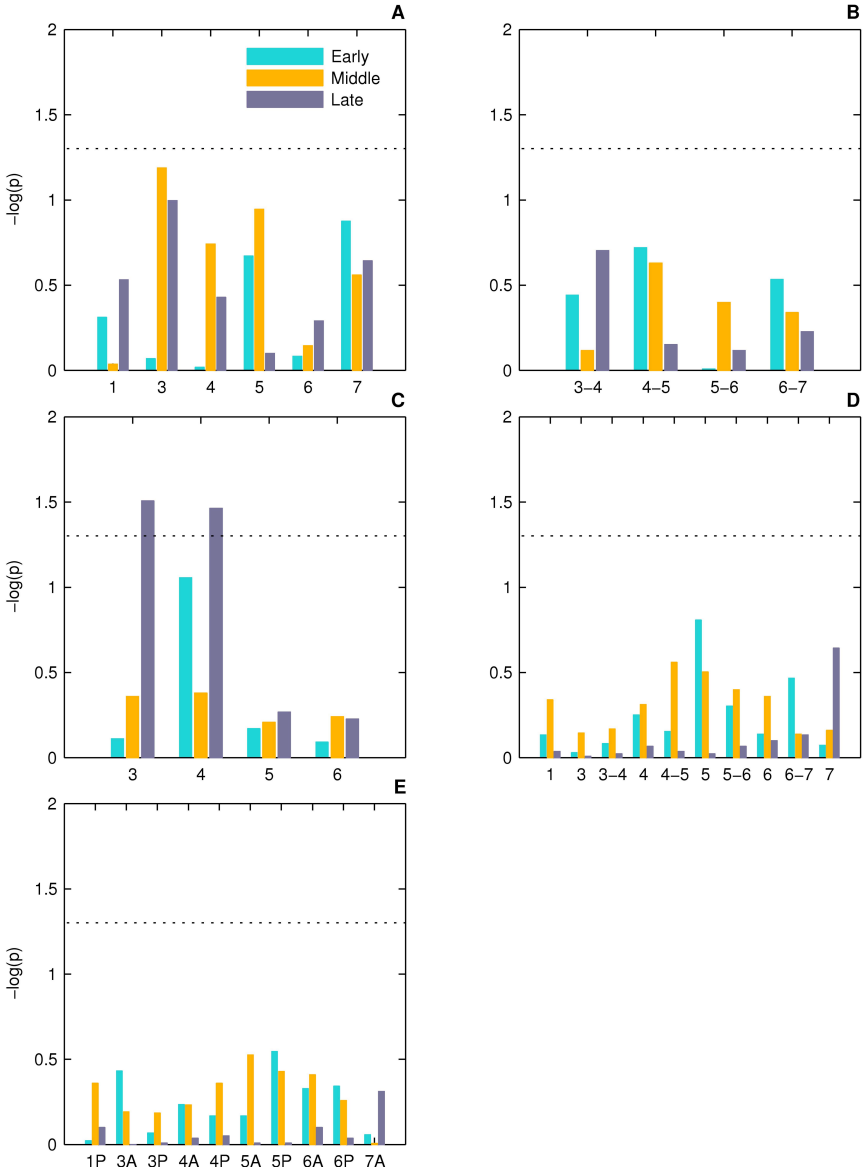

Figure S1 Statistical tests for sex-bias in the expression features of stripes 1, 3-7 in w^{1118}. *p*-values of Wilcoxon ranksum test between males and females for features of stripes 1, 3-7 are plotted as negative logarithms to base 10. Dotted line is at *p=0.05*. **A**, Peak expression; labeled with stripe number. **B**, interstripe expression; n-m is the interstripe between stripes n and m. **C**, relative repression; calculated as the ratio of the heights of anterior and posterior borders as in Fig. 1. **D**, positions of extrema; labeled with stripe number for peaks and the interstripe number for troughs. **E**, positions of borders; labeled with stripe number and either “A” or “P” for anterior or posterior borders respectively. Peak expression is the fluorescence at the peak of a stripe.

Interstripe expression is the fluorescence at the trough between two stripes. Peak and interstripe expression were normalized to mean fluorescence in each embryo. Only relative repression of stripe 3 and 4 (panel **C**) show sex bias. Sample size is the same as Fig. 1E.

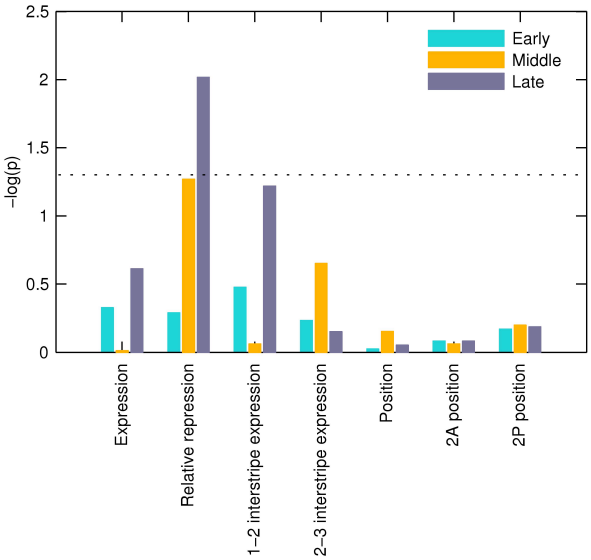

Figure S2 Relative repression is the only sex-biased stripe 2 feature in w^{1118}. The negative logarithm to base 10 of *p*-values of the Wilcoxon ranksum test between males and females. Dotted line is at *p=0.05*. Expression is the fluorescence at stripe peak. Relative repression is defined in Fig. 1. Interstripe expression is the fluorescence at the troughs between stripes 1 and 2 (1-2) and 2 and 3 (2-3). Peak and interstripe expression were normalized to mean fluorescence in each embryo. Position is the position of stripe 2 peak. 2A and 2P positions are the positions of the anterior and posterior borders respectively. Sample size is the same as Fig. 1E.

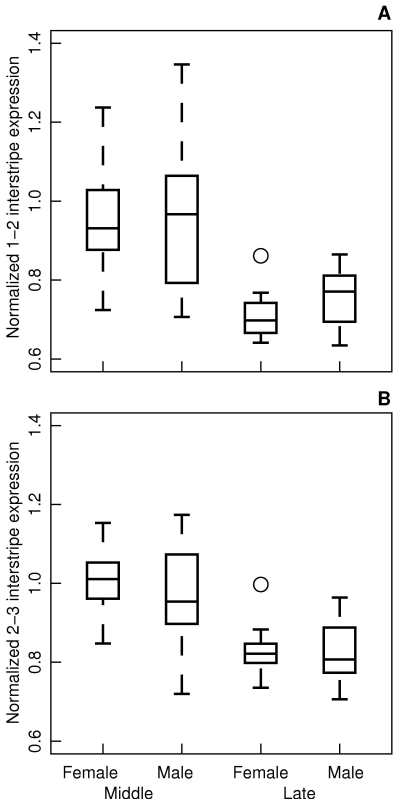

Figure S3 Interstripe 1-2 expression is elevated in w^{1118} males. Boxplots show normalized Eve expression in the 1-2 (**A**) and 2-3 (**B**) interstripes. See legend of Fig. 1 for an explanation of boxplot. Note that *greater* expression in an interstripe implies less repression, that is, *lower* values of relative repression. Sample size is the same as Fig. 1E.

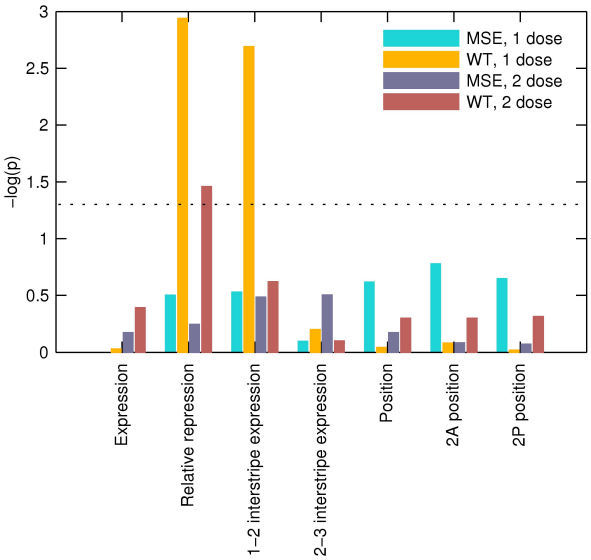

Figure S4 Relative repression is the only sex-biased stripe 2 feature in WT, while MSE lacks sex bias in all stripe 2 features. The negative logarithm to base 10 of *p*-values of the Wilcoxon ranksum test between males and females. Dotted line is at *p=0.05*. Expression is the fluorescence at stripe peak. Relative repression is defined in Fig. 1. Interstripe expression is the fluorescence at the troughs between stripes 1 and 2 (1-2) and 2 and 3 (2-3). Peak and interstripe expression were normalized to mean fluorescence in each embryo. Position is the position of stripe 2 peak. 2A and 2P positions are the positions of the anterior and posterior borders respectively. Sample size is the same as Fig. 2.

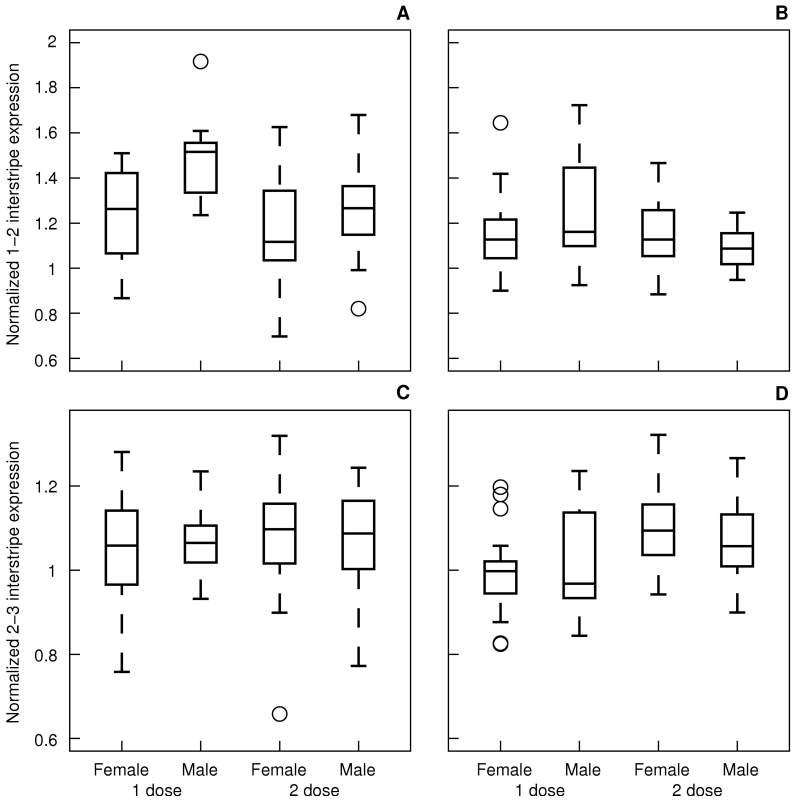

Figure S5 Interstripe 1-2 expression is elevated in WT, but not MSE males. Plots show normalized Eve expression driven by the WT or MSE transgenes in the 1-2 (**A**,**B**) and 2-3 (**C**,**D**) interstripes. See legend of Fig. 1 for an explanation of boxplot. **A**,**C**, WT. **B**,**D**, MSE. Note that *greater* expression in an interstripe implies less repression, that is, *lower* values of relative repression. Sample size is the same as Fig. 2.

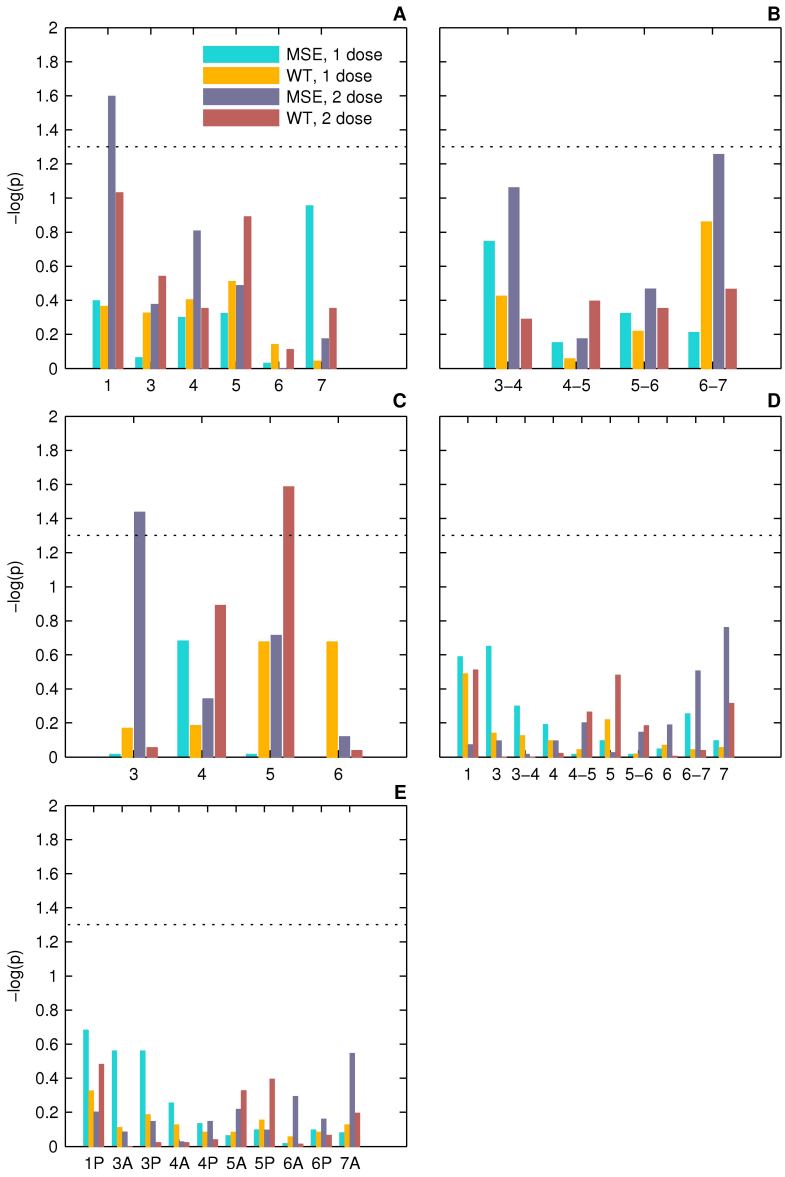

Figure S6 Statistical tests for sex-bias in the expression features of stripes 1, 3-7 when Eve expression is driven by WT or MSE. *p*-values of Wilcoxon ranksum test between males and females for features of stripes 1, 3-7 plotted as negative logarithms to base 10. Dotted line is at *p=0.05*. **A**, Peak expression, **B**, interstripe expression, **C**, relative repression, **D**, positions of extrema, and **E**, positions of borders. See Fig. S1 for an explanation of these phenotypes. Sample size is the same as Fig. 2.

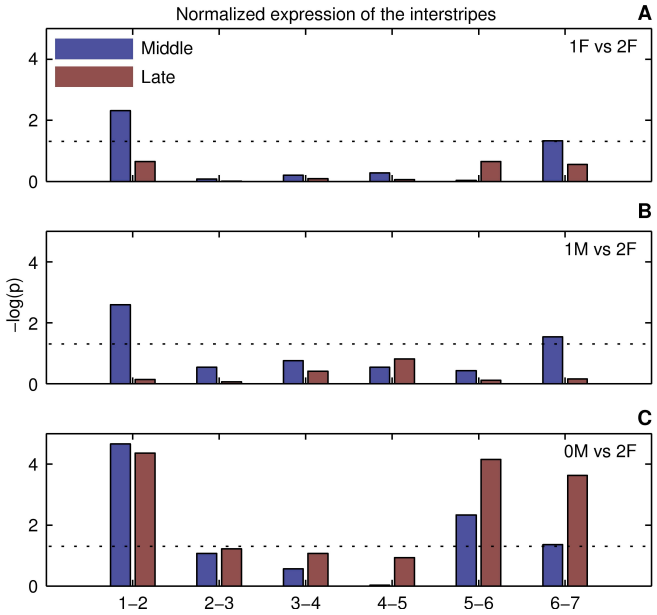

Figure S7 *gt* dose affects expression at the 5-6 and 6-7 interstripes. *p*-values of the Wilcoxon ranksum test on normalized interstripe expression between 1F (**A**), 1M (**B**), or 0M (**C**) and 2F genotypes are plotted as negative logarithms. Dotted line corresponds to *p=0.05*. Both 1M and 1F have the same pattern of differential expression, differing from 2F in the 1-2 and 6-7 interstripes but not elsewhere. 0M (gt^{-}) also differs from 2F in the same pattern except that 5-6 interstripe expression is also affected. Sample size is the same as Fig. 3B.

File S1

Supplementary Materials and Methods

Sequence of SYFP2 (Drosophila codon usage adjusted)

ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGCGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTGAACGGCCACAAGTTCAGCGTGC

GCGGCGAGGGCGAGGGCGACGCCACCAACGGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCT

CGTGACCACCCTGGGCTACGGCGTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAGGG

CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCACCTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACC

GCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGCCACAAGCTGGAGTACAACTTCAACAGCCACAACGTCTACATC

ACCGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACGTGGAGGACGGCGGCGTGCAGCTCGCCGACCACTACC

AGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCAAGCTGAGCAAGGACCCCAACGAG

AAGCGCGACCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGCATCACCCACGGCATGGACGAGCTGTACAAGTAA

Sequence of genomic *giant* 26149bp

Drosophila melanogaster Reference Sequence Release 5.30

Start X: 2312831

End X: 2338979

Primers for recombineering

***gt –YFP* fusion**

Primer_gtCYFP_F:

CCCTCAAGGTCCAGCTGGCCGCCTTCACCTCCGCCAAAGTAACCACCGCCGATTATGATATTCCAACTACTGCAAGCATGGTGAGCAAGGGCG

AG

Primer_gtCYFP_R:

ACATACGATTCGGATCCTCGCGTTCAACGCATCAAGAGAGGAGTGGACCTTTACTTGTACAGCTCGTCCATGC

For cloning from BAC to attB_3xP3_DsRed_P15A-amp

gt_intF2:

TATCTCAATAATACACATCTAGTTTCGGATCCTTAAGTCTACTTGAAACTCAGGCATTCAAATATGTATCC

gt_intR2:

CGTCATAAATGGCAGTGTCTTAAATTACAATCCTTCCCTTGGTCTTCCTCGTCGACGATGTAGGTCACG

For verification of the insertion of the vector attB into attP2 landing site

See Ludwig *et al.* (2011).

Primers for genotyping

***p[hb-lacZ]* marker (800bp) for balancer second chromosome**

Z353 CTGCCAGTTTGAGGGGACGACGACA

hb32 ACCAACGTAATCCCCATAGAAAA

Positive marker (80bp) of PCR genotyping reaction

sna-F CCCACGTGGACGTCAAGAA

sna-R GAGCGACATCCTGGAGAAAGA

Male fertility factor *kl5* gene on the Y chromosome (240bp)

y6527 GGCCTAATTGGAGACCTGTTTC

y6749 CTGGTTTTGGTATGTCTTGTTA

***p[GAL4-Kr.C]* > *p[UAS-GFP.S65T]* on the X chromosome (600bp)**

Pry1 CCTTAGCATGTCCGTGGGGTTTGAAT

5542GFP TTGCATCACCTTCACCCTCTCCCCT

Table S1 Mutant genotypes

Name	Description
w^{1118}	*white*$^{-}$
WT	attP2[$S2E^{wt}EVE^{YFP}$] 16.4kb *eve* locus with wild type stripe 2 enhancer (S2E) and a YFP tag at the C-terminus of the *eve* coding region, integrated on the third chromosome
MSE	attP2[$S2E^{MSE}EVE^{YFP}$] 16.4kb *eve* locus with 244bp deleted from S2E (Fig. 2A) and a YFP tag at the C-terminus of the *eve* coding region, integrated on the third chromosome
eve^{R13}	Null allele carrying a coding point mutation
gt^{X11}	Null allele
gt^{YFP}	attP2[GT^{YFP}] 26.2kb region of the *gt* locus with a YFP tag at the C-terminus of the *gt* coding region, integrated on the third chromosome
$eve^{\Delta MSE}$	Native locus in which the MSE region of the stripe 2 enhancer is deleted and replaced with w^{+} sequence (Ludwig *et al*., 2005)

Table S2 Rescue of gt^{X11} by the gt^{YFP} transgene

Genotype[†]	Sex	*gt* copies	Number of eclosed adults
$gt^{X11}/+;gt^{YFP}/+$	FEMALE	1 endogenous + 1 transgene	623
$FM7c/+;gt^{YFP}/+$	FEMALE	2 endogenous + 1 transgene	535
$FM7c/Y;gt^{YFP}/+$	MALE	1 endogenous + 1 transgene	352
$gt^{X11}/Y;gt^{YFP}/+$[††]	MALE	0 endogenous + 1 transgene	261

[†] Offspring of a cross between 8 $gt^{X11}/FM7c$ females and 8 gt^{YFP} males at 25°, scored as described previously (Ludwig et al., 2011).

[††] The rescue potency of gt^{YFP} is estimated to be ~40% as the ratio between the number of adults of the $gt^{X11}/Y;gt^{YFP}/+$ *and* $gt^{X11}/+;gt^{YFP}/+$ genotypes. The rescued males were healthy and fertile.