List of Supplementary Items

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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 log₂ scale (log₂ 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.

**Step 1: annotate P-element insertions.** For each DGRP line, we aligned P-element-derived reads to dm6 reference genome as well as X-TAS (MAPQ >=20 and edit distance < 4). We required at least 6 read pairs to call an insertion. 6528 of non-TAS insertions were called.

**Step 2: construct reference with pseudo genomes.** For each DGRP line, we constructed pseudo genomes for all non-TAS P-element insertions.

**Step 3: estimate P-element insertion frequency.** For each DGRP line, we mapped all paired-end reads to a custom reference combining dm6, X-TAS, and all annotated P-element insertions in the given genome. We calculated the frequency of each insertion based on the number of reads aligning to the pseudogenome and number of reads aligning to the reference genome (MAPQ >= 20 and edit distance < 4).

**Step 4: remove false positive insertions.** We removed 239 insertions with < 6 supporting reads and 900 singleton insertions with estimated frequencies < 80%.

**Step 5: generate combined reference genome.** We constructed a reference combining dm6, X-TAS, as well as 3861 pseudogenomes corresponding to the combined set of annotated insertions in all DGRP genomes. Insertions 1 nucleotide apart were combined into a single insertion.

**Step 6: identify false negative insertions.** We mapped paired-end reads to the combined reference, counted the number of reads supporting each insertion and calculated the frequency of each insertion, as in step 3. We identified 156 false negative insertions, which were supported >= 6 reads (MAPQ >=20, edit distance < 4, frequency > 50%) and were not identified in an individual genome in step 1.
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.