



Figure S1. Model for gap repair in *Drosophila*. This modified version of SDSA is based on references cited in the text.

In Tables S1 and S2, each row lists the number of progeny from one vial (five female and three male parents per vial) for non-crossover (NCO), single crossover (SCO), and multiple crossover (double, triple, and quadruple) classes. The bottom row lists the total number of progeny in each class, summed over all vials. For NCO and SCO, the + and – symbols indicate wild-type or mutant, respectively, for each marker, in the order along the chromosome (*net dpp^{d-ho} dp wg^{Sp-1} b pr cn*). Intervals for double crossovers are given in the columns to the right, with interval I being *net* to *dpp^{d-ho}*, etc. Triple and quadruple crossovers intervals are listed below. Analysis of these data is given in Table S3.

Triple and quadruple crossovers:

Wild-type females

Vial 1: II, IV, V
 Vial 5: II, III, IV
 Vial 6: II, III, V
 Vial 7: II, III, IV
 Vial 10: II, III, IV (twice)
 Vial 12: III, IV, V, VI
 Vial 18: II, III, IV
 Vial 19: II, III, IV

mus309 females

Vial 6: III, IV, VI
 Vial 10: IV, V, VI
 Vial 15: IV, V, VI
 Vial 17: III, IV, VI
 Vial 18: IV, V, VI
 Vial 26: I, III, VI

Table S3. Crossover rates in wild-type and *mus309* mutant females.

| Interval | I | II | III | IV | V | VI | I - VI |
|-----------|------|------|------|-------|------|------|--------|
| Size (Mb) | 2.4 | 2.1 | 2.8 | 6.5 | 6.3 | 6.6 | 26.7 |
| COs | 69 | 165 | 249 | 477 | 189 | 50 | 1199 |
| map units | 2.75 | 6.57 | 9.91 | 18.98 | 7.52 | 1.99 | 47.71 |
| m.u./Mb | 1.1 | 3.0 | 3.4 | 2.9 | 1.2 | 0.3 | 1.8 |
| COs | 116 | 135 | 175 | 328 | 302 | 261 | 1317 |
| map units | 2.25 | 2.62 | 3.40 | 6.37 | 5.87 | 5.07 | 25.59 |
| m.u./Mb | 0.9 | 1.2 | 1.2 | 1.0 | 0.9 | 0.8 | 1.0 |

This table lists the total number of crossovers (including those recovered in single and multiple crossover classes) in each of the six intervals assayed from *net* to *cn*. Map units (m.u.) are calculated by dividing the number of crossovers by the total number of progeny (2513 for wild type; 5146 for *mus309*) and multiplying by 100. Physical distances are taken from Flybase, using *Drosophila* genome build 5.1. Pericentromeric heterochromatin between *pr* and *cn* is not included. Map units per megabase (Mb) are represented graphically in Figure 3.

Table S4: Results of P{w^a} assays.

| Genotype | Vials | Progeny | Mean % Red | Mean % Yellow | Mean % SDSA |
|--------------|-------|---------|-------------|---------------|--------------|
| +/+ | 239 | 6236 | 4.21 (0.32) | 5.0 (0.40) | 41.6 (2.6) |
| <i>D3/D2</i> | 149 | 5282 | 0.13 (0.09) | 14.1 (0.73) | 0.87 (0.432) |
| <i>D3/N2</i> | 94 | 3025 | 0.22 (0.11) | 18.4 (0.99) | 0.86 (0.43) |
| <i>N1/D2</i> | 128 | 5503 | 0.90 (0.20) | 8.0 (0.53) | 9.1 (1.9) |
| <i>N2/D2</i> | 133 | 5422 | 0.95 (0.21) | 9.1 (0.57) | 6.6 (1.4) |
| <i>N2/N2</i> | 72 | 3270 | 1.59 (0.40) | 11.9 (0.64) | 10.0 (2.1) |
| +/ <i>D3</i> | 111 | 3433 | 2.22 (0.36) | 7.3 (0.54) | 18.7 (2.6) |
| +/ <i>D2</i> | 119 | 4768 | 3.60 (0.34) | 3.9 (0.52) | 44.5 (3.6) |

Each vial, containing one male parent, is counted as an independent experiment. The number of vials and the total number of female progeny (summing all vials) is given for each genotype. For each vial, the percentage of daughters that had red eyes, the percentage that had yellow eyes, and the percentage SDSA (red / [red+yellow]) was calculated (vials in which [red+yellow]=0 were counted as SDSA=0). This table lists the mean of these percentages across all vials (n = number of vials), with standard error of the mean in parentheses. Because percent SDSA was calculated separately for each vial, Mean % SDSA does not necessarily equal (Mean % Red) / (Mean % Red + Mean % Yellow). Genotypes are listed with the maternally-inherited allele on the left. The +/+ and *D3/D2* data are from Adams *et al.* (2003). The numbers here differ slightly from those reported previously. Previously, we reported percentages based on summing the total number of progeny from all vials. Here, we counted each vial as an independent experiment, but excluded vials in which the number of progeny was more than two standard deviations from the mean number of progeny per vial (fewer than 8-11 progeny, depending on the genotype).

Table S5: Statistical analysis of frequencies of repair by SDSA.

| | <i>N2/N2</i> | <i>N2/D2</i> | <i>N1/D2</i> | <i>D3/N2</i> | <i>D3/D2</i> | <i>+D3</i> | <i>+D2</i> |
|--------------|--------------|--------------|--------------|--------------|--------------|------------|------------|
| <i>+/+</i> | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | 0.4930 |
| <i>+D2</i> | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | |
| <i>+D3</i> | 0.1517 | 0.0022 | 0.0068 | <0.0001 | <0.0001 | | |
| <i>D3/D2</i> | 0.0004 | 0.0101 | 0.0075 | 0.7979 | | | |
| <i>D3/N2</i> | 0.0020 | 0.0353 | 0.0273 | | | | |
| <i>N1/D2</i> | 0.2601 | 0.8308 | | | | | |
| <i>N2/D2</i> | 0.1818 | | | | | | |

Genotypes are listed across the top and in the left column. Two-tailed *p* values from a Mann-Whitney test are given. Comparisons that are not significantly different ($P > 0.05$) are shaded.

Genotypes fell into four groups:

Wild-type or heterozygous for null allele: *+/+* and *+D2*

Heterozygous for antimorphic allele: *+D3*

Heteroallelic with antimorphic allele: *D3/D2* and *D3/N2*

Heteroallelic for null alleles: *N1/D2*, *N2/D2*, and *N2/N2*

There were no significant differences between genotypes in the same group. All comparisons between genotypes from different groups were significantly different, with the exception of *+D3* versus *N2/N2*.

Table S6: Analysis of synthesis tracts in S+EJ repair events.

| Genotype | <i>n</i> | lethal (%) | | viable | deletions (%) | | synthesis from right end (%) | | | |
|--------------|----------|------------|------|--------|---------------|-------|------------------------------|---------|----------|----------|
| | | | | | left | right | ≥5 bp | ≥920 bp | ≥2420 bp | ≥4674 bp |
| +/+ | 71 | 1 | (1) | 70 | 10 | 4 | 96 | 80 | 69 | 20 |
| <i>D3/D2</i> | 147 | 40 | (27) | 107 | 50 | 27 | 73 | 21 | 6 | 0 |
| <i>D3/N2</i> | 89 | 19 | (21) | 70 | 46 | 21 | 79 | 27 | 6 | 4 |
| <i>N1/D2</i> | 119 | 62 | (52) | 57 | 51 | 19 | 81 | 54 | 39 | 21 |
| <i>N2/D2</i> | 92 | 33 | (36) | 59 | 41 | 19 | 81 | 39 | 10 | 5 |
| <i>N2/N2</i> | 73 | 15 | (21) | 58 | 38 | 16 | 84 | 57 | 34 | 2 |
| <i>+/D3</i> | 92 | 0 | (0) | 92 | 3 | 3 | 97 | 75 | 46 | 14 |

n is the number of independent yellow-eyed females that were analyzed for each genotype.

The number of male-lethal repair events, indicating a deletion large enough to extend into one or both *sd* exons, is given, along with percentage of events this represents. All *mus309* heteroallelic or homozygous mutant genotypes resulted in an increase in frequency of male-lethal deletions. For events that were male viable, PCR was done to detect smaller deletions to the left of the insertion site and to the right of the insertion site, and to determine the percentage of that had synthesis tracts from the right end of the $P\{w^{\Delta}\}$ element of at least 5 bp, 920 bp, 2420 bp, and 4674 bp. The frequency of deletions detected by PCR was increased for every *mus309* heteroallelic or homozygous mutant genotype, and tract lengths were decreased, with the exception of *mus309^{N1}/mus309^{D2}*. This might be due to the unusually large number of male-lethal deletions recovered from this genotype.

Table S7: Mitotic crossovers.

| Genotype | Dose | Vials | Progeny | | | | Total | % CO (SEM) | |
|--------------|------|-------|---------|-------------|------------|-------------|-------|------------|--------|
| | | | + + | <i>st e</i> | + <i>e</i> | <i>st</i> + | | | |
| +/+ | 0 | 26 | 1300 | 1256 | 0 | 1 | 2557 | 0.03 | (0.03) |
| <i>N1/D2</i> | 0 | 18 | 656 | 389 | 10 | 11 | 1066 | 1.69 | (0.56) |
| <i>N2/D2</i> | 0 | 14 | 580 | 386 | 7 | 8 | 981 | 1.49 | (0.42) |
| <i>D3/D2</i> | 0 | 19 | 1264 | 1367 | 24 | 41 | 2696 | 2.34 | (0.46) |
| +/+ | 200 | 25 | 1170 | 1080 | 1 | 1 | 2252 | 0.05 | (0.04) |
| <i>N1/D2</i> | 200 | 17 | 913 | 506 | 51 | 49 | 1519 | 5.98 | (1.13) |
| <i>N2/D2</i> | 200 | 12 | 603 | 432 | 22 | 27 | 1084 | 4.61 | (0.68) |
| <i>D3/D2</i> | 200 | 19 | 1205 | 1280 | 91 | 61 | 2637 | 5.58 | (0.75) |
| +/+ | 500 | 23 | 1152 | 1015 | 0 | 5 | 2172 | 0.35 | (0.32) |
| <i>N1/D2</i> | 500 | 19 | 943 | 565 | 65 | 69 | 1642 | 8.10 | (1.15) |
| <i>N2/D2</i> | 500 | 20 | 876 | 570 | 60 | 55 | 1561 | 6.86 | (0.88) |
| <i>D3/D2</i> | 500 | 13 | 668 | 694 | 74 | 52 | 1488 | 8.51 | (0.84) |
| +/+ | 1000 | 24 | 2026 | 1871 | 18 | 2 | 3917 | 0.66 | (0.32) |
| <i>N1/D2</i> | 1000 | 19 | 764 | 524 | 108 | 92 | 1488 | 14.14 | (2.09) |
| <i>N2/D2</i> | 1000 | 14 | 635 | 459 | 75 | 68 | 1237 | 11.26 | (1.47) |
| <i>D3/D2</i> | 1000 | 11 | 522 | 495 | 66 | 52 | 1135 | 11.21 | (1.91) |

Percentages given are means, counting each vial as an independent experiment, with standard error of the mean in parentheses. Each vial is counted as a separate experiment because crossovers may occur pre-meiotically, and therefore all crossovers from a single male are not necessarily independent. Genotypes are listed with the maternally-inherited allele on the left. Doses are in rads (centigrays). For unknown reasons, the *st e* chromosome was often underrepresented in the progeny, relative to the + + chromosome. Crossover classes were usually similar in number, except in +/+ at 500 and 1000 rads. In these experiments, clusters of the same crossover class, presumably derived from a pre-meiotic event, were readily apparent.