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

Supplementary material

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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-S7 and Figure 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.

The recombination compartment

The female recombination compartment

When we initiated the first generation of experimental evolution for each of the sex-limited populations, we placed 12 females with the *FLA/FLA* genotype into individual food vials, each containing a FLA/ap^{XA} male. We allowed them ~24 hours to mate, then discarded the males and pooled the 12 females into a single vial. After ~72 hours we discarded the females and allowed their offspring to develop. The aim of this breeding design was to minimise selection acting on the FLA/ap^{XA} males, since each male simply needed to survive and then fertilise one randomly assigned virgin female. From the progeny, we collected 24 virgin female offspring with the genotype *FLA/FLA*, where recombination had occurred between the homologous *FLA* chromosomes. Half of these females were used to establish the next iteration of the recombination compartment, where they were again individually mated to 12 FLA/ap^{XA} males sourced from the progeny

of the main population. The 12 remaining *FLA/FLA* females, which carried one set of recombined *FLA* autosomes inherited from their mother, were randomly substituted for 12 females in the main breeding population. Therefore, 6% of the females in each generation came from the recombination compartment (Figure 1).

The male recombination compartment

At the start of the first generation for each sex-limited population, we placed 12 females with genotype MLA/ap^{XA} into a single food vial along with 12 MLA/ap^{XA} males. We allowed ~24 hours for the males to compete for fertilisations, after which we discarded the males and moved the females into individual food vials to oviposit. We collected one female and one male *MLA/MLA* offspring from each of the oviposition vials. This rearing protocol minimised selection on females, since females were reared mostly in a competition- and harassment-free environment, and we equalised fitness by collecting a standard number of progeny from each female. The 12 males were randomly substituted for 12 males in the main breeding population breeding population (carrying recombined *MLA* autosomes inherited from their mother), and the 12 females were used in the next iteration of the male recombination compartment. Therefore, 6% of the males in each generation came from the recombination compartment (Figure 1).

We also included a female and male recombination compartment for each of the four control populations. The process was identical as for the sex-limited populations, except that control autosomes can be substituted in the above descriptions for female- or male-limited autosomes.

Creating populations homozygous for sex-limited autosomes

After 20 generations of experimental evolution, the Covid-19 pandemic necessitated that we cease regular laboratory work. In order to preserve the genetic composition of the evolved populations in a form that did not require intensive maintenance, we conducted crosses which converted our 8 replicate populations into 12 populations carrying only one chromosome type (*FLA*, *MLA*, or *CA*). To create the 4 *FLA* populations, we crossed 200 male and 200 female FLA/ap^{XA} individuals from each of the 4 sex-limited populations, producing FLA/ap^{XA} and *FLA/FLA* offspring. These offspring were tipped into new food vials and allowed to breed freely, and a sample of the resulting population was transferred to new food vials every two weeks (at a population size of 13 vials each containing 8 females and 8 males, keeping the population size of chromosomes the same – offspring were pooled across the vials when setting up the next generation). We similarly created and propagated four populations carrying *MLA* and four carrying *CA* alleles. The ap^{XA} balancer chromosomes are deleterious or lethal, and so the balancer was rapidly lost from all 12 populations. We maintained each line under these conditions for 16 generations (i.e. until the easing of Covid-19 lockdown restrictions in Melbourne) at 18 C and a further four generations at 25 C in preparation for the start of our fitness assays. We believe that this period of relaxed selection between the experimental evolution phase and our fitness assays should make it more difficult to detect a fitness difference between the three chromosome types, but could not create any spurious differences between them since all three types were handled similarly.

Creating the genotypes used for experimental evolution

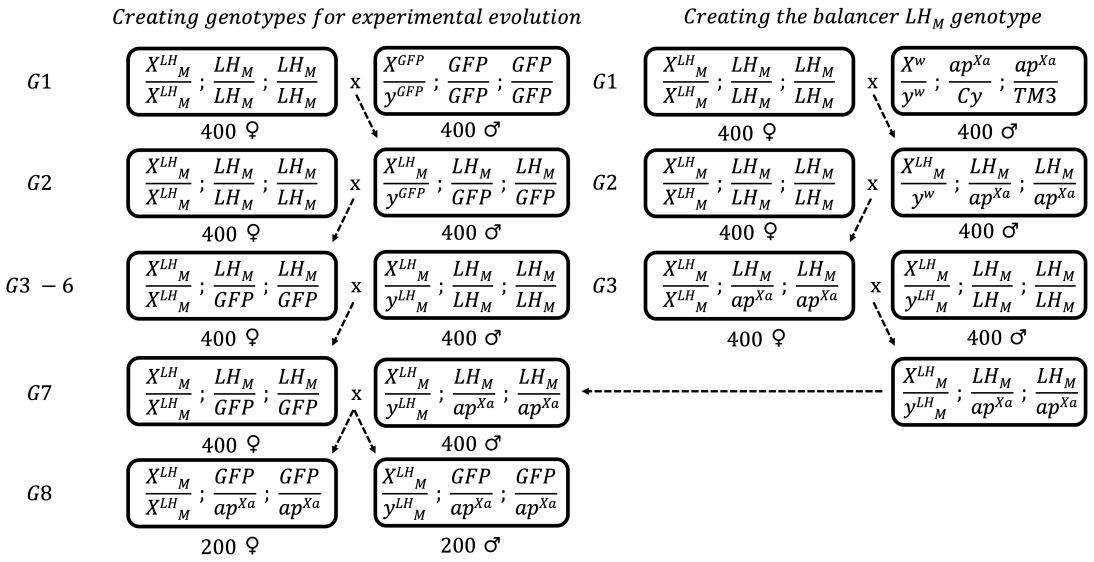

Figure S1. Crossing scheme used to integrate the GFP constructs and ap^{XA} marked translocated second and third chromosome balancers into the LH_M genetic background. We replicated the crosses 12 times to supply the flies used in generation zero of experimental evolution; 6 times using the *Ubi* GFP construct and 6 times with the *3xP* GFP construct. We performed each cross across 25 vials, with 16 females and 16 males in each, to preserve genetic variation in our evolving populations. 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	0.785
Female-limited	0.783	0.749	0.814
Male-limited	0.785	0.752	0.815

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	3.32	-0.25	7.11
Female inherited - Control	3.09	-0.56	6.84
Female inherited - Male inherited	-0.23	-3.7	3.2

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	13.59	10.1	17.08
Block 1 - Block 3	13.27	10.35	16.32
3xP - UBI	6.48	3.11	9.84

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	0.679	0.572	0.771
Female-limited	0.763	0.67	0.84
Male-limited	0.702	0.598	0.792

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	8.33	-2.44	19.22
Male inherited - Control	2.26	-9.22	13.44
Female inherited - Male inherited	6.08	-4.42	16.8

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	-16.76	-23.17	-10.91
Block 1 - Block 3	0.75	-5.25	6.8
3xP - UBI	7.62	-2.31	17.49