Additional file 5 — The alignment of the genomic sequences homologous by position to exon 8 in *D. pseudoobscura* and *D. persimilis.* Only the focal part of this alignment immediately at and near exon 8 is presented. Genomic fragments were amplified and sequenced from the corresponding *Drosophila* species, except for the sequence of *D. willistoni*, which were downloaded from FlyBase.org (see test for details). Exon 8 is highlighted in grey. The splicing sites around exon 8 are underscored. While all *Drosophila* species shared both 3' and 5' SS flanking exon 8, the region corresponding to exon 8 did not encode a polypeptide chain in any tested species except *D. pseudoobscura* and *D. persimilis.* Notably, sequence changes that capacitated exon 8 in the *obscura* group were highly focal and could not be attributed to duplication-translocation of existing exons [e.g., 1, 2], retrotransposition [e.g., 3] or exaptation of repetitive elements [e.g., *Alu* element, 4, 5]. Therefore, the functional ORF of exon 8 was created *de novo* from intronic sequence between the existing SS.

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D. simulans          1 AATCATTATACCCTTTTCT-TTTGTCGTTACAGGTATGTAAAAA---------------A
D. melanogaster      1 AATCATCATACCCTTTTCT-TTTGTCGTTACAGGTATGTAAAAAGAA------------A
D. erecta            1 AATCATAATACCCTTTTCTCTTTGTCTTTACAGGTATGTACAA-----------------
D. ananassae         1 TCTAAATGTATTCTTATCCTTCTCTCTTTTCAGGTATGTTGAA----------------A
D. pseudoobscura     1 TAAATCTGATTTTCCCTCTCTCTATCTTTGCAGGTATGTACTA---------------CA
D. persimilis        1 TAAATCTGATTTTCCCTCTCTCTATCTTTGCAGGTATGTACTA---------------CA
D. willistoni        1 TAACCTCTATTTTCTTTTCTCTTATCGTTTCAGGTATGTAACAAAAAAAAAGTGATGGGA
D. mojavensis        1 TATCACTTCTCTTCTTCTTCTTTATCGTTGCAGGTATGTACCAACAACAA--------CA
D. virilis           1 TCATATTTATTCTCTTCTTCTTTATCTTTACAGGTATGTACTA---------------CA

D. simulans         45 AA-GGTCTTAACCCTCGAGATAAT-CAATCCT----------------------------
D. melanogaster     48 AA-GGTCTAAACCCTCGAGATAAT-CAATCCT----------------------------
D. erecta           44 AA-GGTCCTAACCCCCAGGATAAT-CAATCCT----------------------------
D. ananassae        45 AATTAGCATAACACAAT-AATAA-CCAATCGG----------------------------
D. pseudoobscura    46 AATTTGCATAGCTCCAAGGAT-AT-CAATCCTTT--GCCCTATT----------------
D. persimilis       46 AATTTGCATAGCTCCAAGGAT-AT-CAATCCTTT--GCCCTATT----------------
D. willistoni       61 AATTTGCATAAATCTCTCTCGAATCCAACCCTTTTTTTCCTATTCCCTACTATCCCACAC
D. mojavensis       53 AATTTGCATAACACAATGCAT----GAAAAAAATAGAAAAAAAGTAATCGA--GTAAAAA
D. virilis          46 AATTTGCATAACACGA-----------------------------------GCAAAGA

D. simulans         75 ------------TAAAGTGTTCC-----GTAAGTTCAAGTGCCTTTGTCTTGTCC
D. melanogaster     78 ------------TAAAGTGTTCC-----GTAAGTTCAAGTGCCTTTGTCTTGTGC
D. erecta           74 ------------TAAAGTGTTCC-----GTAAGTTCAAGTGCCTTTGTCTTGTCC
D. ananassae        75 ------------TCAAGTTTTCC-----GTAAGTTCAAGTGCCGTTGTCTGGCGG
D. pseudoobscura    86 ------------------CTGGC-----GTAAGTTCAAGTGCCTTTATCTTGTCC
D. persimilis       86 ------------------CTGGC-----GTAAGTTCAAGTGCCTTTATCTTGTCC
D. willistoni      121 ACTATATATCCAACAAATGCTCC---AAGTAAGTTCAAGTGTCTCTTCTATTCTG
D. mojavensis      107 AGAACC-CTTTCGAGGGGCATCCTTTATGTAAGTGCAGTGTCGCAGACAAATCCT
D. virilis          69 ATAACCCCTTT---GGGGCATCCTTTATGTAAGTGCAGTGTCCCAGACGAATCCT
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