

## Additional file 2 — Experimental results

Additional file 2 is a PDF file containing a description of an application of the methods to real *Drosophila* data.

We applied Algorithm 2 to reconstruct an ancestral chromosome for the chromosome 2 of *Drosophila yakuba* (*D.yak*) (Muller's Elements B and C). The dataset is based on 14 synteny blocks shared with the chromosome 2 of *Drosophila melanogaster* (*D.mel*), and 13 duplicated segments identified at breakpoints in (*D.yak*) [4]. Figure 3 and Table S5 in [4] provide the following arrangement of the synteny blocks, numbered from 14 to 36 according to their order in *D.mel*, and the 13 duplicated segments (numbered from *A* to *M*) in the chromosome 2 of *D.yak*:

( $\circ$  14 *A* -17 19 -*B* -15 -*A* 18 -16 -*B* 20 *C* 27 *D* -21 -*C* -26 -*E* -*F* -28 -*D* 22 *G* -*H* -23 -*G* *H*  
24 *I* -*J* -29 -*F* -*E* -25 -*I* *J* 30 *K* -33 -*L* 35 -*M* -31 -*K* 34 -*L* -32 -*M* 36  $\circ$ ).

After discarding the synteny blocks from the sequence, the following totally duplicated sequence with  $n = 13$  duplicated markers is obtained:

$G = (\circ A -B -A -B C D -C -E -F -D G -H -G H I -J -F -E -I J K -L -M -K -L -M \circ)$ .

Figure 3 and 4 gives the adjacency graph and the overlap graph of  $G$ . They show that  $G$  is an oriented valid-path genome whose adjacency graph contains  $C = 3$  cycles. Therefore, the reversal distance is  $d_{rev}(G) = n - C = 13 - 3 = 10$ .

Algorithm 2, applied to the four oriented connected components of the overlap graph, provides the following reversal scenario of length 10 where reversed segments are underlined:

( $\circ$  *A* -B -A -*B* *C* *D* -*C* -*E* -*F* -*D* *G* -*H* -*G* *H* *I* -*J* -*F* -*E* -*I* *J* *K* -*L* -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* -B *C* *D* -*C* -*E* -*F* -*D* *G* -*H* -*G* *H* *I* -*J* -*F* -*E* -*I* *J* *K* -*L* -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *D* -*C* -*E* -*F* -*D* *G* -H -*G* *H* *I* -*J* -*F* -*E* -*I* *J* *K* -*L* -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *D* -*C* -*E* -*F* -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* -*L* -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *D* -C -*E* -*F* -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* -*L* -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *D* -C -*E* -*F* -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* -*L* -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* -*L* -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* -*L* -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* -*L* -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* -L -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -*L* -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -*M* -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -*K* -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -*L* -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -*M*  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J* *J* *K* *K* -M -L -M -K -L -M -L -L -L -L -L -L -L -L -L  $\circ$ )  
( $\circ$  *A* *A* *B* *B* *C* *C* -D -*E* -F -*D* *G* *G* *H* *H* *I* *I* *E* *F* *J*

The corresponding reversal scenario on the arrangement of the 14 synteny blocks is the following scenario, in which the reversal are denoted using the nomenclature of [4]:

- (o 14 17 19 -15 18 -16 20 27 -21 -26 -28 22 -23 24 -29 -25 30 -33 35 -31 34 -32 36 o)
  - $\downarrow 2L(1)$
- (o 14 15 -19 17 18 -16 20 27 -21 -26 -28 22 -23 24 -29 -25 30 -33 35 -31 34 -32 36 o)
  - $\downarrow 2L(2)$
- (o 14 15 16 -18 -17 19 20 27 -21 -26 -28 22 -23 24 -29 -25 30 -33 35 -31 34 -32 36 o)
  - $\downarrow 2L(6)$
- (o 14 15 16 -18 -17 19 20 27 -21 -26 -28 22 23 24 -29 -25 30 -33 35 -31 34 -32 36 o)
  - $\downarrow 2LR(7)$
- (o 14 15 16 -18 -17 19 20 27 -21 -26 -28 22 23 24 25 29 30 -33 35 -31 34 -32 36 o)
  - $\downarrow 2LR(4)$
- (o 14 15 16 -18 -17 19 20 21 -27 -26 -28 22 23 24 25 29 30 -33 35 -31 34 -32 36 o)
  - $\downarrow 2LR(8)$
- (o 14 15 16 -18 -17 19 20 21 -27 -26 -25 -24 -23 -22 28 29 30 -33 35 -31 34 -32 36 o)
  - $\downarrow 2LR(5)$
- (o 14 15 16 -18 -17 19 20 21 22 23 24 25 26 27 28 29 30 31 -33 35 -31 34 -32 36 o)
  - $\downarrow 2R(9)$
- (o 14 15 16 -18 -17 19 20 21 22 23 24 25 26 27 28 29 30 31 32 -34 33 34 -32 36 o)
  - $\downarrow 2R(10)$
- (o 14 15 16 -18 -17 19 20 21 22 23 24 25 26 27 28 29 30 31 32 -34 -33 35 36 o)
  - $\downarrow 2R(11)$
- (o 14 15 16 -18 -17 19 20 21 22 23 24 25 26 27 28 29 30 31 32 -34 33 35 36 o)

The reversals 2L(6), 2LR(8) 2LR(7) are 2-BD-reversal, and the seven other reversals of the scenario are 1-BD-reversal. In good agreement with biological results, the proposed scenario consists of 10 out of the 11 reversals proposed in the litterature to explain the evolution of the chromosome 2 of *D.mel* and *D.yak* [4]. Thus, the proposed ancestor of *D.yak* chromosome 2 is 10 reversals far from *D.yak*, and only 1 reversal far from *D.mel*. This results strongly supports the hypotheses of a slow evolution in the *melanogaster* species tree compared to the evolution in the *yakuba* species tree, raised by biological studies [15].