Experimental sex-limited evolution in *Drosophila*: No evidence for intralocus sexual conflict

Supplementary material

Click **here** to view the HTML report, which serves as online supplementary material for the associated manuscript (*insert DOI*), in review at *American Naturalist*. 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.

Creating the genotypes used for experimental evolution

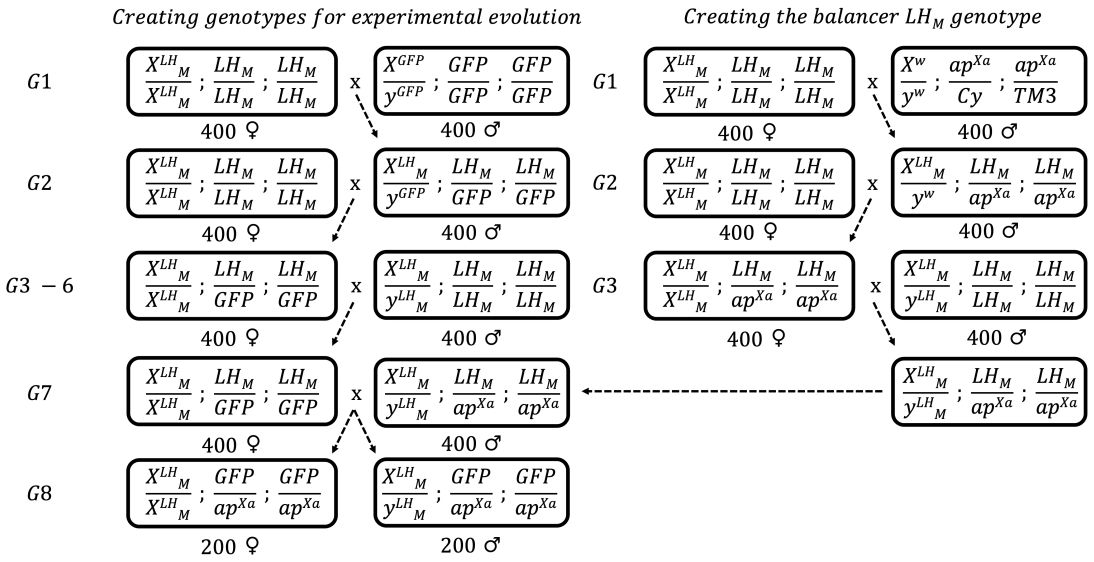

Figure S1. Crossing scheme used to integrate the GFP constructs and ap^{XA} marked translocated second and third chromosome balancers into the LH_M genetic background. We replicated the crosses 12 times to supply the flies used in generation zero of experimental evolution; 6 times using the *Ubi* GFP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

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.

Modelling the effect of the relaxed selection phase

Relaxation of sex-limited inheritance may have allowed sexually antagonistic allele frequencies to rebound back to their original pre-treatment equilibria, nullifying any effect of our experiment. To assess the effect of the relaxed selection phase on our experiment, we built a single-locus, two-allele population genetic model. The model contains three phases: in phase one, we recapitulated prior theory to find the conditions where intralocus sexual conflict is expected to maintain polymorphism. These conditions provide a baseline expectation for the characteristics of the alleles targeted by our expeirmental evolution treatments. Then, in phase two, we modelled how allele frequencies are expected to change under 20 generations of sex-limited evolution. Finally, in phase three, we modelled rebound change in allele frequency when sex-limited evolution was relaxed.

In each phase, the model follows the dynamics of a single autosomal locus with two alleles, A_f and A_m, which have frequencies p and 1 - p, respectively. For simplicity, we assume an infinite population size, discrete, non-overlapping generations and panmixia with respect to the focal locus. Following previous models of sexual antagonism (Kidwell et al, 1977; Rice, 1984; Connallon *et al*, 2009; Patten, *et al*, 2010), we create intralocus sexual conflict by coding the A_f allele to have greater fitness than the A_m allele when expressed in females, while the A_m allele has greater fitness than the A_f allele when expressed in males. The fitness benefits of being homozygous for the 'correct' allele in the 'correct' sex are represented by s_f and s_m (previous models have generally considered the cost of being homozygous for the wrong allele; we make this small change to improve the conceptual flow between the three phases of our model). In heterozygotes, these benefits are moderated by the sex-specific dominance coefficients, h_f and h_m (following Curtsinger *et al* 1994). When $h_f = 0$, the A_f allele is fully recessive when expressed in females, and fully dominant when $h_f = 1$. Modelling

Table S2. Effects of genotype on sex-specific fitness

	Genotypes		
Sex	A_fA_f	A_fA_m	A_mA_m
Female	$1+s_f$	$1+s_fh_f$	1
Male	1	$1+s_mh_m$	$1+s_m$

sex-specific dominance allows us to explore dominance reversals, where the A_f allele is dominant in one sex but recessive in the other, a mechanism that has previously been shown to facilitate balancing selection (reviewed in Connallon and Chenoweth, 2019). Table S2 shows the sex-specific fitness of each genotype.

Phase one

To find regions of stable polymorphism created by intralocus sexual conflict, we first find the within generation change in allele frequency following selection. For females the recursion equation is

$$p'_f = \frac{p^2(1+s_f) + \frac{1}{2}[2p(1-p)](1+s_fh_f)}{p^2(1+s_f) + [2p(1-p)](1+s_fh_f) + (1-p)^2} \tag{1}$$

and for males

$$p'_m = \frac{p^2 + \frac{1}{2}[2p(1-p)](1+s_mh_m)}{p^2 + [2p(1-p)](1+s_mh_m) + (1-p)^2(1+s_m)} \tag{2}$$

Assuming an even primary sex ratio and mendelian inheritance, the A_f allele frequency in the following generation is

$$p = \frac{p'_f + p'_m}{2} \tag{3}$$

We then used equation 3 to numerically determine the selective conditions required for polymorphism at this locus. We explored a parameter space of varying values of s_f, s_m, h_f and h_m, as these parameters have previously been found to be important factors in determining polymorphism (Kidwell *et al*, 1977; Curtsinger *et al*, 1994). We set the initial frequency of the A_f allele to 0.01 and iterated until 10000 generations had elapsed.

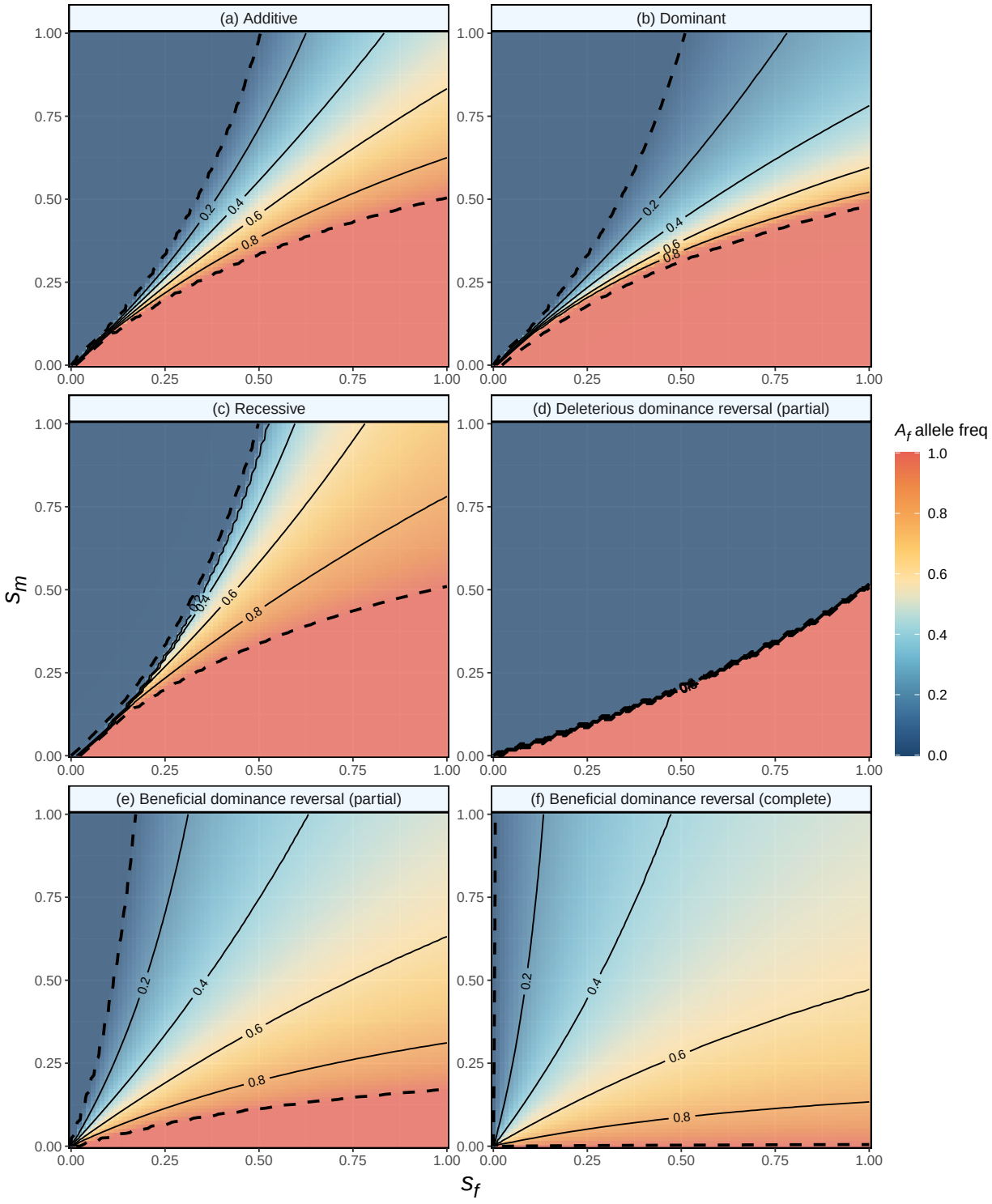

Figure S2: Predicted equilibrium frequency of the A_f allele, calculated from the population genetic model. The plot shows how varying the strength of the selection coefficient in females (s_f) and males (s_m) affects the fate of the A_f allele. The dashed lines enclose the parameter space where the A_f allele is predicted to stabilise at an intermediate frequency. Areas above and to the left of the upper dashed line indicate conditions where the allele is purged from the population, and areas below and to the right of the lower line indicate conditions where the allele spreads to fixation. Six dominance conditions are plotted. **a** additive

expression, where $h_f = h_m = 0.5$. **b** dominant expression of the A_f allele, where $h_f = 1$ and $h_m = 0$. **c** recessive expression of the A_f allele, where $h_f = 0$ and $h_m = 1$. **d** partial deleterious dominance reversal, where the A_f allele is recessive in females ($h_f = 0.25$) and dominant in males ($h_m = 0.25$, where this is the dominance of the A_m allele). **e** and **f** show cases of beneficial dominance reversal, where the A_f allele is dominant when expressed in a female and recessive when expressed in a male. **e** shows an intermediate case where dominance is incomplete $h_f = 0.75$ and $h_m = 0.75$, while **f** depicts the parameter space for polymorphism when beneficial dominance reversal is complete ($h_f = 1$ and $h_m = 1$).

As found previously, the conditions for polymorphism are very restrictive when selection is weak, but expand significantly when selection is strong and/or there is beneficial reversal of dominance i.e. when the A_f allele is (partially) dominant in females and (partially) recessive in males. Panel **d** shows why deleterious dominance reversal is not expected to be commonly observed in the genome. To contextualise s_f and s_m, a value of 0.5 indicates that the focal allele is 50% fitter than its alternative in that particular environment, while a value of 1 indicates it has twice the fitness of the alternative.

Phase two

To predict the effect of sex-limited experimental evolution, we simulate the evolution of a female-beneficial, sexually antagonistic allele over 20 generations of female-limited inheritance. Our synthetic sex-limited inheritance regimes create haploid populations of autosomes, that are nearly always (see recombination compartment) expressed as heterozygotes with non-evolving, single genotype populations of balancer homologs, with which they cannot recombine. We therefore model the locus as haploid, with the exception that dominance relations can affect the strength of selection. The extent to which dominance matters is contingent upon whether the A_f allele is present at the locus on the non-evolving balancer. If so, the A_f allele will always be expressed, if not, dominance relations matter. We model the homozygous and heterozygous cases using two related equations.

To model evolution under female-limited inheritance when the evolving and non-evolving allele are homozygous, we use equation 1 presented in Dapper *et al* (2023). The A_f allele experiences conditions akin to a maternally inherited cytoplasmic allele. The change in allele frequency due to selection per generation is now

$$\Delta p = \frac{s_f pq}{W_{f-limited}} \tag{4}$$

where $W_{f-limited} = 1 + s_f p$.

We then model the effect of female-limited inheritance when the balancer does not carry the A_f allele. The new equation takes the form

$$\Delta p = \frac{s_f h_f pq}{W_{f-limited}} \tag{5}$$

where $W_{f-limited} = 1 + s_f h_f p$.

Equation 5 helps point out that our design targets only those alleles that are expressed against the balancer genetic background. We therefore expect no response to selection for fully recessive alleles heterozygous with alternative alleles carried by the balancer chromosomes.

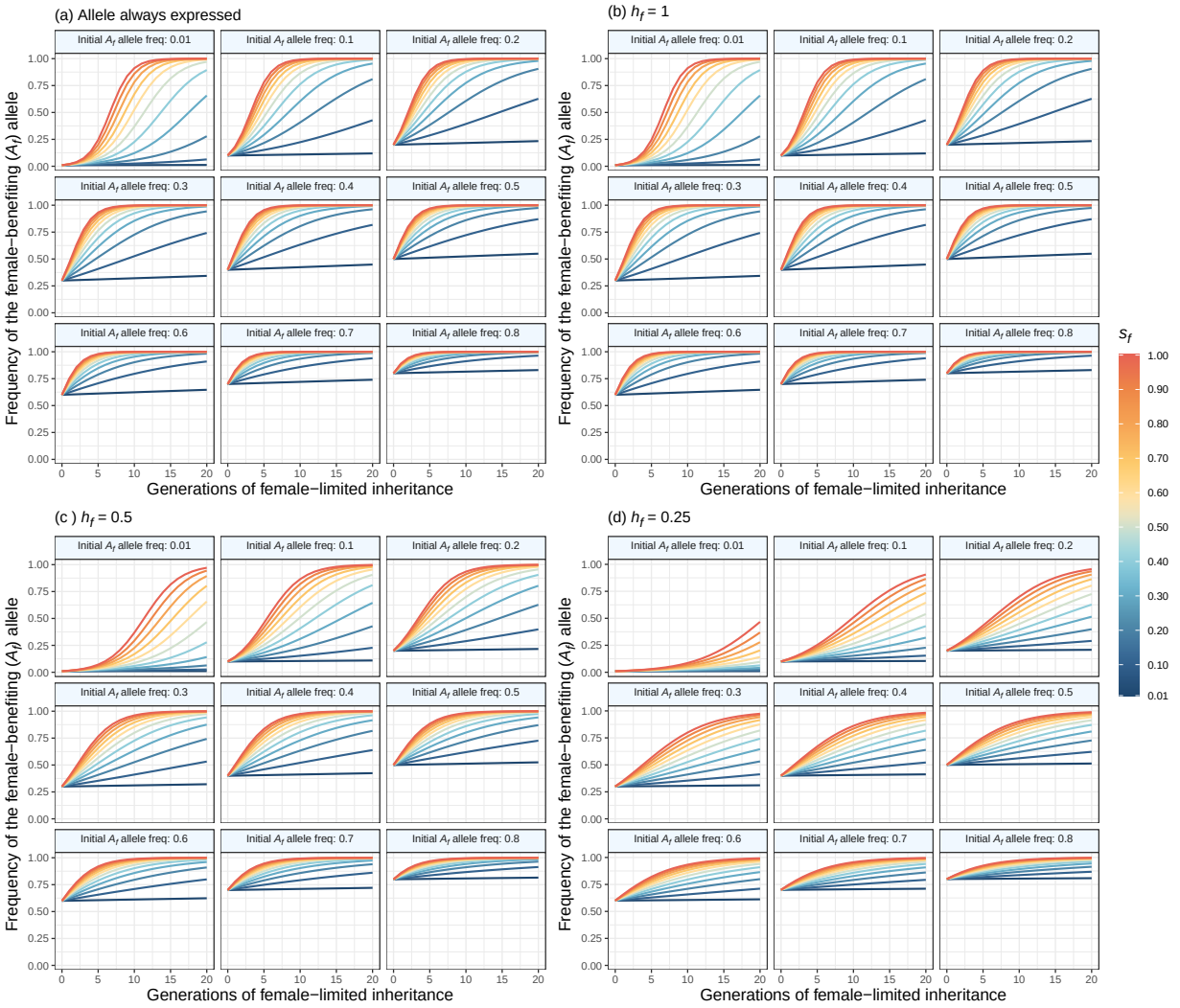

Figure S3: The increase in frequency of the female-beneficial allele across 20 generations of female-limited inheritance. Curves show how varying the female selection coefficient (s_f; increasing by increments of 0.1) for the allele changes the evolutionary trajectory, while comparing panels shows how this is affected by the initial A_f allele frequency. **a** the allele is always expressed, as would occur when the balancer homolog allele is also the A_f allele. **b-d** show cases where the balancer genotype carries the alternative allele to the A_f allele, where dominance relations matter.

Figure S3 shows that when selection is strong ($s_f > 0.4$), the A_f allele is very likely to reach fixation after 20 generations, except when the allele is rarely expressed. Even when $s_f = 0.1$, the A_f allele can reach high frequencies after 20 generations of sex-limited evolution. The figure also illustrates that allele frequency change is non-linear. At intermediate frequencies change is fastest, whereas it is much slower at the floor or ceiling. Therefore, evolution from intermediate to high frequencies should result in greater change than evolution from high to intermediate frequencies.

Phase three

Now we can return to equations 1-3 to assess the likelihood of alleles evolving back to their pre-experimental evolution frequencies. This time, we run the simulation for 20 generations and calculate ΔA_f, the change in allele frequency from generation 0 to 20 of relaxed biparental inheritance conditions that were enforced prior to the fitness assays.

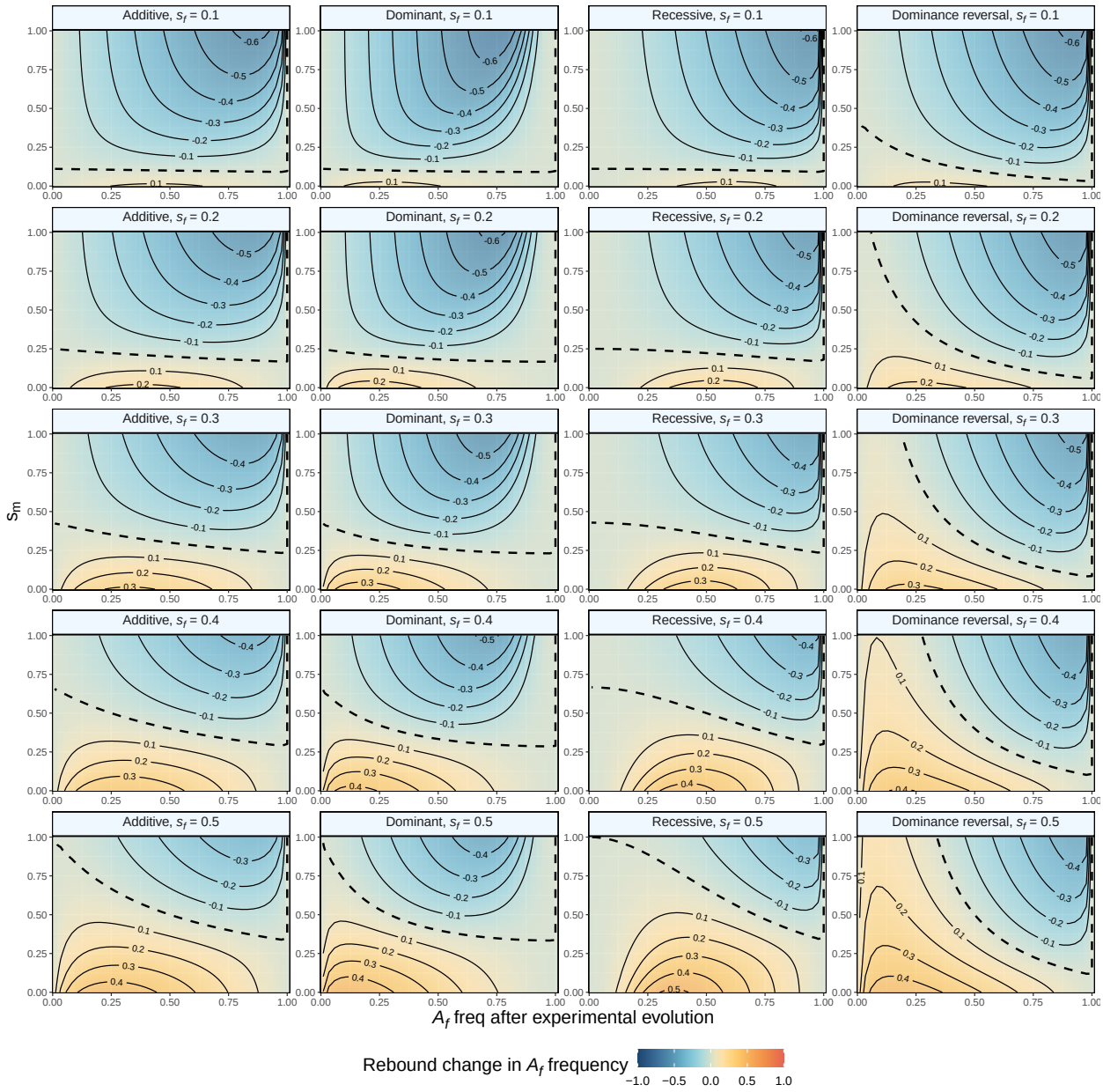

Figure S4: Predicted rebound in the equilibrium frequency of the A_f allele, after 20 generations of selection in both sexes, calculated from the population genetic model. The plot shows how varying the strength of the selection coefficient in males (s_m) and the frequency of the A_F allele at the end of experimental evolution affect the fate of the A_F allele. The dashed line indicates the conditions where no change is predicted for the frequency of the A_F allele. Contours show increments of 0.1 change in frequency, with warm regions indicating positive change and cool regions indicating a decline in frequency. Four dominance conditions are plotted across the columns: additivity, complete dominance, complete recessiveness and beneficial dominance reversal, where the A_F allele is partially dominant when expressed in a female ($h_f = 0.75$) and partially recessive when expressed in a male ($h_m = 0.75$, where this refers to the dominance of the M allele in males). The selection coefficient in females (s_f) increases down the rows. Only cases where s_f tangibly changes allele frequencies while plausibly maintaining polymorphism during the sex-limited evolution phase are plotted.

Figure S4 shows that the A_f allele can decline in frequency when s_m is large relative to s_f. However, as indicated by the dashed line's position on the x axis in many of the panels, when selection is relatively

equal between the sexes, very little change in frequency is predicted. Even when the A_m allele is dominant in males (e.g. Figure S4 columns 3-4), movement from high to intermediate frequencies requires s_m to be substantially stronger than s_m.

Combination of the phases

To produce final predictions for the effect of enforced relaxation of sex-limited inheritance on our experiment, we integrate the three phases into a single simulation. To start the simulation we provide an initial parameter space of varying s_f, s_m, h_f and h_m and track allele frequencies across the three phases of the model. We run phase one for 10000 generations and phases two and three for 20 generations each, simulating our experimental conditions.

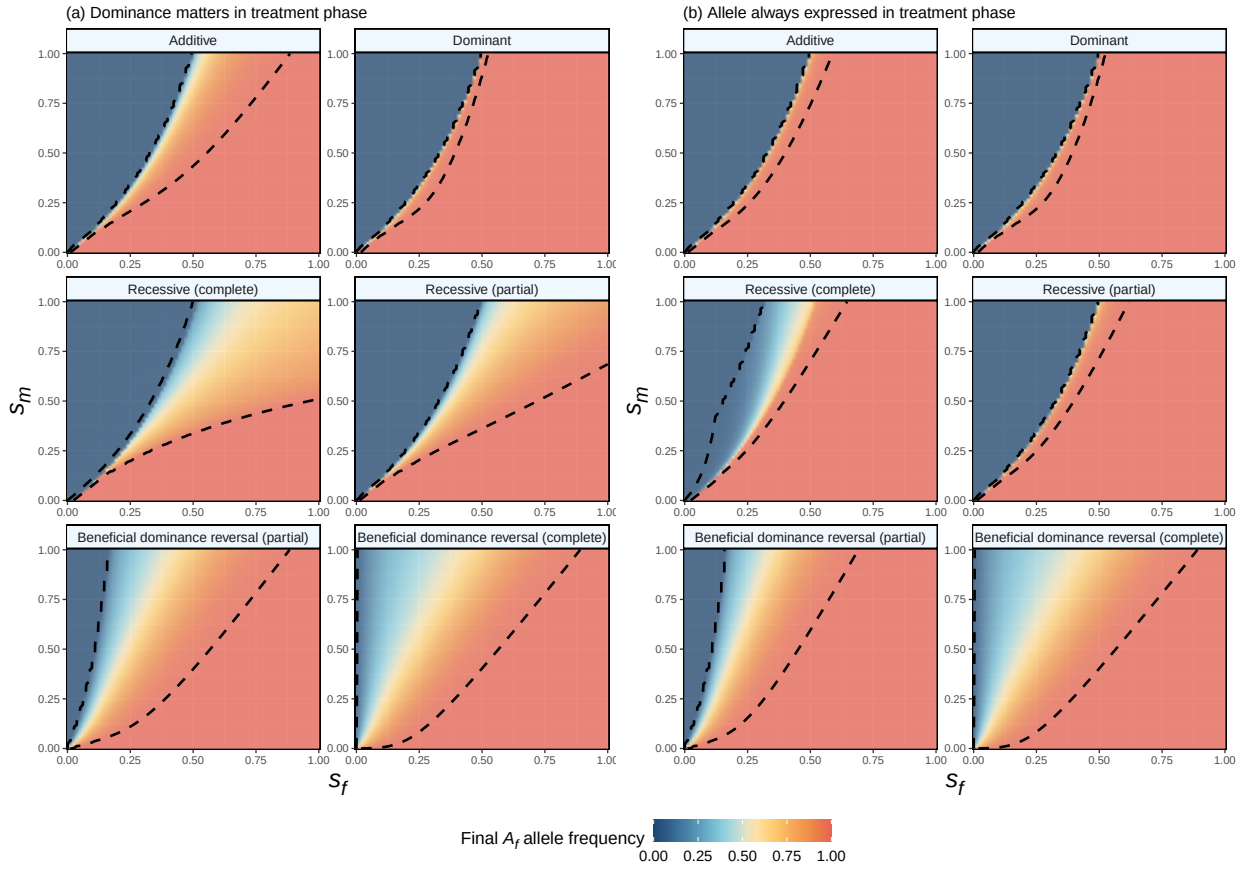

Figure S5: the final frequency of the A_f allele following sex-limited evolution and subsequent relaxation of sex-limited inheritance. The dashed lines bound the parameter space where polymorphism is still predicted. Note that compared with Figure S2, the parameter space for polymorphism is reduced, as the A_f increases to fixation in many cases during the sex-limited evolution phase. **a** the allele is always expressed during the sex-limited inheritance phase, as would occur when the balancer homolog allele is also the A_f allele. **b-d** show cases where the balancer genotype carries the alternative allele to the A_f allele, where dominance relations matter during the sex-limited inheritance phase.

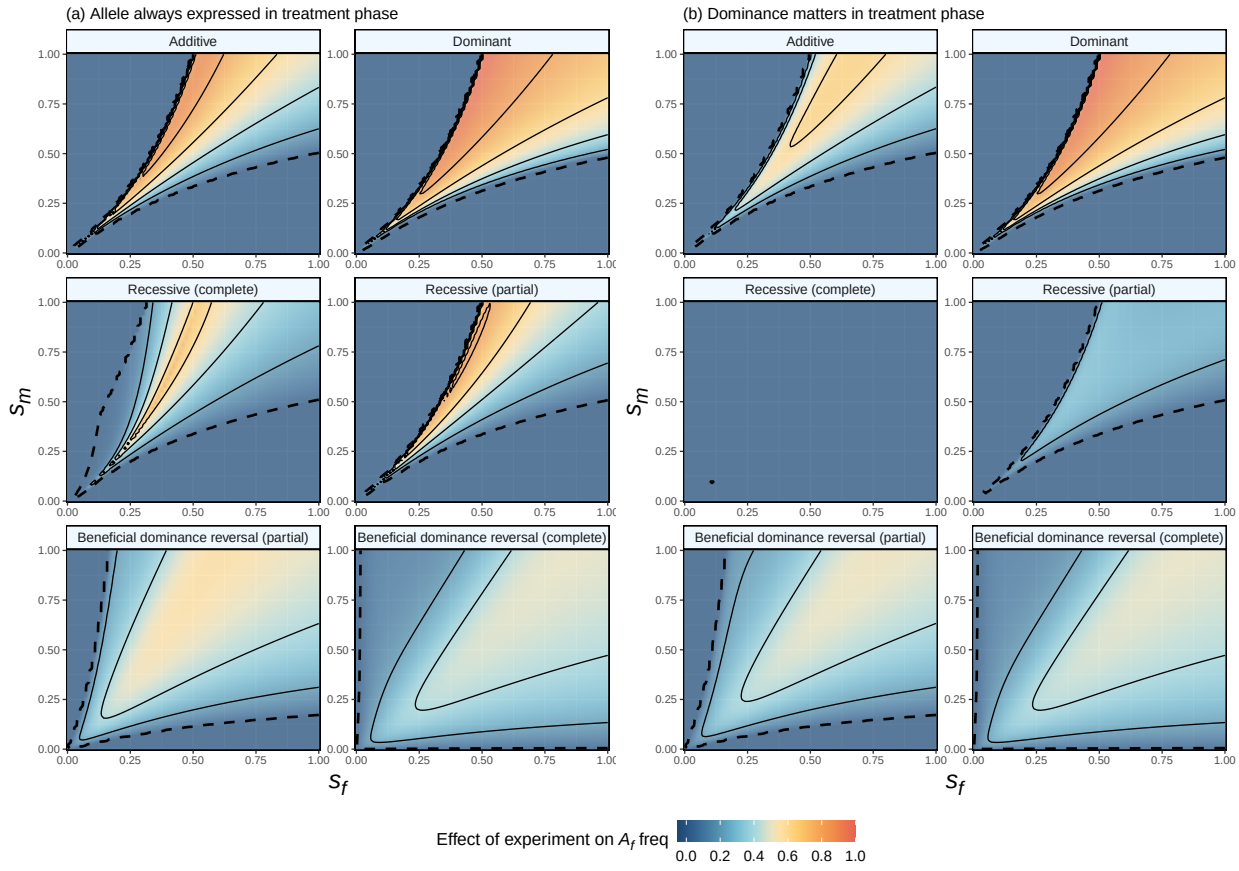

Figure S6: The effects of sex-limited inheritance for 20 generations, followed by 16 generations where a selection response could occur in either sex. The conditions where the A_f allele is expected to increase in frequency are bounded by the dashed lines, outside of which change = 0. Contour lines enclosed within the dashed lines indicate intervals of 0.2, increasing with the warmth of the colour displayed. **a** the allele is always expressed during the sex-limited inheritance phase, as would occur when the balancer homolog allele is also the A_f allele. **b-d** show cases where the balancer genotype carries the alternative allele to the A_f allele, where dominance relations matter during the sex-limited inheritance phase.

The combined simulation predicts that the relaxed selection phase should allow a partial shift back to previous frequencies for a subset of sexually antagonistic loci, but that the majority should retain a signature of sex-limited evolution. Areas of no change occur because the allele is predicted to either be fixed or purged from the population prior to the start of the experiment.

References

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Supplementary results tables

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