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

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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

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
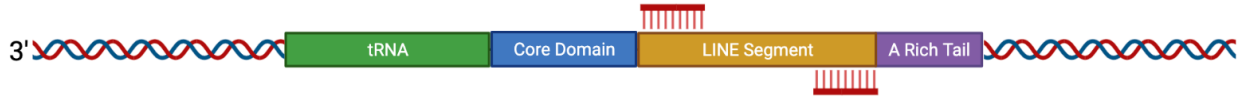
Primer Set 1

Primer Set 2

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
2. Tropheus_moorii
3. Chilotilapia_rhoadesii
4. Dimidiochromis_compressiceps
5. Haplochromis_laparogramma
6. Julidochromis_transcriptus
7. Labidochromis_caeruleus
8. Lepidiolamprologus_elongatus
9. Melanochromis_auratus
10. Neochromis_nigricans
11. O.aureus_estrogen_receptor_gene_(exon_10_3_end
12. Oreochromis_niloticus
13. Petrochromis_macrognathus
14. Ptyochromis_xenognathus
15. Pundamilia_nyererei
16. Taeniochromis_holotaenia

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 six generations of guppies, this allow us 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.

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

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.