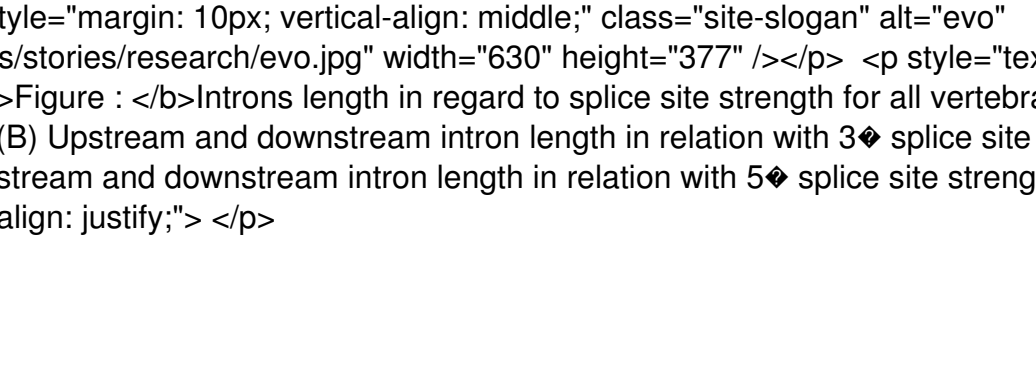
In order to comprehend the influence of evolutionary changes in gene structure on the alternative splicing mechanism, we work on three related subjects: (i) conservation of exonintron sequences, (ii) splice site score changes and (iii) length data changes, across the vertebrate tree. We have analyzed conservation levels of human Alternative cassette exons (ACEs) and human constitutive exons (const exons) across the vertebrate tree. We have also constructed datasets of the orthologous exons for each alternativeconstitutive exon, as well as for its flanking intronic regions. Thus, we were able to compare conservation of orthologous splicing signals across vertebrate evolution. Changes that accumulated within orthologous splice sites during vertebrate evolution might suggest changes in the mode of splicing, such as a shift from constitutive to alternative selection, or changes in the inclusion level. We have also identified point mutations, along the orthologous sequences, which represents a shift in the evolutionary rate (Rate Shift - RS). The RS mutations were found using the Covarion program which was constructed by Tal Pupko's bioinformatics Lab. Next, we produced algorithms in search of ESRs (Exonic Splicing regulators) in those rate shifting areas. Those ESRs could have formed a newly acquired splicing functionality and therefore became more conserved. This also enabled us to discern between highly functional ESR groups and less functional ones.

We have also identified the changes in splicing signals, across the evolutionary tree, which resulted in Alternative Splicing. We have analyzed splice site strength of ACEs and constitutive exons between 17 vertebrate genomes. we calculated 5'ss and 3'ss score and compared between the orthologous 5'ss and 3'ss. With the added aspect of constitutive to alternative differences, the results help us shed light on the prerequisites for the constitutive to ACEs transition.

To further advance our research, we attempt to link between changes in exon and intron length and other splicing signals through the vertebrate tree, which will enable us to find the major interactions that lead to Alternative Splicing. Parallel to the sequence length analysis, we also performed a reconstruction of the length data for all tested genomes in order to assume the direction of evolutionary changes in length, for all tested regions. Please see Figure 1 for preliminary results concerning the change in splice sites strength in regard to intron length, throughout the vertebrate evolution.

    Figure : Introns length in regard to splice site strength for all vertebrates tested.(A)+(B) Upstream and downstream intron length in relation with 3' splice site strength. (C)+(D) Upstream and downstream intron length in relation with 5' splice site strength.