Experimental enforcement of sex-limited autosome inheritance does not reveal intralocus sexual conflict

Supplementary material

Thomas Keaney, Heidi Wong, Theresa Jones and Luke Holman

Click **here** to view the HTML report, which serves as online supplementary material for the associated manuscript (*insert DOI*), in review at *Insert Journal*. The report inlcudes the supplementary methods, documents our empirical analysis (contains raw data and R-script) and provides all supplementary figures and tables.

In an attempt to future proof the availability of our supplementary material, we also include the supplementary methods, Table S1-XX and Figures S1 in this document. Additionally, our raw data is deposited in the Dryad database **update when appicable**.

Supplementary methods

LH_M culturing

We maintained this LH_M stock in our laboratory for 32 generations prior to creating the genotypes required for experimental evolution. We cultured LH_M at 25C, with a 16-8 light-dark cycle and reared in vials (95mm x 25mm) on a corn-meal, yeast and dextrose-based diet (recipe in Table S1; ~8cm3 of food medium per vial) supplemented with dried yeast, at a population size of at least 800 breeding individuals across 25 vials (16 flies of each sex per vial, following Rice *et al.* 2005). Each generation begins by pooling the offspring produced across the 25 vials and randomly assorting 16 female-male pairs to 25 new vials. We then allow these breeding individuals 48 hours to interact and mate, before transferring them to another set of new vials. After 24 hours of egg-laying, we discard all adults, and allow juveniles 12 days to compete for resources, pupate and eclose as adults. We then iteratively repeat this process each generation to maintain the population.

Creating the genotypes used for experimental evolution

We performed the following crosses on mass to preserve genetic variation in our evolving population. **XX individuals were used...**

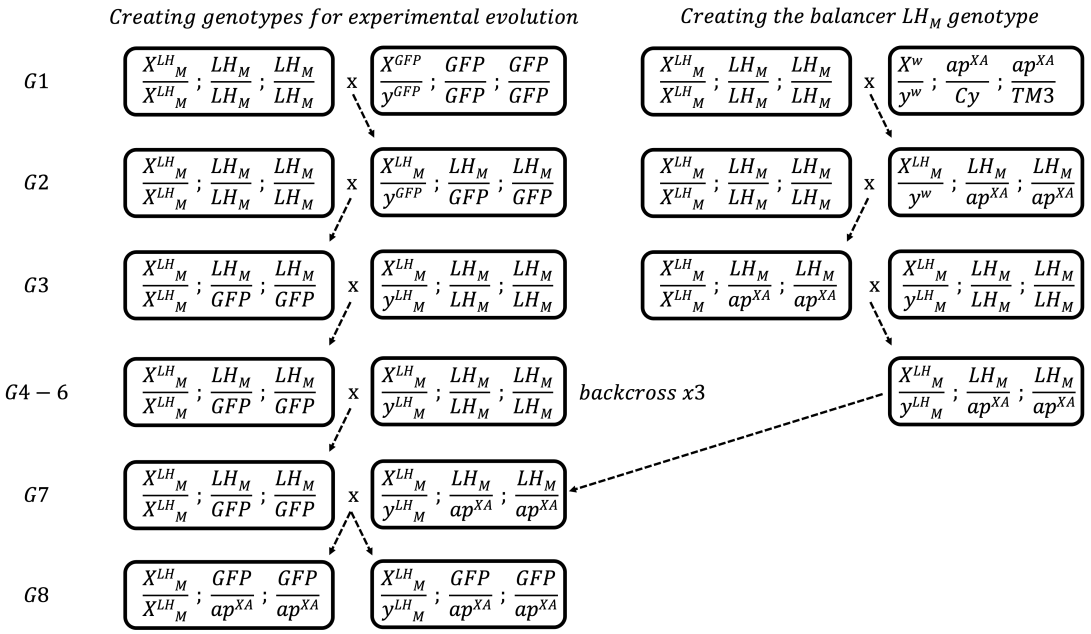

Figure S1. Crossing scheme used to integrate the GFP constructs and ap^{XA} marked translocated second and third chromosome balancers into the LH_M genetic background. We replicated the crosses 12 times (for XX individuals) to supply the flies used in generation zero of experimental evolution; 6 times using the *Ubi* GFP construct and 6 times with the *3xP* construct. G = generation.

Table S1. Recipe for food medium used in our experiment. The provided quantities make ~ 1 litre of food.

Ingredients	Quantity
Soy flour	20 g
Cornmeal	73 g
Yeast	35 g
Dextrose	75 g
Agar	6 g
Water	1000 mL
Tegosept	17 mL
Acid mix (4 mL orthophosphoric acid, 41 mL propionic acid, 55 mL water to make 100 mL)	14 mL

Table S2. Estimated female fitness for flies carrying autosomes derived from each of the three inheritance regimes.

Inheritance treatment	Estimated prop. of offspring produced	2.5%	97.5%
Control	0.752	0.715	0.786
Female-limited	0.782	0.747	0.812
Male-limited	0.785	0.753	0.814

Table S3. Differences in female fitness between each of the three inheritance regimes.

Contrast	Diff in offspring produced per 100	2.5%	97.5%
Male inherited - Control	3.28	-0.33	6.88
Female inherited - Control	2.99	-0.87	6.73
Female inherited - Male inherited	-0.29	-3.8	3.06

Table S4. The effects of the fixed predictor variables on female fitness that are not directly related to intralocus sexual conflict. Female fitness measured in Block 1 was higher than that measured in Blocks 2 and 3. Females carrying autosomes marked with 3xP GFP had higher fitness than those expressing UBI GFP.

Contrast	Diff in offspring produced per 100	2.5%	97.5%
Block 1 - Block 2	13.58	10.02	17.1
Block 1 - Block 3	13.26	10.21	16.37
3xP - UBI	6.46	3.06	9.83

Table S5. Estimated male fitness for flies carrying autosomes derived from each of the three inheritance regimes.

Inheritance treatment	Estimated prop. of offspring sired	2.5%	97.5%
Control	0.68	0.574	0.772
Female-limited	0.762	0.662	0.841
Male-limited	0.702	0.594	0.794

Table S6. Differences in male fitness between each of the three inheritance regimes.

Contrast	Diff in offspring produced per 100	2.5%	97.5%
Female inherited - Control	8.2	-2.6	18.61
Male inherited - Control	2.2	-9.15	13.3
Female inherited - Male inherited	6.01	-4.48	16.79

Table S7. The effects of the fixed predictor variables on male fitness that are not directly related to intralocus sexual conflict. Male fitness measured in Block 2 was higher than that measured in Blocks 1 and 3. There was no effect of GFP transgene on male fitness.

Contrast	Diff in offspring produced per 100	2.5%	97.5%
Block 1 - Block 2	-16.71	-23.09	-10.79
Block 1 - Block 3	0.74	-5.37	6.61
3xP - UBI	7.6	-2.63	17.84