Figure S1   Point mutation rate estimates for each MA sub-line.

D. R. Schrider *et al.*
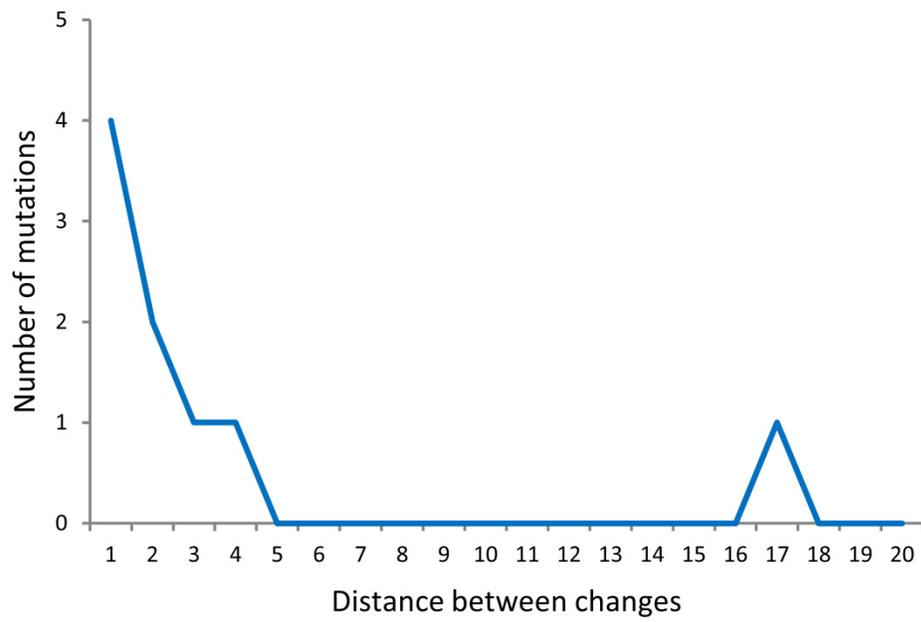
FigureS2 Distance between base changes for multinucleotide mutations affecting two sites.

Tables S1 and S2 are available for download at http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.113.151670/-/DC1.

Table S1   Point mutations occurring during mutation accumulation. Positions of mutations validated by Sanger sequencing are marked with asterisks.

Table S2   Small indels occurring during mutation accumulation. Positions of mutations validated by Sanger sequencing are marked with asterisks.

D. R. Schrider *et al.*

Table S3   Multinucleotide mutations occurring during mutation accumulation, all confirmed by Sanger sequencing.

Chromosome arm	Coordinates	Mutation	Mutant line
chr2L	20628928, 20628930	G->A;T->G	33-27
chr3R	2065681, 2065685	G->A;C->A	33-27
chr2L	7161924, 7161927	G->A;A->T	33-45
chr2R	11166158, 11166159	G->A;C->A	33-45
chr3R	18338533, 18338550	T->C;G->T	33-45
chr2L	19248550, 9248551	A->T;G->T	33-5
chr3R	19467135, 19467136	A->T;T->A	33-55
chrX	5940420, 5940434, 5940463	G->A;G->A;T->C	33-55
chr3L	13034276, 13034277	T->A;A->T	39-51
chrX	15730179, 15730181	T->A;T->A	39-58

Table S4   Complex mutations occurring during mutation accumulation, all confirmed by Sanger sequencing.

Coordinates	Mutation	Mutant line
chr2R:3026177	GGCTATCTTTCTTTCGGAACATTACC→GTATTATTCCT	33-27
chrX:19646896	GCAGG→GAAAAGCA	33-27
chr2R:17877493	GTGCC→GGGT	33-55
chr2L:9526339	CTATATATGTAG→CATCC	39-51
chr3R:27194562	CC→CAG	39-51
chrX:3816859	ATTTT→ATGT	39-51
chr2L:7806673	AGATAGGC→AAT	39-58

D. R. Schrider *et al.*

Table S5   Mutation rate estimates for different minimum read-depth cutoffs.

Depth cutoff	No. of mutations in Line 33	Line 33 rate	95% CI	No. of mutations in Line 39	Line 39 rate	95% CI
5	514	7.71×10^{-9}	$7.06 \times 10^{-9} - 8.40 \times 10^{-9}$	218	3.27×10^{-9}	$2.85 \times 10^{-9} - 3.73 \times 10^{-9}$
6	511	7.77×10^{-9}	$7.11 \times 10^{-9} - 8.47 \times 10^{-9}$	216	3.28×10^{-9}	$2.86 \times 10^{-9} - 3.75 \times 10^{-9}$
7	503	7.80×10^{-9}	$7.13 \times 10^{-9} - 8.51 \times 10^{-9}$	212	3.28×10^{-9}	$2.86 \times 10^{-9} - 3.76 \times 10^{-9}$
8	487	7.75×10^{-9}	$7.08 \times 10^{-9} - 8.47 \times 10^{-9}$	207	3.29×10^{-9}	$2.86 \times 10^{-9} - 3.77 \times 10^{-9}$
9	474	7.81×10^{-9}	$7.12 \times 10^{-9} - 8.54 \times 10^{-9}$	200	3.29×10^{-9}	$2.85 \times 10^{-9} - 3.77 \times 10^{-9}$
10	454	7.83×10^{-9}	$7.12 \times 10^{-9} - 8.58 \times 10^{-9}$	193	3.31×10^{-9}	$2.86 \times 10^{-9} - 3.81 \times 10^{-9}$
11	420	7.67×10^{-9}	$6.95 \times 10^{-9} - 8.43 \times 10^{-9}$	182	3.29×10^{-9}	$2.83 \times 10^{-9} - 3.80 \times 10^{-9}$
12	394	7.72×10^{-9}	$6.97 \times 10^{-9} - 8.52 \times 10^{-9}$	174	3.35×10^{-9}	$2.87 \times 10^{-9} - 3.89 \times 10^{-9}$
13	365	7.79×10^{-9}	$7.01 \times 10^{-9} - 8.63 \times 10^{-9}$	160	3.33×10^{-9}	$2.83 \times 10^{-9} - 3.89 \times 10^{-9}$
14	323	7.64×10^{-9}	$6.82 \times 10^{-9} - 8.51 \times 10^{-9}$	144	3.28×10^{-9}	$2.77 \times 10^{-9} - 3.86 \times 10^{-9}$
15	285	7.59×10^{-9}	$6.74 \times 10^{-9} - 8.53 \times 10^{-9}$	132	3.34×10^{-9}	$2.79 \times 10^{-9} - 3.96 \times 10^{-9}$
16	249	7.62×10^{-9}	$6.71 \times 10^{-9} - 8.63 \times 10^{-9}$	115	3.28×10^{-9}	$2.71 \times 10^{-9} - 3.94 \times 10^{-9}$
17	214	7.68×10^{-9}	$6.69 \times 10^{-9} - 8.79 \times 10^{-9}$	99	3.23×10^{-9}	$2.63 \times 10^{-9} - 3.94 \times 10^{-9}$
18	179	7.70×10^{-9}	$6.61 \times 10^{-9} - 8.92 \times 10^{-9}$	91	3.46×10^{-9}	$2.79 \times 10^{-9} - 4.25 \times 10^{-9}$
19	142	7.49×10^{-9}	$6.31 \times 10^{-9} - 8.83 \times 10^{-9}$	73	3.29×10^{-9}	$2.58 \times 10^{-9} - 4.14 \times 10^{-9}$
20	115	7.61×10^{-9}	$6.29 \times 10^{-9} - 9.14 \times 10^{-9}$	60	3.26×10^{-9}	$2.49 \times 10^{-9} - 4.20 \times 10^{-9}$