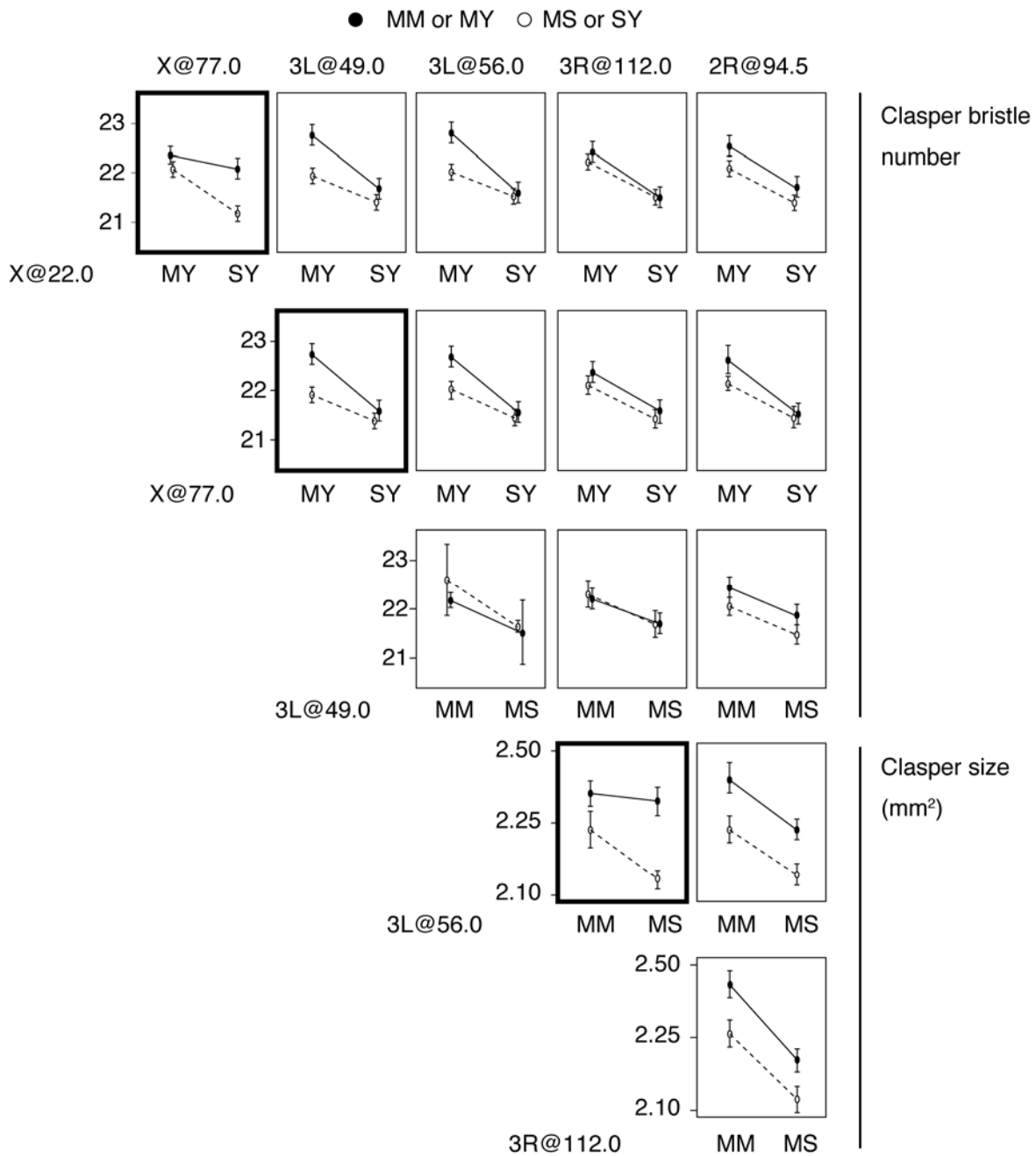
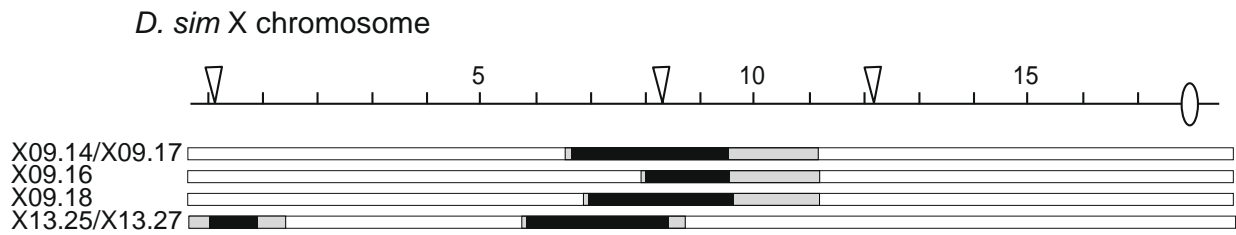


**Figure S1** Claspers (top panel), posterior lobes (middle) and anal plates (bottom panel) of F1 (D/w501), D and Q introgression lines and *D. simulans* w501. Green shading represents the area measured for each trait of introgression lines. The cuticle on the proximal end of the claspers, typically harbouring four bristles has a tendency to fold in when mounted on a slide in *D. simulans* but not in *D. mauritiana*. Therefore, to standardise the measurement of clasper area, we traced a closed contour of each clasper starting at the 4<sup>th</sup> proximal bristle and finishing in the distal limit of the structure. The area of posterior lobes was determined from an outline of the structure delimited by an artificial base line at its proximal end. Since the anal plates are closed structures, the measurement of their area was defined simply by their contour. Finally, the yellow dashed outline in the top left panel indicates the clasper size measurement used in the QTL mapping analysis. The scale bar corresponds to 50  $\mu\text{m}$  in all images.



**Figure S2** Interaction between QTL. Genetic interactions between QTL peaks are shown for clasper bristle number and clasper size. Graphs enclosed by bold frames indicate significant interactions as shown in Table 1. M and S represent *D. mauritiana* and *D. simulans* alleles, respectively. Genotypes of the left QTL peaks are shown under the boxes. Open circles (MS or MY) and black-coloured circles (MM or MY) represent genotypes at the top QTL peaks.



**Figure S3** X chromosome introgression lines. The X chromosomes are shown with *D. simulans* coordinates in Mb. Open triangles indicate the position of the visible markers (along the chromosome, *y,v,f*). The centromere position is indicated by an open oval. Introgression chromosomes are shown with black and white shading indicating DNA from *D. mauritiana* or *D. simulans* respectively. Grey shading indicates where the precise breakpoints of the introgressions have not yet been resolved.

**Tables S1-S8**

Available for download as Excel files at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.174045/-/DC1>

**Table S1. Phenotypic measurements**

**Table S2. Molecular markers**

**Table S3. Principal Components in the elliptic fourier analysis for posterior lobe shape**

**Table S4. QTL mapping file formatted for import into R/qtl.**

**Table S5. Tukey's test for multiple comparisons between homozygous D introgression lines.**

**Table S6. Test of dominance**

**Table S7. Interaction test by two-way ANOVA**

**Table S8. RNAi Screen**