

**Table S1 *ald* allele selection.** Each *yw; ald<sup>excision</sup> / TM3, Sb; pol* stock was crossed to *FM7, y w B / y<sup>+</sup>Y; ald<sup>1</sup> / TM3, Sb; pol* males. Trans-heterozygous *ald<sup>1</sup> / excision* female progeny were crossed to males from the tester stock and progeny scored for NDJ (see Methods). Previous sequencing characterized *ald<sup>excision-25</sup>* as a precise excision while *ald<sup>excision-23</sup>* retains a 130-nt fragment of *P* sequence in the 5' UTR (GILLILAND *et al.* 2007). Calculated NDJ rates are presented here, in ascending *X* rate order. The eight lines with asterisks were those selected for the main experiment to provide the greatest range of NDJ rates; the bottom three alleles were not used as they proved to be semilethal over *Df(3R)AN6*.

Excision Line	<i>X</i> NDJ (%)	4 NDJ (%)	N
*25	2.3	2.6	344
*30	6.2	7.7	1157
2	8.7	7.4	1172
38	9.3	5.7	1427
*1	9.8	12.4	266
22	12.1	3.8	943
34	12.8	3.5	313
*26	13.0	3.1	1058
5	13.3	5.0	813
31	13.9	3.7	374
18	14.8	4.2	1097
*15	14.9	8.8	308
21	15.2	2.1	1292
17	15.5	3.6	982
20	15.9	12.5	893
*14	18.6	15.0	506
*4	25.9	32.9	85
35	26.4	12.2	492
36	28.6	16.2	748
13	28.9	15.1	166
*23	29.7	9.0	619
11	32.6	14.3	926
29	37.2	22.0	468
6	41.0	22.5	356

**Table S2 Nondisjunction count data.** For each excision line, single *FM7, y w B / y w; ald<sup>Excision</sup> / Df(3R)AN6; pol* hemizygotes were crossed to *attached-XY, v f B / ∅; C(4)RM, ci ey<sup>R</sup>* males in vials, and progeny up through day 18 were scored. *Progeny* indicates the phenotype of each progeny class, while *Sperm* and *Oocyte* indicates the inferred chromosome content of the gametes. The three *y w ♂* columns include both *FM7, y w B / ∅* and *y w / ∅* males, which were not scored separately.

Progeny	<i>y w ♂</i>	<i>B ♀</i>	<i>y w; ci ey<sup>R</sup> ♂</i>	<i>B; ci ey<sup>R</sup> ♀</i>	<i>y w; pol ♂</i>	<i>B; pol ♀</i>	<i>v f B ♂</i>	<i>y w ♀</i>	<i>v f B; pol ♂</i>	<i>y w; ci ey<sup>R</sup> ♀</i>	<i>v f B; ci ey<sup>R</sup> ♂</i>	<i>y w; pol ♀</i>
Sperm	∅ 44	XY 44	∅ 44	XY 44	∅ ∅	XY ∅	XY 44	∅ 44	XY ∅	∅ 44	XY 44	∅ ∅
Oocyte	X 4	X 4	X ∅	X ∅	X 44	X 44	∅ 4	XX 4	∅ 44	XX ∅	∅ ∅	XX 44
Excision	Normal		4-only NDJ				X-only NDJ		X & 4 Double NDJ			
1	1093	1149	14	41	36	63	71	42	23	10	1	0
4	1697	1929	36	60	61	115	90	92	28	27	1	1
14	879	1026	29	44	40	49	47	38	18	17	5	1
15	2019	2196	37	52	51	90	75	83	19	18	1	3
23	601	691	85	98	98	174	200	189	72	71	25	20
25	1041	1412	1	1	2	0	2	2	3	2	0	0
26	887	941	19	43	40	61	63	57	24	12	1	2
30	940	1120	19	30	46	58	62	40	12	12	0	2

**Table S3 Cytological count data.** Virgin *FM7, y w B / y w; ald<sup>Excision</sup> / Df(3R)AN6; pol* females were aged for 4 days post eclosion with yeast and no males, and Immuno-FISH labeled oocytes were scored for metaphase arrested chromosome coorientation. “Heterologous” segregation is when both *X* homologs segregate away from both *4s*, while “Non-Heterologous” segregation is when all *X* and *4* homologs go to the same pole. Note that the order of columns here corresponds to the order of columns in Table S2, which are further divided depending on which pole becomes the egg pronucleus and the sperm genotype.

Line	Normal Coorientation	4-only Malorientation	X-only Malorientation	X-4 Heterologous	X-4 Non-Heterologous
	<i>X4</i> ↔ <i>X4</i>	<i>X44</i> ↔ <i>X</i>	<i>XX4</i> ↔ <i>4</i>	<i>XX</i> ↔ <i>44</i>	<i>XX44</i> ↔ ∅
1	160	13	19	9	3
4	178	8	21	10	1
14	192	10	17	10	2
15	175	9	13	5	2
23	103	35	44	19	13
25	201	0	0	0	0
26	193	12	22	15	8
30	178	4	16	6	2

**Table S4 *nod* progeny count data.** Experimental females were crossed to tester males in vials, brooded once on day 5, and progeny scored as for *ald* alleles. Columns are the same as Table S2; however note that these flies carried isosequential X chromosomes which recombine normally, so NDJ was mainly restricted to chromosome 4. The *nod/+* heterozygous control exhibited 0.12% X and 0.29% 4 NDJ, close to expected wildtype background rates (ZHANG and HAWLEY 1990) indicating that the mutant allele is fully recessive. The *nod* homozygotes exhibited 2.2% X and 78.4% 4, with nullo progeny accounting for 50% of X and 95.1% of 4 NDJ.

Progeny	<i>y w</i> ♂	<i>B</i> ♀	<i>y w; ci ey<sup>R</sup></i> ♂	<i>B; ci ey<sup>R</sup></i> ♀	<i>y w; pol</i> ♂	<i>B; pol</i> ♀	<i>vf B</i> ♂	<i>y w</i> ♀	<i>vf B; pol</i> ♂	<i>y w; ci ey<sup>R</sup></i> ♀	<i>vf B; ci ey<sup>R</sup></i> ♂	<i>y w; pol</i> ♀
<b>Sperm</b>	∅ 44	XY 44	∅ 44	XY 44	∅ ∅	XY ∅	XY 44	∅ 44	XY ∅	∅ 44	XY 44	∅ ∅
<b>Oocyte</b>	X 4	X 4	X ∅	X ∅	X 44	X 44	∅ 4	XX 4	∅ 44	XX ∅	∅ ∅	XX 44
	<b>Normal</b>		<b>4-only NDJ</b>				<b>X-only NDJ</b>		<b>X &amp; 4 Double NDJ</b>			
<i>nod<sup>d</sup> / +</i>	954	772	0	2	1	0	0	0	0	1	0	0
<i>nod<sup>d</sup></i>	419	290	1279	1218	71	51	5	8	3	10	11	1

**Table S5 *yw nod<sup>d</sup>* cytological data.** All 20 possible configurations are listed, with the number (N) of each observed, and the expected number of NDJ events each configuration would produce. For each oocyte, direction of the poles was determined by the paired 2L-3L probe signals, and the homologs associated with each pole were identified. The columns on the right are the proportion of normal, nullo or diplo progeny expected from that class, assuming the two poles are equally likely to become the pronucleus and that all chromosomes not touching the main autosomal mass will be lost. These proportions are multiplied by N for each row to predict the number of nullo and diplo X and 4 events (in parentheses). The numbers of events are then totaled, yielding a prediction of 3% X NDJ and 88% 4 NDJ. Nullo progeny accounted for 75% of X and 98% of 4 NDJ events. As a control, *yw / yw nod<sup>d</sup>* heterozygous females were also assayed, with 200/200 oocytes showing proper  $X4 \leftrightarrow X4$  coorientation.

<i>Class</i>	<i>N</i>	<i>Normal X</i>	<i>Nullo X</i>	<i>Diplo X</i>	<i>Normal 4</i>	<i>Nullo 4</i>	<i>Diplo 4</i>
$\emptyset \leftrightarrow \emptyset$	2	0	1 (2)	0	0	1 (2)	0
$\emptyset \leftrightarrow X$	0	0.5	0.5	0	0	1	0
$\emptyset \leftrightarrow 4$	0	0	1	0	0.5	0.5	0
$\emptyset \leftrightarrow XX$	3	0	0.5 (1.5)	0.5 (1.5)	0	1 (3)	0
$X \leftrightarrow X$	143	1 (143)	0	0	0	1 (143)	0
$\emptyset \leftrightarrow 44$	0	0	1	0	0	0.5	0.5
$4 \leftrightarrow 4$	0	0	1	0	1	0	0
$\emptyset \leftrightarrow X4$	2	0.5 (1)	0.5 (1)	0	0.5 (1)	0.5 (1)	0
$4 \leftrightarrow X$	0	0.5	0.5	0	0.5	0.5	0
$\emptyset \leftrightarrow XX4$	0	0	0.5	0.5	0.5	0.5	0
$4 \leftrightarrow XX$	0	0	0.5	0.5	0.5	0.5	0
$X \leftrightarrow X4$	39	1 (39)	0	0	0.5 (19.5)	0.5 (19.5)	0
$\emptyset \leftrightarrow X44$	0	0.5	0.5	0	0	0.5	0.5
$X \leftrightarrow 44$	0	0.5	0.5	0	0	0.5	0.5
$4 \leftrightarrow X4$	0	0.5	0.5	0	1	0	0
$X \leftrightarrow X44$	7	1 (7)	0	0	0	0.5 (3.5)	0.5 (3.5)
$4 \leftrightarrow XX4$	0	0	0.5	0.5	1	0	0
$\emptyset \leftrightarrow XX44$	0	0	0.5	0.5	0	0.5	0.5
$44 \leftrightarrow XX$	0	0	0.5	0.5	0	0.5	0.5
$X4 \leftrightarrow X4$	4	1 (4)	0	0	1 (4)	0	0
<b>Total</b>	<b>200</b>	<b>194</b>	<b>4.5</b>	<b>1.5</b>	<b>24.5</b>	<b>172</b>	<b>3.5</b>