

Table S1 FDR adjusted P-values for Tukey post-hoc comparisons of sex-ratios sired by males with X^{SR} and several different Y chromosomes

	114	145	189	123	41	166	33	52	96	168	138	171	173	79	162	107	177	159	62
114															*	*		***	***
145																		***	***
189																		***	***
123																		***	***
41																		***	***
166													*		*	*		***	***
33																		***	***
52																	*	***	***
96																		***	***
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79																		***	***
162																		***	***
107																		***	***
177																		***	***
159																			*
62																			

Table S2 Generalized linear model assuming a Poisson distribution for number of successful matings by each genotype

Factor	df	Sum Sq.	Mean Sq.	F value	P
X chromosome	1	236.95	236.95	166.749	<0.0001
Y chromosome	2	100.88	50.44	35.497	<0.0001
X-Y interaction	2	68.31	34.16	24.037	<0.0001
Block	1	0.50	0.50	0.351	0.56
Residuals	109	154.89	1.42		

Table S3 Negative binomial generalized linear model for number of offspring produced in the “first” (most successful) by each genotype

Factor	df	Sum Sq.	Mean Sq.	F value	P
X chromosome	1	23123	23123	92.51	<0.0001
Y chromosome	2	6512	3256	13.03	<0.0001
X-Y interaction	2	8861	4430	17.73	<0.0001
Block	1	8863	8863	35.46	<0.0001
Residuals	109	27244	250		

Table S4 Negative binomial generalized linear model for total offspring produced by each genotype

Factor	df	Sum Sq.	Mean Sq.	F value	P
X chromosome	1	340587	340587	114.954	<0.0001
Y chromosome	2	65817	32908	11.107	<0.0001
X-Y interaction	2	53297	26648	8.994	<0.0001
Block	1	88712	88712	29.942	<0.0001
Residuals	109	322948	2963		

Table S5 Distance matrix between strain 141.02 (X^{ST}), two sex-ratio strains (X^{SR1} and X^{SR2}) and *D. pseudoobscura* for a 286bp region of *Runt* including 249 bases of intronic sequence and the first 37 bases of exon 2. Above diagonal represents percent identity, below diagonal represents number of differences.

	<i>D. pseudoobscura</i>	<i>D. affinis</i> 141.02 (X^{ST})	<i>D. affinis</i> X^{SR1}	<i>D. affinis</i> X^{SR2}
<i>D. pseudoobscura</i>	-	76.81	77.17	77.17
<i>D. affinis</i> 141.02 (X^{ST})	64	-	97.86	99.64
<i>D. affinis</i> X^{SR1}	63	5	-	98.22
<i>D. affinis</i> X^{SR2}	63	1	6	-

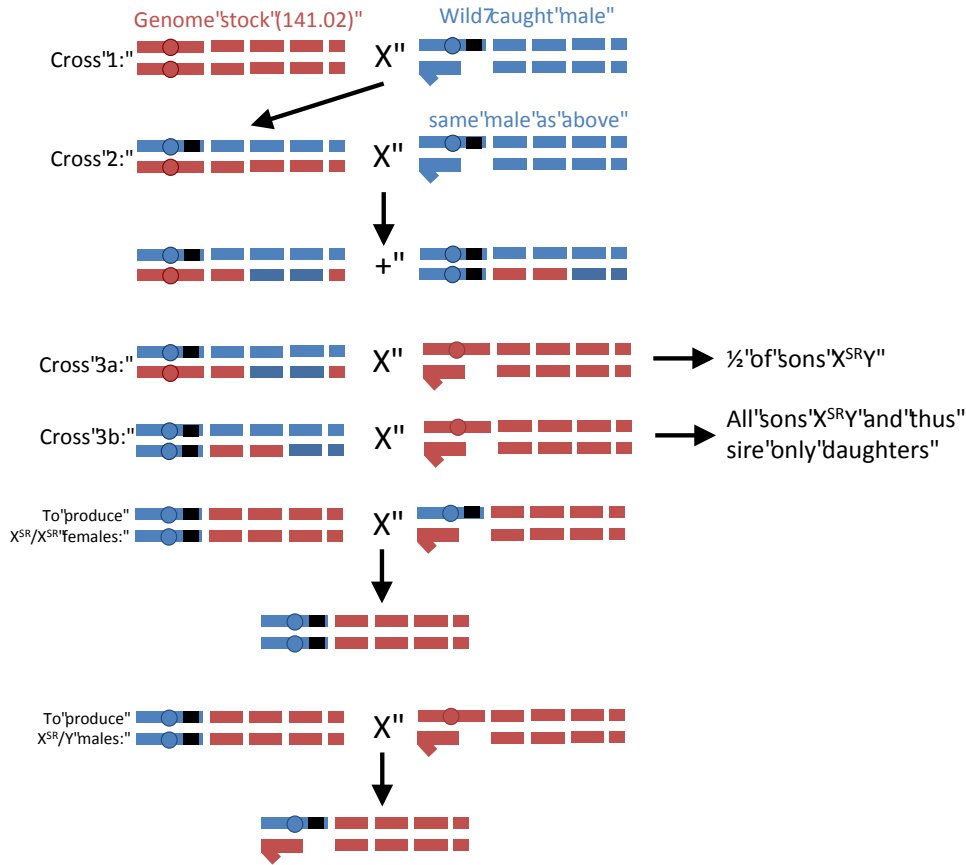


Figure S1 Crossing scheme to generate X^{SR} replacement lines. Cross 1: Wild-caught males crossed to lab stock (141.02) and sex-ratio of offspring noted. All lines with skewed sex-ratio were retained. Cross 2: Same wild-caught male as in Cross 1 mated to his daughters. Most offspring from Cross 2 were be female and assuming equal viability, 50% were homozygous for X^{SR} and 50% were heterozygous (X^{SR}/X^{ST}). Cross 3: Virgin females from Cross 2 were mated to standard males (141.02). If these Cross 3 females were heterozygous for the driver, half of sons sired female-biased sex-ratios (3a). If they were heterozygous, all sons sired female-biased sex-ratios (3b). Males from Cross 3b were mated to virgin females from Cross 2 (their aunts) assuring that the male was $X^{SR}Y$ and the female was $X^{SR}X^{SR}$. To generate new $X^{SR}X^{SR}$ females, virgin $X^{SR}X^{SR}$ females were mated to $X^{SR}Y$ males. To generate new $X^{SR}Y$ males, virgin $X^{SR}X^{SR}$ females were mated to $X^{ST}Y$ males (141.02). This crossing scheme replaces 50% of the genome each generation with 141.02 genetic material and therefore over several generations, the wild autosomes and Y chromosome are replaced with the lab stock (141.02).

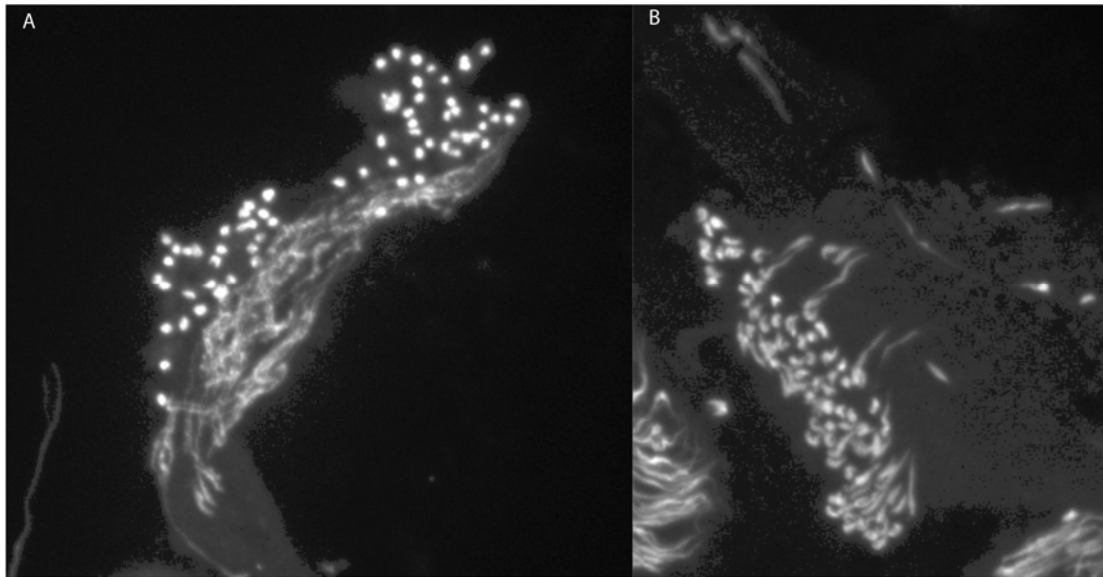


Figure S2 Phenotypes from X^{SRYSUS} testes are variable. (A) 64 round-headed spermatozoa in the same cyst as fully elongated spermatozoa. (B) A cyst of elongating spermatozoa where some spermatozoa heads are malformed.

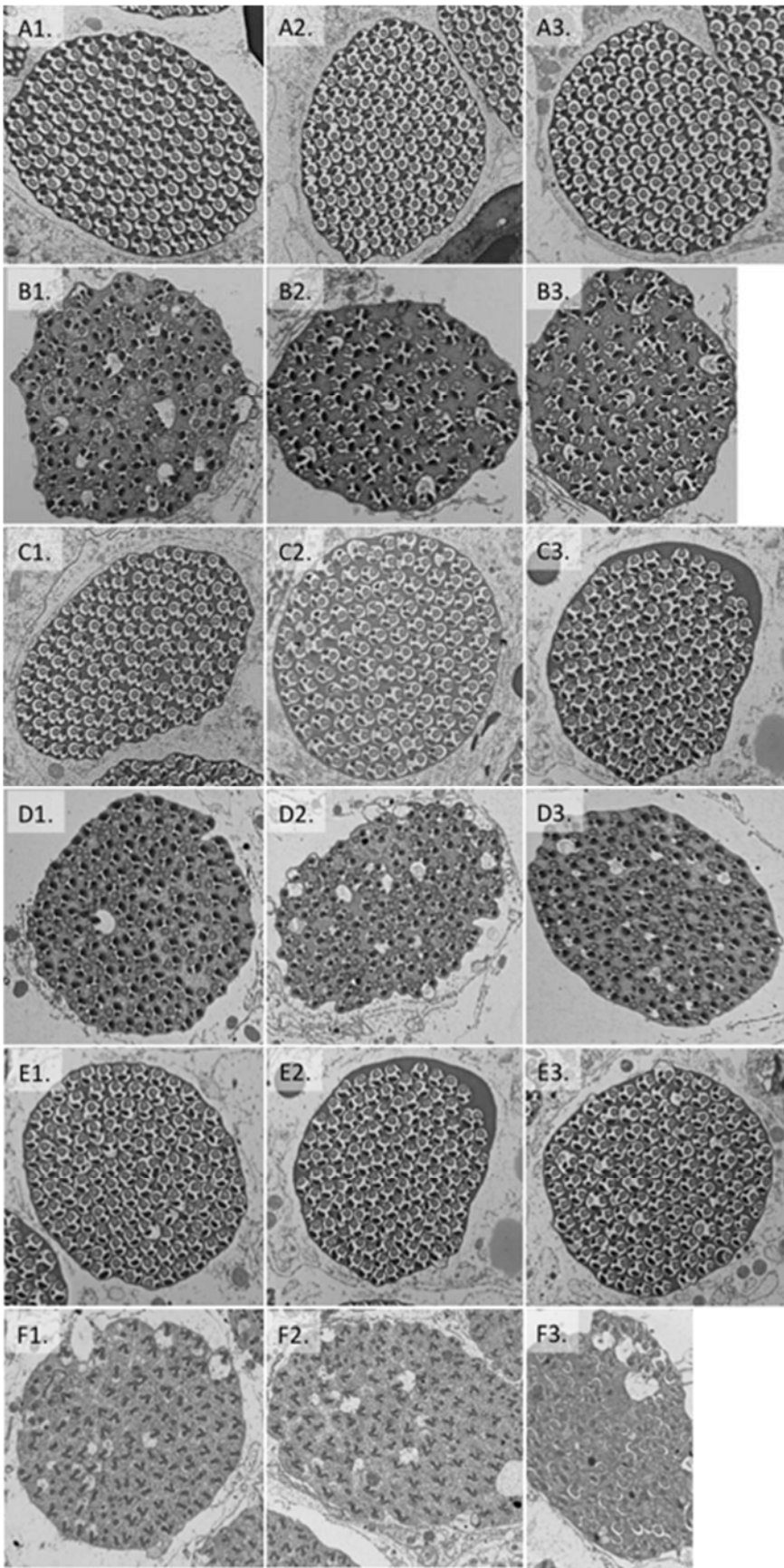


Figure S3 Variation in sperm bundle morphology for each genotype, see corresponding Figure 4 in main text. A1-A3)

X^{STYsus} , B1-B3) X^{SRYSus} , C1-C3) X^{STYres} , D1-D3) X^{SRYres} , E1-E3) X^{STO} and F1-F3) X^{SRO} .