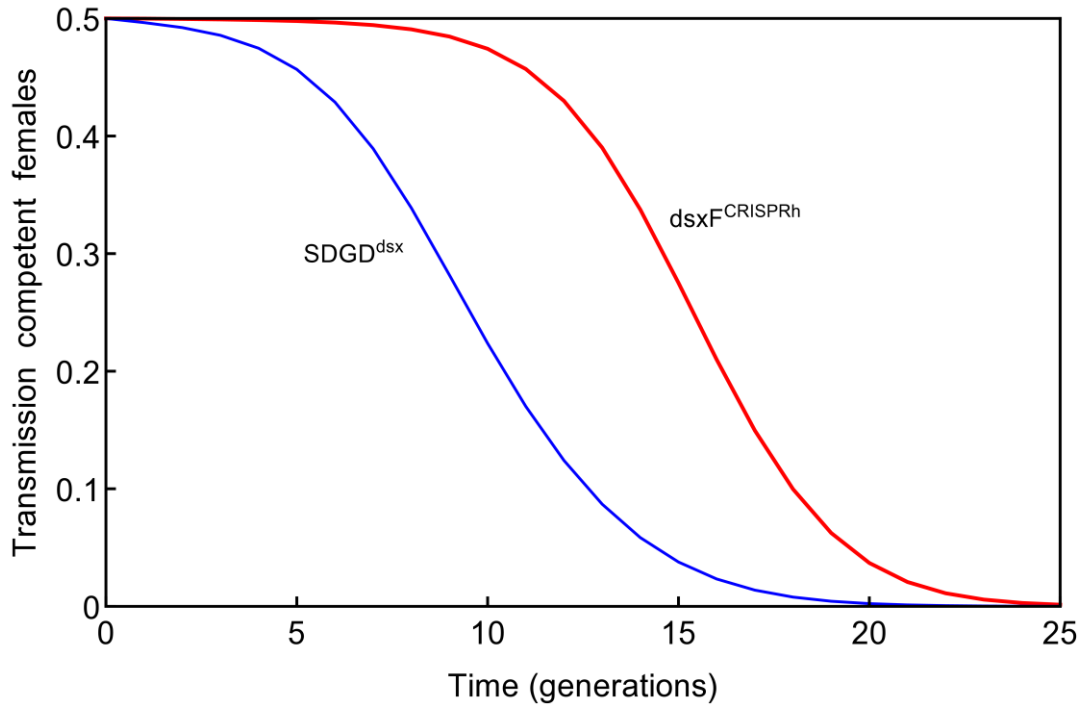


In the format provided by the authors and unedited.

A male-biased sex-distorter gene drive for the human malaria vector *Anopheles gambiae*

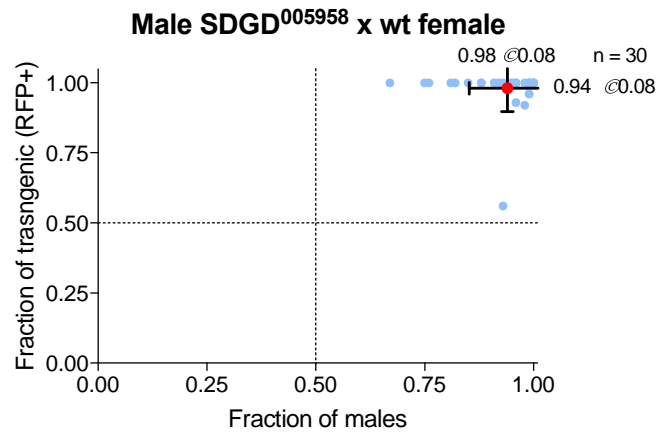
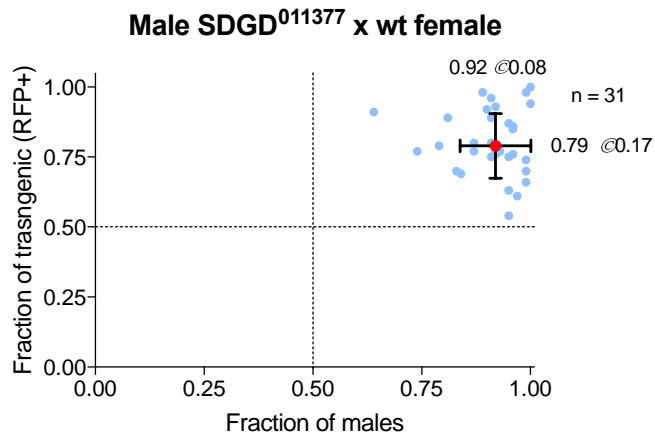
In the format provided by the authors and unedited



Supplementary Figure 1

Model prediction of the reduction in abundance of transmission competent females.

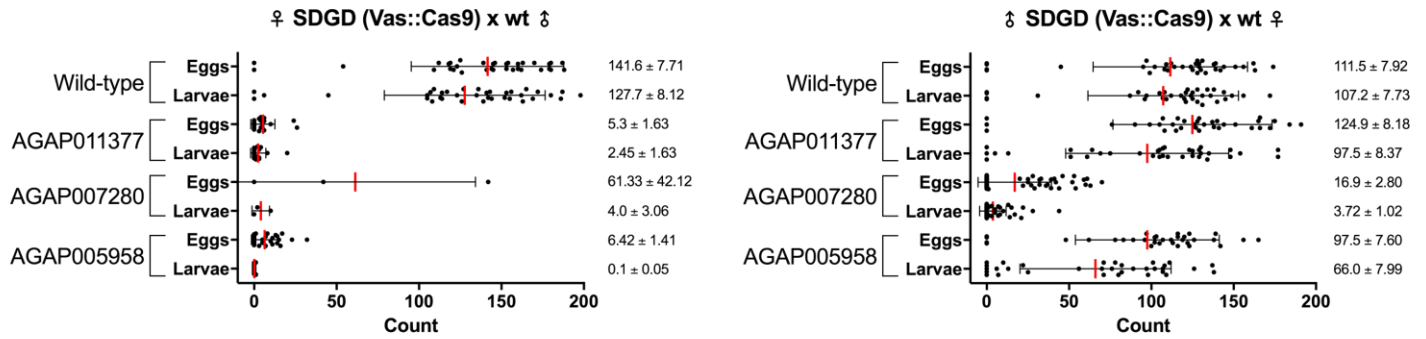
Model prediction of the reduction over time in abundance of transmission competent (i.e. biting) females, normalised by the initial total population size, for the SDGD^{dsx} construct compared to dsxF^{CRISPRh} (Kyrou et al., 2018), using a continuous-time population dynamics model (see Supp. Modelling Methods) for 'field' release of 1% heterozygous transgenic males in the male population. The SDGD^{dsx} construct is predicted to suppress the population of transmission competent females faster than the dsxF^{CRISPRh} mainly due to the creation of a male bias in the population by the sex distorter. Parameters used for SDGD^{dsx} are in Table S2; dsxF^{CRISPRh} parameters were estimated from Kyrou et al. (2018) using an average W/D female fitness of 0.4335; for both, R_m (intrinsic growth rate per generation) = 6. At long times (not shown), the SDGD^{dsx} population rebounds to an intermediate equilibrium (suppressed) population.



Supplementary Figure 2

Sex and inheritance bias caused by SDGD⁰¹¹³⁷⁷ and SDGD⁰⁰⁵⁹⁵⁸ males.

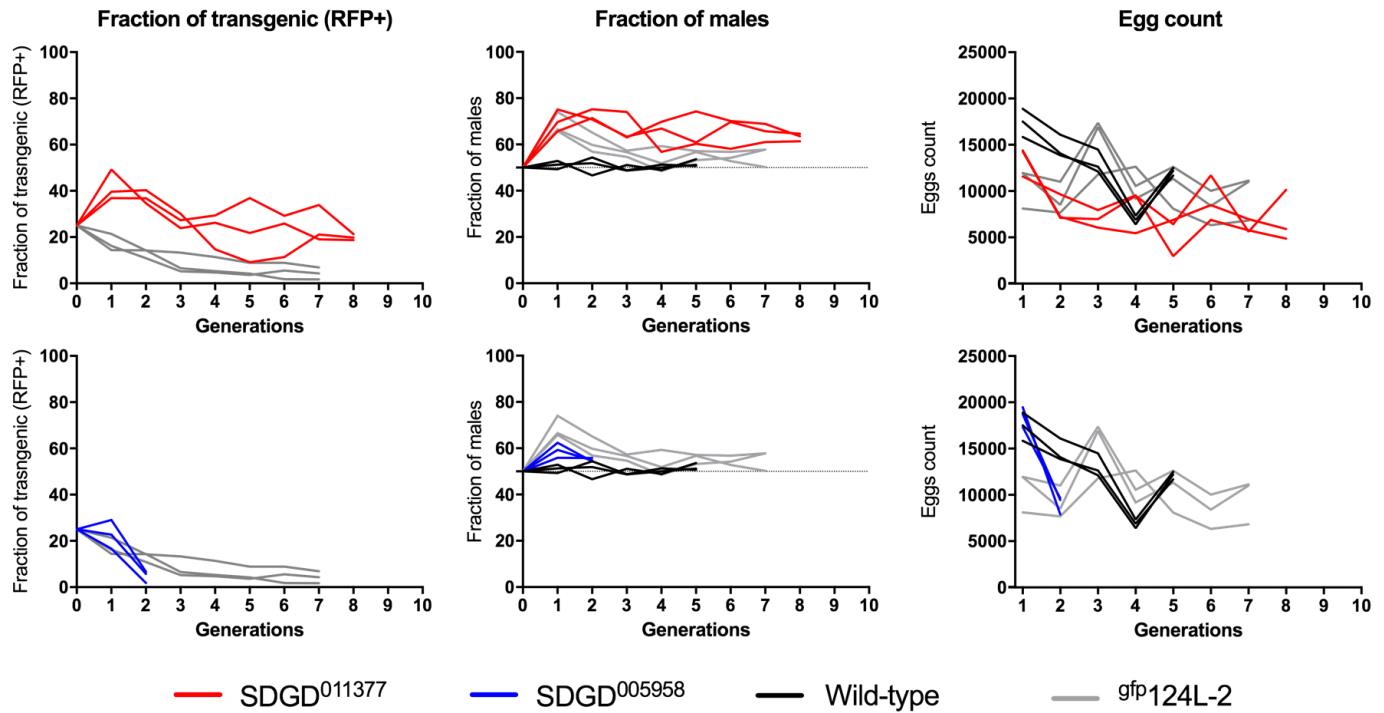
Scattered plots showing the fraction of transgene inheritance (y-axis) against male bias (x-axis) in the progeny of heterozygous male SDGD⁰¹¹³⁷⁷ (left-hand panel) SDGD⁰⁰⁵⁹⁵⁸ and (right-hand panel) crossed to wild type females. Individual coloured dots represent the progeny derived from a single female and the red dots indicate the average of the population (with respective values indicated next to the plot \pm s.e.m.). Error bars indicate standard deviation. SDGD at both loci showed a high transmission rate of the transgene determined by scoring in the progeny the presence of RFP marker that is linked to the SDGD allele. The progeny of SDGD/+ at both loci showed a strong sex ratio distortion towards males. Dotted lines indicate expected Mendelian inheritance.



Supplementary Figure 3

Fecundity phenotype of SDGD targeting 3 different fertility loci in *An. gambiae*

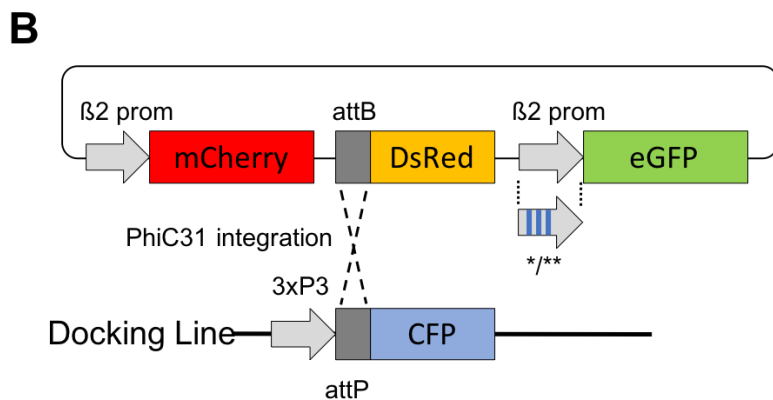
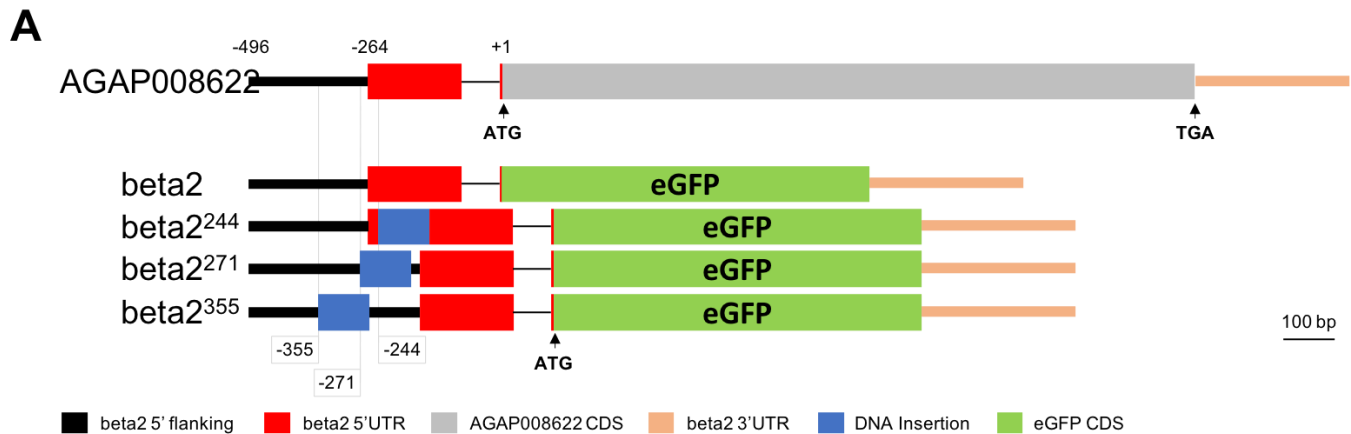
SDGD constructs expressing the Cas9 nuclease under the control of the *Vas2* promoter were generated targeting the fertility loci *AGAP011377*, *AGAP007280* and *AGAP005859* (as indicated). SDGD heterozygous male and female were crossed to wild-type counterparts. Each dot represents progeny of individual females. Fecundity was measured by counting the number of eggs per female and the hatched larvae. Values on the right represent average \pm s.e.m. A strong fertility effect was observed in heterozygous SDGD females at 3 loci, while male fecundity was strongly impaired by targeting 7280 and 5859 loci. Vertical red bars indicate average count, and error bars indicate standard deviation. A minimum of 20 females were analysed for each cross.



Supplementary Figure 4

Kinetics of SDGD⁰¹¹³⁷⁷ and SDGD⁰⁰⁵⁹⁵⁸ spread in target mosquito populations.

In these experiments 100 heterozygous transgenic males were introduced into a population of 100 wild-type males and 200 wild-type females (transgenic allele frequency of 12.5%). The frequency of the transgene was monitored every generation together with the fraction of males in the population and the total number of eggs laid. Each consecutive generation was established by collecting 450 eggs. The frequency of the SDGD⁰¹¹³⁷⁷ (red lines) and SDGD⁰⁰⁵⁹⁵⁸ (blue lines) was compared to that of the autosomal self-limiting sex-distorter *gfp124L-2* (grey lines) (Galizi et al., 2014) as well as to that of wild-type populations (black lines) as control. Each genotype was tested in triplicate cages. The SDGD⁰⁰⁵⁹⁵⁸ allele disappeared from the populations at generation 2 due to the strong fertility effects. The SDGD⁰¹¹³⁷⁷ alleles persisted in the populations despite the fertility effects but failed to increase over the frequency of release on subsequent generations. The fraction of males in the population was stably biased to about 65%.



C

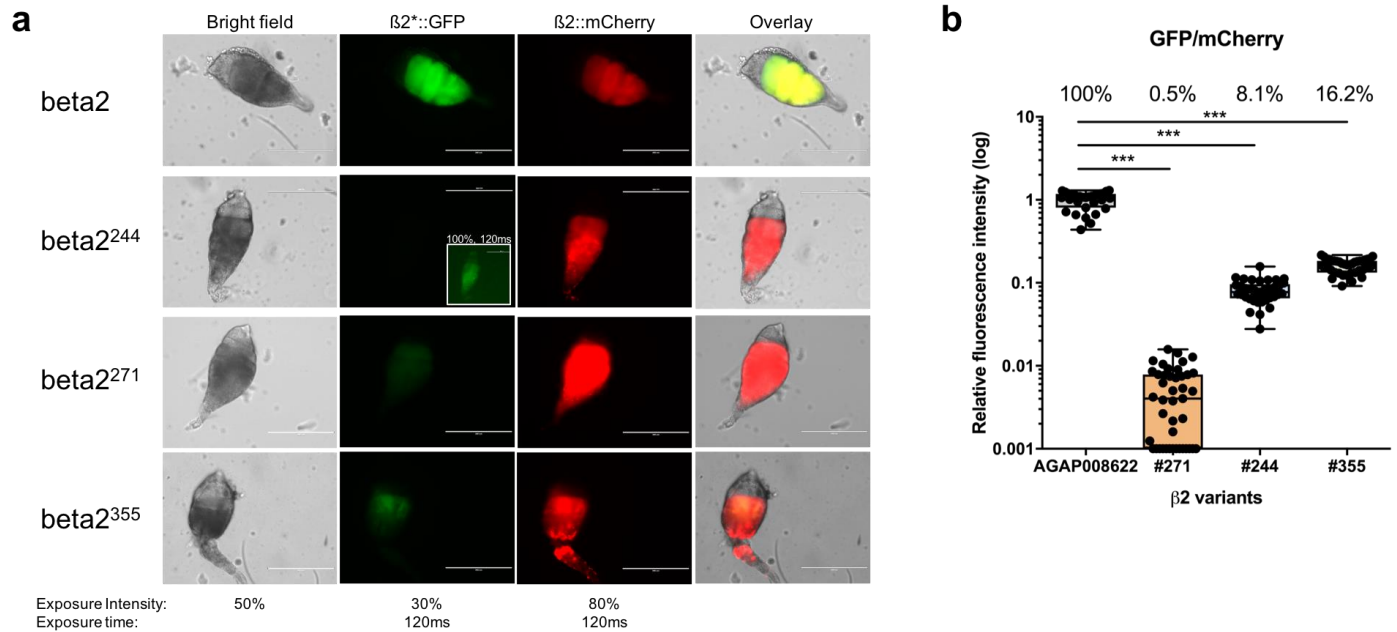
DNA spacer

```
GACTCGGACCCGAGTTGGCCAACGACCCACGGGCGGAG
TTAGGGCGGATGGTGAGAAGTGCGCGTCTCGTTCCCGC
AGCTCGCCAGCACTCTCAGACTCA
```

Supplementary Figure 5

Mutagenesis of the *beta2* tubulin promoter.

A) Schematic overview of the *AGAP008622* (*beta2-tubulin*) locus and the three *beta2* promoter variants generated by placing a 100bp GC-rich DNA sequence (blue rectangle) upstream of the start codon at position 244, 271 and 355. B) The double-fluorescence reporter assay developed to detect the effects of the *beta2* promoter modifications on its transcription level. A construct for each modification was generated harbouring the modified *beta2* promoter (stars key) driving an eGFP marker, while a second, unmodified, *beta2* promoter was driving a mCherry protein. The constructs were integrated within the same autosomal docking line by PhiC31-mediated integration by replacing a 3xP3::CFP cassette with a 3xP3::DsRed as integration marker. C) The sequence of 100bp GC-rich DNA region inserted at the 5' of the *beta2* promoter.

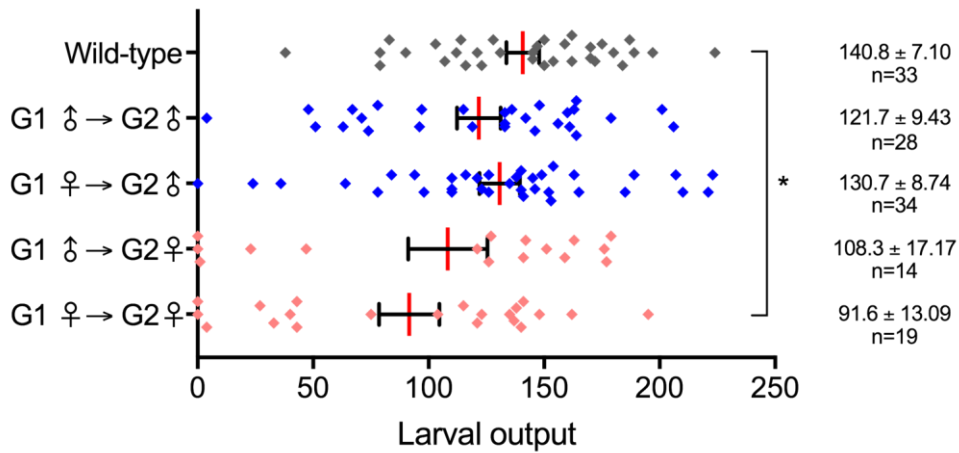


Supplementary Figure 6

GFP and mCherry signal quantification from mosquito testes transformed with modified *beta2* promoters.

a) Dissected testis from $\beta 2^{wt}$, $\beta 2^{271}$, $\beta 2^{244}$ and $\beta 2^{355}$ transgenic lines containing a single heterozygous insertion were microphotographed under the same exposure settings (as indicated), using mCherry as internal control. Line $\beta 2^{271}$ showed GFP fluorescence intensity comparable to background; GFP expression was detectable at increased exposure (100% gain, 120ms, inset). Scale bar, 200 μm . b) Quantification of fluorescence intensity as GFP/mCherry ratio, normalized to the $\beta 2^{wt}$ control (100%). Average relative intensity is indicated above the bars. *** indicates P value < 0.001 (ordinary One-way ANOVA). A minimum of 31 testes were analysed from individual expressing each promoter variant.

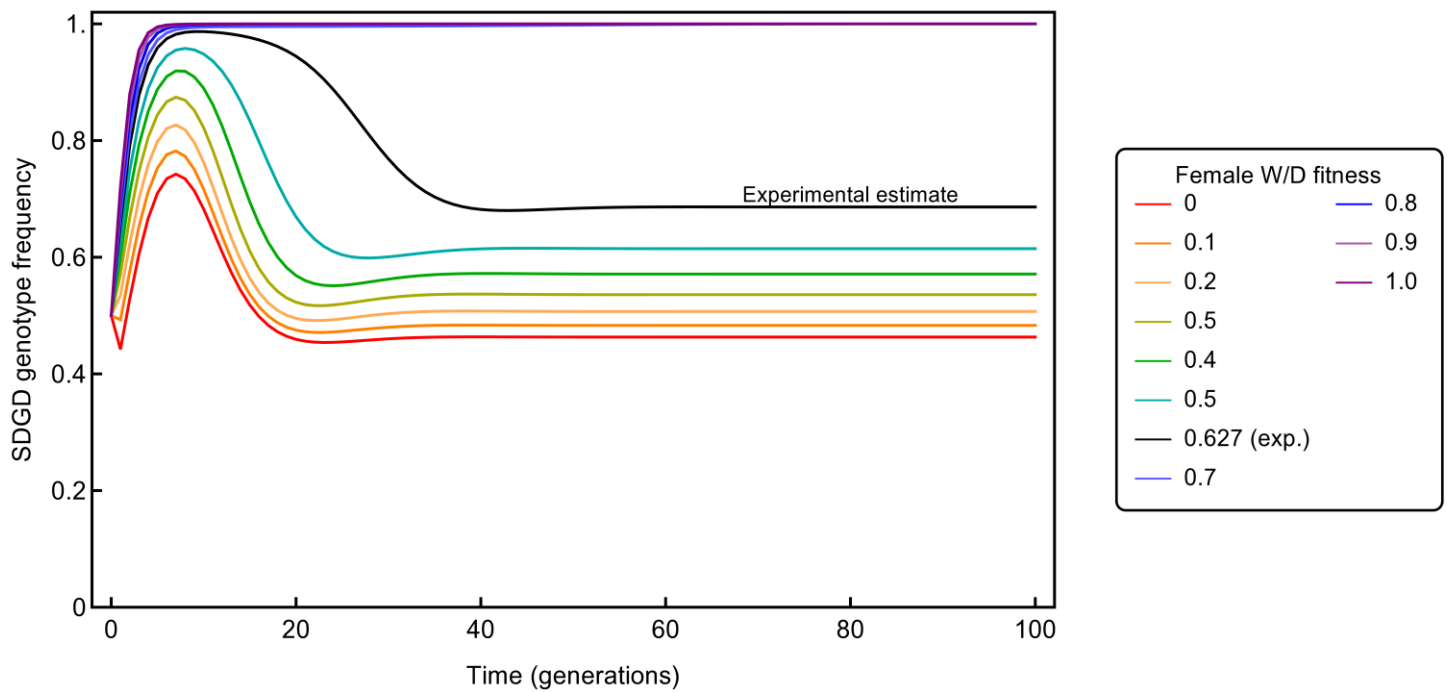
SDGD^{dsx} x wt



Supplementary Figure 7

Maternal or paternal contribution to the fecundity of the SDGD^{dsx} allele

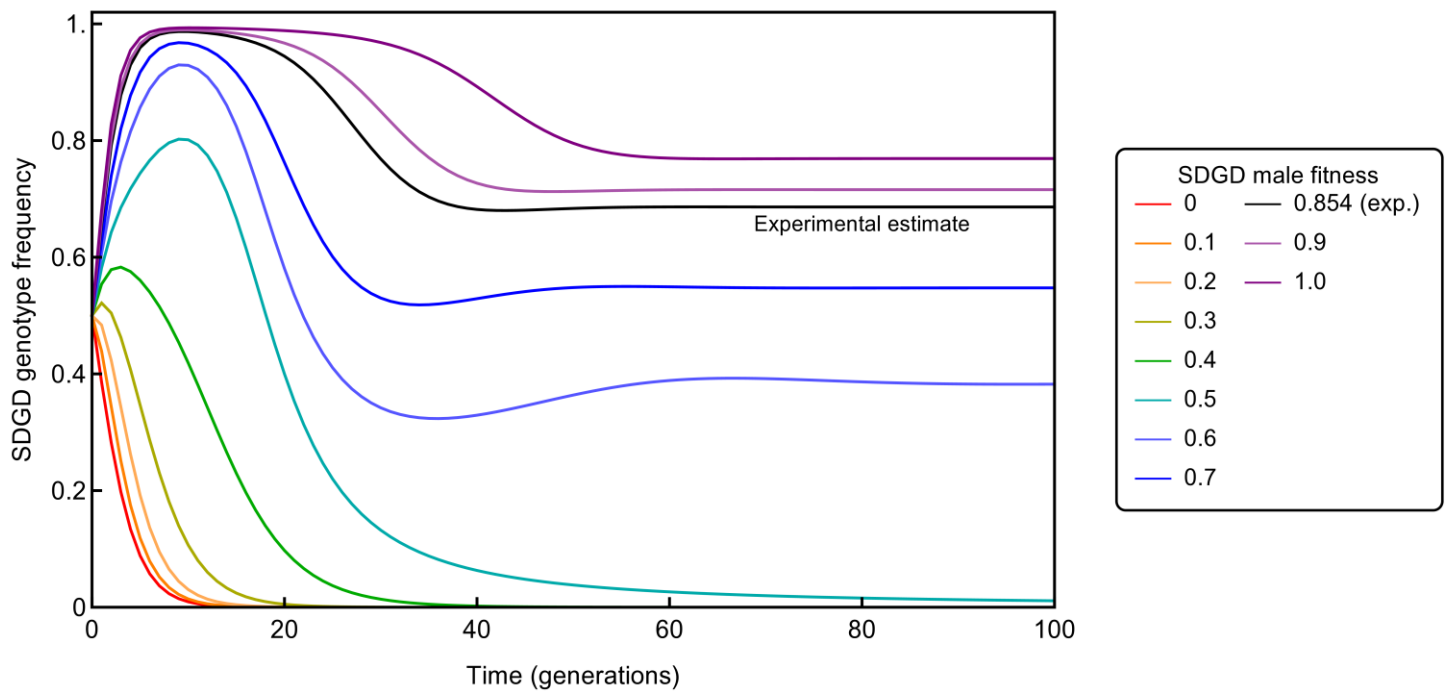
Male and female SDGD^{dsx} heterozygotes that had inherited a maternal or paternal copy of the SDGD^{dsx} allele were crossed to wild type counterparts and assessed for fecundity. The total larval output is plotted for individual females (dots). Red bars indicate the average and the mean count (± s.e.m.) is shown. Females inheriting the transgene from the mother (G1♀ → G2♀) have significantly lower larval progeny (* $P = 0.0256$, Kruskal-Wallis test) compared to wild-type control.



Supplementary Figure 8

Time dynamics of the frequency of $SDGD^{dsx}$ as function of female fitness

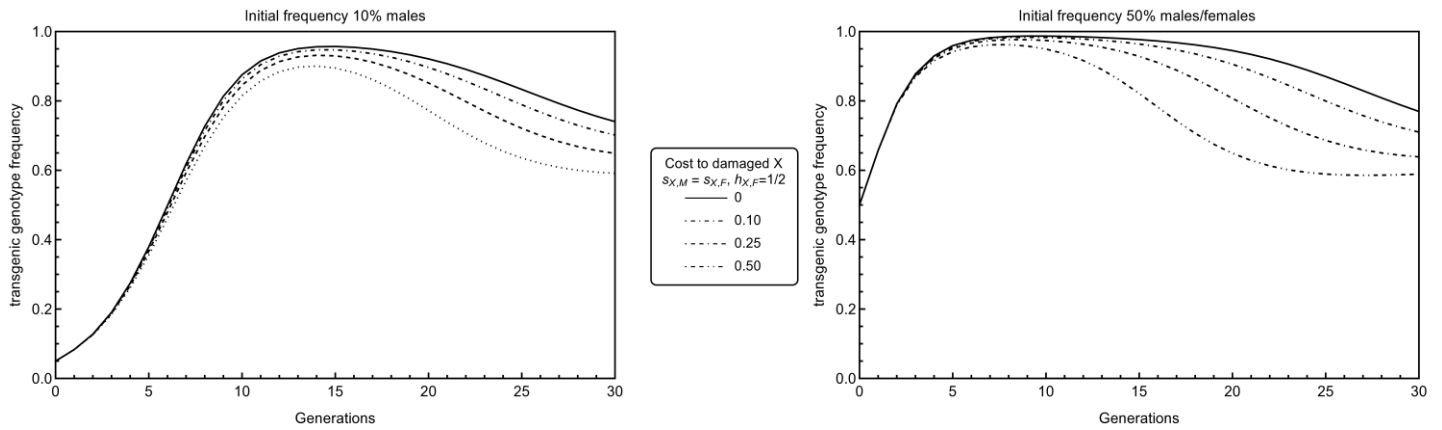
Time dynamics of the frequency of $SDGD^{dsx}$ transgenic individuals in the population as a function of W/D (where D represent the SDGD allele and W the wild-type allele) female fitness ($w_{WD,xx}$), as predicted by the deterministic discrete-generation model at 25% initial allelic frequency. The graph shows the frequency of SDGD heterozygote males and females as a proportion of the male (or female) population, with other parameter estimates and baseline values given in Supp Table 2 (SDGD male fitness = 0.854; m (sex distortion) = 0.93). The predicted outcome at high W/D female fitness is elimination of the population, and at lower fitness, an intermediate equilibrium with W, R and D alleles. The result (black line) for the experimental estimate for female SDGD heterozygote fitness, $w_{WD,xx} = 0.627$, is in a parameter region where even a small (positive) change leads to a prediction of population elimination instead of suppression.



Supplementary Figure 9

Time dynamics of the frequency of $SDGD^{dsx}$ as function of male fitness

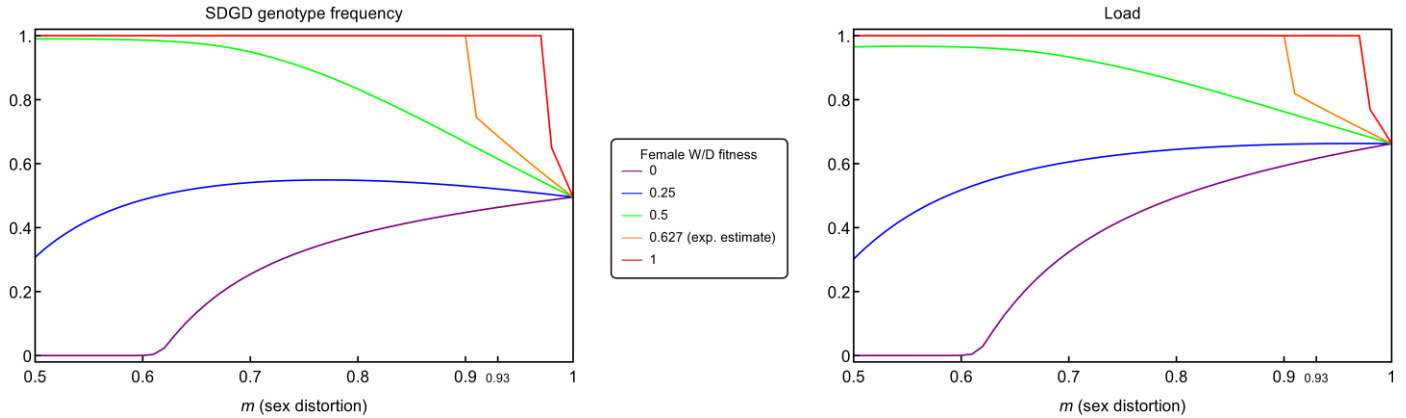
Time dynamics of the frequency of $SDGD^{dsx}$ transgenic individuals in the population as a function of the fitness of SDGD males (assume $w_{WD,XY} = w_{DR,XY} = w_{DD,XY}$) as predicted by the deterministic discrete-generation model. Initial release is 50% of SDGD heterozygote males and females as a proportion of the male (or female) population, with other parameter estimates and baseline values given in Supp Table 2 (W/D female fitness $w_{WD,XX} = 0.627$; $m = 0.93$). For low SDGD male fitness ($< \approx 0.5$), the construct is eventually lost.



Supplementary Figure 10

Impact of fitness due to damaged X chromosome.

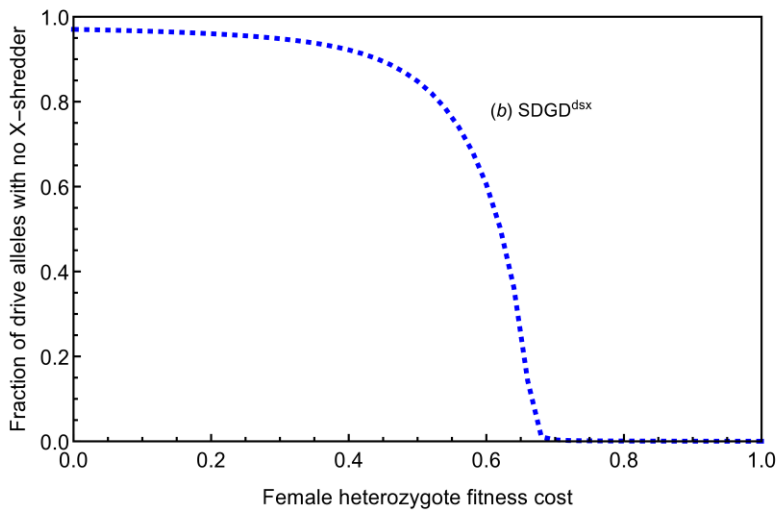
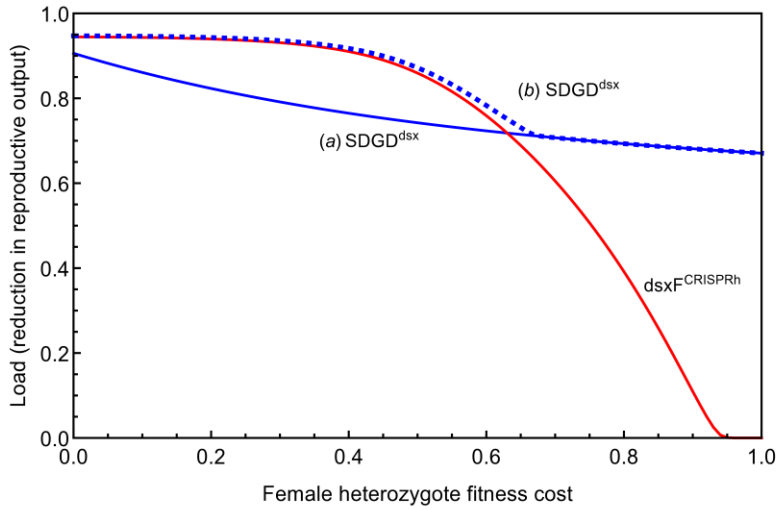
Modelling the impact of fitness reduction, due to the inheritance of damaged X chromosomes passed through X-shredding in a SDGD male, on the spread of the SDGD transgenics as predicted by the deterministic discrete-generation cage model. For simplicity, we assume that the additional cost to carrying one damaged X chromosome in males is the same as the cost in females that carry two copies of the damaged X ($s_{X,f} = s_{X,m}$), and females with one damaged X and one wildtype X chromosome have only half the fitness cost of females with two copies (dominance coefficient $h_{X,f} = 1/2$). Estimates used for other parameters given in Supplementary Table 2.



Supplementary Figure 11

Effect of female fitness on SDGD frequency and population load.

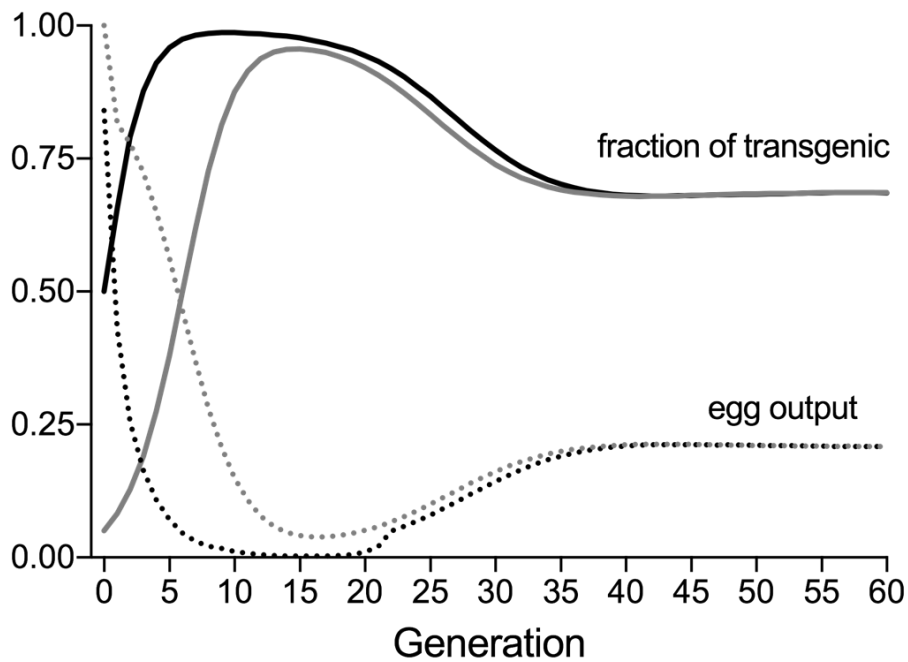
Effect of female W/D heterozygote fitness (D refers to the SDGD^{dsx} allele) on the SDGD genotype frequency (i.e., individuals with at least one copy of the SDGD) and load after 200 generations, as predicted by the deterministic discrete-generation model. Parameter estimates and baseline values given in Supp Table 2 (SDGD male fitness = 0.854). The possible outcomes (load = 1 and population elimination; intermediate equilibrium with W, D and R; or load = 0 and construct lost) depend upon the sex distortion m (0.5 [no sex bias] to 1 [only male progeny]) and the female W/D heterozygote fitness ($0 \leq w_{\text{WD},\text{xx}} \leq 1$). For low female fitness, adding an X-shredder (increasing sex distortion m) is predicted to beneficially increase the load on the population. For complete female heterozygous sterility (purple line), the drive construct disappears from the population if there is no sex distortion ($m = 1/2$), whereas sufficiently increasing the sex distortion allows the SDGD^{dsx} to spread and impose a load on the population. The presence of the X-shredder keeps the construct predominantly in males, therefore mitigating the heterozygous female sterility effect. By contrast if female fitness is sufficiently high (lines of fitness 0.5 or greater on plot), the load decreases at high sex distortion because due to male bias, SDGD^{dsx} males replace high-fitness W/D heterozygote females which decreases the ability of the construct to spread. For a complete male sex bias, $m = 1$, no W/D heterozygote females are created (and therefore no female/male SDGD^{dsx} homozygotes), since all X chromosomes are shredded and SDGD^{dsx} males have no female progeny; thus the load at $m = 1$ does not depend on the fitness of female W/D heterozygote individuals since none are present. Only SDGD^{dsx} heterozygous males can pass on the construct, with the SDGD^{dsx} allele present in reduced frequency in an intermediate equilibrium with wildtype and resistance alleles and a load less than one for these parameters. (In general, the amount of reduction in SDGD^{dsx} frequency and load at $m = 1$ will depend on parameters such as the homing rate, here less than 100%, the rate of resistant R mutation, and the relative fertility of the R and SDGD^{dsx} allele).



Supplementary Figure 12

Comparison of the predicted equilibrium for release of SDGD^{dsx} or dsxF^{CRISPRh} into a wild-type population

(Top panel) Comparison of the predicted equilibrium load (i.e., the reduction in reproductive output by the population after 400 generations) for release of SDGD^{dsx} or dsxF^{CRISPRh} into a wild-type population, varying the fitness cost to heterozygote W/D females. The discrete generation model predicts that the SDGD^{dsx} construct (blue solid line) is more robust to reductions in female heterozygote fitness compared to dsxF^{CRISPRh} (red line), still maintaining a substantial load even at 100% reduction in female heterozygote fitness (i.e. females heterozygous for the drive are completely non-viable). We also consider the possibility that the X-shredder component may be lost from the SDGD^{dsx} construct during homing (bottom panel, blue dashed line), such that out of the drive alleles transmitted from female or male W/D individuals, 0.01% will not have a functioning X-shredder component. For low to mid-fitness costs, the predicted load is similar to that of the dsxF^{CRISPRh} drive-only construct since the fraction of drive individuals without an intact X-shredder is high; for high fitness costs, the load merges with that of intact SDGD^{dsx} (blue solid line) since almost all drive individuals have an intact X-shredder. We use representative parameters for both constructs for comparison (drive transmission $a_f = a_m = 0.95$ for both males and females; rate of resistance $u_f = u_m = 0.5$; X-shredding parameter for SDGD^{dsx} is $m=0.95$; no reduction in fitness for heterozygote males).



Supplementary Figure 13

Time dynamics of the frequency of $SDGD^{dsx}$ transgenic individuals and relative egg output

Time dynamics of the frequency of $SDGD^{dsx}$ transgenic individuals (solid lines) and relative eggs output (dotted lines) in the population as predicted by the deterministic discrete-generation model using experimental parameters given in Supp Table 2 and assuming two initial releases of 50% of $SDGD$ heterozygote males and females (black line) or 10% $SDGD$ heterozygous males only (grey line). Independently of the release scenarios, for these parameters, the frequency of transgenic individuals reaches an intermediate equilibrium while W , R and D alleles and the egg output is reduced (population suppressed).

Supplementary Tables

Supplementary Table 1 | Summary of values (\pm s.e.m.) obtained from crossed of the different SDGD developed in this study.

Target gene	Promoter Cas9	Promoter I-Ppol	Transgenic parent	N	Average eggs per female	Hatched larvae	Hatching rate	Transmission rate (RFP+)	Fraction of Males
AGAP007280	vasa2	beta2	Male x wt	23	16.9 \pm 2.80	3.72 \pm 1.02	0.23	na	na
			Female x wt	2	61.3 \pm 42.12	2.45 \pm 1.63	0.07	na	na
AGAP011377	vasa2	beta2	Male x wt	35	124.9 \pm 8.18	97.5 \pm 8.37	0.78	0.79 \pm 0.17	0.92 \pm 0.08
			Female x wt	20	5.3 \pm 1.63	2.45 \pm 1.03	0.47	0.69 \pm 0.07	0.48 \pm 0.13
AGAP005958	vasa2	beta2	Male x wt	33	97.5 \pm 7.61	66.0 \pm 7.99	0.68	0.94 \pm 0.08	0.94 \pm 0.08
			Female x wt	32	6.42 \pm 1.41	0.1 \pm 0.05	0.014	na	na
AGAP004050 (dsx)	zpg	beta2 ²⁴⁴	Male x wt	71	130.2 \pm 7.72	126.7 \pm 6.39	0.84 \pm 0.02	0.96 \pm 0.01	0.93 \pm 0.08
			Female x wt	44	115.1 \pm 10.2	98.8 \pm 10.4	0.67 \pm 0.05	0.99 \pm 0.01	0.48 \pm 0.14

Supplementary Table 2 | Model parameters for Sex Distorter Gene Drive (SDGD^{dsx})

Parameter	Estimate	Method of estimation
Mating probability [†]	0.85	Estimated from Hammond et al. 2017; Kyros et al. 2018
Egg production, wildtype female crossed with wildtype male [†]	150	From assays of mated females
Egg production, W/D female crossed with wildtype male [†]	115 (relative to 150 for WT = 0.77)	From assays of mated females
Egg production, wildtype female crossed with W/D male [†]	132 (relative to 150 for WT = 0.88)	From assays of mated females
Hatching probability, wildtype [†]	0.88	From assays of mated females
Hatching probability, W/D heterozygous female crossed with wildtype male [†]	0.72 (relative to 0.88 for WT = 0.81)	From assays of mated females
Hatching probability, W/D heterozygous male crossed with wildtype female [†]	0.86 (relative to 0.88 for WT = 0.97)	From assays of mated females
Fertility of W/D female (relative to WT, for deterministic model), ($w_{WD,xx}$)	0.627	From assays of mated females
Fertility of W/D male (relative to WT, for deterministic model)*	0.8536	From assays of mated females
Additional cost to females due to carrying two damaged X-chromosomes ($s_{x,f}$)	0	Baseline
Dominance coefficient for damaged X in females ($h_{x,f}$)	½	Baseline
Additional cost to males due to carrying damaged X-chromosome ($s_{x,m}$)	0	Baseline
Transmission of drive by W/D heterozygous females, (d_f)	1	Observed fraction transgenic from assays
Transmission of drive by W/D heterozygous males, (d_m)	0.96	Observed fraction transgenic from assays
Male sex bias of progeny of W/D heterozygous males ($m = m_1 = m_2$)	0.93	Observed from assays
Meiotic EJ parameter (fraction non-drive alleles that are resistant) ($u = u_f = u_m$)	0.469	Estimated from Hammond et al. 2016; Kyros et al. 2018
Probability of emergence from pupa (survival from larva) [†]	0.89	Estimated from Kyros et al. 2018
Initial population (zero generation) [†]	50% release: 150 WT males/females, 150 W/D heterozygous males/females; 10% release: 270 WT males, 30 W/D heterozygous males, 300 WT females	Following cage experiments

[†] For stochastic model. Note values are averages if there are parental effects (in fitnesses, drive).

*This fertility is assumed in the model for all males with one or two copies of the SDGD allele ($w_{WD,XY}, w_{DD,XY}, w_{DR,XY}$).

Supplementary Table 3 | Analysis of SDGD^{dsx} females with and without a copy of “modified” X chromosome.

Cross	Eggs ¹	% eggs hatching ²	Transgenic rate ³	Male progeny (% of sum ± SD) ⁴	Male progeny (average of % ± SEM) ⁴	P value
X ^{mod} /X;SDGD ^{dsx} /+ ♀ x wild-type ♂	130.7 ± 15.2 (n=19)	69.8% n= 16 (2484)	100% (509)	47.1 ± 0.17 n=13 (1261)	0.41 ± 0.05, n=13 (1261)	n.s. (P= 0.242, t=1.203, df=21.09)
X/X;SDGD ^{dsx} /+ ♀ x wild-type ♂	103.2 ± 13.1 (n=25)	74.6% n=21 (2580)	100% (728)	50.5 ± 0.13 n=19 (1190)	0.48 ± 0.03, n=19 (1190)	

Average number of eggs laid by n number of females analysed (±SEM). (2) Average percentage of larvae hatching from the eggs, from n females analysed. (3) Average percentage of transgenic progeny (RFP positive) out of total larvae screened. (4) Average percentage of males in the progeny (±SD) established from n females. Significance (Welch's t-test, two-tailed, unpaired two-sample with unequal variance) was tested comparing logit transformed percentages observed from the progeny of n females of the two groups. The total number of eggs or individuals counted in each experiment is given in parentheses.

Supplementary Table 4 | The fitnesses of the 18 female genotypes and the proportion of each type of egg produced by them.

Female genotype	Fitness	Eggs produced					
		W, x	D, x	R, x	W, X	D, X	R, X
WW, xx	1	1	0	0	0	0	0
WD, xx	$w_{WD,xx}$	$(1 - d_f)(1 - u_f)$	d_f	$(1 - d_f)u_f$	0	0	0
WR, xx	$w_{WR,xx}$	1/2	0	1/2	0	0	0
DD, xx	$w_{DD,xx}$	0	1	0	0	0	0
DR, xx	$w_{DR,xx}$	0	1/2	1/2	0	0	0
RR, xx	$w_{RR,xx}$	0	0	1	0	0	0
WW, xX	$1 - h_{x,f}s_{x,f}$	1/2	0	0	1/2	0	0
WD, xX	$w_{WD,xx}(1 - h_{x,f}s_{x,f})$	$1/2(1 - d_f)(1 - u_f)$	$d_f/2$	$1/2(1 - d_f)u_f$	$1/2(1 - d_f)(1 - u_f)$	$d_f/2$	$1/2(1 - d_f)u_f$
WR, xX	$w_{WR,xx}(1 - h_{x,f}s_{x,f})$	1/4	0	1/4	1/4	0	1/4
DD, xX	$w_{DD,xx}(1 - h_{x,f}s_{x,f})$	0	1/2	0	0	1/2	0
DR, xX	$w_{DR,xx}(1 - h_{x,f}s_{x,f})$	0	1/4	1/4	0	1/4	1/4
RR, xX	$w_{RR,xx}(1 - h_{x,f}s_{x,f})$	0	0	1/2	0	0	1/2
WW, XX	$1 - s_{x,f}$	0	0	0	1	0	0
WD, XX	$w_{WD,xx}(1 - s_{x,f})$	0	0	0	$(1 - d_f)(1 - u_f)$	d_f	$(1 - d_f)u_f$
WR, XX	$w_{WR,xx}(1 - s_{x,f})$	0	0	0	1/2	0	1/2
DD, XX	$w_{DD,xx}(1 - s_{x,f})$	0	0	0	0	1	0
DR, XX	$w_{DR,xx}(1 - s_{x,f})$	0	0	0	0	1/2	1/2
RR, XX	$w_{RR,xx}(1 - s_{x,f})$	0	0	0	0	0	1

Supplementary Table 5 | The fitnesses of the 12 male genotypes and the proportion of each type of sperm produced by them.

Male genotype	Fitness	Sperm produced								
		W, x	R, x	W, X	D, X	R, X	W, Y	D, Y	R, Y	
WW, xY	1	1/2	0	0	0	0	1/2	0	0	
WD, xY	$w_{WD,xY}$	0	0	$(1 - m_1)(1 - d_m)(1 - u_m)$	$(1 - m_1)d_m$	$(1 - m_1)(1 - d_m)u_m$	$m_1(1 - d_m)(1 - u_m)$	$m_1 d_m$	$m_1(1 - d_m)u_m$	
WR, xY	$w_{WR,xY}$	1/4	1/4	0	0	0	1/4	0	1/4	
DD, xY	$w_{DD,xY}$	0	0	0	$(1 - m_2)$	0	0	m_2	0	
DR, xY	$w_{DR,xY}$	0	0	0	$(1 - m_1)/2$	$(1 - m_1)/2$	0	$m_1/2$	$m_1/2$	
RR, xY	$w_{RR,xY}$	0	1/2	0	0	0	0	0	1/2	
WW, XY	$1 - s_{X,m}$	0	0	1/2	0	0	1/2	0	0	
WD, XY	$w_{WD,xY}(1 - s_{X,m})$	0	0	$(1 - m_1)(1 - d_m)(1 - u_m)$	$(1 - m_1)d_m$	$(1 - m_1)(1 - d_m)u_m$	$m_1(1 - d_m)(1 - u_m)$	$m_1 d_m$	$m_1(1 - d_m)u_m$	
WR, XY	$w_{WR,xY}(1 - s_{X,m})$	0	0	1/4	0	1/4	1/4	0	1/4	
DD, XY	$w_{DD,xY}(1 - s_{X,m})$	0	0	0	$(1 - m_2)$	0	0	m_2	0	
DR, XY	$w_{DR,xY}(1 - s_{X,m})$	0	0	0	$(1 - m_1)/2$	$(1 - m_1)/2$	0	$m_1/2$	$m_1/2$	
RR, XY	$w_{RR,xY}(1 - s_{X,m})$	0	0	0	0	1/2	0	0	1/2	

Supplementary Note

Full sequence of following vectors (in GenBank format)

<i>pB\</i> (b2-mCherry)-attB-dsRFP-244beta2-GFP vector.....	6
<i>pB\</i> (b2-mCherry)-attB-dsRFP-271beta2-GFP.....	11
<i>pB\</i> (b2-mCherry)-attB-dsRFP-355beta2-GFP.....	16
<i>pB\</i> (b2-mCherry)-attB-dsRFP-WTbeta2-GFP_v2.....	20

***pB*(b2-mCherry)-attB-dsRFP-244beta2-GFP vector**

```
LOCUS          pB\ (b2-mCherry)-attB-dsRFP-244beta2-GFP          7976 bp    DNA    circular UNA 05-APR-
2017
DEFINITION    Mars-244.
ACCESSION    urn:local...z-781tst0
VERSION      urn:local...z-781tst0
KEYWORDS     .
SOURCE
  ORGANISM   .
FEATURES             Location/Qualifiers
   rep_origin        21..327
                     /label="f1_origin"
   CDS                complement(471..614)
                     /modified_by="User"
                     /label="lacZ_a"
   promoter          600..616
                     /label="M13_forward20_primer"
   promoter          626..644
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ORIGIN

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pB\ (b2-mCherry)-attB-dsRFP-271beta2-GFP

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2017
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VERSION    urn.local...10-781tst0
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SOURCE     .
ORGANISM   .
FEATURES   Location/Qualifiers
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    promoter         626..644
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pB\ (b2-mCherry) -attB-dsRFP-355beta2-GFP

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