

**Table S17:HKA test for Old and Young high sequence coverage sites**

Species	Groups compared	Polymorphism		Divergence		Sum of Deviations (X <sup>2</sup> )	P value
		Observed	Expected	Observed	Expected		
<i>D.mel/Dsim</i>	Old	3377	3289.8	5416	5503.2	996.5	P<0.001
	Young	351	438.6	810	722.4		
	Old	3377	3395.1	5416	5397.9	1124.6	0.15<P<0.19
	Small intron	1147	1128.8	1886	1904.2		
	Young	351	370.9	810	790.1		
	Small intron	124	104.1	215	234.9		
<i>D.mel/D.yak</i>	Old	3386	3310.1	12256	12332.0	1071.0	P<0.001
	Young	352	428.2	1647	1570.8		
	Old	3386	3407.8	12256	12234.2	1334.2	0.03<P<0.04
	Small intron	1099	1076.7	4115	4137.3		
	Young	352	404.1	1647	1594.9		
	Small intron	116	110.9	469	474.2		

Note: the observed and expected number of polymorphic and divergence sites are summarized over all sites within each group, while the value of sum of deviation (X<sup>2</sup>) is calculated by adding values of deviation for each loci together. The P value is calculated based on 1000 iterations of colacence simulation. All results shown here are obtained after filtering sites with input sequence coverage <0.5. When compared to Old sites and neutral small introns, the group of Young sites show significant reduced polymorphism, suggesting directional positive selection on them.