

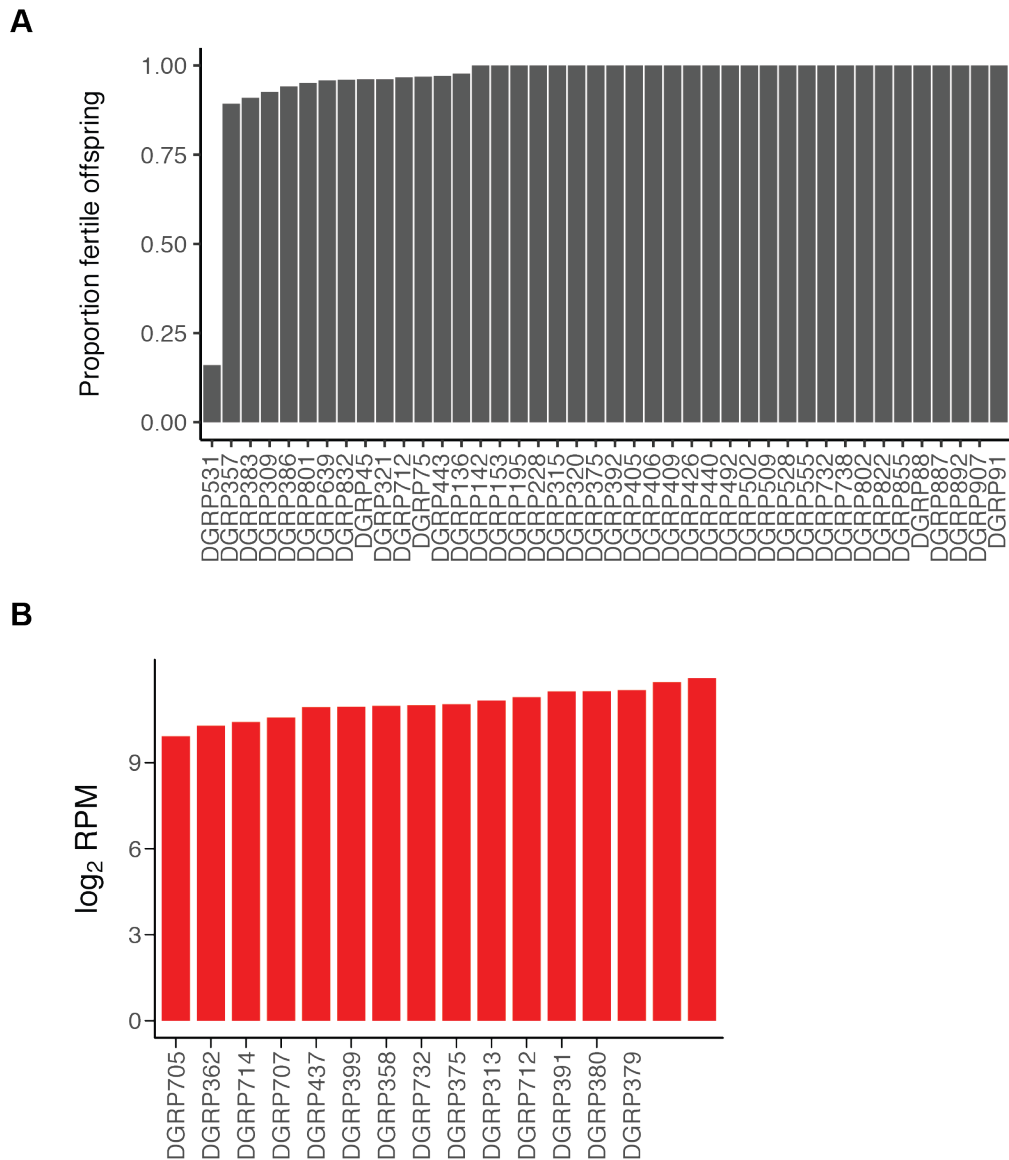
## List of Supplementary Items

Supplemental Figure S1: Maternal *P*-element repression and piRNA production among DGRP genomes.

Supplemental Figure S2: Flowchart of annotation pipeline for non-TAS insertions.

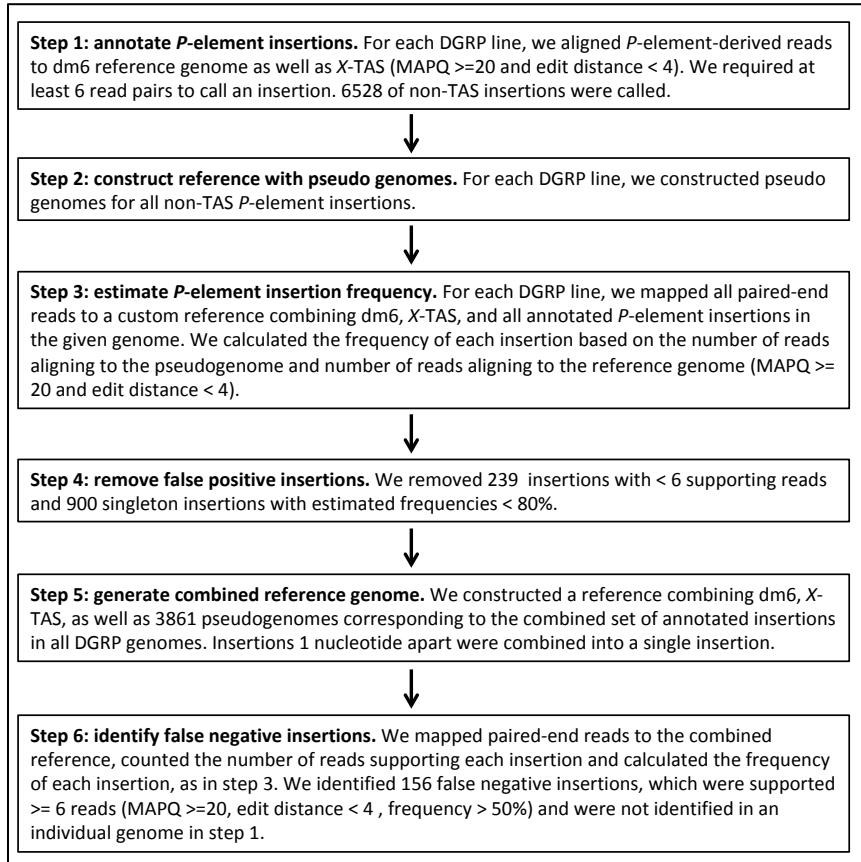
Supplemental Figure S3: Histogram of the of *P*-element insertions in piRNA clusters across DGRP genomes.

Supplemental Figure S4: Maternal repression of hybrid dysgenesis among strains lacking a *P*-element insertion in an ancestral piRNA cluster.

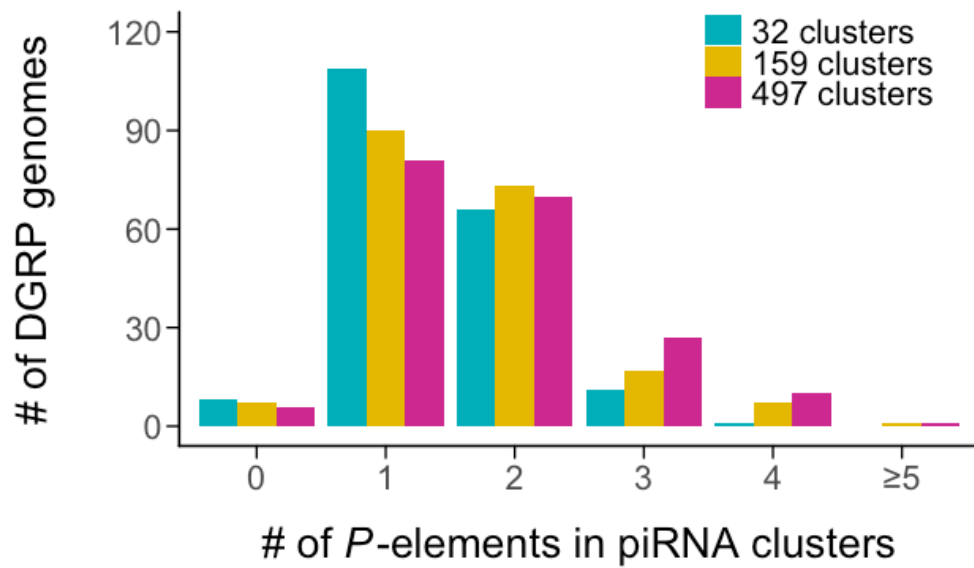


**Supplemental Figure S1.** Maternal *P*-element repression and piRNA production among DGRP genomes. (A) Maternal repression of *P*-element activity was measured for 42 randomly selected DGRP genotypes through dysgenic crosses with males from the reference *P* strain Harwich. The proportion of F1 offspring from each cross is represented, with high fertility indicating maternal repression. All genomes contain annotated *P*-element insertions in TAS piRNA clusters, except for DGRP509 that contains an insertion in a high-confidence non-TAS ancestral piRNA cluster, DGRP91 that contains an insertion in a medium-confidence non-TAS piRNA cluster, and DGRP 153, 357 and 386, whose sequencing reads are too short to annotate *P*-elements. (B) *P*-element derived piRNA production for 16 DGRP genomes, based on small RNA libraries from Song *et al.* (Song *et al.* 2014). piRNA density is measured in Reads Per Million mapped piRNA reads (RPM) and transformed to log<sub>2</sub> scale (log<sub>2</sub> RPM). All genomes contain annotated *P*-element insertions in TAS piRNA

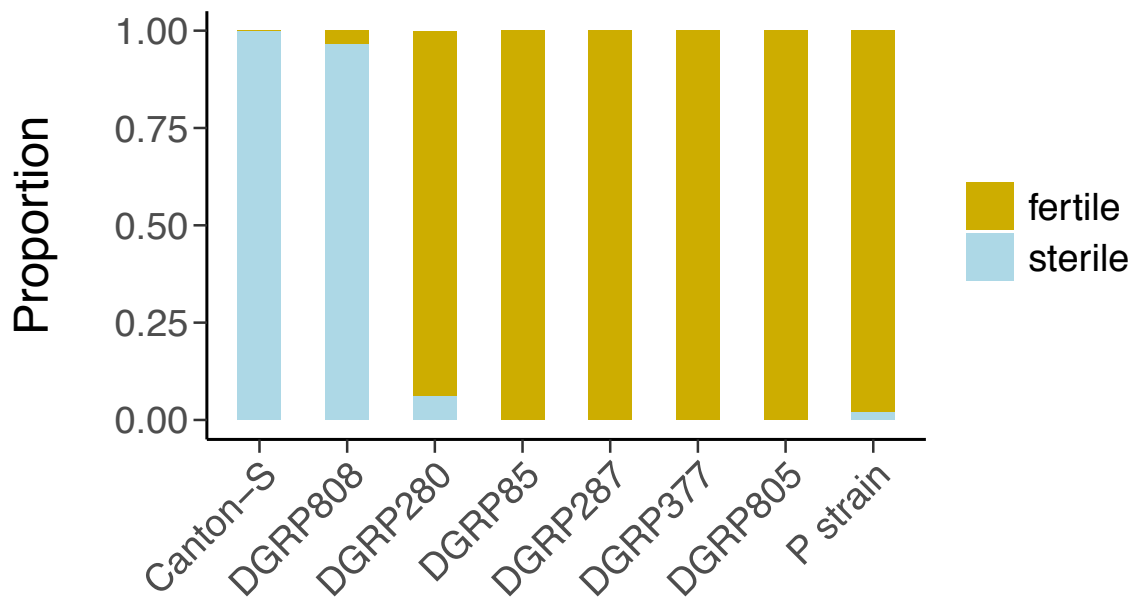
clusters, except for DGRP 313, 379 and 427, whose sequencing reads are too short to annotate P-elements.



**Supplemental Figure S2.** Flowchart of annotation pipeline for non-TAS insertions.



**Supplemental Figure S3.** Histogram of the of *P*-element insertions in piRNA clusters across DGRP genomes. Counts are reported for all three stringencies of piRNA cluster annotation. Regardless of the stringency of piRNA cluster annotation, more than 90% DGRP lines had at least one *P*-element insertion an ancestral piRNA cluster.



**Supplemental Figure S4.** Maternal repression of hybrid dysgenesis among strains lacking a *P*-element insertion in an ancestral piRNA cluster. Proportional fertility (ovaries not atrophied) is reported for F1 offspring of dysgenic crosses between DGRP females and Harwich (P strain) males. Crosses involving Canton-S and P-strain females are provided as negative and positive controls for the absence and presence of repression, respectively.