

**Table S16:HKA test for Old and Young 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             | 3496         | 3395.9   | 5684       | 5784.1   | 1036.4                              | P<0.001      |
|                    | Young           | 391          | 491.0    | 925        | 825.0    |                                     |              |
|                    | Old             | 3496         | 3491.4   | 5684       | 5688.6   | 1156.9                              | 0.15< P<0.16 |
|                    | Small intron    | 1145         | 1148.1   | 1973       | 1969.9   |                                     |              |
|                    | Young           | 391          | 405.8    | 925        | 910.2    | 172.4                               | P<0.001      |
|                    | Small intron    | 135          | 120.3    | 273        | 287.7    |                                     |              |
| <i>D.mel/D.yak</i> | Old             | 3504         | 3413.3   | 12784      | 12874.7  | 1114.2                              | P<0.001      |
|                    | Young           | 388          | 478.7    | 1872       | 1781.3   |                                     |              |
|                    | Old             | 3504         | 3510.6   | 12784      | 12777.4  | 1324.7                              | 0.04<P<0.05  |
|                    | Small intron    | 1108         | 1101.8   | 4283       | 4289.2   |                                     |              |
|                    | Young           | 388          | 417.5    | 1872       | 1842.5   | 202.5                               | P<0.01       |
|                    | Small intron    | 145          | 115.6    | 510        | 539.4    |                                     |              |

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 coalescence simulations. When compared to Old sites and neutral small introns, the group of Young sites show significant reduced polymorphism, suggesting directional positive selection on them.