Supporting Information

Sun et al. 10.1073/pnas.1305638110

SI Materials and Methods

***Drosophila* Stocks and Crosses.** Mutations, genes, and chromosomal balancers are described in Flybase. All flies were cultured on cornmeal dextrose medium at 20 °C. Larval metafemales (XXX), females, and males were obtained for RNA-sequencing from crosses of C(1)DX, y w f/Y females with y^+ w^+/Y males based on the phenotype of y^+. The *miniwhite* reporter metafemale adults were collected from the crosses (Fig. S1) according to the phenotype of y^+ f^+; the control female and male flies were collected from the same cultures for eye color comparisons with the exception of heterozygous females, which were produced in parallel crosses. Detachments of the compound X occur rarely to produce a y^+ f^+ female, but these can be distinguished by a normal abdominal and wing phenotype rather than those characteristic of metafemales.

Eye Color. Because we use *miniwhite* as the reporter gene, we could easily evaluate the expression level of *white* based on eye colors of the adult flies. Newly emerged adult flies were collected and aged 1 d before photography. The eye pictures were taken using a digital camera with a dissecting microscope in white light with a blue background. The same intensity of white light and the same zoom were used for all pictures.

Illumina TruSeq RNA Library Preparation and Sequencing. RNA isolation, library preparation, and sequencing were performed as described (1). Single end reads (50 bp) were performed.

RNA Sequencing Analyses. Sequence analysis and ratios distribution determinations were performed as described (1).

Relative Quantitative PCR. cDNA was synthesized using the SuperScript III first Strand Kit from Invitrogen. Exogenous of HeLa total RNA (0.5 μg) was added into each sample to serve as a control. β-Actin was the exogenous standard, using primers 5′-GCTCG TCGTC GACAA CGGCT C-3′ and 5′-CAAAC ATGAT CTGGG TCATC TTCTC-3′ to generate a 353-bp product. The real-time PCR reactions were processed using the diluted cDNA (1/50) described above with the Power SYBR Green PCR Master Mix Kit (ABI). Three biological and technical repeats were applied to each pair of primers. The individual female samples were applied as the calibrator in the comparisons. The relative quantification for each pair of primers was measured based on the ΔCt analysis according to the instructions from the manufacturer (7300 Real-time PCR system, sequence detection software version 1.3.1; Applied Biosystems). The primers used in relative quantitative PCR are listed below.

Primers used in relative quantitative PCR

Gene names	Locations	Left primer	Right primer
Ssp3	2L	ACAATCCGCCAGCCAGAGA	GTCTTTGGTGCGGCTGTTTATC
Jet	2L	GAACTCCACCAGCTTGCTTT	AACGAATGCGTCAACATCAC
Ssrp	2R	GGATCCAAGGAGAGCACAAA	CGGTCGCTATGACATCAAGA
Magu	2R	TACATCTGCGTCCAAAGGC	GAATCCGAGAGAGATGGCTG
Lysp	3L	CCCTCCTAACCGACGACATC	CCGCTGCAGTACTTCCATGTG
CG16758	3L	GGTATTCGCCTTCAGCCTCAT	GTTCGCAGCAGGCCTTTTG
Oxt	3L	CCAACTTGTCGAGGGTCTTC	GGAAGCGATTCTCCACCATA
Kap-α1	3L	TTGACCTGCATTAGCCACCT	CATGATAACCATCAGCTGCG
Ac76E	3L	TGGTGCCCAGAAACCTCAGT	CCTCGGCACTCGCACTCATA
Sp7	3R	AGCACACGAATGGCAATCAA	GATCCAATCGCTGCTTTATGG
Gish	3R	AAATCCCTAACTGGAACGGC	TGTATCAGCCTTTAGGCCTTG
Nup358	3R	GCGAACTTCTGGTGAAGGAG	CTCTTTCGCCGTTACTGTCC
Rpl3	3R	CACCGTACCGAGATCAACAAGAA	ATGGGCGTGATGCTCTTGTC
Sw	X	CAGGTTGGGATTGAACTTGG	TCGTACCACAACAACGAGGA
Out	X	GTGAGAAGATGGTGCGGAAT	TGAAGCTGGATAACCGTGTG
Ag5r2	X	GATTCTGGCCGGAGTACTTG	ACGATTCCAATCACAATGCC
TLK	X	TCTCCTCTTTGCGGTTCATT	CATATGATGTTGATTTCGGGG
Ing3	X	CCACCAGCTCGTGTATCTGA	CCCACATGTTCTTCAAGCAG
CG9577	X	CGTCGCCGTAAAGACAACCA	CACAGCCTGGGCGAAGTC
Myb	X	TTGAAATGCGGTCCGATAAT	GGGACAGAACAAAAGCGGTA
Eo-ry	X	CGTGGTGGACGAAAGATTGA	TCCCGATCGTCGAGAATCAT
fog	X	TGCGGCTCGATCACTTCAC	TGTTGGCCCCTATGAAAACC
Karl	X	GAGAAGCTCGTGGGCAACAT	GTGTCGTAGTCCGTGTCCAGAA
CG15771	X	TCTATGCCGCCTGCAACTTT	TGCCACGTCTTCTGGAAACC

1. Sun L, et al. (2013) Male-specific lethal complex in Drosophila counteracts histone acetylation and does not mediate dosage compensation. *Proc Natl Acad Sci USA* 110(9): E808–E817.

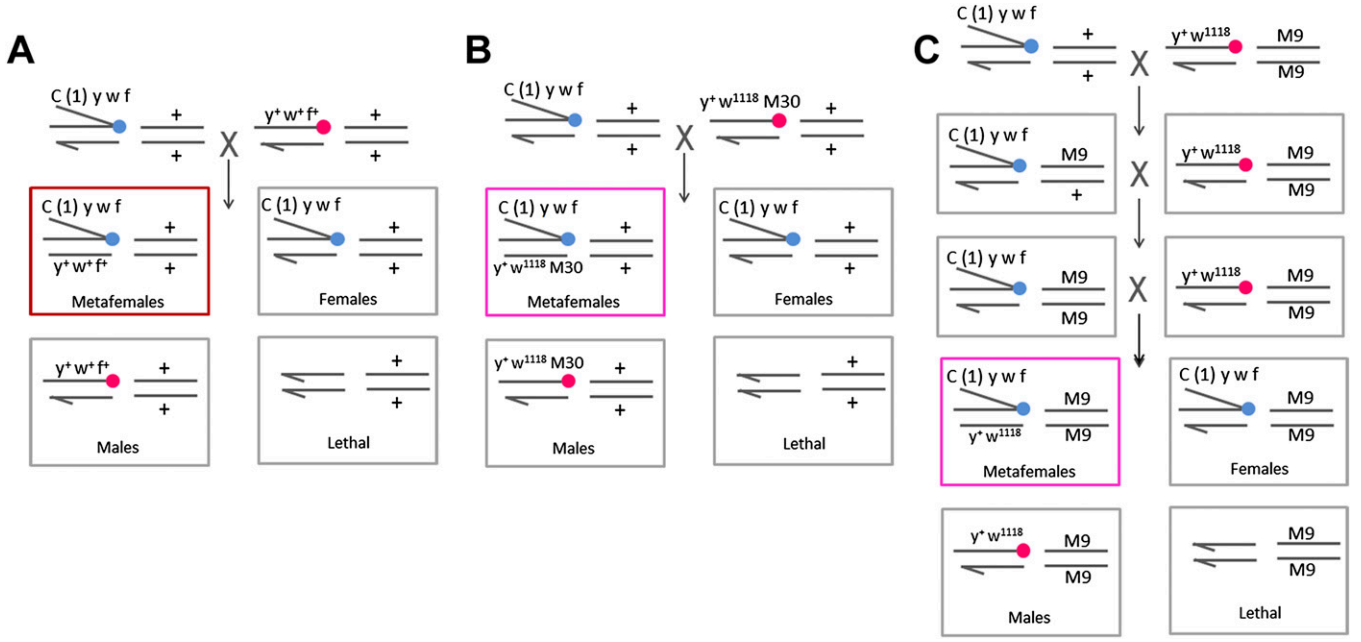

Fig. S1. Genetic crosses to generate metafemales. (*A*) Cross of C(1)DX, y w f/*Y* females with y^+ w^+/*Y* males to produce metafemale C(1)DX, y w f/y^+ larvae (shown in red box), which were collected based on the phenotype of y^+. The females and males from the same cross were collected as controls. (*B*) Cross of C(1)DX, y w f/*Y* females with y^+ w^{1118} M30/*Y* males to obtain one copy of M30 in metafemales C(1)DX, y w f/M30, which were separated by the phenotype of y^+ f^+ (shown in pink box). (*C*) Cross of C(1)DX, y w f/*Y* females with y^+ w^{1118}; M9/M9 males to generate the F1 females C(1)DX, y w f/*Y*; M9/+ with a heterozygous reporter gene. These were backcrossed to the paternal stock to obtain the F2 homozygous M9 reporters according to the eye color differences. In the final stock with C(1)DX, y w f/*Y*; M9/M9 females and y^+ w^{1118}; M9/M9 males, the metafemales with homozygous M9 reporters in the F3 generation were separated and collected based on the phenotype of y^+ f^+ (shown in pink box). The blue dots represent the compound X chromosome. The red dots represent the normal X chromosome.

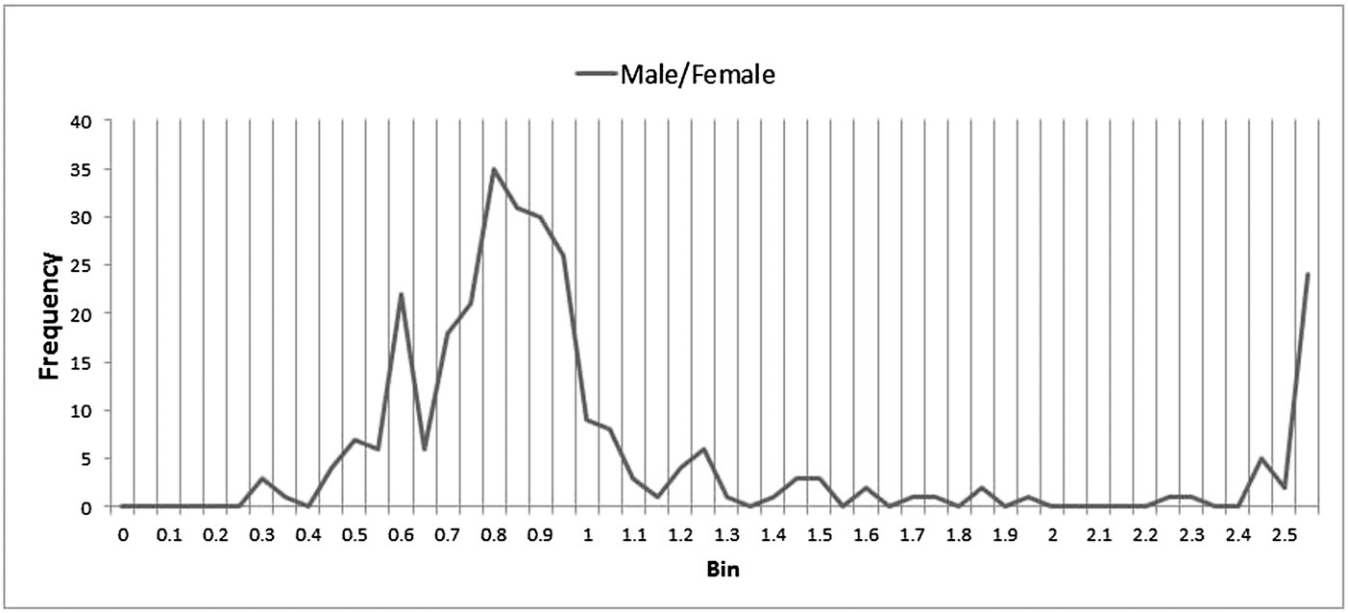

Fig. S2. Distribution of male/female ratios corresponding to X-linked genes in the metafemale distribution in the range of 0.90–1.10. This distribution is significantly different from the total male/female X chromosome distribution (Kolmogorov–Smirnov test, $P < 5.0 \times 10^{-5}$).

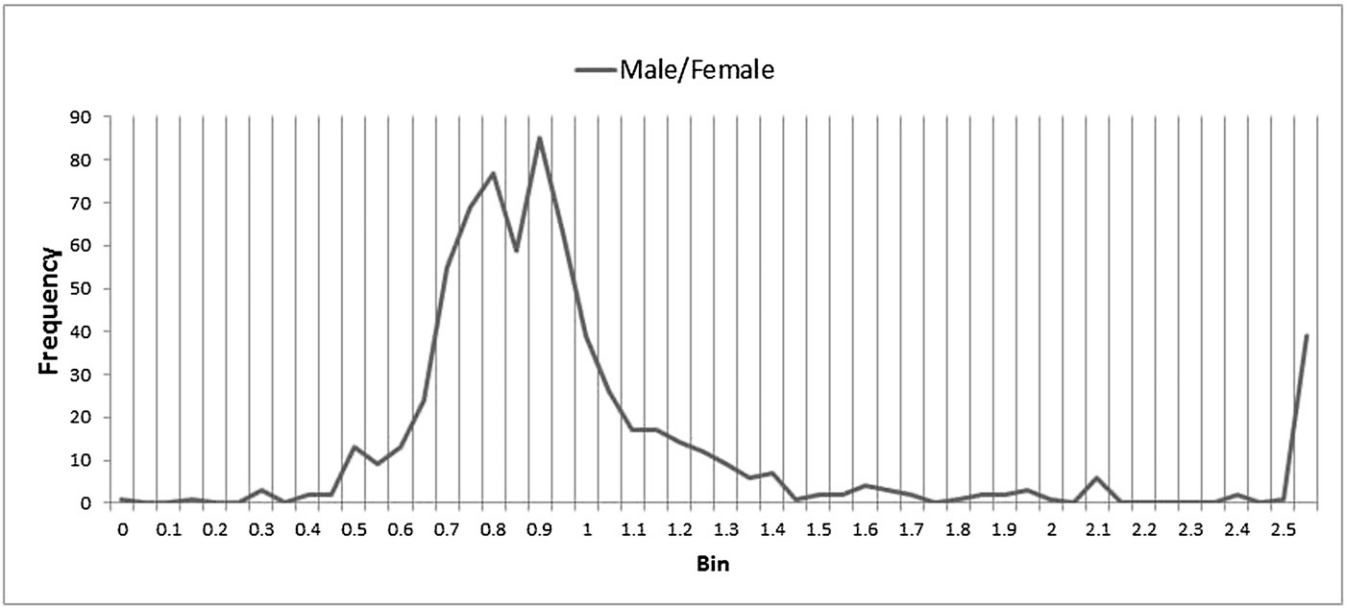

Fig. S3. Distribution of male/female ratios corresponding to X-linked genes in the metafemale distribution in the range of 1.40–1.60. Some genes do not exhibit compensation in the male/female ratios (0.40–0.60), but this distribution is not significantly different from the total male/female X chromosome distribution (Kolmogorov–Smirnov test, $P = 0.10$).