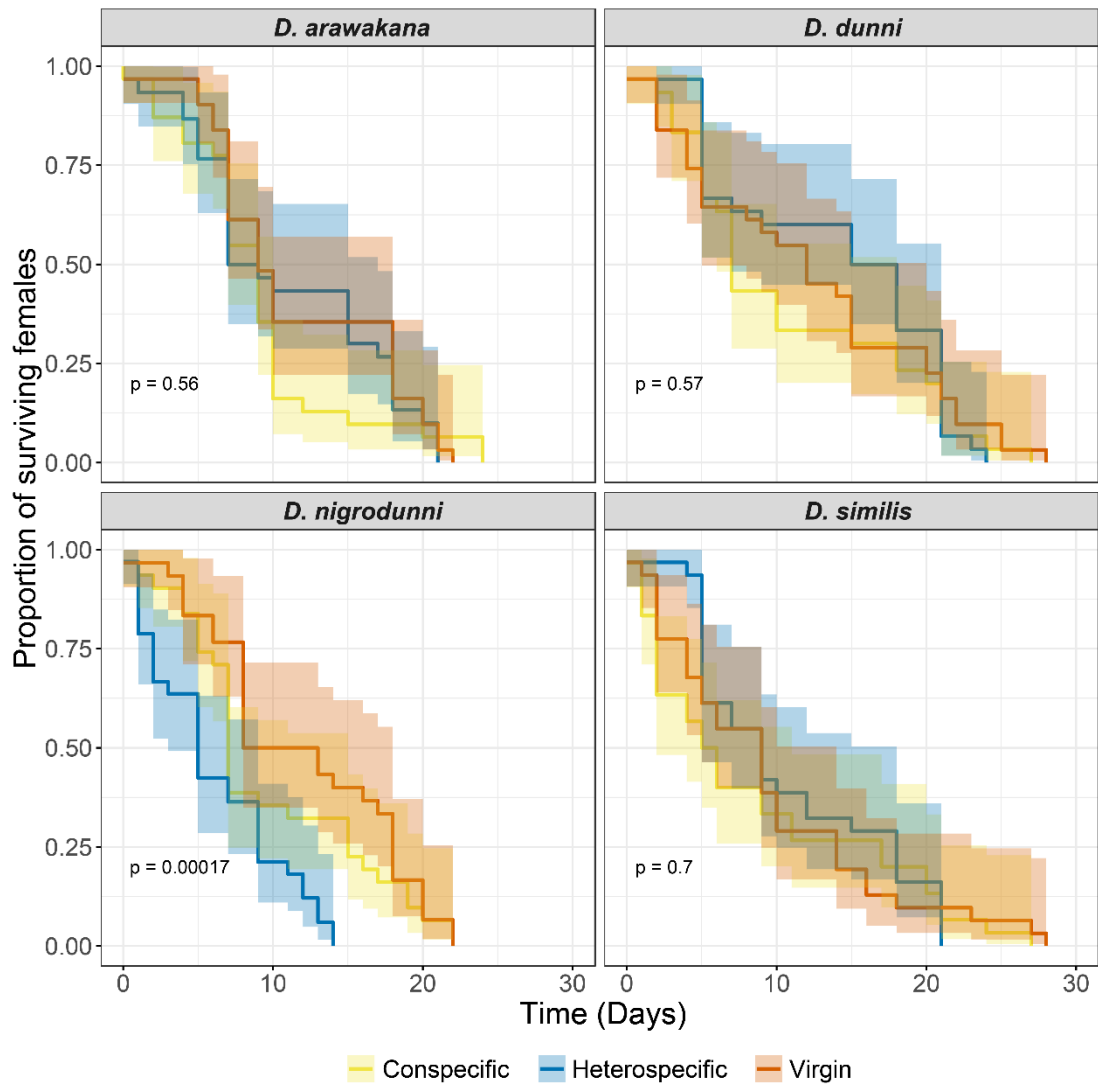**Supplementary Figure 1: Phylogeny of the *dunni* group relative to other *Drosophila* species.**

Phylogeny was calculated using PhyML (GUINDON *et al.* 2010), finding the consensus of 100 genes, with bootstrap values (the number that match out of 100) shown at nodes. Scale bar shows the branch length of 0.2 substitutions per site.
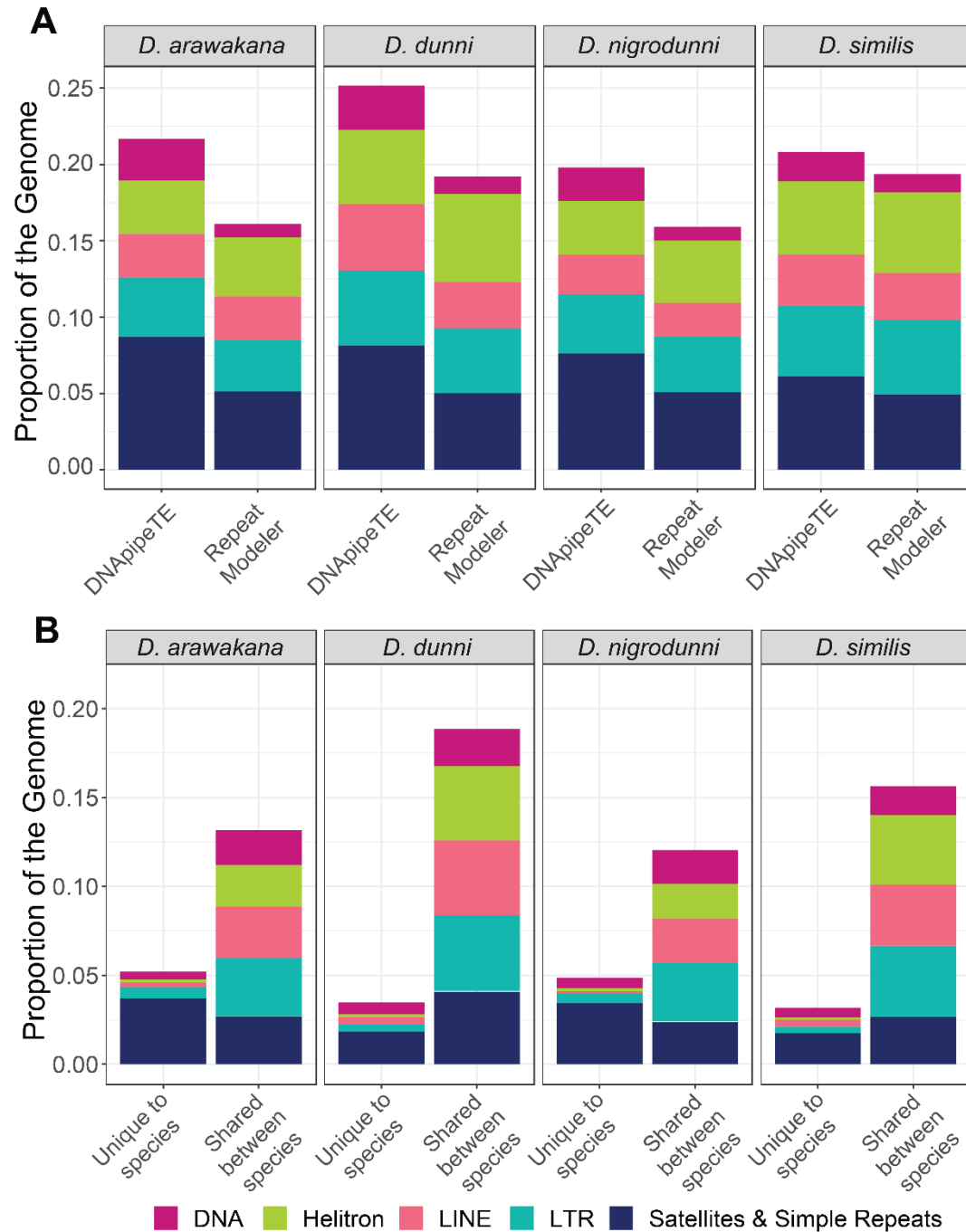
Supplementary Figure 2: Proportion of females surviving each day, for each species used in each cross, compared to virgin female survival. Crosses following curing of the strain with Tetracycline-Hydrochloride. Females are separated by species, and grouped as unmated (virgins, red), conspecific crossed (crossed to own species, blue), heterospecific crossed (crossed to a different species, yellow). In the case of heterospecific crosses, *D. arawakana* is only crossed to *D. nigrodunni* and *D. dunni* is only crossed to *D. similis*. The shaded regions of each line represent the standard error of the survival curve.
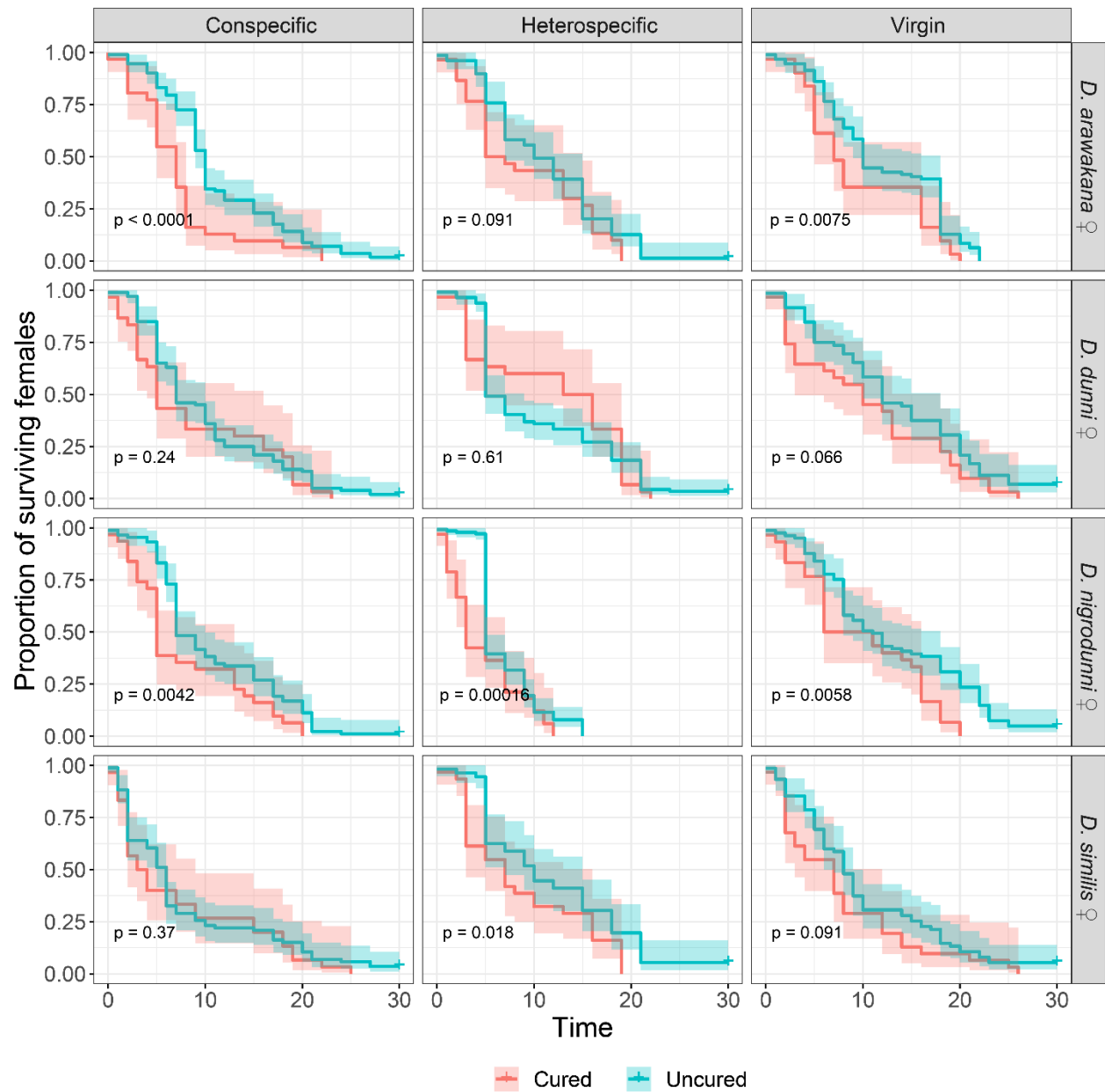
Supplementary Figure 3: Difference in survival for different sets of crosses, comparing between survival of females before and after curing with Tetracycline-Hydrochloride. The shaded regions of each line represent the standard error of the survival curve.
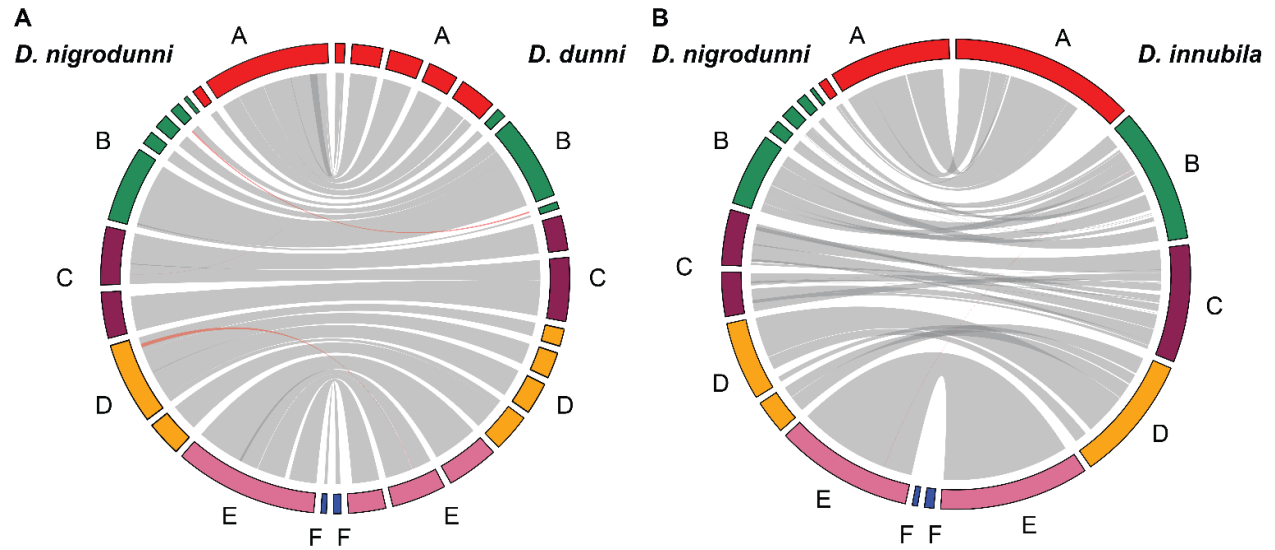
Supplementary Figure 4: Orthologous regions between the *D. nigrodunni* genome, *D. dunni* genome and *D. innubila* genome. Syntenic regions on the same chromosome (shown as Muller elements, A-F) are labelled with grey ribbons, while syntenic regions between difference chromosomes are labelled in red.

Supplementary Figure 5: Proportion of each genome made up of repetitive content (colored by classification of repetitive content) For simplicity, the satellite category contains Satellites, microsatellites, simple repeats tandem repeats and low complexity regions. **A.** Comparison of TE annotation between two tools, DNApipeTE and Repeatmodeler. **B.** Comparison of TE content across species and if that content is shared between species or is unique to one species.
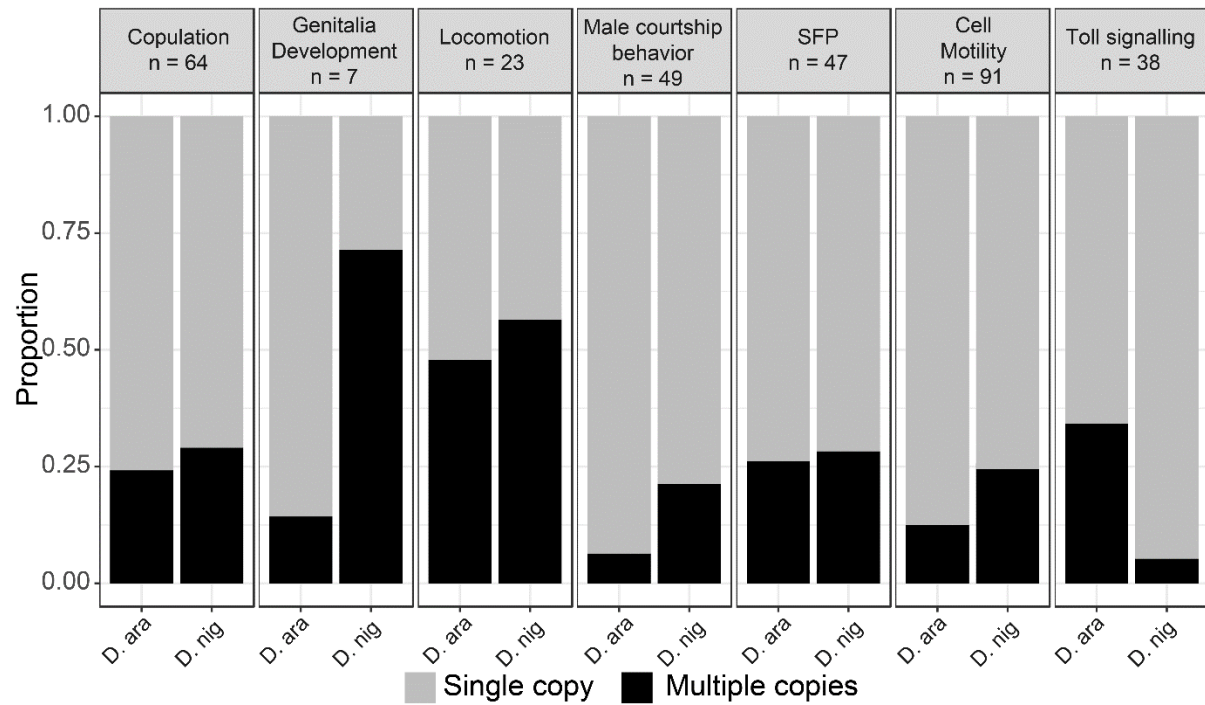
Supplementary Figure 6: Proportion of gene categories with enrichments of duplications in *D. nigrodunni* (D. nig), *D. arawakana* (D. ara) or both species. N = number of genes per category.