Centromeric Determinants of Host Tolerance to Transposable Elements

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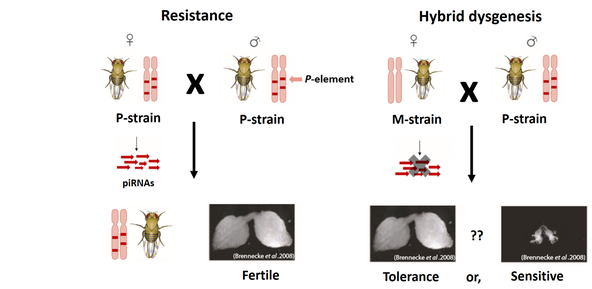
Hybrid dysgenesis reveals Tolerance

- Hosts can reduce fitness costs of TEs through resistance, in which TE proliferation is regulated, or through tolerance, where the fitness consequences of TEs are minimized without impacting their activity.
- Resistance is conferred by maternally deposited piRNAs, absence of which results in hybrid dysgenesis (Kidwell et al. 1977 , Brennecke et al. 2008). But host factors conferring cellular tolerance is not known.
- The severity of hybrid dysgenesis, either complete sterility or fertility, reveals the host's ability to tolerate the fitness consequences of TEs, allowing us to study tolerance.

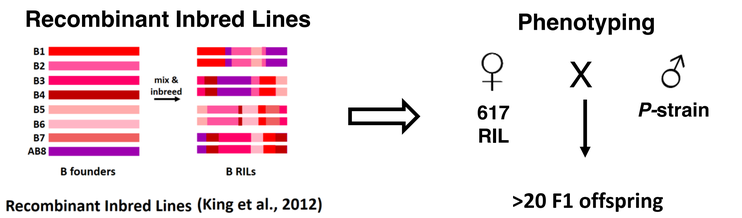


Objective : To find the genetic variants conferring germline tolerance to TE activity.

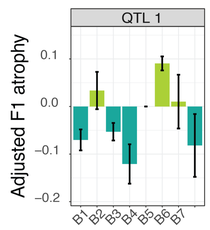
Methods

- We used recombinant inbred lines (RILs) from Drosophila synthetic population (DSPR) (King et al., 2012) for QTL mapping of genetic variation in tolerance.
- All founder genotypes used to establish the DSPR were M strains, and therefore produce dysgenic offspring when crossed to *P*-strain males.

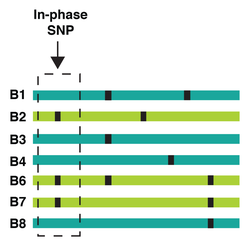
1. QTL mapping



2. Identifying sensitive & tolerant alleles

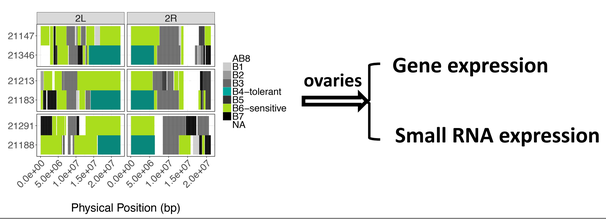


3. Identifying Inphase SNPs



4. Expression Profiling

- We performed small RNA and total RNA sequencing on ovarian tissues derived from pairs of RILs that differ predominantly in the QTL region, either carrying a tolerant or a sensitive QTL allele.



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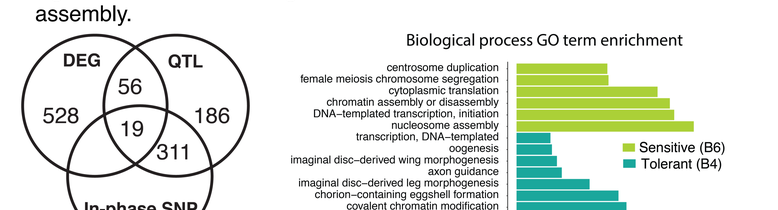
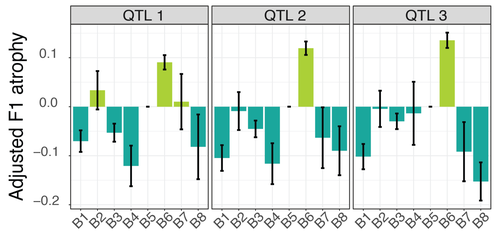
QTL mapping tolerance

- QTL analysis revealed a complex QTL peak including 3 discrete sub-peaks located in the centromeric and pericentromeric region of chromosome 2.
- Pericentric heterochromatin (under the QTL peak) contains many protein coding genes but is also rich in satellite repeats, TEs and piRNA clusters.



- Phasing of founder alleles at each QTL sub peak show evidence for the presence of two allelic classes: tolerant and sensitive

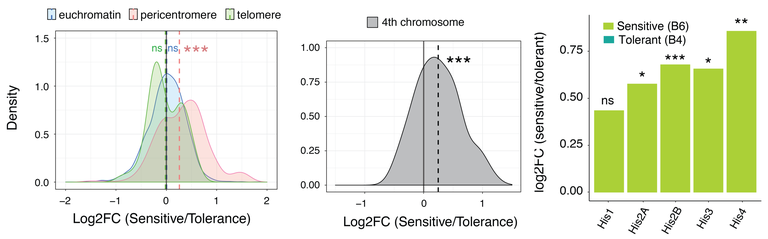


Sensitive and tolerant alleles exhibit differential expression of chromatin packaging genes

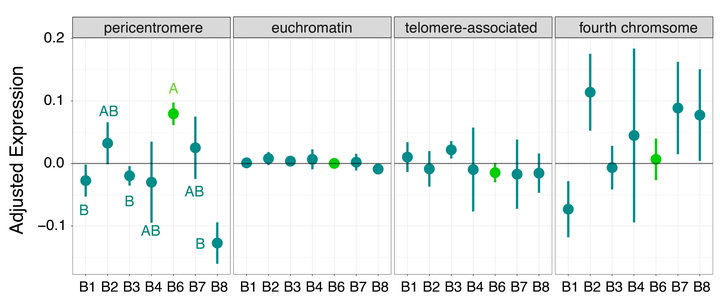
- 603 genes are differentially expressed between the tolerant (B4) and sensitive (B6) alleles. 75 of these genes are found within the QTL, of which 19 have in-phase SNPs.
- GO analysis on complete set of differentially expressed genes revealed significant enrichment for genes involved in chromatin and nucleosome assembly.

Sensitive alleles exhibit upregulation of pericentromeric genes

- Pericentromeric gene expression is sensitive to the abundance of chromatin factors (Elgin & Reuter 2013).
- Sensitive alleles are associated with increased expression of pericentromeric genes, as well as genes on the highly heterochromatic 4th chromosome.
- Sensitive alleles also show upregulation of histone gene cluster that resides in QTL region and is sensitive to chromatin changes.
- These results suggests sensitive allele is associated with reduced heterochromatin formation

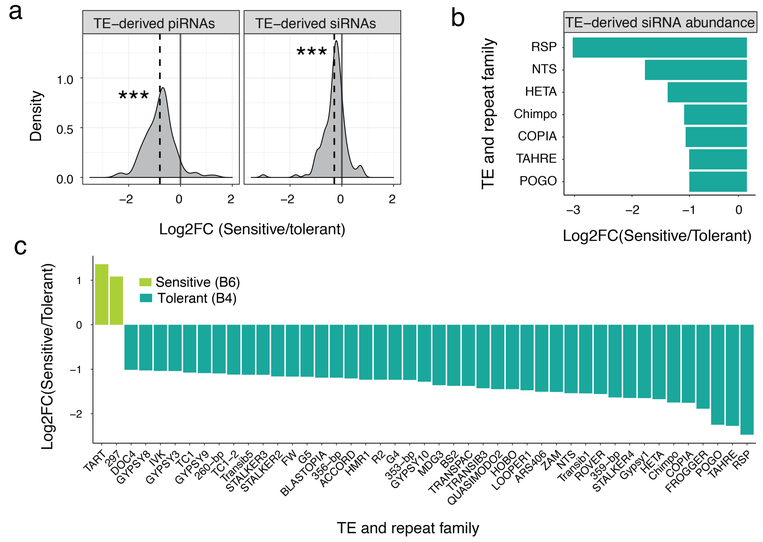


- Sensitive alleles also showed increased expression of pericentromeric genes in a previously published microarray dataset from head tissue (King et al., 2014).

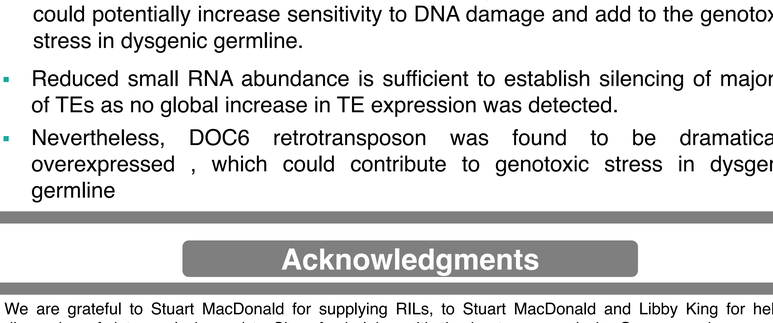


Sensitive centromeres exhibit reduced piRNA abundance

- Consistent with reduced heterochromatin formation, sensitive alleles are associated with significantly reduced TE-derived piRNAs and siRNAs (fig a).
- 43 out of 45 differentially abundant TEs and repeats are more abundant among small RNA pools of tolerant alleles (fig b and c).



- There was no corresponding global increase in TE expression in sensitive alleles carrying RILs (fig a).
- However, we did identify four TEs with higher expression levels in sensitive NILs, with DOC6 non-LTR retrotransposons being dramatically overexpressed (~15 fold, fig b).
- Increased DOC6 expression could enhance dysgenesis if it contributes to genotoxic stress in early stages of oogenesis like *P*-elements.



Summary

Conclusion

- Natural variation in host tolerance of TE activity is abundant, and arises from multiple loci near the second chromosome centromere.
- Relative to tolerant alleles, sensitive alleles exhibit increased pericentromeric gene expression as well as reduced small RNA abundance, both suggesting reduced heterochromatin formation.
- Sensitive alleles are also associated with upregulation of histone genes, which could potentially increase sensitivity to DNA damage and add to the genotoxic stress in dysgenic germline.
- Reduced small RNA abundance is sufficient to establish silencing of majority of TEs as no global increase in TE expression was detected.
- Nevertheless, DOC6 retrotransposon was found to be dramatically overexpressed , which could contribute to genotoxic stress in dysgenic germline

Acknowledgments

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