

Supporting Information

Lemos et al. 10.1073/pnas.1010383107

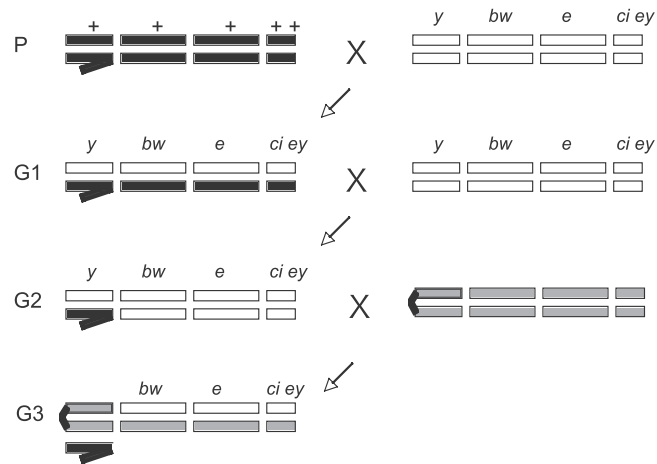


Fig. S1. Crossing scheme for obtaining population-specific Y chromosome substitution lines. This was done independently for each Y chromosome lineage to be introgressed such that males from generation G2 have different Y chromosomes introgressed into a common isogenic background. XXY females from generation (G3) also have different Y chromosomes in a background of attached X chromosomes and autosomes that is identical across lineages.

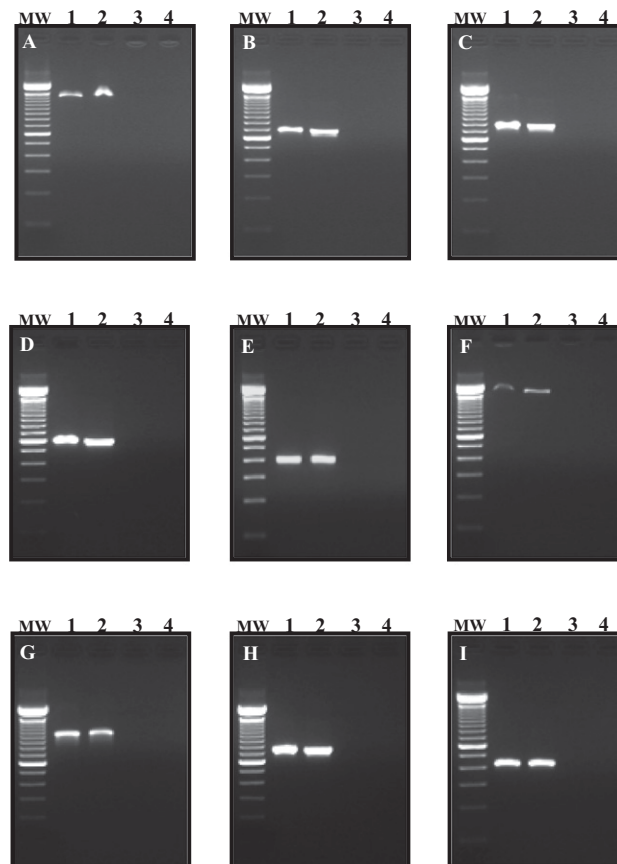


Fig. S2. Protein-coding Y-linked genes are not expressed in XXY females. RT-PCR was performed to detect the transcription of Y-linked genes in XY males and XXY females. Lane 1: *Y[Ohio]*. Lane 2: *Y[Congo]*. Lane 3: *XXY[Ohio]*. Lane 4: *XXY[Congo]*. (A–C) *kl-2* gene. (D–F) *kl-3* gene. (G–I) *kl-5* gene. MW, 100-bp DNA ladder (Invitrogen).

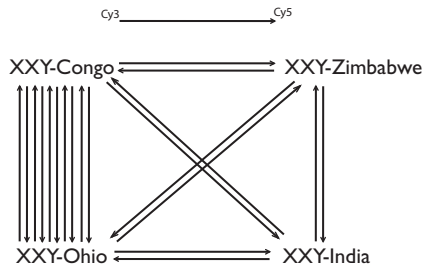


Fig. S3. Experimental design for collecting genome-wide gene expression data across XXY female genotypes. Each line denotes two hybridization reactions with Cy3 and Cy5 dyes swapped, for a total of 20 hybridizations.

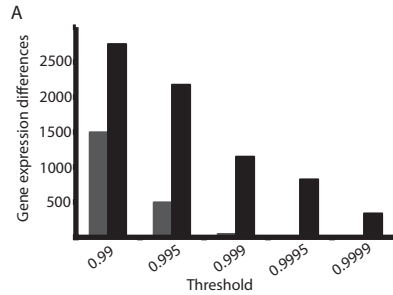


Fig. S4. Widespread effect of polymorphic Y chromosomes in XXY female genotypes of *Drosophila*. Number of statistically significant gene expression differences between XXY[Congo] and XXY[Ohio] females (black bars) as a function of the Bayesian posterior probability of differential expression. Gray bars indicate the estimated number of genes expected by chance after permutation of the data.

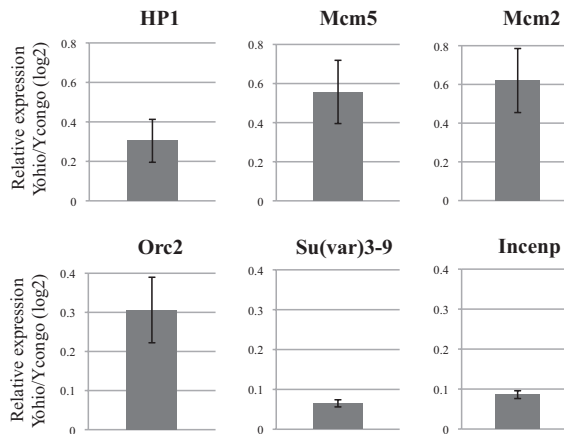


Fig. S5. Chromatin-associated proteins are differentially expressed between Y[Congo] and Y[Ohio] males. Ratios of Y[Ohio] and Y[Congo] expression obtained with quantitative real-time PCR are shown.

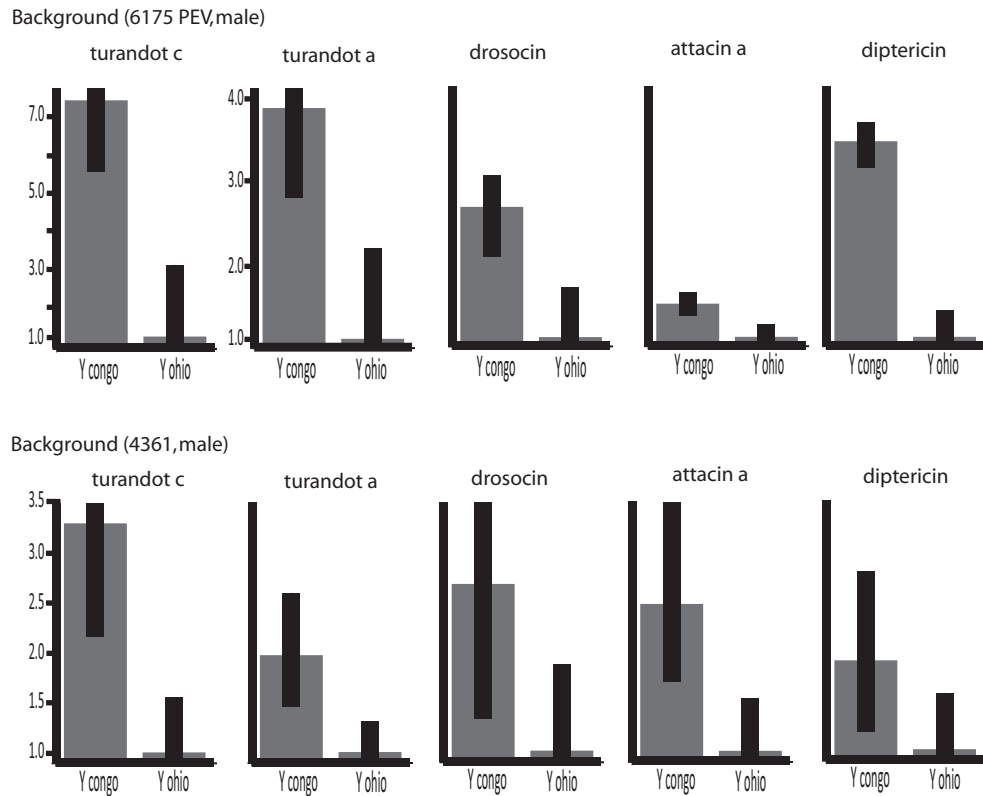


Fig. S6. Examples of immune response genes expressed more highly in *Y[Congo]* males relative to *Y[Ohio]* males in two different backgrounds of autosomes and the X chromosome. Background 4361 is the same inbred background used in the Y chromosome substitution lines reported by Lemos et al. (1). Background 6175 results from the cross of males from the Y-chromosome substitution lines in the 4361 background with females carrying the PEV marker *w[m4h]*.

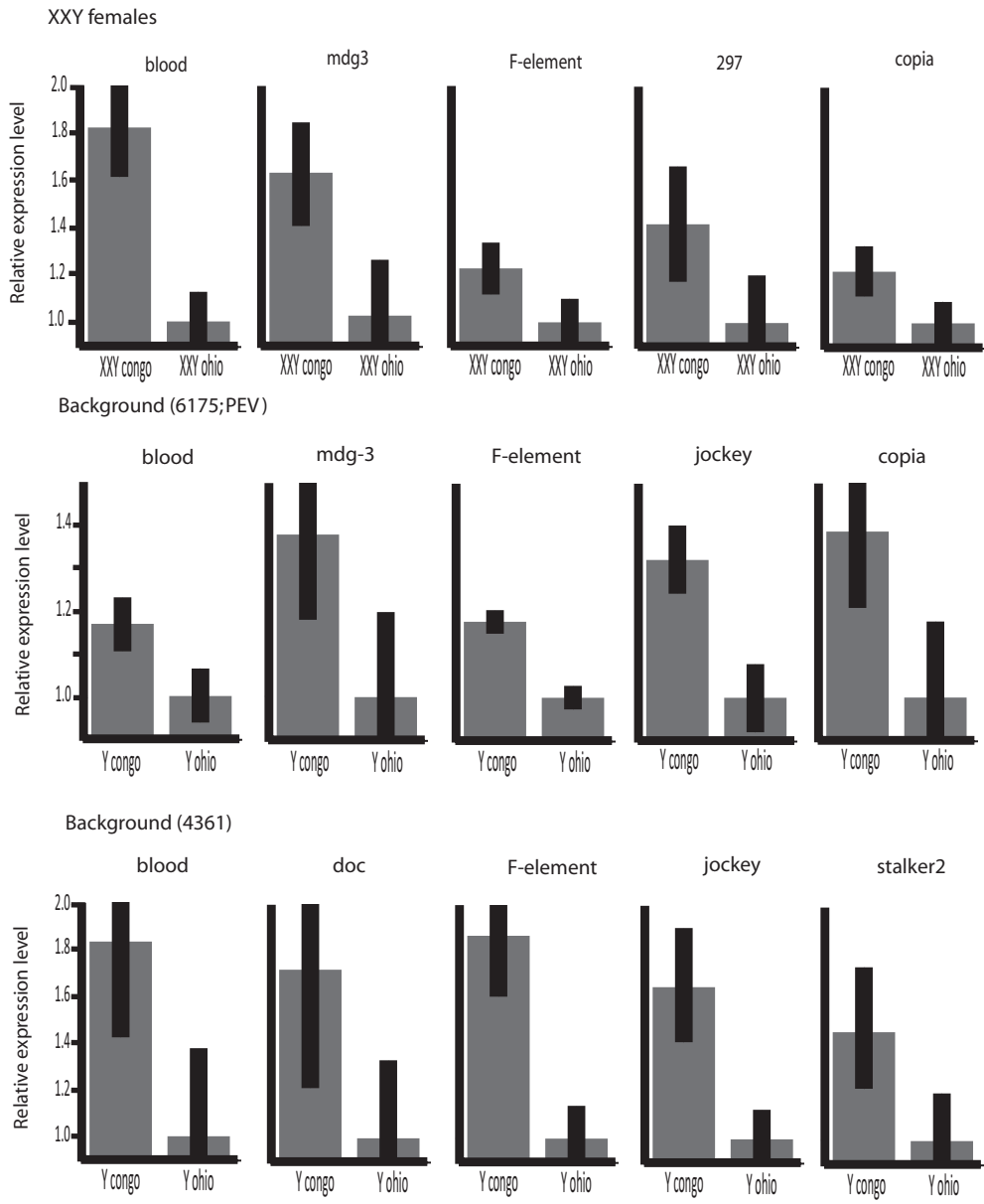


Fig. S7. Examples of transposable elements more highly expressed *Y[Congo]* relative to *Y[Ohio]* in XXY females and in two different male backgrounds of autosomes and the X chromosome. Background 4361 is the same inbred background used in the Y chromosome substitution lines reported by Lemos et al. (1). Background 6175 results from the cross of males from the Y chromosome substitution lines in the 4361 background with females carrying the PEV marker *w[m4h]*.

1. Lemos B, Araripe LO, Hartl DL (2008) Polymorphic Y chromosomes harbor cryptic variation with manifold functional consequences. *Science* 319:91–93.

Table S1. Variation in eye pigmentation in stocks carrying the X chromosome PEV marker *w[m4h]* and Y chromosomes sampled from diverse localities worldwide

Strain	Mean	SEM
<i>Y[Zimbabwe]</i>	NA	NA
<i>Y[Congo-1]</i>	0.0563	0.0052
<i>Y[Bogota, Colombia]</i>	0.0940	0.0073
<i>Y[Connecticut, USA]</i>	0.1087	0.0056
<i>Y[Cape Town, South Africa]</i>	0.1200	0.0061
<i>Y[Arizona, USA]</i>	0.1270	0.0101
<i>Y[Athens, Greece]</i>	0.1310	0.0090
<i>Y[LE Reduit, Mauritius]</i>	0.1470	0.0082
<i>Y[Congo-k]</i>	0.1605	0.0314
<i>Y[Mumbai, India]</i>	0.1707	0.0153
<i>Y[Kuala Lumpur, Malaysia]</i>	0.1755	0.0049
<i>Y[Massachusetts, USA]</i>	0.1898	0.0199
<i>Y[Ohio, USA]</i>	0.1934	0.0077
<i>Y[Captain Cook, Hawaii, USA]</i>	0.2057	0.0415
<i>Y[Congo-11]</i>	0.2473	0.0126
<i>Y[Congo-10]</i>	0.2625	0.0192
<i>Y[Congo-4]</i>	0.3103	0.0030

Eye pigmentation was assessed by spectrophotometric analysis at an optical density of 480 nm.

Table S2. Number of genes up-regulated and down-regulated in *Y[congo]* relative to *Y[Ohio]* in XXY females across four Gene Ontology categories and a range of significance thresholds for differential expression

	Up-regulated in <i>Y[Congo]</i>	Down-regulated in <i>Y[Congo]</i>	Percent down-regulated in <i>Y[Congo]</i>	Percent up-regulated in <i>Y[Congo]</i>
Chromatin silencing				
<i>P</i> < 0.01	7	94	93%	
<i>P</i> < 0.005	5	71	93%	
<i>P</i> < 0.001	3	34	92%	
Mitochondrion				
<i>P</i> < 0.01	65	28		70%
<i>P</i> < 0.005	49	16		75%
<i>P</i> < 0.001	29	8		78%
Electron transport				
<i>P</i> < 0.01	51	14		78%
<i>P</i> < 0.005	38	11		78%
<i>P</i> < 0.001	23	6		79%
Defense response and Immunity				
<i>P</i> < 0.01	43	9		83%
<i>P</i> < 0.005	38	7		84%
<i>P</i> < 0.001	31	2		93%

The excess of down-regulated genes in *Y[Congo]* belonging to the chromatin-silencing class is statistically significant (*P* < 0.01; Fisher's exact test). The excess of up-regulated genes in *Y[Congo]* localized to the mitochondrion, involved in electron transport, or associated with defense response and immunity is also statistically significant (*P* < 0.01, Fisher's exact test).