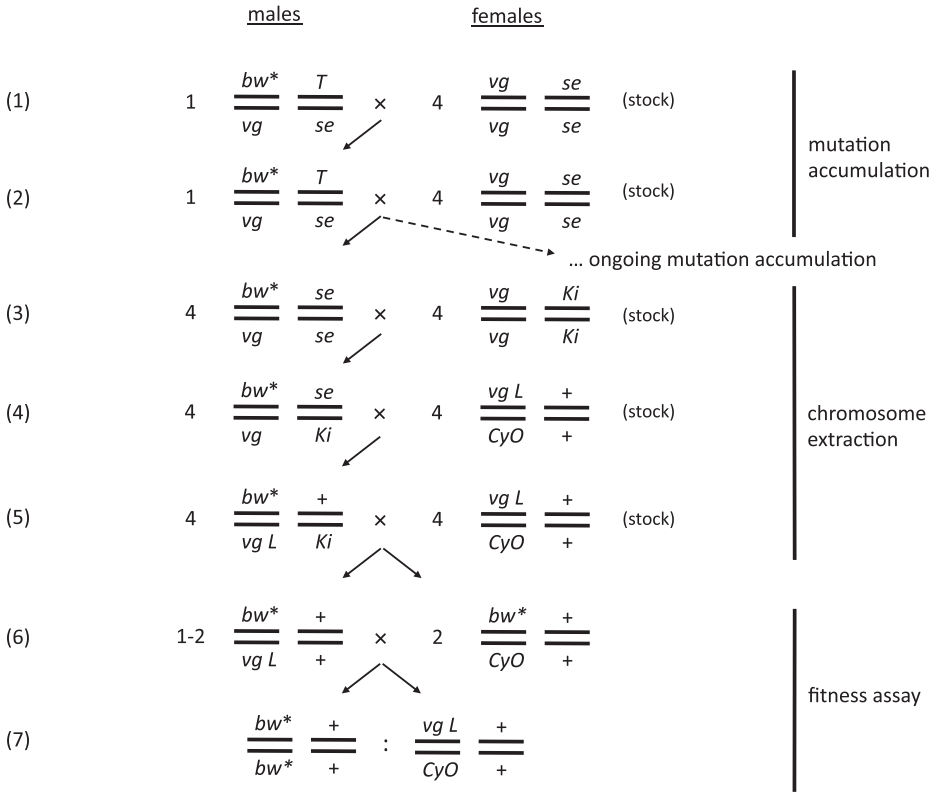
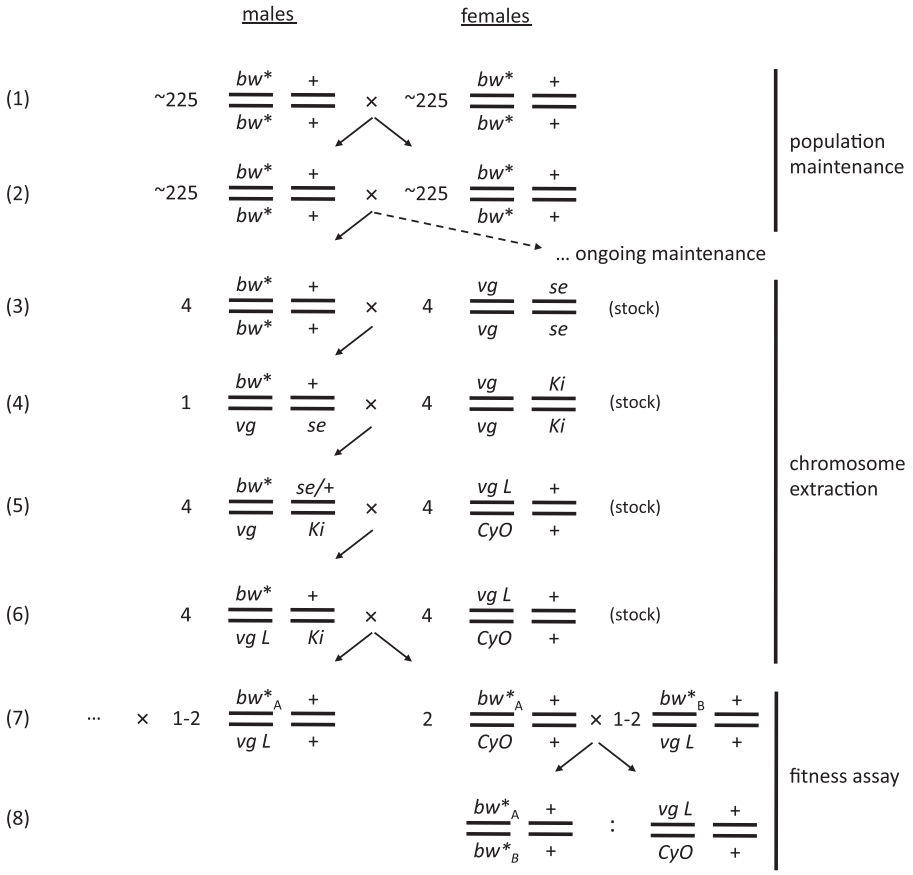
Supporting Information

Sharp and Agrawal 10.1073/pnas.1118918109



Symbols	
+	wild-type (outbred, unmarked)
*bw**	focal second chromosome, carrying the recessive phenotypic marker bw^1 (*brown*)
T	treatment third chromosome, carrying 0, 1, or 2 treatment mutations
vg	recessive phenotypic marker vg^1 (*vestigial*)
se	recessive phenotypic marker se^1 (*sepia*)
Ki	dominant phenotypic marker Ki^1 (*Kinked*)
L	dominant phenotypic marker L^2 (*Lobe*)
CyO	balancer chromosome to suppress recombination, carrying the dominant phenotypic marker *Cy* (*Curly*)

Fig. S1. Crosses for mutation accumulation (MA) lines and fitness assays. Only the two major autosomes, chromosomes 2 and 3, are shown; all females were virgins before crossing; numbers of flies used in each cross for a given line are shown to the left of each genotype image. Males lack recombination in this species; the balancer chromosome *CyO* was used to suppress recombination of the second chromosome in females as needed. Further details are provided in the text. During MA (steps 1 and 2), individual males were crossed to females from a standard stock, using recessive phenotypic markers to identify offspring of the appropriate genotype. In addition to ongoing maintenance of the MA lines (not shown), focal second chromosomes were "extracted" for fitness assays at three time points. We first conducted several crosses (steps 3–5) to place focal second chromosomes on a standard WT background, involving females from standard stocks bearing phenotypic markers and balancer chromosomes. Finally, males and females with the focal second chromosome were crossed to one another (step 6), and we scored each replicate vial (step 7) for the fraction of offspring that were homozygous for the focal second chromosome (*bw*/bw**) relative to those of a standard genotype (*vg L/CyO*).



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CyO	balancer chromosome to suppress recombination, carrying the dominant phenotypic marker *Cy* (*Curly*)

Fig. S2. Crosses for control populations and fitness assays. Only the two major autosomes, chromosomes 2 and 3, are shown; all females were virgins before crossing, except in steps 1 and 2; numbers of flies used in each cross for a given line are shown to the left of each genotype image. Males lack recombination in this species; the balancer chromosome *CyO* was used to suppress recombination of the second chromosome in females as needed. Further details are provided in the text. Three control populations consisting of flies homozygous for the focal second chromosome were maintained throughout the experiment by using 450 flies each generation (~225 of each sex). In addition to ongoing maintenance of these populations (not shown), focal second chromosomes from each control population were sampled for fitness assays at three time points. We first conducted several crosses (steps 3–6) to place focal second chromosomes on a standard WT background, involving females from standard stocks bearing phenotypic markers and balancer chromosomes. Note that the use of a single male at step 4 ensures that only one focal second chromosome copy is sampled per replicate line (for some assays, this bottleneck was done at step 5). Finally, flies carrying a given focal second chromosome (e.g., bw^*_A) were crossed to flies with a different focal second chromosome sampled from the same control population (e.g., bw^*_B) to create heterozygous controls. These crosses were performed in a replicated "round-robin" fashion, using males and females with a given focal second chromosome for different crosses (only one direction is shown here). We scored each replicate vial (step 8) for the fraction of offspring with two focal second chromosome copies (e.g., bw^*_A/bw^*_B) relative to those of a standard genotype (*vg L*/*CyO*).

Table S1. Sample sizes and results summary

Treatment	Genotype	N*	$n^{\dagger}$	ΔM (SE)	ΔV (SE)	U_{min}	$\bar{s}_{max}$
Unloaded	*wt*	53, 47, 41	4.1	−0.0014 (0.0007)	0.0004 (0.0001)	0.0033	−0.25
Loaded	$Antp^{Ns}$	21, 15, 13	4.0	−0.0028 (0.0011)	0.0009 (0.0003)	0.0068	−0.28
Loaded	Bsb^{1}	36, 31, 26	3.8	−0.0027 (0.0012)	0.0012 (0.0004)	0.0046	−0.39
Loaded	Dr^{1}	37, 31, 26	4.1	−0.0050 (0.0018)	0.0029 (0.0006)	0.0075	−0.51
Loaded	Gl^{1}	36, 33, 27	3.9	−0.0043 (0.0012)	0.0011 (0.0002)	0.0143	−0.25
Loaded	Ki^{1}	34, 31, 27	3.9	−0.0046 (0.0015)	0.0017 (0.0005)	0.0104	−0.34
Loaded	$sens^{Ly\text{-}1}$	33, 36, 30	4.1	−0.0029 (0.0008)	0.0007 (0.0002)	0.0096	−0.24
Loaded	$Dr^{1}\ Kg^{V}$	38, 32, 25	4.1	−0.0033 (0.0012)	0.0011 (0.0003)	0.0078	−0.30
Loaded	$Bsb^{1}\ sens^{Ly\text{-}1}$	27, 23, 19	3.9	−0.0059 (0.0020)	0.0026 (0.0008)	0.0111	−0.39
Loaded	$Ki^{1}\ k^{D}$	25, 24, 23	4.0	−0.0060 (0.0020)	0.0026 (0.0006)	0.0119	−0.39
Loaded	$Gl^{1}\ H^{2}$	21, 18, 10	3.8	−0.0021 (0.0008)	0.0005 (0.0002)	0.0064	−0.21
Control‡	—	30, 102, 102	4.2	—	—	—	—

*Number of nonlethal lines assayed at generations 16, 30, and 46, respectively.
†Number of within-line replicates averaged over lines and assays.
‡Pooling three control populations; note that control lines were sampled from control populations for each assay separately.

Table S2. Rates of lethal mutation

Treatment	Genotype	Generation	Lethal rate (confidence limits)
Unloaded	*wt*	16	0.0131 (0.0069, 0.0238)
		30	0.0080 (0.0042, 0.0144)
		46	0.0075 (0.0042, 0.0129)
Loaded	$Antp^{Ns}$	16	0.0210 (0.0092, 0.0437)
		30	0.0048 (0.0009, 0.0167)
		46	0.0036 (0.0007, 0.0125)
Loaded	Bsb^{1}	16	0.0093 (0.0037, 0.0216)
		30	0.0059 (0.0023, 0.0135)
		46	0.0057 (0.0025, 0.0120)
Loaded	Dr^{1}	16	0.0131 (0.0061, 0.0266)
		30	0.0072 (0.0031, 0.0153)
		46	0.0036 (0.0013, 0.0091)
Loaded	Gl^{1}	16	0.0074 (0.0025, 0.0189)
		30	0.0032 (0.0008, 0.0094)
		46	0.0026 (0.0007, 0.0075)
Loaded	Ki^{1}	16	0.0099 (0.0039, 0.0230)
		30	0.0072 (0.0031, 0.0153)
		46	0.0055 (0.0024, 0.0115)
Loaded	$sens^{Ly\text{-}1}$	16	0.0060 (0.0016, 0.0176)
		30	0.0061 (0.0026, 0.0131)
		46	0.0078 (0.0040, 0.0142)
Loaded	$Dr^{1}\ Kg^{V}$	16	0.0088 (0.0035, 0.0205)
		30	0.0069 (0.0030, 0.0148)
		46	0.0018 (0.0003, 0.0066)
Loaded	$Bsb^{1}\ sens^{Ly\text{-}1}$	16	0.0128 (0.0051, 0.0292)
		30	0.0064 (0.0023, 0.0159)
		46	0.0051 (0.0018, 0.0126)
Loaded	$Ki^{1}\ k^{D}$	16	0.0109 (0.0038, 0.0273)
		30	0.0029 (0.0004, 0.0106)
		46	0.0010 (-0.0002, 0.0057)
Loaded	$Gl^{1}\ H^{2}$	16	0.0096 (0.0028, 0.0276)
		30	0.0084 (0.0030, 0.0205)
		46	0.0049 (0.0011, 0.0161)