Electronic supplementary material

Title: Meiotic drive reduces egg-to-adult viability in stalk-eyed flies

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Suppmentary Methods - DNA Extraction and Genotyping Protocol

DNA was extracted by isopropanol precipitation in 96-well plates. Half a fly thorax was added to a well containing 4ul Proteinase K (10 mg.ml-1) and 100ul DIGSOL (25mM NaCl, 1mM EDTA, 10mM Tris–Cl pH 8.2), mechanically lysed, and incubated overnight at 55C. The following day, 35ul of 4M ammonium acetate was added and plates were left on ice for 5 minutes before being centrifuged at 4500RPM at 4C for 40 minutes. 80ul of supernatant was then aspirated into a new 96-well plate containing 80ul of isopropanol. The precipitate was discarded. Samples were then centrifuged again at 4500RPM and 4C for 40 minutes to precipitate the DNA. The supernatant was then discarded, 100ul 70% ethanol was added, and samples were spun again at 4500RPM and 4C for 20 minutes. The supernatant was once again discarded and plates were left to air-dry for 45 minutes at room temperature. Finally, 30ul of Low TE (1mM Tris-HCL pH8, 0.1mM EDTA) was added to elute the DNA. DNA was PCR-amplified in 96-well plates, with each well containing 1ul of dried DNA, 1ul of primer mix (consisting of the forward and reverse primers of comp162710 at a concentration of 0.2uM) and 1ul of QIAGEN Multiplex PCR Mastermix (Qiagen). The length of amplified fragments was determined by gel electrophoresis. A 3% agarose gel was made using 3g of molecular grade agarose, 100ml of 0.5x TBE buffer (45mM Tris (pH 7.6), 45mM boric acid, 1mM EDTA), and 4ul ethidium bromide. PCR products were diluted with 3ul ultrapure water and 2ul of gel loading dye was added. 4ul of this mixture was loaded into each well and assessed for size against a ladder made from the PCR-amplified DNA of multiple heterozygous drive females. comp162710 is an indel marker with small alleles (201bp) indicating the presence of the drive chromosome and large alleles (286bp) indicating the presence of the standard chromosome (GS Wilkinson, personal communication; Meade et al. 2019).

Model outputs

Supplementary table S1

The effect of food condition on egg-to-adult viability in males:

```
m1 <- lmer(data=Male_Survival, formula = W ~ Genotype*Condition +
             (1|Cage_ID) + (1|Collection_Date))
```

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	0.3828775	0.0545171	55.57003	7.0230708	0.0000000
GenotypeXY	0.1790490	0.0654798	79.00000	2.7344174	0.0077113
ConditionL	0.0769641	0.0720155	147.04295	1.0687149	0.2869495
ConditionM	0.0308253	0.0730934	148.96913	0.4217254	0.6738334
GenotypeXY:ConditionL	-0.1157585	0.0969522	79.00000	-1.1939743	0.2360609
GenotypeXY:ConditionM	-0.0157272	0.0980011	79.00000	-0.1604799	0.8729127

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Genotype	0.7431435	0.7431435	1	79.00000	11.1821885	0.0012649
Condition	0.0144249	0.0072124	2	72.97766	0.1085266	0.8972995
Genotype:Condition	0.1066840	0.0533420	2	79.00000	0.8026450	0.4517624

Supplementary table S2

The effect of food condition on egg-to-adult viability in females:

```
m1 <- lmer(data=Female_Survival, formula = W ~ Genotype*Condition +
             (1|Cage_ID) + (1|Collection_Date))
```

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	0.4577565	0.0710439	127.3557	6.4432926	0.0000000
GenotypeSRX	0.0785903	0.0942983	116.0000	0.8334220	0.4063195
GenotypeXX	0.2052136	0.0942983	116.0000	2.1762178	0.0315662
ConditionL	0.0185047	0.0972369	165.6508	0.1903051	0.8493031
ConditionM	0.0041773	0.0984081	165.5148	0.0424482	0.9661925
GenotypeSRX:ConditionL	-0.0260082	0.1317608	116.0000	-0.1973899	0.8438679
GenotypeXX:ConditionL	-0.0958206	0.1317608	116.0000	-0.7272316	0.4685493
GenotypeSRX:ConditionM	0.0442427	0.1333579	116.0000	0.3317589	0.7406700
GenotypeXX:ConditionM	-0.0240328	0.1333579	116.0000	-0.1802124	0.8573003

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Genotype	0.8327350	0.4163675	2	116.00000	4.6824068	0.0110758
Condition	0.0275940	0.0137970	2	53.53907	0.1551592	0.8566625
Genotype:Condition	0.0727005	0.0181751	4	116.00000	0.2043948	0.9355153

Supplementary table S3

As food condition did not affect egg-to-adult viability, condition was removed from subsequent analysis. Below are the full model results from linear mixed effect models examining the effect of genotype on egg-to-adult

viability.

The effect of genotype on egg-to-adult viability in males:

```
m1 <- lmer(data=Male_Survival, formula = W ~ Genotype +
             (1|Cage_ID) + (1|Collection_Date))
```

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	0.4167260	0.0390008	16.94126	10.685053	0.0000000
GenotypeXY	0.1375502	0.0401625	81.00000	3.424845	0.0009681

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Genotype	0.7757225	0.7757225	1	81	11.72957	0.0009681

Supplementary table S4

The effect of genotype of egg-to-adult viability in females:

```
m1 <- lmer(data=Female_Survival, formula = W ~ Genotype +
             (1|Cage_ID) + (1|Collection_Date))
```

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	0.4654582	0.0424106	29.18295	10.975046	0.0000000
GenotypeSRX	0.0841424	0.0532743	120.00000	1.579420	0.1168722
GenotypeXX	0.1643466	0.0532743	120.00000	3.084916	0.0025278

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Genotype	0.8239569	0.4119784	2	120	4.759265	0.0102556

Supplementary table S5

The viability of both male genotypes was estimated directly from the model output of the more simplified linear model below.

```
m1 <- lm(data=Male_Survival, formula = W ~ Genotype)
```

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.4063265	0.0307031	13.234068	0.0000000
GenotypeXY	0.1375502	0.0434207	3.167849	0.0018358

Here the X^{SR}/Y genotype is used as the comparison, so its egg-to-adult viability is the model intercept term, 0.40633. The viability of X^{ST}/Y (labelled as simply GenotypeXY in the model), is calculated by adding the intercept term and the effect term together: 0.40633 + 0.13755 = 0.54388.

Supplementary table S6

The viability of each female genotype was estimated in the same way as above:

```
m1 <- lm(data=Female_Survival, formula = W ~ Genotype)
```

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	0.4649979	0.0395727	11.750485	0.0000000
GenotypeSRX	0.0841424	0.0559642	1.503505	0.1344614
GenotypeXX	0.1643466	0.0559642	2.936639	0.0037515

Supplementary table S7

To determine if the three female genotypes had significantly different viabilities, we used a Tukey's post-hoc comparison test:

	diff	lwr	upr	p adj
SRX-SRSR	0.0841424	-0.0481157	0.2164006	0.2916928
XX-SRSR	0.1643466	0.0320885	0.2966048	0.0104345
XX-SRX	0.0802042	-0.0520539	0.2124623	0.3260922

Fertility trial - Supplementary table S8

Below are the results of a trial designed to test the fertility of eggs laid by X^{SR}/X^{ST} females crossed to X^{SR}/Y (Cross A) and X^{ST}/Y (Cross B) males. One day old eggs were collected and counted, then allowed to develop for a further five days. After five days of development, the vast majority of fertilised eggs have hatched, and the remainder of show clear signs of development (eg segmental striations, darker colouration, development of mouthparts, etc.). At this time, the number of hatched/fertilised eggs were counted, along with the number of unfertilised eggs. In this trial, eggs were not inspected for signs of development before they were collected, and yet fertility remains high. There is no obvious difference in the fertility of Cross A and Cross B.

Date	Cross	Pot.ID	Total.eggs	Unfert	Fert	Percent.Fert
15-Nov	A	A1	12	3	9	0.7500000
15-Nov	A	A2	131	12	119	0.9083969
15-Nov	A	A3	76	6	70	0.9210526
15-Nov	B	B1	81	8	73	0.9012346
15-Nov	B	B2	67	6	61	0.9104478
15-Nov	B	B3	40	4	36	0.9000000
21-Nov	A	A1	43	4	39	0.9069767
21-Nov	A	A2	89	4	85	0.9550562
21-Nov	A	A3	76	3	73	0.9605263
21-Nov	B	B1	85	8	77	0.9058824
21-Nov	B	B2	105	8	97	0.9238095
21-Nov	B	B3	34	3	31	0.9117647
23-Nov	A	A1	90	0	90	1.0000000
23-Nov	A	A2	69	3	66	0.9565217
23-Nov	A	A3	43	3	40	0.9302326
23-Nov	B	B1	57	4	53	0.9298246
23-Nov	B	B2	49	0	49	1.0000000
23-Nov	B	B3	42	0	42	1.0000000
17-Dec	A	A1	59	2	57	0.9661017
17-Dec	A	A2	69	2	67	0.9710145
17-Dec	A	A3	35	0	35	1.0000000
17-Dec	B	B1	84	0	84	1.0000000
17-Dec	B	B2	58	1	57	0.9827586
17-Dec	B	B3	52	3	49	0.9423077
19-Dec	A	A1	47	0	47	1.0000000
19-Dec	A	A2	134	4	130	0.9701493
19-Dec	A	A3	13	2	11	0.8461538
19-Dec	B	B1	99	8	91	0.9191919
19-Dec	B	B2	29	3	26	0.8965517
19-Dec	B	B3	34	0	34	1.0000000

Cross	Total.eggs	Total.Unfertilised	Fertility
A	986	48	0.9513185
B	916	56	0.9388646

Data accessibility

Raw and processed data are available on the Dryad Digital Repository: doi:10.5061/dryad.kc49jk1