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What Causes Variation in Peacock Feather Colors?

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Honors Research Project

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What Causes Variation In Peacock Feather Color?

Abstract

Sexual selection favoring the most attractive peafowl is very well understood, but the answer to what drives the variation in peacock feather coloration remains a mystery. The goal of this experiment is to determine the precise physical mechanism used by peafowl to generate such elaborate color variations in the eyespots of their tail feathers among males within the species. Barbs from eyespots of *Pavo cristatus* were embedded in an epoxy resin, sectioned on a microtome, and TEM images of the nanostructure within each barbule were analyzed. Results indicate that melanosome diameter is a significant positive predictor of iridescence and that as melanosome diameter increases and spacing decreases, brightness will increase. Variation in melanosome size does not predict physiological superiority among peafowl, but because melanin production is established genetically, females choosing to mate with more iridescent males may receive an additional benefit of a higher quality mate.

1. Introduction

The use of complex coloration strategies has been employed by a great variety of species and is more often than not used for communication (4, 2). Most of the time, these elaborate colors are designed to achieve one of two tasks: to maintain a certain level of subtlety through the use of camouflage thereby avoiding predation or, as is the case with *Pavo cristatus,* to be used in displaying behavior in an effort to increase copulatory success through sexual selection (5, 13). We are specifically interested in the efficiency of the coloration design which is of utmost importance for any species which relies on displaying vibrant colors to enhance mating success. Due to the potentially large impact on fitness, an understanding of the variation in peacock coloration is essential (7, 10). The vast arrays of colors observed in nature are typically generated by pigmentation or structural coloration. With pigmentation, longer wavelengths of light are capable of generating colors in the yellow to red ranges. With structural coloration , the melanosomes (organelles containing melanin) direct the flow of light and can drastically increase the range of colors to encompass nearly the entire visible spectrum of *Pavo cristatus* (12). The creation of these vibrant structural colors is achieved through three variables: refractive index, organization, and size of the melanosomes (11). Specific to peafowl, a two-dimensional photonic crystal organization is utilized while variation in the sizes of the melanosomes (or surrounding air pockets) is also observed (20). Due to the fact that variation in photonic crystals leads to a broad range of colors, this is an ideal structure to study in *Pavo cristatus.*

2. Materials and Methods:

2.1. Study System

To investigate the physical mechanism used by peafowl to generate such elaborate color variations in the tail feather eyespots, we collected eyespots from nineteen male peafowl (*Pavo cristatus*) during 2009 from a feral population of male peafowl from the state of California (6).

2.2. Barbule Nanostructure

2.2.1. Embedding

To begin the experiment, we removed one green colored barb from the lower left region of the eyespot of each male using a scalpel under a Leica 58AP0 dissection scope (Leica Microsystems GmbH, Wetzlar, Germany). Once separated from the eyespot, the green barbs were divided into four smaller but equal sections and each sample was placed in individual glass tubes. Next, a weeklong embedding process began to encase the barb sections in an amber colored polymer called Epon using techniques established by Shawkey et al (18). This step enables a more effective means of orienting the barb sections for the rest of the experiment. In short, each glass vial containing four equally divided pieces of the barb were carefully bathed in a solution of 0.1% Tween-20 and 0.25M NaOH. Following dehydration with 100% ethanol, the barb pieces were fully submerged in increasing concentrations of the Epon resin for lengths of time ranging from two to twenty-four hours. Each solution change was accomplished using a pipette. Once completed, the resin-hardened barbs were placed in small rubber wells lying end to end and fully encased with Epon. These samples were then cured in an oven at 60 degrees Celsius for two days and the resulting plastic blocks were removed.

2.2.2. Trimming

Following this embedding process, the small plastic blocks were shaved down with a razor blade and mounted lengthwise into a Leica S6 EM-Trim 2 (Leica Microsystems GmbH, Wetzlar, Germany). From here, we used a diamond tipped drill bit to slowly taper down the leading face of the block into a shape similar to a blunt four sided pyramid. Care was taken to slowly polish smooth the leading face of the block with the drill to ensure no damage was done to the diamond blade when sectioning.

2.2.3. Sectioning

Next we mounted the samples onto a Leica UC-6 ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany). Small 70um sections of this block were then sectioned off from the sample, providing perfect cross sectional slivers of the barbs. An individual hair was used to corral the floating sections and with the help of a copper grid, sections were lifted out of the water. After air drying, sections were ready to image.

2.3. TEM Imaging

Cross sections were then taken and imaged on a JEOL JEM-1230 transmission electron microscope (TEM). Two to three images of the barbules were taken from each of the nineteen samples. ImageJ was used to measure five parameters (1). To begin,

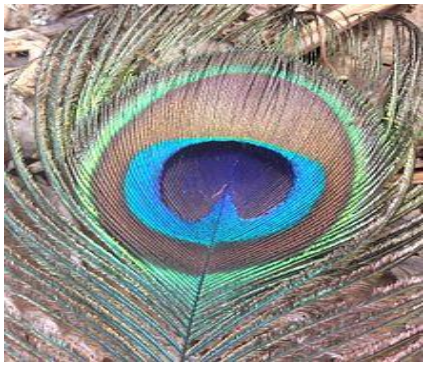


Figure 1. Typical eyespot found among males of peafowl species. Red arrow indicates the blue-green region in which barbs were removed for embedding.

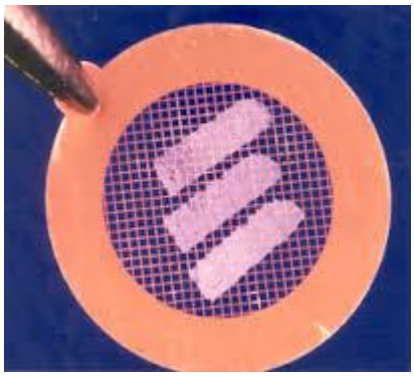


Figure 3. Typical copper grid used to lift sectioned samples out of the water after sectioning on ultramicrotome. Grids will then be placed directly into microscope for TEM images.

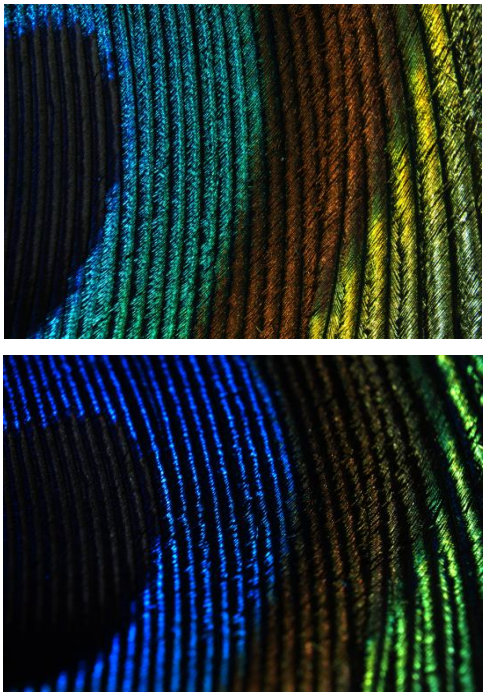
twenty-five melansosmes were tagged for measuring by moving in an "L" shaped pattern to ensure complete coverage of all melanosomes imaged within the barbule. The diameter of each melanosome was then measured from left to right and again from bottom to top. Following this, the spacing between adjacent melanosomes was determined by measuring the distance between their centers following the same pattern, left to right, bottom to top. Cortex thickness was also measured at ten different locations along the length of the barbule. Measurements began at the edge of the cortex and terminated on the edge of the

Figure 2. Samples now encased in hardened block of Epoxy resin. After removal from rubber wells, blocks were trimmed and sectioned.

closest melanosome directly above. The number of melanosomes per layer was then counted for ten different periods containing at least three to four melanosomes ordered along the same axis. Lastly, for two of the nineteen samples, size of the air pockets was also measured. This was done by first tagging twenty-five melanosomes. Again the distance between adjacent air pockets was recorded following the predetermined left to right, bottom to top pattern, but in addition, the air pocket which occupied the "right angle" formed by the three adjacent melanosomes was selected as the air pocket to measure. Air pockets were measured precisely according to the same pattern.

2.4. Reflectance Measurements

We used a microspectrophotometer (CRAIC Microsystems) to obtain reflectance curves for all nineteen samples. A mirror was used as a reference to ensure no saturation would result. In total, nine measurements were taken from the barbs immediately adjacent to the original barb cut from the eyespot. These measurements were then averaged for each sample and curves were smoothed to minimize noise generated by the machine (17). From this, brightness (maximum reflectance) and hue (wavelength at



Figures 4 and 5. Apparent color change observed on goniometer as eyespot is perpendicular to (4) and tilted (5) with respect to the camera.

maximum reflectance) were determined. To measure how color of the eyespots changes with angle, samples were placed in a lab-made goniometer (16). The angle of incidence and reflectance was then varied and recorded at 5 degree increments beginning at ten degrees from the vertical and ending at sixty degrees from the vertical. A total of eleven measurements for each sample were obtained.

3. Results

3.1. Barbule Morphology

Within the *Pavo cristatus* population, cross sectional TEM images confirmed that the melanosomes were in fact arranged in the familiar two dimensional square crystal

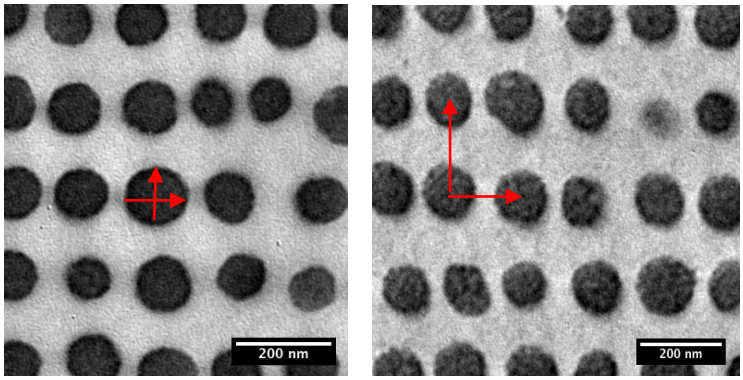


Figure 6. Two examples of the square color-producing nanostructure in peacock feathers, illustrating extremes in melanosome size. Red arrows indicate location of measurements taken.

lattice (Figure 6) and covered by a cortex composed of keratin. Melanosome radius displayed a high degree of variability but averaged approximately 105nm. Similarly, melanosome spacing (center to center distance) averaged 175nm. Cortex thickness averaged roughly 119nm. Lastly, number of melanosomes within any particular layer averaged 9 each.

3.2. Reflectance Measurements

3.2.1. Brightness

Among the samples, data indicates a positive correlation between melanosome diameter and brightness. That is, as the diameter of the melanosome increases, so too does the brightness of the feather ($p = 0.0033$). Therefore larger melanosomes equate to brighter *Pavo cristatus* eyespots. In addition, there is a negative correlation between melanosome spacing (lattice constant) and brightness of feather ($p = 0.0074$) as indicated in figure 7. As spacing increases, brightness diminishes.

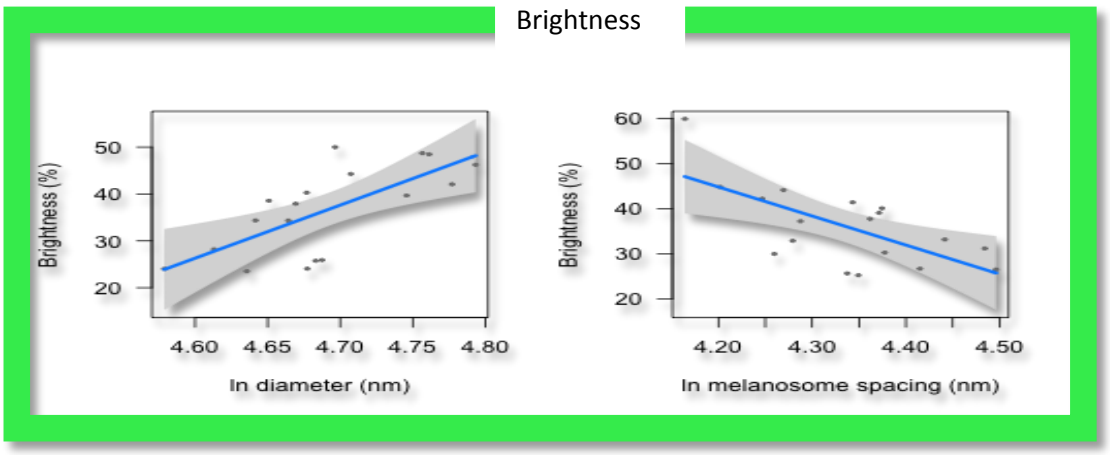


Figure 7. Brightness of feathers correlates positively with melanosome diameter (p = 0.0033) and negatively with melanosome spacing (p = 0.0074).

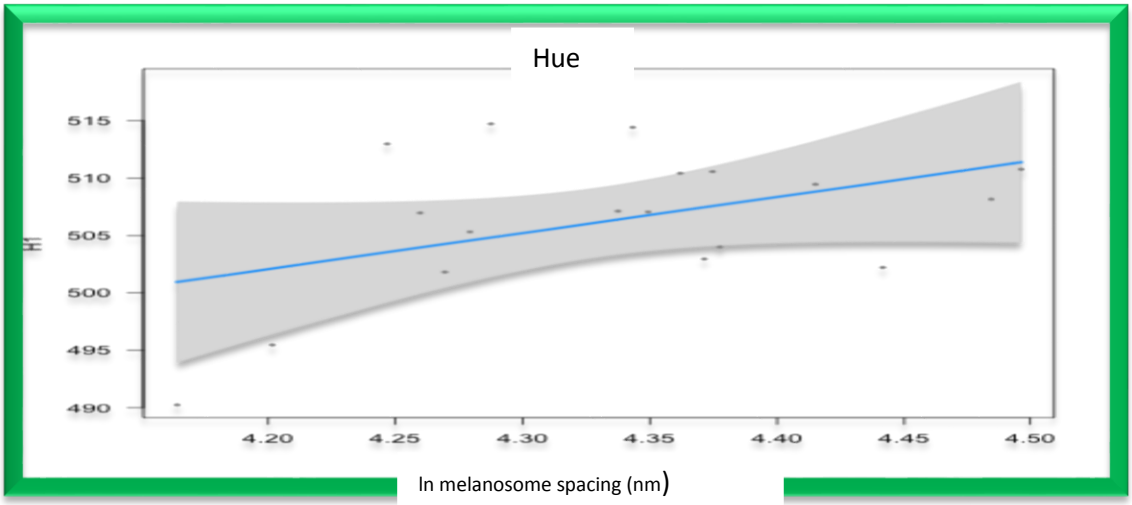


Figure 8. Melanosome spacing did not significantly predict hue (p = 0.10).

3.2.2. Hue

Figure 8 indicates that melanosome spacing is not a statistically significant predictor of hue (color) within *Pavo cristatus* (p = 0.10).

3.2.3. Iridescence

Data from the lab designed goniometer indicate that as the angle of incidence changes from 10° to 60°, hue shifts toward UV (Figure 9). In other words, peacock eyespots appear to transition from bright green color to the shorter blue wavelengths as the angle of incidence increases (see figures 4 and 5). Plotting these values onto a tetrahedral avian color space and measuring the difference between the incidence angles, the degree of iridescence can be measured (Figure 10).

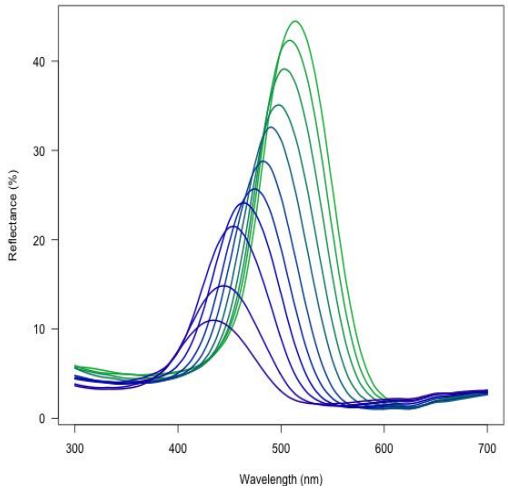


Figure 9. Example of hue change caused by varying angles of incidence for one sample.

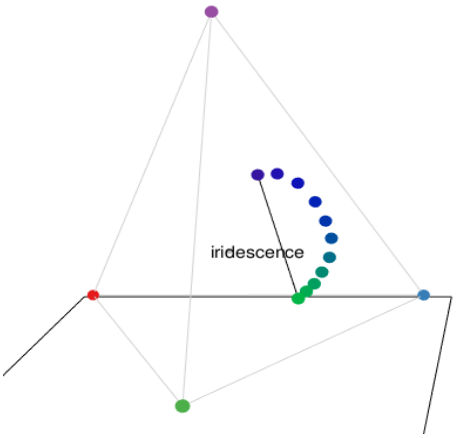


Figure 10. Avian tetrahedral color space.

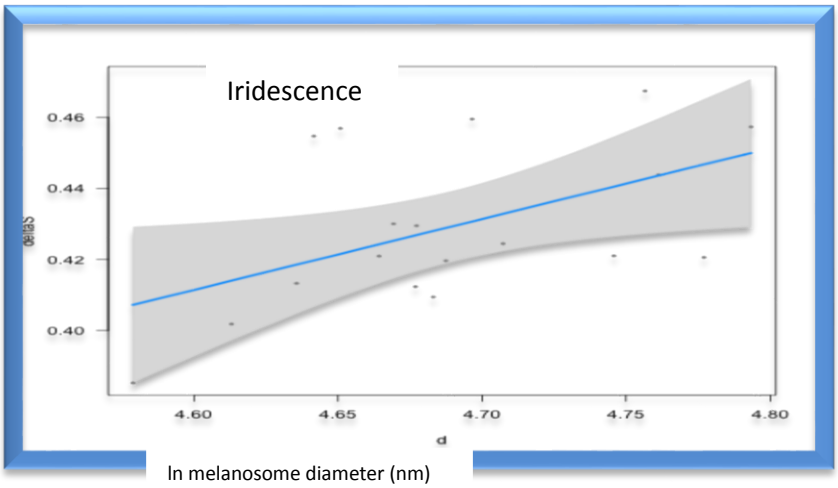


Figure 11. Melanosome diameter is positively correlated with degree of iridescence (p = 0.029).

Finally, data reveals that as melanosome diameter increases, iridescence will also increase (figure 11). Therefore we can affirm that melanosome diameter is a significant positive predictor of iridescence (p=0.029).

4. Discussion

Results indicate that the iridescent colors of peafowl are produced by a two-dimensional organization of melanosomes into a square lattice framework. It is also evident that slight differences in melanosome size and spacing have the potential to generate a considerable degree of variation among members of *Pavo cristatus.* With regards to brightness of the eyespots, larger melanosome diameters were found to yield brighter peafowl eyespots. Similarly, as the spacing between melanosomes increased,

brightness diminished. These findings mutually support one another. Due to the fact that a barbule may only grow so large, available space within the structure is finite and eventually an upper limit will be reached, limiting potential color ranges in the process (9). For this reason, we expect that as melanosome size within the barbules increases, available space between adjacent melanosomes will decrease. Conversely, as melanosomes decrease in size the spacing between adjacent structures will expand. Since no two melanosomes may occupy the same location, the packing of melanosomes will eventually become physically limited (3). Therefore we expect this physical conformation to eventually limit the variability observed and restrict the maximal brightness of eyespots.

Interestingly, the square lattice nanostructure organization of melanosomes is only one of several different ways to generate iridescence. For example, in many duck species two-dimensional melanosomes are organized into a hexagonal lattice. This appears to resemble the square lattice but instead each row of melanosomes is offset and the rods occupy the space directly above the gaps between adjacent melanosomes (9). By packing their melanosomes in this hexagonal lattice, these duck species have been able to achieve a highly stable and energetically favorable structure (15). If structurally colored feathers do develop through self assembly as is suggested by the literature and observed in lab experiments (8, 14), it would make sense that hexagonal packing would be the final result. Why then is the square lattice framework observed in *Pavo cristatus,* or any other peafowl for that matter? Since hexagonal packing appears to be the most efficient way to stack spherical and rod-like objects, the very existence of a square lattice suggests an energetic cost to the peafowl associated with maintaining this particular two-dimensional lattice. Perhaps this energetic cost serves to maintain signal honesty among peafowl (19)? More research should be done to determine the exact energetic demands placed on species utilizing the various melanosome packing strategies.

Despite limitations on color production, a large degree of variation exists between members of *Pavo cristatus*. Observational research on a population of peafowl from France studied by Loyau *et al* has provided convincing evidence that eyespot brightness is correlated with mating success and suggests that the evolution of these iridescent colors is achieved through sexual selection. (13). As previously mentioned, iridescent eyespots serve as a strong visual signal during pre-copulatory courtship displays. Studies show that the male peafowl has a rather intricate set of display patterns which it uses to garner attention from mates. Most importantly would be a train-rattling display pattern in which the male peafowl orients itself at a 45-degree angle to the right of the sun with the female facing directly ahead. The rattling of erect feathers is believed to serve as a means of enhancing the iridescent color signal. Furthermore, peafowl have been shown to identify the correct location of the sun even on overcast days and can do so independent of geographic location. This strict adherence to mating ritual is most likely a product of sexual selection and would doubtfully be maintained if it failed to influence female mate selection (6).

In short, nanostructural variation in the size and spacing of melanosomes is capable of contributing to significant differences in the brightness of eyespots on male peafowl trains. The female eye is capable of detecting these differences and using this information,

in part, to select a partner. Therefore, nanostructural differences provide the variation necessary for iridescent eyespots to be acted upon by sexual selection.

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