship between the curves is not present, we cannot conclude that the structured layers are the mechanism that aids in the production of the iridescent color. These structures may contribute to the color production in an unpredictable way that could not be concluded upon in
this study. In addition, because human hair has been found to be composed of similar layers around the circumference of the cross section, we hypothesize that these layers can’t be the only mechanism causing the iridescent color (Khumalo 2005).

Mammal hair has been found to produce more glossiness based on the orientation of the hair profile. Hairs with a smoother surface and less protruding of the scales tend to formulate a higher hair gloss. In addition, hairs that exhibit flatter scales also exhibit a thinner cuticle (Chernova 2002). The shine of hair depends on how the light interacts with the surface. If the cuticle reflects more light, then the more intense the luster will be of the hair (McMichael 8).

The SEM pictures showed a significant difference between the iridescent and non-iridescent golden mole hairs and the common mole hairs. The iridescent hair profile showed a smoother surface with scales that were slightly elevated above the surface. The non-iridescent hairs show a rougher surface with visible scales. Like the non-iridescent hairs, the common mole hair profile showed scales that had elevated height off the surface. The thick and curly segment of the hair both showed these prominent scales. Therefore, the straightness or curly morphology of the hairs does not play a trivial difference between the iridescent and common mole hairs’ surface. The smoothness or the roughness of the hair surface seems to point out a very important difference between the hairs. Since smooth hair is known to increase gloss of hair, it makes sense that the iridescent hairs would exhibit this same characteristic. We hypothesize that the golden mole’s smooth surface contributes to the iridescent color.

We conclude that a flat morphology may be significant for the iridescent hair to increase surface area exposed to the light, but the smooth surface is essential to produce the iridescent color and shine. We cannot conclude that the layers in the cross sections produce the iridescent color. The golden mole produces iridescence that is not very vibrant unlike many birds and insects; therefore the morphology, smoothness, and three distinct layers of the hair may together produce the faint iridescence in the golden mole. Other than the structured layers, there is no other organization observed such as with the melanosomes. For instance, the icterid species in birds exhibits organization of melanosomes, which is believed to contribute to their iridescent color (Shawkey et al. 2006). We observed that the golden mole has melanosomes or melanin granules, but they are not oriented in an organized manner. The common mole hairs are also composed of melanin granules. The golden mole hair has melanin granules distributed randomly along the inside, but the common mole has granules concentrated in the center. The golden moles appear to have developed a separate mechanism through evolution that produces the iridescent color of their hair. Overall we can conclude: 1) The structured layers may contribute to color, but some of the assumptions in our model (refractive index, mode of color production) may not be correct or 2) The layers do not contribute to the color, but the color is produced by an unknown way. We hypothesize that the color might vary along the hair, and therefore the place of the cross section may affect the outcome of the data. In addition, the shape of the cross section may vary among the length of the hair. In our study, we could not cut the hairs at the same point on every hair and therefore, this may be a source of error.
The question to be considered past these conclusions is why the golden mole developed the iridescent color through evolution. In many animals iridescent color aids in mating, in the peacock, the mating success of the male has been linked to the intensity of the iridescent color (Loyau et al. 2007). The golden mole however does not rely on sight for mating. Perhaps the glossiness of the coat aids in the moisture proof coat and the mole’s tunneling to find food.

REFERENCES


R Development Core Team See http://r-project.org/.

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