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Analysis of Photoreceptor Development in Pekin Duck

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Abstract

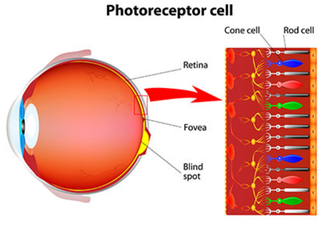
The Pekin duck is a valuable agricultural commodity in the U.S. Pekin ducks are seasonal breeders; they are sensitive to light and thus, research on the neuroendocrine and behavioral responses are needed to overcome technological limitations. There is compelling evidence that specific wavelengths of light are required to improve the growth and welfare of meat (grow out) ducks. For example, blue light may not be ideal for grow out ducks due to considerably increased motor activity, significantly decreased body weight and increased serum corticosterone (cort) levels. Therefore, our objective is to determine the role of both deep brain photoreceptors (DBPs) and retina photoreceptors (RPs) during duck development. Two groups of ducks were raised with and without light over 21 days from egg laying, embryonic day zero. We then collected brain and retinal tissues of ducks at embryonic days 3, 7, 11, 16, and 21. To examine DBPs, we designed and created primers for 3 genes: OPN4, VAL-opsin, and OPN5. For RPs, we designed and created primers for genes responsible for both cones (RH2, SWS1, SWS2, LWS opsins) and rods (rhodopsin, MAFA, IRBP) in duck eye development. qRT-PCR was performed utilizing listed primers for DBP, RP rods, RP cones and reference gene, using 10 samples each for ducks raised in both light and dark conditions. The housekeeping genes GAPDH, RPS13, and SDHA were used to normalize all qRT-PCR data. RNA was then extracted from the tissue collected and qRT-PCR was performed. Understanding when these specific genes are upregulated across development will help the husbandry of duck by providing an ideal time for light usage during duck development. Future directions will determine which wavelength of light, at a specific time of development, is most suitable for grow out ducks.

Why is this important?

- In the Midwestern United States, leading companies contract Amish farmers to produce approximately 80% of U.S. ducks.
- Pekin ducks are seasonal breeders and as such, very sensitive to artificial and natural light. Technological limitations of the lack of electric lights on traditional, non-electric Amish duck farms can lead to a 10-30% reduction in fertility of Pekin ducks during shorter day lengths.
- Determining the neuroendocrine and reproductive development responses to specific wavelengths of light will allow us to utilize this information to enhance lighting systems on Midwestern Amish farms that will work within their technological limitations.
- An increase in fertility and reproductive development in size of the ducks could result in more meat, therefore benefitting producers and consumers in the poultry industry.

Retina and Brain DBs Interactions

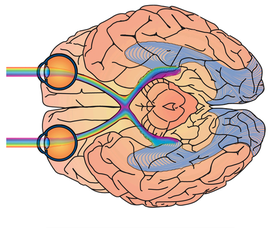
Retina



Genes

Cones	Rods
• RH2	• Rhodopsin
• SWS1	• MAFA
• SWS2	• IRBP
• LWS opsins	

Deep Brain Photoreceptors (DBS)



Genes

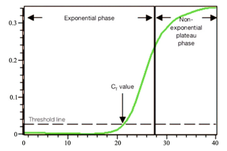
- OPN4
- VAL-opsin
- OPN5

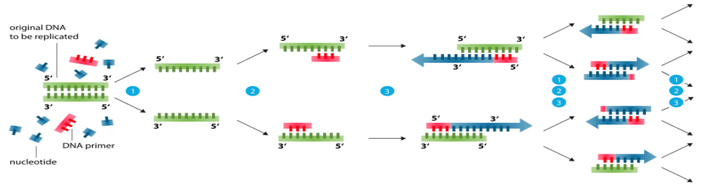
Hypothesis

Removing light during development of visual perception will decrease the amount of muscle produced in the adult Pekin duck.

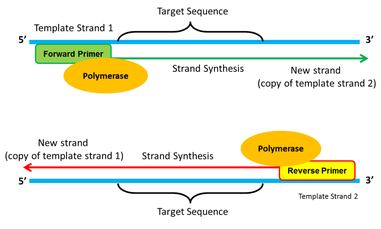
What is qPCR?

Quantitative Polymerase Chain Reaction (qPCR) is a technique that can be used to amplify specific target regions of mRNA.





What is a primer?



Primers are segments of either RNA or DNA nucleotides that can be used as templates required for replication. Primers act as targeting sequences, helping to determine which part of the RNA strand gets amplified.

Our Primer Design

	Classification		Examples
	Good	Bad	Good
Hairpin	Most +ΔG	Most -ΔG	≥-1
Self-dimer	Most +ΔG	Most -ΔG	≥-41 / ≥-5
% Self-dimer	Smallest %	Biggest %	≤12%
Hetero-dimer	Most +ΔG	Most -ΔG	≥-41/ ≥ -8
% Hetero-dimer	Smallest %	Biggest %	≤12%

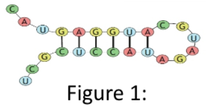


Figure 1: Hairpin

```
5'          CGGATGGGAGACACTGGATG
                 ||
3' GTAGGTCACAGAGGGTAGGC
```

Figure 2: Homo-dimerization

```
5'          CGGATGGGAGACACTGGATG
                 :  |||  :
3' TAAAGGGGGTAACCAGTGGG
```

Figure 3: Hetero-dimerization

Good Primer

structure	Image	ΔG (kcal.mole^{-1})
1		-0.1

Delta G: -1.47 kcal/mole Base Pairs: 2

```
5'          ATGATGAGGGAGAGGGGGAC
                ||
3' CAGGGGGAGAGGGAGTAGTA
```

Bad Primer

structure	Image	ΔG (kcal.mole^{-1})
1		-2.06

Delta G: -6.59 kcal/mole Base Pairs: 4

```
5'          GGGAAAATTCCAGGATCTAT
                ||||  ::::
3' TATCTAGGACCTTAAAAGGG
```

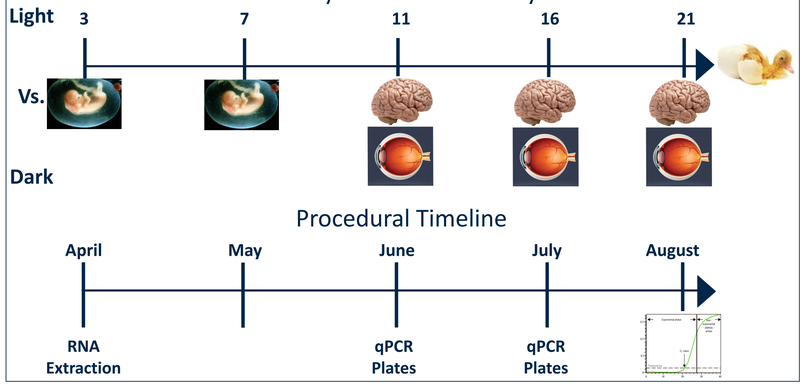
Developmental Time Course of Visual Perception

	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
			Rods Identified	Rhod-opsins	TULP gene expressed	Cone layers and rod somas			Cones and rods forming axons	
								Cones and rods forming axons		
					Rhod-opsins forming cells					
		X	X	Rods form? X	X	Cones form?	X	Axons?	Axons? X	

% = percent of development from fertilization (0%) to eye opening (100%); Ex: 80%= Embryonic Day 10

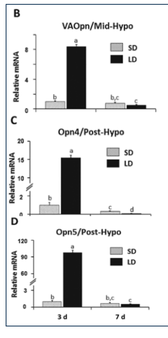
The Big Experiment

Embryonic Extraction Day



Procedural Timeline

Expected Results



	GENES	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
Rods	Rhodopsin										
	Green Opsin										
Cones											
DB	OPN5										
	VAopsin										

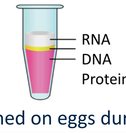
Rods: Darker color = Higher gene expression in developmental timeline %

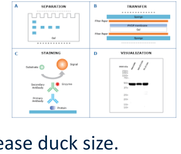
Deep Brain: Darker color = higher gene expression in percentage of developmental timeline in long day vs. short day (10% being shortest day)

Future Directions

1. Asses Protein Level

- RNA extraction with Trizol
- Western Blot





2. Light & Egg Development

What color light should be shined on eggs during development to increase duck size.

Acknowledgements

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