Assessing the Correlation Between DNA Methylation Levels of the ednRB gene using Bisulfite Sequencing and Morphological Color Change of *Astatotilapia Burtoni*

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Abstract

Epigenetics is the study of how the surrounding environment affects gene function. The changes caused by epigenetic mechanisms are reversible and they affect gene expression, but not the organism's DNA sequence. DNA methylation is a process that regulates gene expression by the covalent transfer of a methyl group to the C-5 position on the cytosine ring of DNA. When portions of DNA are methylated, transcription is silenced and expression of that portion of the gene is reduced. *Astatotilapia burtoni*, a model species of the African cichlid fish, displays wide variations in morphology and behavior, despite having little to no intraspecies genetic differences. Specifically, there are two typical color morphs: blue and yellow, that can be induced in a laboratory setting, but they are also found in the wild. Previous research in a laboratory setting has demonstrated that this color change is associated with different levels of DNA methylation of the EdnRB gene. This study will assess if this color change also occurs naturally in the species home environment of Lake Tanganyika, one of the African Great Lakes. We will assess the percent amount of methylation at this single locus and see how it corresponds to the color morphology of the fish.

Background

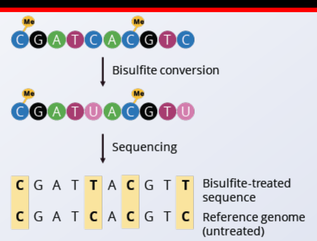

Figure 1: Bisulfite conversion is a process that deaminates the unmethylated cytosines in a strand of DNA, converting them to uracil (U). Methylated cytosines (C) will be unaffected, enabling the ability to differentiate and detect methylated versus unmethylated cytosines.

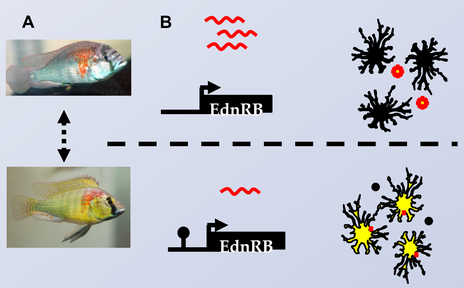

Figure 2: A) Examples of the yellow and blue color morphs of *Astatotilapia burtoni*. B) EdnRB is a single gene locus that affects color change. The gene is overexpressed when the color morphology of the fish is blue, and gene expression is reduced when the color morphology of the fish is yellow

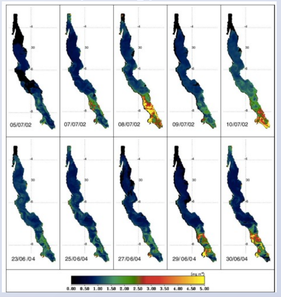

Figure 3: MODIS daily chlorophyll-a concentrations for Lake Tanganyika during the dry season. First row: from 07/05/02 to 07/10/02. Second row: From 06/23/04 to 30/06/04. The variable environment causes morphological color changes in *Astatotilapia burtoni*.

Materials and Methods

- 231 fish samples from the species *Astatotilapia burtoni* were caught and sacrificed on the shore of Lake Tanganyika in Africa. The caudal fin of each fish was removed, labeled, and suspended in ethanol until they were brought back to the lab. They were then washed and placed in individual 1.5 Eppendorf tubes and stored at -80ºC until the following step.

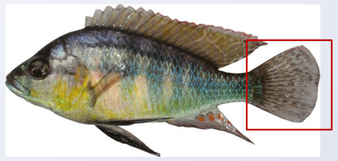

Figure 4: *Astatotilapia burtoni* fish. The caudal fin is indicated by the red box.

- The caudal fin was crushed, and the DNA was extracted. It was then stored in 1.5 Eppendorf tubes at -80ºC until the following step.
- The extracted DNA was deaminated using a sodium bisulfite reaction mixture. The converted DNA was then stored in 1.5 Eppendorf tubes at -80ºC until the following step.
- PCR amplification of the converted DNA was performed at 60ºC using the forward (for) and reverse (rev) ednRB primers listed in Table 1.
- A gel was run to confirm that the primers bound correctly to the converted DNA. This would be portrayed by bands that show up on the gel at the correct base pair lengths for the fragmented DNA.
- Bisulfite sequencing of the converted DNA will be performed using pyrosequencing technologies. The percentage of methylated to unmethylated DNA can then be quantified, as well as how it corresponds to the morphological color of the fish.

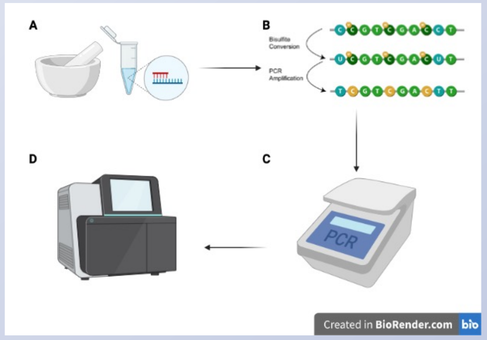

Figure 5: Methods used in their correct order. A) Fin crushing and DNA extraction. B) Bisulfite Conversion of the extracted DNA. C) PCR amplification of the converted DNA using the primer sequences listed below. D) Bisulfite sequencing of the amplified DNA at the ednRB locus.

Primer	Sequence	Tm
EdnRB qPCR for	TGTGCAGAAATCCTCAGTGG	58
EdnRB qPCR rev	CATGGTGATCATGTCGAAGG	
EdnRB in situ for	TGTGCAGAAATCCTC	60
EdnRB in situ rev	GGAACTCCGATCCCTTTGAT	
S EdnRB Luc for	AAGCTTGGGAGTGAGGGAAGATAGGC	60
S EdnRB Luc rev	GGATCCACAAAACCACAGCGTGAACA	
AS EdnRB Luc for	GGATCCGGGAGTGAGGGAAGATAGGC	60
AS EdnRB Luc rev	AAGCTTACAAAACCACAGCGTGAACA	
R1 bis EdnRB for	ACCATTTTTTTGGAGTTTGTGTGCAGACTCT	60
R1 bis EdnRB rev	GTGAGGGAAGATAGGCAGGA	
R1S (-86,-124,-159)	GGAAGATAGGTAGGATG	
R2 bis EdnRB for	GTTTTTTTTTTTTTAATAAGGATAAATATGT	58
R2 bis EdnRB rev	CCCTCTAACATCTTAAAAATTAAAACTTAT	
R2S (-1317,-1270)	AAAACTTATCCAATAATTATCAA	
R3 bis EdnRB for	GTTTGGAAAGGTTGTAGAGAGAA	60
R3 bis EdnRB rev	ACTTTAACAAAACCAAAATTAAACTAAACT	
R3 S (-1678, -1709, -1760, -1771)	AAGTATTTTATATTATTTGTTAAG	
R4 bis EdnRB for	TGGGTTAGGTGTATATGGGTGGTAG	60
R4 bis EdnRB rev	ACCCCAAAAACCTCTCACTATTACC	
R4 S (+1265,+1249,+1229,+1226, +1223,+1202, +1197,+1181,+1174)	GTATATGGGTGGTAGG	
R5 bis EdnRB for	ATAATGGATGAGTGAAAGAAGATATAAATA	60
R5 bis EdnRB rev	CAAACTCTTAAATATACACCCAAACT	
R5 S (+255,+264,+354)	AAAAATTACTAAAAAACTTTAAAAC	

Table 1: List of primer sequences designed for the ednRB locus

Preliminary Results

Sample #	1	2	3	4	5	6
Conc (ng/μl)	307.0	443.0	664.9	522.7	449.4	350.6
260/280	1.86	1.80	1.81	1.84	1.85	1.84
260/230	2.19	2.37	2.38	2.33	2.27	2.28

Table 2: Concentration of the DNA extracted from the caudal fins of fish samples 1-6. The concentration is measured on a scale of nanogram (ng) per microliter (μl). The 260/280 and 260/230 values indicate the purity of the sample. A ratio ~1.8 for the 260/280 value is considered pure. A ratio ~2.0-2.22 for the 260/230 value is considered pure.

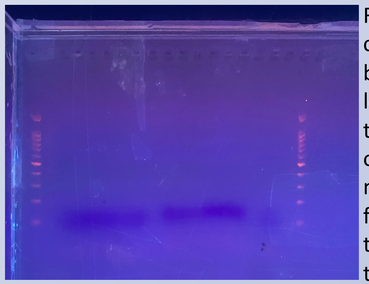

Figure 6: Gel assay of the PCR amplified converted DNA for samples 1-6. The ladder bands on the side correspond to base pair lengths with the longest base pairs closer to the top as they move the slowest. No bands of the DNA are shown on this gel which may be because the DNA was too fragmented during the bisulfite conversion that the pieces are too small to appear on the gel.

Future Directions

- These results can be used to further understand and dissect the function of the ednRB gene.
- Do other species have this conserved locus but also display morphological variation in color? If so, can the variation also be explained by DNA methylation patterns?
- When the DNA is methylated, the promotor is unable to bind and begin the process of transcription. A further study can test the promotor with a promotor reporter assay to confirm that DNA methylation effectively blocks transcription.

Acknowledgements

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Unpublished Data from Sebastian G Alvarado.