Table S1.
Mapping of Y chromosome genes to fertility factor intervals.

Region [b]	Size [c]	Fertility Factor [a]	Known Genes
A	14%	*kl-5*	*PP1-Y1* *kl-5* *PRY*
B	8%	*kl-3*	*PRY* *kl-3*
C	5%	*kl-2*	*kl-2* *ARY* *Su(Ste)* [d] *Ppr-Y*
D	3%	*kl-1*	*Ppr-Y* *WDY* *Su(Ste)* [d]
E	10%		*CENTROMERE* *FDY* *Mst77* [d] *Pp1-Y2*
F	5%	*ks-1*	*ORY*
G	3%	*ks-2*	*CCY*

[a] Fertility factors were originally defined by X-ray mutagenesis (Brosseau 1960).
[b] Regions were designated A-G by Hardy et al (Hardy *et al.* 1981) and are defined by segmental deletions (Kennison 1981).
[c] Size estimates are based on a “breakage map” or the fraction of translocation breakpoints that fall within the region (Kennison 1981).
[d] *Su(Ste)* and *Mst77* are multi-copy genes. *CG41561* is a single copy Y-linked gene but has not been mapped to a fertility factor interval.

Table S2.
Fly stocks used in this study.

Fly Lines	Genotype	Source
Canton S	wildtype	lab stock
Oregon-R modENCODE	wildtype	BL# 25211
$\widehat{XX}$	*FM7a/Y/C(1)M4,y*	BL# 988
$\widehat{XY}$	*C(1;Y)1,y/0; ry*	BL# 4408
Bam-Gal4	*w Protamine-GFP,w+ ;; Bam-Gal4, UAS-Dicer*	See references [a]. Combined into one stock by Caroline Sartain.
vig2	*w; FRT2A FRT82B PBac{GAL4D,EYFP}vig2[PL00470]*	BL# 19518

[a] (Chen and McKearin 2003; Manier *et al.* 2010)

Table S3.
Primer sequences used in this study.
SgRNA sequences are underlined, Cas9 cut sites are indicated with an asterisk, and extra Gs that were added for the optimal T7 promoter are indicated in red.

Gene	Primer	Sequence	Notes
CCY	Forward	ATCGCGTTTTGTTGCCGATTTAGTGGC	914bp WT PCR product, touchdown PCR protocol used
	Reverse	TCTCTAATCCTTTCGCTTTGTCAACT	
	Guide 1	GAAATTAATACGACTCACTATAGGCTATCGAG CCGGCATC*GAAGTTTTAGAGCTAGAAATAGC	extra G (in red) added for optimal T7 promoter
	Guide 3	GAAATTAATACGACTCACTATAGGCTACAATC GAGATAAT*GCTGTTTTAGAGCTAGAAATAGC	extra G (in red) added for optimal T7 promoter
	Guide 4	GAAATTAATACGACTCACTATAGGCTGAATAT CGTTTATG*TATGTTTTAGAGCTAGAAATAGC	extra G (in red) added for optimal T7 promoter
	Forward RT	GAGAGCTCACCTACATGCAT	200bp WT cDNA PCR product
	Reverse RT	GGTATCGCAACAAAACGTGG	
PRY	Forward	TCTCTGTAAGTGCTGGTCCCAGTAGAG	971bp WT PCR product
	Reverse	AAAATGCCATGCATGTGCTAACCCTGG	
	Guide 2.7	GAAATTAATACGACTCACTATAGGCAGCATGA CGCGTAGTGTCGTTTTAGAGCTAGAAATAGC	extra G (in red) added for optimal T7 promoter
	Guide 2.3	GAAATTAATACGACTCACTATAGGTTGAGGGT TTACCTGTGGAGTTTTAGAGCTAGAAATAGC	extra G (in red) added for optimal T7 promoter
	Forward RT	CGGAAGGTGATCCAATAAATGAGC	89bp WT cDNA PCR product, touchdown PCR protocol used
	Reverse RT	GACATTTTCGATATCTTCCTCGGGG	
FDY	Forward	CCCTGTTCAAAAACCAACAACCGCTCA	953bp WT PCR product, 5 bases of forward and 1 base of reverse primer differ in *vig2* sequence
	Reverse	TGTTGCCAAGGGTTGCGTTGTCTCTAT	
	3' Forward	AACTTCAATGATGGCCGCAAGGTCG	1175bp WT PCR product, 1 base of forward differs in *vig2* seq, reverse primer is not similar
	3' Reverse	CGGGAGCGAGCGGAAAGAGAGCA	
	Guide 1.12	GAAATTAATACGACTCACTATAGGAGCAACTT ACGCACCA*CGCGTTTTAGAGCTAGAAATAGC	extra G (in red) added for optimal T7 promoter, identical sequence in v*ig2*
	Guide 2.1	GAAATTAATACGACTCACTATAGGACCACCAC GCGTCCG*TTCGTTTTAGAGCTAGAAATAGC	1 base is different in *vig2* sequence

Table S3 *continued*.

Gene	Primer	Sequence	Notes
Vig2	Forward	TCAGGCCGAAGAGGACAGAGAATTTCG	1579bp WT PCR product, 3 bases of reverse primer differ in *FDY* seq, forward primer is not similar
	Reverse	GTCTGTTTTCATAGCCGTTGCTGGGTC	
	Forward RT	GGATCCGATAGAACTGGCGTCAAG	105bp WT cDNA PCR product, 1 base differs from *FDY* in both forward and reverse primer
	Reverse RT	CCCGGTCGTCTTTAAGTCCTCA	
Generic	Reverse - guide synthesis	AAAAAAAGCACCGACTCGGTGCCACTT TTTCAAGTTGATAACGGACTAGCCTTAT TTTAACTTGCTATTTCTAGCTCTAAAAC	Used together with guide primers
Actin5c	Forward RT	AGCGCGGTTACTCTTTCACCAC	103bp WT PCR product
	Reverse RT	GTGGCCATCTCCTGCTCAAAGT	
RpL32	Forward RT	CACCAGTCGGATCGATATGC	120bp WT PCR product
	Reverse RT	CGATCCGTAACCGATGTTG	
Pp1-Y2	Forward	TGTTTTCATGCTTGCCTTTGGACGACG	1128bp WT PCR product
	Reverse	CCCCCAAAGAAGCGCAATATGTCACTG	
WDY	Forward	TGAGATGGTATCTTGCGTTTACTTTTC	837bp WT PCR product
	Reverse	ACTTCTTGGTCTGGCATTATACTCATA	
ARY	Forward	GCTTCGTGGATATCAATGCTCGGCAGC	775bp WT PCR product
	Reverse	TGACTTGCAGAGCGCTTGTACTTCTT	

Table S4.
Number of females and mean counts from each *PRY* group (Allele x Repeat).

	PRY						**Control for *PRY***					
Allele	G5.7		G54.1		G54.7		A1		A2		B1	
Repeat	1	2	1	2	1	2	1	2	1	2	1	2
# Eggs / Female												
Day 1	45.83	38.18	44.07	41.39	45.31	30.27	41.80	46.00	51.29	46.05	53.00	39.75
Day 2	46.92	42.06	44.20	38.61	45.77	41.67	48.47	49.81	60.71	48.09	58.50	50.10
Day 3	53.67	39.12	45.40	39.22	47.38	42.33	54.00	56.44	47.14	45.50	61.10	59.40
Day 4	43.25	43.94	43.87	37.89	45.08	50.67	52.93	50.88	47.00	39.14	53.80	54.95
Day 5	39.58	34.41	48.20	36.89	49.62	41.47	50.87	37.31	54.29	29.64	53.20	52.65
Total	**229.25**	**197.71**	**225.73**	**194.00**	**233.15**	**206.40**	**248.07**	**240.44**	**260.43**	**208.41**	**279.60**	**256.85**
# Pupa / Female												
Day 1	5.08	4.65	12.60	6.28	7.77	5.80	38.33	35.25	48.00	34.23	46.00	36.80
Day 2	0.67	0.88	0.73	1.22	0.38	1.00	45.67	41.00	52.71	34.73	54.20	42.70
Day 3	0.25	0.12	0.13	0.67	0.38	0.47	35.67	28.06	42.00	30.45	50.60	38.70
Day 4	0.17	0.12	0.00	0.56	0.08	0.00	29.60	19.00	39.71	18.55	32.20	30.35
Day 5	0.00	0.12	0.33	0.00	0.00	0.00	24.20	9.00	50.14	12.09	23.40	18.70
Total	**6.17**	**5.88**	**13.80**	**8.72**	**8.62**	**7.27**	**173.47**	**132.31**	**232.57**	**130.05**	**206.40**	**167.25**
N	12	17	15	18	13	15	15	16	7	22	10	20

Table S5.
Number of females and mean counts from each *FDY* group (Allele x Repeat).

	FDY						**Control for *FDY***					
Allele	L3.2		L64.3		L71.4		A		C		E	
Repeat	1	2	1	2	1	2	1	2	1	2	1	2
# Eggs / Female												
Day 1	40.61	44.85	41.53	35.57	43.44	39.86	37.13	46.20	43.23	45.62	43.07	35.82
Day 2	25.28	37.23	22.82	25.29	27.56	30.93	20.07	27.47	27.38	21.69	35.43	30.94
Day 3	18.50	34.54	18.88	19.29	21.75	21.00	20.33	34.73	28.23	18.23	44.93	38.53
Day 4	15.17	28.00	16.18	19.71	17.63	18.57	13.93	32.60	17.46	19.77	36.93	49.24
Day 5	12.17	17.31	15.88	5.71	20.56	11.00	8.67	17.40	15.46	12.00	39.57	30.12
Total	**111.72**	**161.92**	**115.29**	**105.57**	**130.94**	**121.36**	**100.13**	**158.40**	**131.77**	**117.31**	**199.93**	**184.65**
# Pupa / Female												
Day 1	36.39	39.38	38.94	33.43	40.38	35.50	33.00	42.07	41.23	43.31	40.93	30.53
Day 2	25.33	33.54	22.06	24.79	26.50	28.00	18.93	28.13	27.38	21.46	34.00	28.47
Day 3	15.78	27.92	17.35	17.36	19.13	17.79	18.27	31.93	24.00	17.31	43.29	33.88
Day 4	12.28	23.92	15.00	17.07	16.56	16.86	12.40	29.20	15.23	18.54	36.29	38.71
Day 5	10.06	14.54	15.59	5.21	18.94	7.86	7.73	14.53	13.69	10.54	32.64	25.41
Total	**99.83**	**139.31**	**108.94**	**97.86**	**121.50**	**106.00**	**90.33**	**145.87**	**121.54**	**111.15**	**187.14**	**157.00**
N	18	13	17	14	16	14	15	15	13	13	14	17

Table S6.
p-values from Kruskal-Wallis Rank Sum Test to compare the independent alleles of each genotype.

		Control for *PRY*	*PRY*	Control for *FDY*	*FDY*
# Eggs / Female	**Day 1**	0.65762	0.30374	0.71674	0.80181
	Day 2	0.92863	0.75028	0.22442	0.56031
	Day 3	0.11878	0.96518	0.02269 *	0.92516
	Day 4	0.15507	0.62343	0.00142 ***	0.82582
	Day 5	0.03306 *	0.50622	0.00036 ***	0.52639
	Total	0.70840	0.22076	0.51583	0.83841
# Pupa / Female	**Day 1**	0.64033	0.55454	0.34498	0.74190
	Day 2	0.29890	0.33600	0.02213 *	0.95450
	Day 3	0.23414	0.33668	0.02693 *	0.96757
	Day 4	0.75713	0.22503	0.00092 ***	0.87822
	Day 5	0.20480	0.95535	0.02507 *	0.84271
	Total	0.43658	0.08452	0.06744	0.95793

* $p<0.05$, *** $p<0.01$

Table S7.
p-values from Wilcoxon Rank Sum Test to compare the mean fertility and fecundity of mutants compared to controls.

		***PRY* vs Control**	***FDY* vs Control** (all)	***FDY* vs Control** (Control E omitted)
# Eggs / Female	**Day 1**	0.06106	0.74746	0.59194
	Day 2	0.01374 *	0.70744	0.22769
	Day 3	0.00544 ***	0.01536 *	0.33008
	Day 4	0.13900	0.00852 ***	0.41788
	Day 5	0.24883	0.03361 *	0.95870
	Total	0.02499 *	0.06369	0.63515
# Pupa / Female	**Day 1**	0.00000 ***	0.68494	0.57829
	Day 2	0.00000 ***	0.92065	0.42998
	Day 3	0.00000 ***	0.00995 ***	0.23949
	Day 4	0.00000 ***	0.02292 *	0.36537
	Day 5	0.00000 ***	0.04824 *	0.95859
	Total	0.00000 ***	0.06968	0.55841

* $p<0.05$, *** $p<0.01$

Table S8.
p-values from Levene Test for homogeneity of variance around the mean of mutants compared to controls.

		***PRY* vs Control**	***FDY* vs Control** (all)	***FDY* vs Control** (Control E omitted)
# Eggs / Female	**Day 1**	0.67016	0.79047	0.24197
	Day 2	0.00885 ***	0.39501	0.04996 *
	Day 3	0.00000 ***	0.00989 ***	0.23477
	Day 4	0.03562 *	0.00025 ***	0.58438
	Day 5	0.02306 *	0.00573 ***	0.38211
	Total	0.00000 ***	0.65741	0.08378
# Pupa / Female	**Day 1**	0.00000 ***	0.36859	0.03618 *
	Day 2	0.00000 ***	0.00852 ***	0.47207
	Day 3	0.00000 ***	0.00023 ***	0.49856
	Day 4	0.00000 ***	0.02190 *	0.28833
	Day 5	0.00457 ***	0.01266 *	0.95503
	Total	0.00000 ***	0.04587 *	0.47835

* p<0.05, *** p<0.01

Table S9.
Fertility of *FDY–vig2* double mutants.

Genotype	# Males That Produce Progeny At 25°C			# Males That Produce Progeny At 19°C		
	#	%		#	%	
w / Y, Control ;; + ; +	18/19	95%	**Not Sterile**	19/20	95%	**Not Sterile**
w / Y, FDY L3.2 ;; + ; +	14/20	70%	**Not Sterile**	20/20	100%	**Not Sterile**
w / Y, Control ;; vig2	19/19	100%	**Not Sterile**	17/18	94%	**Not Sterile**
w / Y, FDYL 3.2 ;; vig2	18/18	100%	**Not Sterile**	16/18	85%	**Not Sterile**

Table S10.
Summary of all *CCY* alleles with visible INDELs.

Allele	Fertility	BP Inserted	BP Deleted	Total BP Change	Effect	Guide Site Altered
I6.2	not sterile	0	6	-6	In frame	3
I6.3	not sterile	1	10	-9	In frame	3
I6.4	not sterile	2	2	0	In frame	3
I6.6	not sterile	0	6	-6	In frame	3
I8.7	not sterile	0	6	-6	In frame	3
I8.12	not sterile	0	6	-6	In frame	3
I10.2	not sterile	0	6	-6	In frame	3
I15.2	not sterile	3	3	0	In frame	3
I15.10	not sterile	3	3	0	In frame	3
I6.9	sterile	7	18	-11	Out of frame	3
I8.2	sterile	0	5	-5	Out of frame	3
I8.4	sterile	9	5	-4	Out of frame	3
I8.5	sterile	0	5	-5	Out of frame	3
I10.1	sterile	0	10	-10	Out of frame	3
I10.3	sterile	17	7	10	Out of frame	3
I10.4	sterile	18	8	10	Out of frame	3
I15.7	sterile	0	2	-2	Out of frame	3
I17.4	sterile	0	25	-25	Out of frame	3