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The Presence of Transposable Elements in Actinopterygii, The Ray Finned Fish

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The Presence of Transposable Elements in Actinopterygii, The Ray Finned Fish Rock, Matthew T.

Purpose: My project developed after I was introduced to the idea of horizontal gene transfer in various species. I wanted to further understand one of the largest known mechanisms of horizontal gene transfer, SINE and LINE elements. This project is centered around the analysis of Transposable Elements in various Actinopterygii populations. Transposable Elements are extremely common in The Ray Finned Fish, however analysis of their frequency and position has yet to be done in a majority of the class. I plan to identify their presence in three groups of Ray Finned Fish – Atlantic Killifish, African Cichlids, and Guppies - to analyze their impact on the evolution of each species.

Transposable Elements (TEs): Mobile segments of DNA that can rearrange and reposition themselves throughout the genome. TEs are divided into two major classes: Class I TEs retrotransposons and Class II TEs transposons. Class I TEs are transcribed into RNA and the Reverse transcribed back to DNA. The Newly copied DNA is then inserted back into the genome. Class II TEs are transposed into the genome using transcriptase enzymes to copy and paste regions of DNA.

SINEs and LINEs: The two largest subclasses of Class II TEs, and are estimated to represent 30 percent of the Eukaryotic genome. LINEs or Long Interspersed Nucleotide Elements consisting of roughly 7000 base pairs are transcribed into mRNA and translated into reverse transcriptase which can then copy the LINE and place it elsewhere into the genome. SINEs or Short Interspaced Nucleotide Elements consisting of between 100 to 700 base pairs and generally rely on the reverse transcriptase created by LINEs to be copy and pasted elsewhere in the genome.

Typical SINE

3' Core Domain LINE Segment A Rich Tail

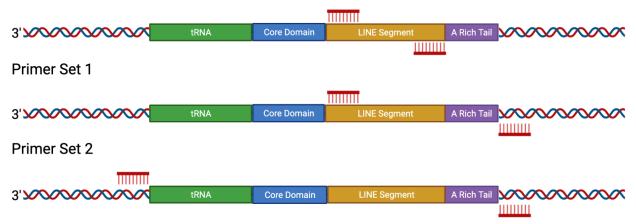
Diagram of a Typical SINE: The first segment of a SINE is a tRNA-derived segment that functions as a promoter for the RNA polymerase and reverse transcriptase. The second component is the Core of the SINEs, a highly conserved region with unknown use. The Third Segment is a portion of the corresponding LINE that the SINE uses to replicate. The Final portion of the SINE is the A rich tail, a region of the SINE that consists primarily of adenine.

Hypothesis: If TE effect gene expression and fitness; then we expect that we will find TE insertions either in gene that influence fitness or their regulatory regions.

Methods: Using the existing literature we identified well known SINEs in class Actinopterygii or Ray-finned fish to test whether we can extract them using PCR technology. Having identified a SINE, we developed 3 set of primers using the program Primer3. The diagram below shows the three different locations for the primers developed. Importantly, we are including some primers in flanking regions of genomic DNA in order to identify specific insertion events in different

individuals and species. These Primers were developed to sequence a SINE present is Cichlids, a member of class Actinopterygii. These sequences were then aligned using MEGA, the 3' and 5' regions of the Alignment are depicted below.

Control



Primer Sets: The Control Primer set is placed to sequence the Line segment of the SINE and is working as our positive control. The Line segment is similar for all SINEs that use the LINE reverse transcriptase thus sequencing all the relevant SINEs in the genome. Primer Set 1 includes the Adenine rich tail and demonstrates whether we have the complete tail of the SINE sequenced. Primer Set 2 attempts the sequence the entire SINE element at a single location in the genome.

Species/Abbrv						• •	••	•	•	• •	•	•	•••	••
1. SINE SEQUENCE				<mark>0</mark>	G G C G A	TCGTGG	С Т С А А -	GAGTTO	GGGAGT	т с <mark>с</mark> с с т	TGTAA	YCGGAA	GGTTGC	C G G T T C G
2. Tropheus_moorii	TAAGGTT	СТАААСС	С Т Т Т <mark>А</mark> Т G [.]	G G	- G <mark>C</mark> G A	TCGTGG	СТСАА-	GAGTTO	G G G <mark>A</mark> G T	тс <mark>сс</mark> т	TGTAA	TCGGAA	GGTTGC	тсстсс
3. Chilotilapia_rhoadesii	TAAGGTT	СТАААСС	CTTTATG	G G	6 - G T G A	TCGTGG	СТСАА-	GAGTTO	G G G <mark>A</mark> G <mark>T</mark>	тсстст	TGTAA	T C G G A A	GGTTGC	с <mark>с с т т с</mark> с
4. Dimidiochromis_compressiceps	AAAAAG	AGACTTT	TCACTGC	A T	- GTGT	TCGTGG	СТСАА-	GAGTTO	GGGAGG	тс <mark>сс</mark> т	TGTAA	T C G G A A	GGTTGC	с с с с т т с с
5. Haplochromis_laparogramma	ATATTTC	ΤΤ <mark>ΓΑ</mark> ΤΤΤ	ACAGCCA	СТТ	TTCGA	тсстсс	СТСАА-	GAGTTO	GGAGT	тсстст	TGTAA	T C G G A A	GGTTGC	сссттус
6. Julidochromis transcriptus			<mark>C</mark> AG	T C A	CACGA	TCGTGG	СТСАА-	GAGTTO	G G A G T	тс <mark>сст</mark>	TGTAA	TCGGAA	GGTTGC	C G G T T C G
7. Labidochromis_caeruleus	TAAGGTT	CTAAC	CTTTATG	G G	GGTGA	TCGTGG	СТСАА-	GAGTTO	G G <mark>A</mark> G T	тсстст	TGTAA	T C G G A A	GGTTGC	C G G T T C G
8. Lepidiolamprologus_elongatus	AAACTTG	GCATTAT	GTCAGGG	G <mark>C</mark> G G G	GGCGA	TCGAGG	СТСАА-	GAGTTO	G G A G T	тс <mark>сс</mark> т	TGTAA	T C G G A A	GGTTGC	сббтттб
9. Melanochromis_auratus	GGAAGTC	A T A T	GCCCAAA	C G G	GGCGA	тсстсс	СТСААА	GAGTTO	GCAGT	тсстст	TGTAA	C C <mark>G G A A</mark>	GGTTGC	сссттсс
10. Neochromis_nigricans	TGTTTT	стттстс	CCTTCGA	GAAAG	GGAGA	тсстсс	СТСАА-	GAGTTO	GGAGT	тс <mark>сс</mark> т	TGTAA	T C G G A A	GGTTGC	сссттсс
11. O.aureus_estrogen_receptor_gene_(exon_10_3_end	d C A T C T G C	TATTCAG	ATGAACA	G T 🖊	AGCGA	тсстсс	СТСАА-	GAGTTO	GGAGT	тс <mark>с</mark> сст	TGTAA	A C G G A A	GGTTAC	сссттсс
12. Oreochromis_niloticus	GGACTTA	ттттст	ATGAAGG	T T T G 🖊	ACTTT	AAATGG	ΤΑΑΑΑΑ	TGGTA	AATGGC	СТСТАТ	TTATA	TAGCGC	ТТТТА С	TAGTCCC
13. Petrochromis_macrognathus	TAAGGTT	СТАААСС	CTTTATG	G G	CGGGA	ТССТСС	СТСАА-	GAGTTO	GGGAGT	тссст	TGTAA	TCGGAA	GGTTGC	тббттсб
14. Ptyochromis_xenognathus	AGAGATC	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	CCCTAAG	G	- GCGA	тсстсс	СТСАА-	GACTTO	G G G A G T	тс <mark>с</mark> сст	TGTAA	TTGGAA	GGTTGC	сссттсс
15. Pundamilia_nyererei	ATATTTC	ΤΤ <mark>ΓΑ</mark> ΤΤΤ	ACAGCCA	C T 1	TTCGA	тсстсс	СТСАА-	GAGTTO	GGAGT	тсстст	TGTAA	TCGGAA	GGTTGC	с с с т т с с
16. Taeniochromis_holotaenia	ATATTTC	ΤΤ <mark>ΓΑ</mark> ΤΤΤ	ACAGCCA	стт	ттсса	тсстсс	СТСАА-	GAGTTO	GGGAGT	тсстст	TGTAA	T C G G A A	GGTTGC	сссттсс
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Species/Abbrv 1. SINE SEQUENCE	ACCA-GA					TTTAYY								
1. SINE SEQUENCE										TGAGT				
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1. SINE SEQUENCE 2. Tropheus_moorii 3. Chilotilapia_rhoadesii	ACTA-GT		ТАТАСАА	A T A C A A T A C A	G G C C A		ATGGG		T C A G	CAAGT	- ССАТ	T T T A C T T T T A C A A C A T T A	AGTAT AGTAT TTTGG	
SINE SEQUENCE Tropheus_moorii Chilotilapia_rhoadesii Dimidiochromis_compressiceps	ACTA-GT ACTA-GT	A A A <mark>G</mark> C <mark>G</mark> C	ТАТАСАА	ATACA ATACA ATACA	G G C C A G G C C A G G C C A	т т т <mark>а с т</mark> т т т <mark>а с т</mark>	A T G G G 1 G T T T T A	СТА	T C A G	CAAGT GCAAG/	- C C A T		AGTAT AGTAT TTTGG TCCTCC	C T C T A A A C T C T A A A T C A C T G G T T G G A T A
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SINE SEQUENCE Tropheus_moori Orbitolipai, phoadesii Orbitolipai, phoadesii Dindidochromis_compressiceps Haplochromis_transcriptus Julidochromis_transcriptus Lepidiochromis_carelueus Lepidiolamprologus_elongatus Melanochromis_auratus	A C T A - G T A C T A - G T A C T A - G T A C T A - G T G T C C C G A A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C C A - G T A C C A - G T A C T A - G T A C A - G T	A A G C G A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C	T A T A C A T A T A C A T A T A T A T G T A T A T A T A A T A T A A T A T A A T A T A A T A T A A T A T A A T A T A A T A T A A T A T A A T A T A A T A T A A	A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T G C A A T G C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A	G G C C A G G C C A G G C C A G G C C A A T C C A G G C C A G G C C A A T C C A A T C C A G G C C A G G C C A G G C C A	T T T A C T T T T A C T T T T A C C N N N N N N T T - A T T T T A C C T T A C C	A T G G G G T T T T A T T T T A T T A T A T T G G A T T G G A T T G G A T T T T A T T T T A T T T T A T T T T	C T A - C C A - C A T - - A T - C T A - C T A - G T C - - A T - G T C - T T T - C A T - C C A T C C A T	- T C A G - T A A A - T T T Y - T A T T - T C A G - C T T C - T - C C C T	C A A G T A G C A A G A A Y G A G A C T T C C A A G T - T G A A C T C A G T C - T A C T T C	- C C A T A G G G G G A G A A T - C C A - A A C A C - A T G T T T C T T	TACTCC TAAGCT CTGTCC TGTCC	C A T A T A	TTGGATA ATTTGCT TAGCAAG TTTTAAG
SINE SEQUENCE Tropheus_moorii Chiotiapia_rhoadesii Dimidiochromis_compressiceps Haplochromis_laparogramma Julidochromis_careruleus Lebidioamorologus_elongatus Melanochromis_auratus No. Neochromis.girgicans Loauratus	A C T A - G T A C T A - G T A C T A - G T A C T A - G T G T C C C G A A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T A - G T A C T G G C A	A A G C G A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C A A G C G C	T A T A C A T A T A C A T A T A C A T G T A C A T G T A C A T A T A C A T A T A C A T A T A C A T A T A C A T A T A C A G T A T A A G A T A C A G A T A C A	A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A T A C A A G G C G	G G C C A G G C C A G G C C A G G C C A A T C C A G G C C A G G C C A A T C C A A T C C A G G C C A G G C C A G G C C A	T T T A C T T T T A C T T T T A C C N N N N N N T T - A T T T T T A C C T T T A C C T T T T A C C T	A T G G G G T T T T A T T T T A T T A T A T T G G A T T G G A T T G G A T T T T A T T T T A T T T T A T T T T	C T A C C A C A T A T C T A G T C A T C T A C T A C T A C T A C T A C T A C C A T A C C A T A C C A C A C A C A C A C A C A C A C A C	T C A G T A A A T T T Y T C A G - C T T C C C C T A A C T A T	C A A G T - G C A A G A A Y G A G A C T T C - C A A G T - T G A A C T C A G T C - T A C T T C	- C C A T A G G G G G A G A A T - C C A - A A C A C - A T G T T T C T T	TACTCC TAAGCT CTGTCC TGTCC	CATAT TGTGAC TCCAG	TTGGATA ATTTGCT TAGCAAG TTTTAAG
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Guppies: We have been developing a low diversity population of *Poecilia reticulata* a member of class Actinopterygii. This population has low heterozygosity and as a result should have a larger fraction of fixed RTEs. Using a program known as RepeatMasker we plan to search the genome of the Guppies for low complexity regions that are highly conserved. From there we can search the promoter region of genes known to impact fitness in the guppy population. We can also search for TE insertions in coding regions that may destroy function. Specifically, we plan to search genes known to impact fertility because we have data containing observations from guppies. six generations of guppies, this allow us



Figure 1"Blue Moscow Guppy (Poecilia Reticulata Var. 'Blue Moscow'), Males and Females" Aquatic Arts, aquaticarts.com/blue-moscowguppies.

to quantify reproductive fitness using as a result. If we can find TEs in the promoter regions of the reproductive fitness genes it could explain the decrease in fitness of the overall population.

Atlantic Killifish: We will search the promoter regions of these fish using the same methods as the guppies. Fundulus heteroclitus is a well studied fish that has adapted to live in heavily polluted environments. Genes have been identified that are believed to explain this adaptation, but the evolutionary details are unknown. If this technology identifies a TE in the promoter regions of the genes it could suggest that the mechanism behind their adaptations was the presence of Transposable Elements. Alternatively, some adaptations in F. heteroclitus are thought to be loss of function mutations.



Figure 2 Clark B. "F. heteroclitus", U.S.E.P.A

African Cichlids: The African cichlids are a well known model for the evolution of species diversity, and several species have whole genomes available. SINE elements are well studied and ubiquitous in this group. We have a large set of population samples from several species and will be searching for TE insertion events that are unique to different lineages



Figure 3 Konigs, Ad. "East African Cichlids." New Markers for New Species: Microsatellite Loci and the East African Cichlids, 2001, doi.org/10.1016/S0169-5347(00)02064-4.