

**Combinatorial effects of transposable elements on gene expression and phenotypic robustness in *Drosophila melanogaster* development.**

Alexa W. Clemmons and Steven A. Wasserman

Section of Cell & Developmental Biology, University of California San Diego La Jolla, California 92093---  
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**Table S1.** Polymorphisms found in *tub<sup>ste</sup>* chromosome within the 25 kb region determined via site-specific recombination to be responsible for the *tub<sup>ste</sup>* variable phenotype. Sequence polymorphisms are relative to the reference genome and a functional *tube* cDNA (Celniker *et al.* 2002; Letsou *et al.* 1993); all were also found in *tub<sup>7</sup>* and *tub<sup>8</sup>*. Locations of site-specific recombination sites and of the beginning and end of the *tube* transcription unit are noted under Reference Points.

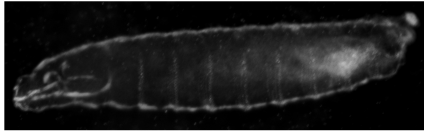
### Polymorphisms

Description	Location
4 bp deletion (TTAG)	3R: 205,032 - 205,035
Single base substitution (A > G)	3R: 206,259
1 bp deletion (T)	3R: 207,180
4 bp deletion (ATTT)	3R: 213,067 - 213,070
7,400 bp insertion ( <i>opus</i> )	3R: 213,161
Single base substitution (T > A)	3R: 219,883
Single base substitution (G > C)	3R: 220,488
Two base substitution (GC > AA)	3R: 223,222 - 223,223

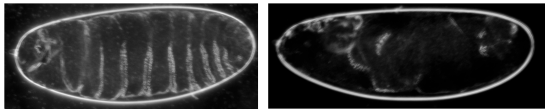
### Reference points

Description	Location
Proximal recombination site	3R: 204,648
<i>tube</i> transcription start site	3R: 213,460
End of <i>tube</i> transcription unit	3R: 215,536
Distal recombination site	3R: 223,611

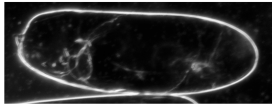
SUPPLEMENTARY FIGURES



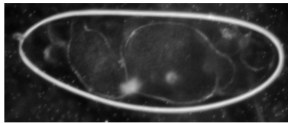
wild-type



moderately dorsalized: D3 (left) and D2 (right)



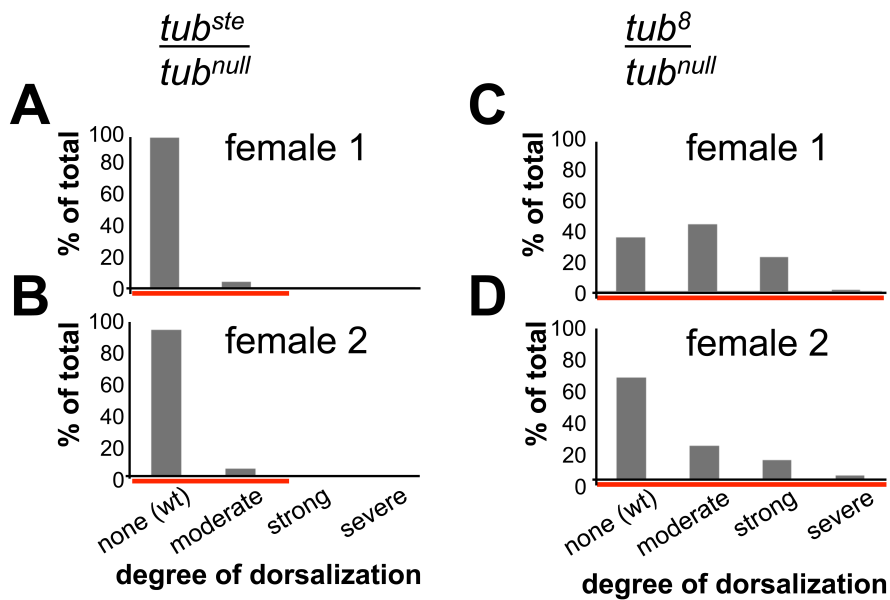
strongly dorsalized: D1



severely dorsalized: D0

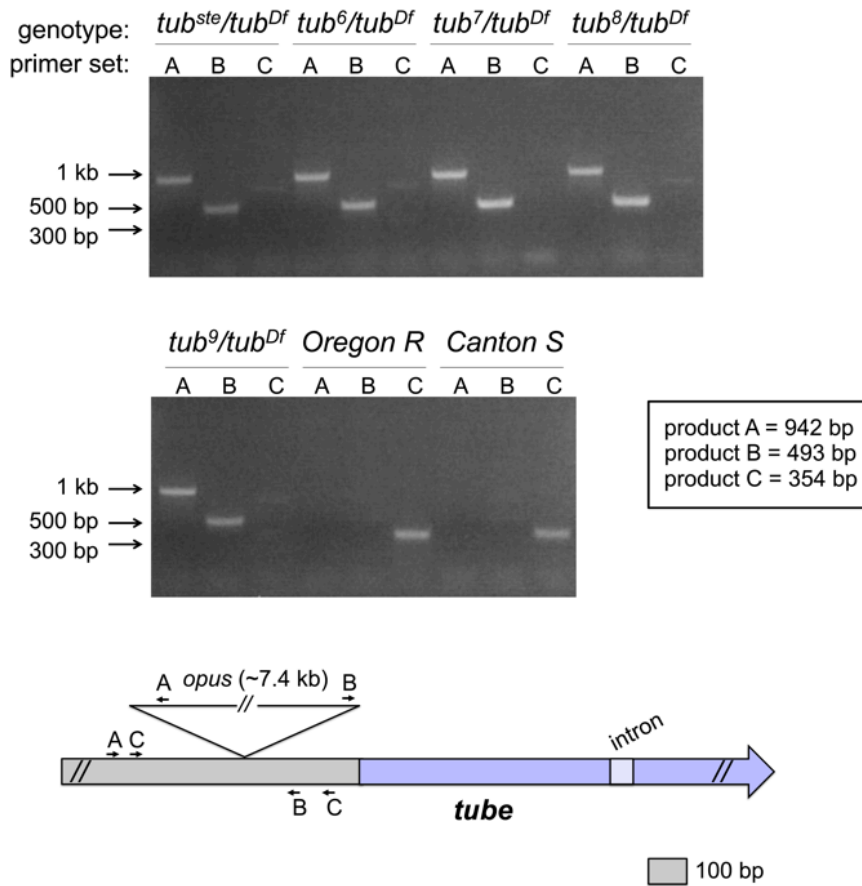
**Figure S1.** The spectrum of embryonic dorsalization phenotypes.

Pictures of embryos representative of the dorsalization phenotypes referred to in this paper. Embryonic phenotypes were scored based on pre-existing scale, D0-D3 and wild-type (Wieschaus and Nüsslein-Volhard 1986).



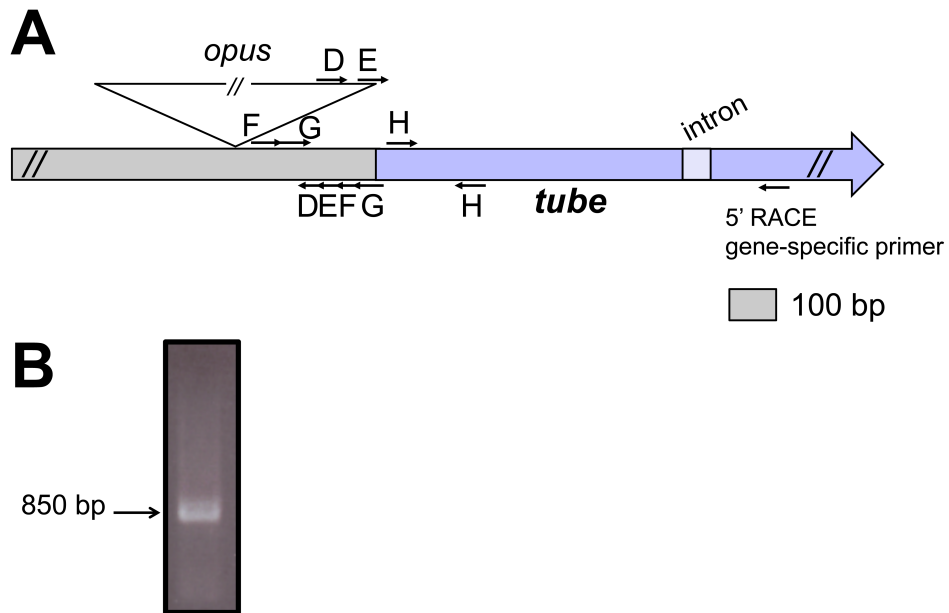
**Figure S2.** Embryos from single females display a range of phenotypes, as is also seen among embryos produced from a population of females of the same genotype.

Single females of specified genotypes were mated with wild-type males. Cuticle phenotypes are represented as the percent of total embryos scored from that female. Compare to Figure 1. **A.**  $tub^{ste}/tub^{null}$  female, n = 156. **B.**  $tub^{ste}/tub^{null}$  female, n = 176. **C.**  $tub^8/tub^{null}$  female, n = 73. **D.**  $tub^8/tub^{null}$  female, n = 80. Red lines highlight range of phenotypes. Dorsalized phenotypes are underrepresented relative to wild-type in this experiment because of the difficulty in recovering small numbers of unhatched embryos.



**Figure S3.** The *opus* insertion is present in all *tub<sup>var</sup>* chromosomes and absent from wild-type chromosomes.

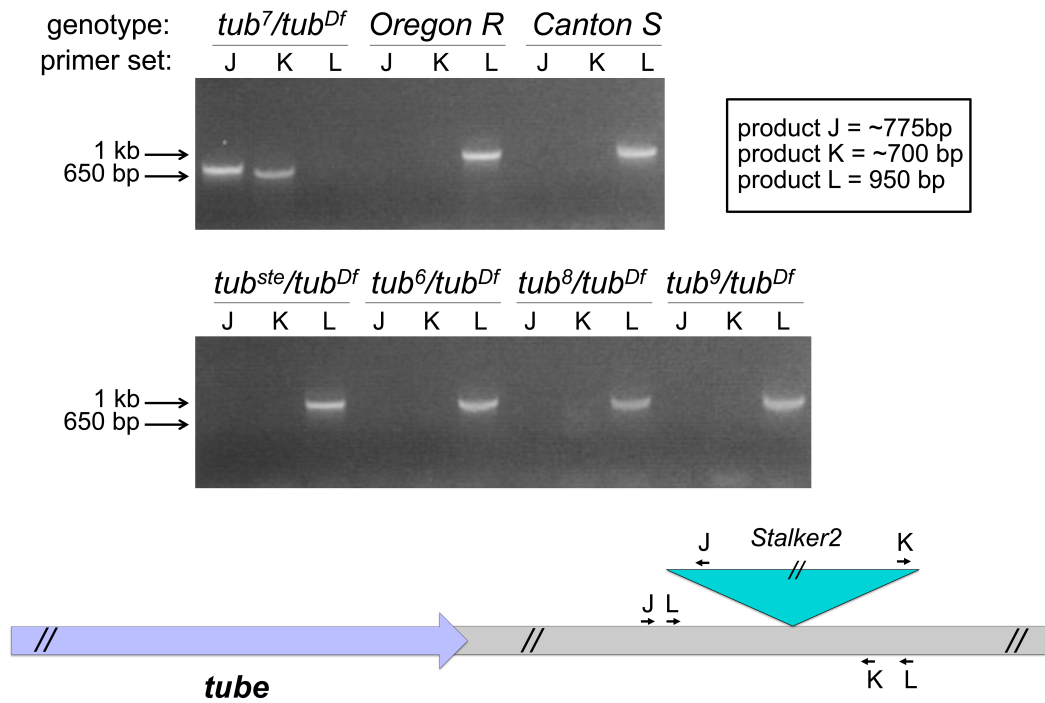
Genomic DNA from flies of the specified genotype was PCR amplified using three different primer sets to assay for the presence of the *opus* insertion (see diagram at bottom). Primer set A spans the 5' insertion junction, primer set B spans the 3' insertion junction, and primer set C spans the entire insertion (>7 kb in *tub<sup>ste</sup>* chromosome, too large to amplify under given cycling conditions).



**Figure S4.** Downregulation of *tube* by *opus* insertion does not depend on an alternative transcription start site.

**A.** Schematic of primer locations for quantitative RT-PCR and 5' RACE to assay for use of an alternative transcription start site in embryos from *tub<sup>ste</sup>/tub<sup>null</sup>* females. Quantitative RT-PCR primer sets are labeled D-H. Products were detected from primer set H, but not from primer sets D-G.

**B.** Results from 5' RACE using RNA prepared from embryos from *tub<sup>ste</sup>/tub<sup>null</sup>* females. Expected size of *tube* transcript originating from wild-type transcription start site was 830 bp.



**Figure S5.** The downstream *Stalker2* insertion appears to be unique to  $tub^7$ .

Genomic DNA from flies of the specified genotypes was PCR amplified using three different primer sets to assay for the presence of the *Stalker2* insertion (see diagram at bottom). Primer set J spans the 5' insertion junction, primer set K spans the 3' insertion junction, and primer set L spans the entire insertion (>8 kb in  $tub^7$  chromosome, too large to amplify using given cycling conditions).