1. Introduction

Birds (Class *Aves*) are one of the most colorful vertebrates and among the most colorful of all metazoans (Scott 2010). Their diversely colored feathers are used for mate attraction and for other communication purposes (Maia et al. 2009). The colors of many feathers are caused by the optical properties of their key components, keratin and melanin. The bulk of all feathers is composed of beta-keratin, a fibrous protein with polypeptide bonds (Feughelman 1996). Melanin is a tyrosine derivative that is manufactured, stored and deposited in organelles called melanosomes (Orlow 1995). The two biochemical forms of melanin, eumelanin and pheomelanin, produce darker colors like blacks, grays, and browns (Scott 2010) through broad-spectrum light absorbance.

Iridescence is a property of objects that change in hue (color) at different angles of observation or illumination (Prum 2006). Iridescence in feathers is caused by thin-film interference from organized layers of melanin, keratin and sometimes air (Prum 1999). In the simplest case, a very thin (~50-650 nm) layer of keratin overlaying a thicker layer of melanin is sufficient to produce iridescent hues (figure 1a). Thus, organization of feather components present in non-iridescent feathers is sufficient to produce iridescent nanostructures (Prum 2006).

However, almost nothing is known of how such organization takes place during feather development. Beta-keratin self-assembles into hierarchical fibers, and recent work suggests that other nanostructures in feathers may form through fundamentally similar processes of self-assembly (Dufresne et al. 2009, Prum et al. 2010). Moreover, iridescent nanostructures, consisting of a “ring” of melanosomes around the edge of feather barbules, bear striking resemblances to patterns formed through “coffee ring” effects of capillary flow during dewetting (Deegan et al. 1997) (figure 1b). We thus hypothesized that the melanosomes spread to the outer
edges of the keratin forming a ring like structure, and tested this hypothesis using dewetting experiments with keratin and melanin extracted from feathers.

2. Methods and Materials

2.1 Extraction
To extract the melanosomes, a similar procedure was used as in the extraction done by Liu and collaborators (2003). Iridescent feathers of wild turkeys (*Melagris gallopavo*) were washed 3 times with acetone, once with dichloromethane, and once with ether. They were then washed once again with acetone, once with high performance liquid chromatography (HPLC) water, and
then again with acetone. Two grams of feathers was used for the extraction. The feathers were cut into smaller pieces to allow for an easier extraction. The pieces were added to a combination of 40 mL of 0.1M phosphate buffer and 0.4g of dithiothreitol (DTT). This was stirred under argon for 23 hours at 37 degrees Celsius. Proteinase K and 0.2g of DTT were added, and this was left overnight. The pellet was centrifuged at 3300g for 10 minutes. It was rinsed 6 times with HPLC water with centrifuging for 10 minutes after every rinse. The pellet was suspended overnight in phosphate buffer with 14mg of Papain and 67mg of DTT. This was centrifuged and washed 6 more times with HPLC water. The pellet was then suspended overnight in phosphate buffer, proteinase K, and 27mg DTT. This was centrifuged for 10 minutes. The pellet was put in deaerated phosphate buffer and 2% Triton X-100 solution and stirred for four hours. This was then ultracentrifuged at 100,000g for 10 minutes. The pellet was washed thoroughly with methanol and HPLC water. Overnight, the pellet was put in a solution of proteinase K, .2g DTT. Finally, the pellet was washed three times with HPLC water and left out to dry.

2.2 Keratin extraction
This procedure was performed similarly to the extraction procedure done by Church and collaborators (2010). A large amount of white, gray, and black feathers from domestic chickens were washed excessively with HPLC water and acetone. The feathers were cut into smaller pieces. 0.16g of active Na$_2$S was mixed with 100mL of water. This was shaken until all of the Na$_2$S was dissolved and it was poured over the feathers. It was placed in the shaker for one hour at 32 degrees Celsius. The mixture was centrifuged at 4000g for 10 minutes and the pellet was removed. The supernatant was kept and poured into Petri dishes in thin layers. The dishes were left out overnight to dry.
2.3 Experiment
The extracted melanosomes were dissolved in water. Drops were then placed on the extracted keratin which was on a glass slide and left to dry in a hood. After the slides were completely dry, photographs were taken under a microscope of both the keratin by itself and the keratin with the melanosomes. The pictures were observed and comparisons were made from the results.

**Results**
When looking at the keratin rings without the melanosomes, they formed multiple defined rings as shown in figure 2. The keratin was denser at the outer region of the circles and less concentrated in the middle of the ring. When looking at the rings with the melanosomes all of the melanosomes congregated where there was less keratin, in the middle of the ring as seen in figure 3 and 4.

![Figure 2: Keratin ring without melanosomes](image)

![Figure 3: 1x -Melanosomes on keratin formed in the middle.](image)

![Figure 4: 5x -Middle of keratin ring where melanosomes lay.](image)
Discussion

When coffee is spilled, it leaves a dark stain around the edge of the spill (Deegan et al. 1997). Although the coffee is originally evenly dispersed throughout the spill, eventually it joins together and forms the ring (Deegan et al. 1997) leaving the inner most part nearly coffee free. This is common among drops which contain a solid when evaporated (Deegan et al. 1997). The evaporation will cause capillary flow toward the edges of the drop; all of the evaporated liquid from the edge gets “replenished” by liquid from the middle carrying all of the solid granules to the edge of the drop (Deegan 1997). The rings seem to form from many different solutes and solvents as long as “the solvent meets the surface at a non-zero angle, the contact line is pinned to its initial position, and the solvent evaporates (Deegan et al. 1997). The higher the initial concentration, the wider the ring the ring will be (Deegan et al. 1997).

We predicted that similar effects would occur when we mixed keratin and melanin; however, they did not. The keratin by itself formed concentric rings while drying leaving the center almost keratin free, but full of melanosomes. This suggests repulsion between the keratin and melanosomes. The melanosomes may be repulsed from the keratin due to differences in charge or other chemical/physical properties. As the keratin dried from the outer edge inward, the melanosomes may be pushed into the area of lowest keratin concentration, in this case the center of the ring. To test this hypothesis, we could analyze the physical and chemical properties of the keratin and melanin to determine why they may repel each other physically. More research on this would allow for us to better understand coloration in bird feathers and the self-assembly of the nano-structures. Such research could include testing iridescent bird feathers to see if the same results occur. We could also test the melanosomes droplets on other non water-resistant substances to see if it is really this property that is causing the repulsion. Finally, we should vary
the concentrations of keratin and melanin during our experiments, predicting that lower concentrations of either material would decrease the repulsive interactions between them. While this experiment did not support our hypothesis, considerable work remains to be done before we can consider it refuted.
References


