The molecular basis of developmental change and evolutionary diversity remains poorly understood for many phenotypes. The purpose of my internship was to investigate the molecular and developmental basis of the head crest in the domestic rock pigeon, a trait characterized by a reversal in the direction of feather growth on the back of the head. Domesticated pigeons make ideal candidates for developmental studies into evolutionary diversity as they display a high degree of diversity analogous to wild Aves, different breeds can be intercrossed in lab, and many protocols established for chicken are easily modified for use in pigeon. Preliminary studies had implicated a nonsynonymous mutation in Ephrin Receptor B2 in causing head crest, and had shown that growth and molecular polarity within the feather buds of the crest region had been reversed.

One goal of my internship was to determine the effect of the crest mutation on the expression of other feather polarity genes. Whole mount in situs on crested and uncrested birds for EphA4, Wnt7a, and Delta1 showed that all were expressed in the opposite orientation in crested birds relative to control. This suggests that the action of the crest mutation is upstream of these genes in regulating feather polarity.

With a postdoc in the Shapiro lab, we conducted experiments swapping the dermal and epidermal layers between crested and uncrested breeds. In situs for molecular polarity of these explants were inconclusive as to which skin layer determines polarity. The procedure of swapping and culturing the recombined samples, while preserving A/P polarity, needs to be optimized.

The third goal of my internship was to illustrate a functional effect of the crest mutation on the activity of EphB2. Our ultimate goal was to perform a kinase assay to determine whether the cr mutation altered catalytic activity of the EphB2 kinase domain. Previous expression studies found that EphB2 protein is highly toxic to E. coli. Thus, we cloned three different insert lengths composed only of the domain in which the mutation is found along with varying portions of the auto-regulating juxtamembrane domain in an attempt to decrease toxicity by decreasing activity. Plasmids free of mutations were extremely difficult to obtain as a result of the toxic nature of EphB2 protein in E. coli. Liquid cultures of bacteria containing inducible constructs of both wt and cr EphB2 alleles grew when EphB2 expression was not induced. However, when expression of EphB2 was induced by the addition of IPTG, bacteria expressing the wt allele of EphB2 died, while bacteria expressing the cr EphB2 allele grew normally. These data suggest that the cr mutation reduces the catalytic activity of EphB2 kinase domain. To circumvent the problem of toxicity, I cloned the kinase domain of EphA4, and made the analogous “cr” mutation. This will allow us to test the effect of the “cr” mutation on catalytic activity of the kinase domain.

Over the course of this internship, I was able to learn many new experimental techniques. Some worked the first time and others still need to be improved. I learned how to handle experiments failing for months at a time, and how to think of ways to work around complications. Currently, I am working to express EphB2 protein in vitro for biochemical analysis and am investigating the developmental interactions of EphB2 in pigeon embryos leading up to head crest formation. The EDEN internship was a wonderful opportunity and has been a fantastic experience.