BMC Ecology and Evolution

RESEARCH

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Investigating the interaction between inter-locus and intra-locus sexual conflict using hemiclonal analysis in *Drosophila melanogaster*

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Abstract

Background: Divergence in the evolutionary interests of males and females leads to sexual conflict. Traditionally, sexual conflict has been classified into two types: inter-locus sexual conflict (IeSC) and intra-locus sexual conflict (IaSC). IeSC is modeled as a conflict over outcomes of intersexual reproductive interactions mediated by loci that are sex-limited in their effects. IaSC is thought to be a product of selection acting in opposite directions in males and females on traits with a common underlying genetic basis. While in their canonical formalisms IaSC and IeSC are mutually exclusive, there is growing support for the idea that the two may interact. Empirical evidence for such interactions, however, is limited.

Results: Here, we investigated the interaction between IeSC and IaSC in *Drosophila melanogaster*. Using hemiclonal analysis, we sampled 39 hemigenomes from a laboratory-adapted population of *D. melanogaster*. We measured the contribution of each hemigenome to adult male and female fitness at three different intensities of IeSC, obtained by varying the operational sex ratio. Subsequently, we estimated the intensity of IaSC at each sex ratio by calculating the intersexual genetic correlation ($r_{w,g,mf}$) for fitness and the proportion of sexually antagonistic fitness-variation. We found that the intersexual genetic correlation for fitness was positive at all three sex ratios. Additionally, at male biased and equal sex ratios the $r_{w,g,mf}$ was higher, and the proportion of sexually antagonistic fitness variation lower, relative to the female biased sex ratio, although this trend was not statistically significant.

Conclusion: Our results indicate a statistically non-significant trend suggesting that increasing the strength of IeSC ameliorates IaSC in the population.

Keywords: Sex ratio, Sexually antagonistic coevolution, Intersexual genetic correlation for fitness, Sexual antagonism, Male and female heritability, Fitness, Intersexual genetic correlation for fitness

Background

Defined for the first time in 1979 [1], the term "sexual conflict" is typically used to describe situations which exhibit a negative covariance for fitness between the sexes, i.e., circumstances that are optimal for the fitness of one sex but detrimental to the fitness of the other sex [2]. Examples of sexual conflict encompass a wide range

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of organisms and traits. They include body size [3], immunocompetence [4–6], parental investment [7, 8], sex ratios and sex allocation [9], mating behavior [10], sperm competition [11], traumatic insemination [12], colour patterns [13], age of maturation [14, 15] and leaf area [16] among others. Conceptually, sexual conflict has been thought to be of two kinds: Inter-locus Sexual Conflict (IeSC) or Intra-locus Sexual Conflict (IaSC) [2].

Typically, IeSC has been mathematically modeled as a conflict over mating rates, with male fitness increasing indefinitely with increasing mating rates, while females having an intermediate optimum mating rate [17, 18]. Mating rates are modeled as a function of male and female traits that are sex-limited in their expression (usually called "persistence" and "resistance" traits, respectively). Therefore, IeSC is a conflict between a set of loci limited to males, and a different set of loci limited to females. IeSC can also be extended to other spheres of reproductive interactions between males and females; for example, the interplay between the female reproductive tract and male ejaculate components [19]. IeSC has been reported in diverse taxa including crickets [20], beetles [21, 22], flatworms [23], snails [24, 25], and even plants [26, 27].

IaSC, on the other hand, is a consequence of males and females sharing the same gene pool while experiencing markedly different selection pressures [2]. IaSC is usually defined for traits that have a common underlying genetic basis in males and females, but have vastly different sex-specific fitness optima [28]. At the level of a locus, IaSC arises when the allele that is favoured in males is different from the one that is favoured in females [29]. Patterns consistent with IaSC have been reported in a wide range of organisms including guppies [30], the bank vole [31], the collared flycatcher [32], the ant *Nylanderia fulva* [33], and even human beings [34].

In their traditional formalisms, IaSC (which deals with traits that are shared between the sexes) and IeSC (which deals with traits that are sex-limited in their expression) are mutually exclusive phenomena. However, there have been strong arguments in favour of an interaction between IaSC and IeSC. Pennell and Morrow [35] argued that IaSC and IeSC could interact in several ways, primarily as a consequence of traits involved in IeSC not being entirely sex-limited in their effects. Traits involved in IeSC could be genetically correlated with traits involved in IaSC. Alternatively, loci involved in IeSC could have pleiotropic effects with fitness consequences in the other sex [36]. Pennell and Morrow also pointed out that processes that resolve IaSC leading to evolution of sexual dimorphism, could trigger IeSC as a result of trait exaggeration. Another useful way of looking at the interaction between IeSC and IaSC is to investigate whether selection gradients on shared traits that mediate IaSC are a function of the intensity of IeSC. If the divergence in sex-specific fitness optima for shared traits is primarily driven by sexual selection [37], experimentally increasing the intensity of IeSC (and by corollary sexual selection) should cause male and female fitness optima to move further apart, yielding a stronger signal of IaSC in that generation. However, it is important to note that, in general, there is no unequivocal theoretical expectation that strengthening IeSC should lead to a strengthening of IaSC in the population. The nature of the interaction between IeSC and IaSC will depend on the genetic architecture of traits involved in the two kinds of sexual conflict (see "Discussion"). Very few empirical studies have investigated the interaction between IaSC and IeSC. Working on *Callosobruchus maculates* isofemale lines, Berger et al. [38] were able to show that multivariate traits associated with high male fitness were genetically associated with a greater drop in line-productivities than could be explained by mate harm (an important aspect of IeSC) or IaSC independently, pointing towards concurrent operation of IaSC and IeSC. However, to the best of our knowledge, no study has yet investigated the consequences of *experimentally* manipulating the intensity of IeSC on the signal of IaSC in the population.

In the present study, we explored the interaction between IeSC and IaSC in a population of *Drosophila melanogaster* maintained in the laboratory for more than 500 generations. *D. melanogaster* is a convenient model organism to address this question as it has been at the forefront of sexual conflict research, primarily because of the tractability of long-term experimental evolution studies, and the development of crucial genetic tools. One such tool, hemiclonal analysis, which was first developed by Rice [39], enables the experimenter to sample hemigenomes from the population of interest and express them in males and females carrying random genetic backgrounds from the population [40]. This allows explicit measurements of various quantitative genetic parameters such as additive genetic variances and covariances between quantitative traits, including Darwinian fitness. Using experimental evolution and special genetic constructs used in hemiclonal analysis (for example, "clone generator" flies; see "Methods"), *D. melanogaster* has been widely used as a model organism to investigate the evolutionary consequences of IeSC on males and females [41], quantify genetic variation for IeSC-related traits [42, 43], estimate the intensity of IaSC [44–46], identify traits involved in IaSC [47] and explore sexually antagonistic fitness consequences of male-limited or female-limited evolution [48–50].

Table 1 ANOVA-like table for random terms in the linear mixed effects model for male and female fitness

	npar	logLik	AIC	LRT	Df	p value
<none>	9	− 1861.4	3740.9			
(1|Hemigenome line)	8	− 1864	3744	5.114	1	**0.0237**
(1|Hemigenome line:Sex)	8	− 1878	3772	33.147	1	**< 0.0001**
(1|Hemigenome line:Sex.Ratio)	8	− 1861.5	3738.9	0.052	1	0.8196
(1|Hemigenome line:Sex:Sex.Ratio)	8	− 1868.2	3752.3	13.479	1	**0.0002**

Statistically significant values are shown in bold

To investigate the interaction between IaSC and IeSC, we sampled a panel of hemigenomes from a large laboratory adapted population of *D. melanogaster*. We measured the reproductive fitness of males and females carrying each hemigenome (expressed in a large number of genetic backgrounds randomly sampled from the source population) at three different adult sex ratios: male biased (strong IeSC), equal (intermediate IeSC) and female biased (weak IeSC). Manipulating operational sex ratios has been one of the two principal techniques of experimentally changing the intensity of IeSC [41, 51–54], the other being experimentally enforcing monogamy [55–60]. First, we examined the relationship between the contribution of each hemigenome to sex-specific fitness at each of the three adult sex ratios. Particularly, we attempted to infer if there were any interactions between hemigenome line, sex and sex ratio for fitness. Subsequently, we estimated the following two parameters corresponding to the strength of IaSC for each sex ratio. First, we calculated the male–female genetic correlation for fitness ($r_{w,g,mf}$), a widely used method of estimating the intensity of IaSC [28] with a highly negative $r_{w,g,mf}$ thought to be indicative of strong IaSC (but see Connallon and Mathews [61]). Second, we estimated the proportion of sexually antagonistic genetic variation, a more recent method that partitions fitness-variance along sexually antagonistic and sexually concordant axes [46, 62].

Results

Using cytogenetic cloning techniques [39], we sampled a panel of 39 hemigenomes from a laboratory adapted population of *D. melanogaster* called LH. This involved using clone generator females (see "Methods") that possess a compound X chromosome and a translocation between the two major autosomes. This allows the sampling and cloning of entire haploid nuclear genomes (with the exception of the dot chromosome). We expressed each of these haploid genomes in males and females carrying the rest of the genome randomly sampled from the LH population. Subsequently, we measured male and female fitness at three different intensities of IeSC obtained by varying the adult sex ratio: male biased sex ratio (24 males: 8 females per vial) where IeSC is expected to be intense, female biased sex ratio (8 males: 24 females per vial) where IeSC is expected to be weak, and equal sex ratio (16 males: 16 females per vial) where the intensity of IeSC is expected to be intermediate. We used competitive fertilization success as the measure of male fitness, and fecundity post strong female-female competition for acquiring live yeast as the measure of female fitness (see the "Methods" section for details).

We fit a linear mixed effects model for standardised fitness to investigate if there were any interactions between sex, sex ratio, and hemigenome line. Next, using two different approaches we measured the intensity of IaSC at each of the three different sex ratios. First, using the sex-specific line averages for fitness at each sex ratio, we estimated the intersexual genetic correlation for fitness ($r_{w,g,mf}$) and the proportion of fitness variation along the sexually antagonistic axis. Second, we used the R package "MCMCglmm" to estimate $r_{w,g,mf}$ as well as male and female heritabilities at each sex ratio. We also estimated the sex-specific genetic correlation for fitness between sex ratios. See the "Methods" section for details on statistical analysis.

Interactions between hemigenome line, sex, and sex ratio

The output of our linear mixed effects model (Table 1) suggested that there was a significant effect of hemigenome line (likelihood ratio test (LRT), $p = 0.0237$), its interaction with sex (LRT, $p < 0.0001$), and the three-way interaction between hemigenome line, sex and sex ratio (LRT, $p = 0.0002$). While all across-sex ratio correlations for both males and females, and all across-sex correlations for all three sex ratios were positive (Table 2A, B; Figs. 1, 2), many hemigenome lines exhibited fitness rank reversals across sex ratios (Fig. 3) or sex (Fig. 4), explaining the interactions observed in the linear mixed effects model.

Table 2 The summary of results from (A) the analysis using hemigenome line averages and (B) the MCMCglmm model

	Sex ratio	**Estimate**	**Lower CL**	**Upper CL**
(A) Using line averages				
Intersexual genetic correlation for fitness ($r_{w,g,mf}$)	Male biased	0.3805	0.2992	0.5283
	Equal	0.4027	0.3140	0.5526
	Female biased	0.2515	0.1198	0.4502
Proportion of sexually antagonistic fitness variation	Male biased	0.3097	0.2358	0.3504
	Equal	0.2986	0.2237	0.3430
	Female biased	0.3742	0.2749	0.4401
	Pairs of sex ratios	**Estimate**	**Lower CL**	**Upper CL**
Genetic correlations for female fitness between pairs of sex ratios	Male biased—female biased	0.7688	0.7442	0.8497
	Male biased—equal	0.7493	0.7213	0.8368
	Female biased—equal	0.8421	0.8403	0.8956
	Pairs of sex ratios	**Estimate**	**Lower CL**	**Upper CL**
Genetic correlations for male fitness between pairs of sex ratios	Male biased—female biased	0.5567	0.4997	0.7262
	Male biased—equal	0.6995	0.6755	0.8018
	Female biased—equal	0.5415	0.4664	0.7417
	Sex ratio	**Estimate**	**Lower CL**	**Upper CL**
(B) Using MCMCglmm				
Intersexual genetic correlation for fitness ($r_{w,g,mf}$)	Male biased	0.5056	0.1418	0.7983
	Equal	0.4999	0.1397	0.7787
	Female biased	0.4462	0.0059	0.8470
Female heritability ($h^2_{w,f}$)	Male biased	0.8702	0.5935	1.1520
	Equal	0.9992	0.7337	1.2696
	Female biased	0.7385	0.5021	1.0539
Male heritability ($h^2_{w,m}$)	Male biased	0.4788	0.2383	0.7303
	Equal	0.5762	0.3192	0.8637
	Female biased	0.2229	0.0495	0.4080
	Pairs of sex ratios	**Estimate**	**Lower CL**	**Upper CL**
Genetic correlations for female fitness between pairs of sex ratios	Male biased—female biased	0.8932	0.6888	0.9996
	Male biased—equal	0.8785	0.7477	0.9994
	Female biased—equal	0.9536	0.8767	0.9995
	Pairs of sex ratios	**Estimate**	**Lower CL**	**Upper CL**
Genetic correlations for male fitness between pairs of sex ratios	Male biased—female biased	0.8932	0.6888	0.9996
	Male biased—equal	0.9438	0.8190	1.0000
	Female biased—equal	0.9010	0.7025	0.9997

Lower and upper CL represent the limits of 95% confidence intervals

Signals of IaSC at male biased, equal, and female biased sex ratios

The analyses using hemigenome line averages suggested that the $r_{w,g,mf}$ for male biased sex ratio (0.3805, 95% CI = [0.2992, 0.5283]) and equal sex ratios (0.4027, 95% CI = [0.3140, 0.5526]) were comparable to each other, but were larger than that for the female biased sex ratio (0.2515, 95% CI = [0.1198, 0.4502]). However, the 95% confidence intervals (CIs) for the difference in the $r_{w,g,mf}$ estimates of male biased and female biased sex ratios (− 0.0721, 0.3507) included 0, suggesting these differences were not statistically significant. The estimates of $r_{w,g,mf}$ from the MCMCglmm model (Table 2B) were slightly higher but the relative trend among sex ratios was similar. The $r_{w,g,mf}$ estimates were comparable for male biased (0.5056, 95% credible intervals (CI) = [0.1418, 0.7983]) and equal sex ratios (0.4999, 95% CI = [0.1397, 0.7787]), while the $r_{w,g,mf}$ estimate for the female biased

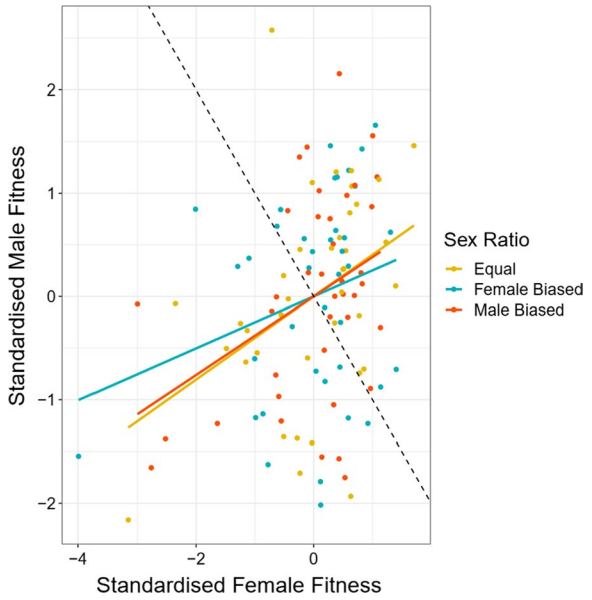

Fig. 1 Scaled and centred male and female fitnesses for each of the 39 hemigenome lines for equal sex ratio (yellow), female biased sex ratio (blue) and male biased sex ratio (red). The solid lines represent the least-squared regression lines for each of the three sex ratios. The dashed line represents the axis of sexually antagonistic fitness variation with male beneficial, female detrimental genotypes to the top-left and female beneficial, male detrimental genotypes to the bottom-right

sex ratio (0.4462, 95% CI = [0.0059, 0.8470]) was lower (Table 2B). However, the credible interval for the difference between the $r_{w,g,mf}$ estimates for male biased and female biased sex ratios (− 0.3788, 0.5561) included 0, suggesting the two were not significantly different.

The proportion of fitness variation along the sexually antagonistic axis (estimated using line averages) too was comparable for male biased and equal sex ratios (0.3097, 95% CI = [0.2358, 0.3504] and 0.2986, 95% CI = [0.2237, 0.3430], respectively). The female biased sex ratio had a higher proportion of sexually antagonistic fitness variation (0.3742, 95% CI = [0.2749, 0.4401]). The 95% CIs for the difference between estimates of proportion of sexually antagonistic fitness variation for male biased and female biased sex ratios (− 0.1753, 0.0360) included 0, suggesting these differences were not statistically significant.

Male and female heritabilities at male biased, equal, and female biased sex ratios

The estimates of female heritabilities for fitness, obtained using the MCMCglmm model, in male biased (0.8702, 95% CI = [0.5935, 1.1520]), equal (0.9992, 95% CI = [0.7337, 1.2696]) and female biased (0.7385, 95% CI = [0.5021, 1.0539]) sex ratios, were higher than the corresponding estimates of male heritabilities at male biased (0.4788, 95% CI = [0.2383, 0.7303]), equal (0.5762, 95% CI = [0.3192, 0.8637]) and female biased (0.2229, 95% CI = [0.0495, 0.4080]) sex ratios. This trend was statistically significant, as the 95% credible intervals for the difference in female and male heritabilities did not overlap with 0 in male biased [− 0.7343, − 0.0207] and equal [− 0.7703, − 0.0852] sex ratios, but not in the female biased sex ratio [− 0.3740, 0.0668]. Additionally, for both males and females, heritabilities were highest under equal sex ratio, and were marginally lower in the male biased sex ratio. Both male and female heritabilities were considerably lower in the female biased sex ratio. The variance estimate for the interaction between day and hemigenome line was 0.0353 (95% CI = [0.0068, 0.0606]).

Discussion

We investigated the interaction between inter- and intralocus sexual conflict in a laboratory adapted population of *D. melanogaster*. We isolated 39 hemigenomes from the LH population and measured the contribution of each hemigenome to the adult fitness of males and females at male biased, equal and female biased sex ratios. Our analyses yielded the following major findings:

(a) At each sex ratio the intersexual genetic correlation for fitness ($r_{w,g,mf}$) was positive. $r_{w,g,mf}$ was smaller and the proportion of fitness variation along the sexually antagonistic axis higher in the female biased sex ratio relative to male biased or equal sex ratios, suggesting an amelioration of IaSC at higher intensities of IeSC. However, it must be noted that these differences were not statistically significant.
(b) Genetic correlations across sex ratios for male and female fitness were strongly positive.
(c) There were significant hemigenome line × sex, and hemigenome line × sex × sex ratio interactions for standardised fitness.
(d) Heritabilities for fitness were the highest in the equal sex ratio, followed by the male biased sex ratio, and were considerably lower in the female biased sex ratio.
(e) Estimates of female heritabilities in all three sex ratios were higher than the corresponding estimates of male heritabilities.

Below, we discuss the potential implications of these findings.

The interaction between IeSC and IaSC can take many different forms, primarily as a consequence of traits involved in one kind of conflict also playing a

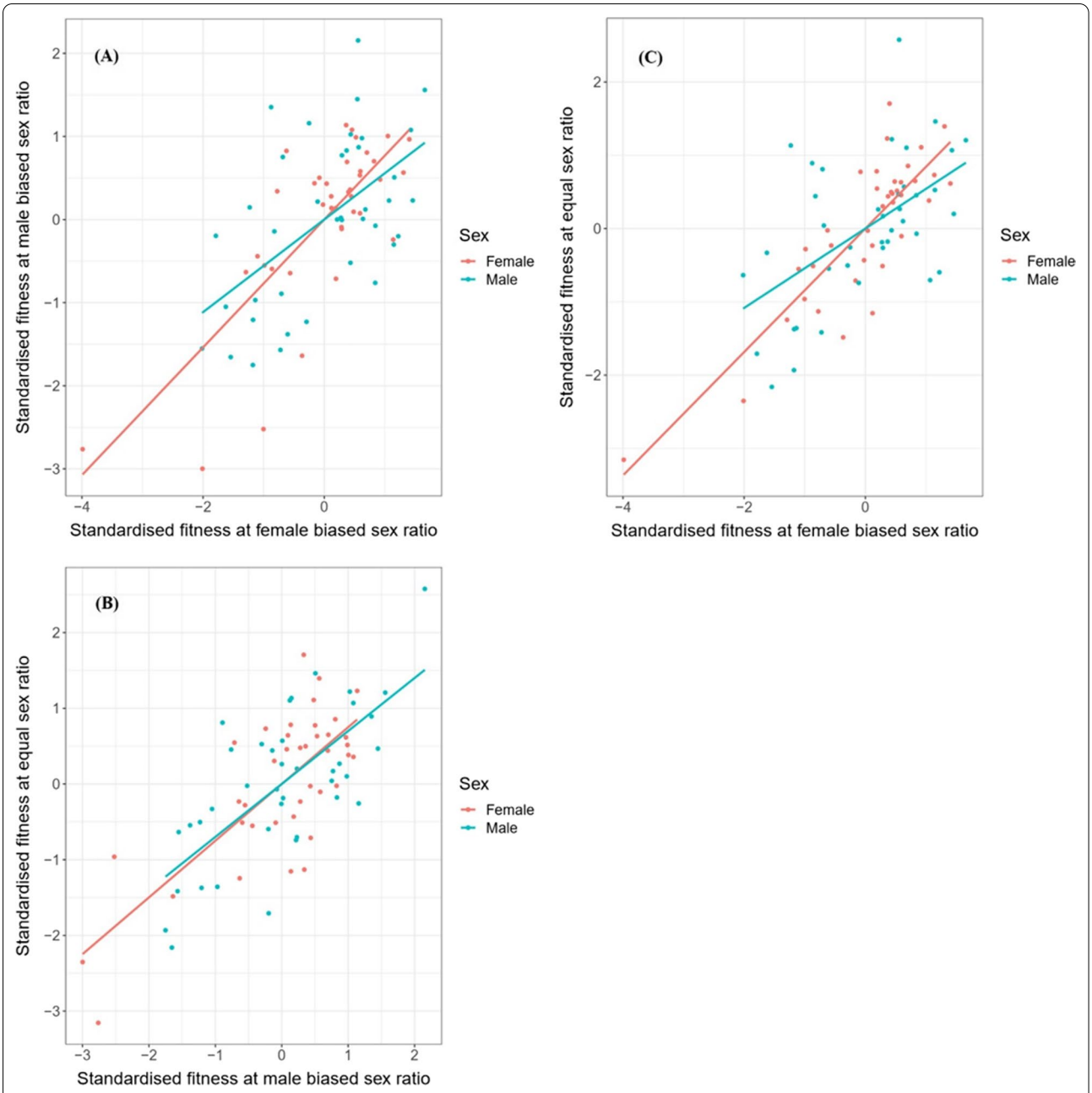

Fig. 2 Scatterplots showing standardised male and female fitnesses for various hemigenome lines between (**A**) male biased and female biased sex ratios, (**B**) equal and male biased sex ratios, and (**C**) equal and female biased sex ratios. Blue represents data for males, and red represents data for females. The solid lines represent least-squared regression lines

role in the other kind of conflict [35]. While there is no universal expectation with respect to the direction in which these interactions should proceed, in some of the cases, IaSC and IeSC are expected to reinforce each other. For example, at higher intensities of IeSC, stronger sexual selection could result in male and female fitness optima for shared traits being further apart leading to a stronger signal of IaSC, relative to lower intensities of IeSC. Similarly, if traits involved in IeSC have negative fitness consequences when expressed in the opposite sex (similar to the assumptions of Pennell et al. [36], experimentally increasing the intensity of IeSC, all else being equal, would lead to an increase in the signal of sexually antagonistic selection (relative to sexually concordant selection). Our results find no evidence that the interaction between

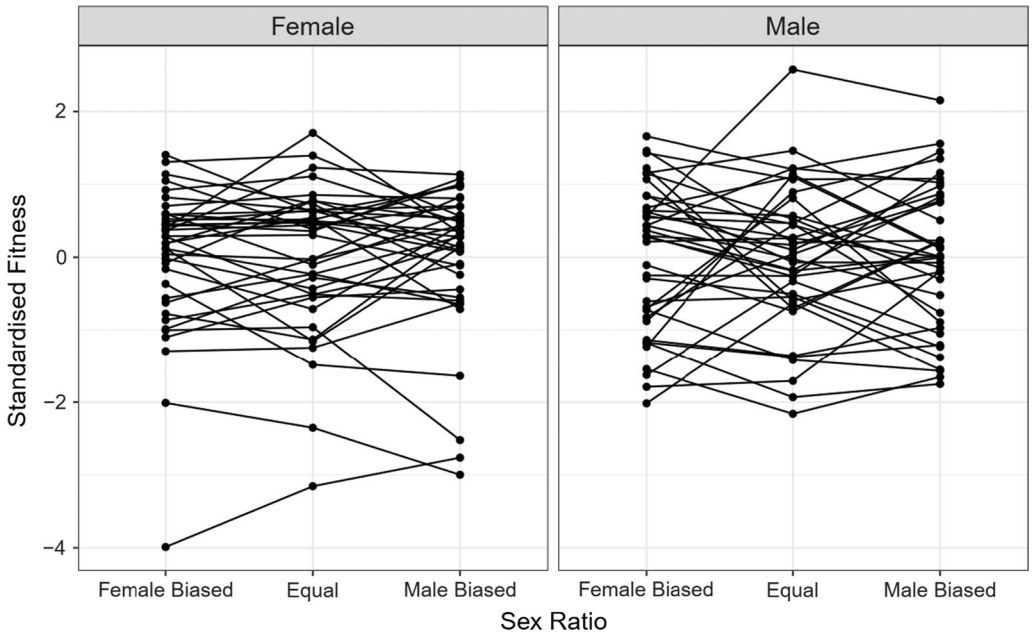

Fig. 3 Interaction plots showing standardised fitnesses for various hemigenome lines expressed as females and males, and assayed at female biased, equal, and male biased sex ratios. Points connected by a line represent a hemigenome line

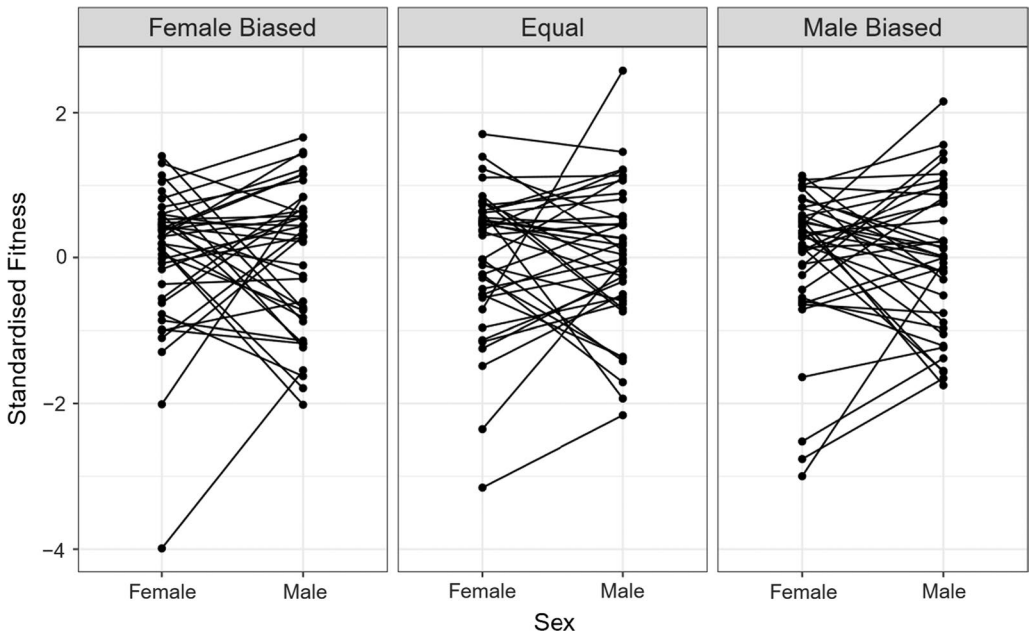

Fig. 4 Interaction plots showing standardised fitness for various hemigenome lines expressed as females and males, and assayed at female biased, equal, and male biased sex ratios. Points connected by a line represent a hemigenome line

IaSC and IeSC manifests along these lines. In contrast, we find a statistically non-significant *decrease* in the signal of IaSC at higher intensities of IeSC. The proportion of sexually antagonistic variation was higher at the female biased sex ratio, compared to the other two sex ratios. While the absolute estimates of $r_{w,g,mf}$ were different between the analysis using line averages and the Bayesian analysis using MCMCglmm, the relative trend among the three sex ratios was identical. Both the analyses suggested a statistically non-significant reduction

in $r_{w,g,mf}$ at the female biased sex ratio compared to the male biased or equal sex ratios, which were comparable to each other.

Both IaSC and IeSC are complex biological phenomena that involve an interplay of a large number of traits. To be able to predict how changing the intensity of one, influences the intensity of the other would, therefore, require an understanding of the genetic architecture of these traits, and the nature of selection acting on each of them. Below, we describe two plausible scenarios under which strengthening the intensity of IeSC could lead to weaker IaSC within the population.

First, as the intensity of IeSC increases, it is possible that selection gradients on traits involved in IaSC change, leading to a change in the intensity of IaSC over those traits. In an extreme scenario, with increase in the strength of IeSC, one of these selection gradients could change signs in one of the sexes resulting in sexually concordant selection on that trait. Given that we found a strong three-way interaction between sex, sex ratio, and hemigenome line for fitness in our linear mixed effects model, this explanation becomes fairly plausible. Below, we use available results about locomotory activity to illustrate our point. Adult locomotory activity has been shown to mediate IaSC in *D. melanogaster* [47], with more active males and less active females enjoying higher fitness. Numerous studies have reported patterns that indicate that *D. melanogaster* males that tend to be more active enjoy greater mating success [63–66]. On the other hand, female activity stimulates male courtship in *D. melanogaster* [67]. Therefore, active females are thought to attract more courtship from males, resulting in diversion of resources away from egg-production. While a substantial fraction of fitness costs of male–female interactions to females are pre-mating [68], several studies have highlighted post-mating fitness costs to females [69–71]. Therefore, it is possible that in an environment where IeSC is intense (for example, the male biased sex ratio in our experiments), where male-courtship is guaranteed regardless of female activity, selection on females to reduce the number of matings might be stronger than avoiding courtship per se. As a corollary, in an environment with extremely elevated levels of male-courtship, more active females might enjoy higher fitnesses by virtue of their ability to reject male mounting attempts. Therefore, at higher intensities of IeSC, the selection on adult locomotory activity might become sexually concordant reducing the intensity of IaSC. Nandy et al. [41] and Nandy [72] evolved replicate populations of *D. melanogaster* at male biased, equal and female biased sex ratios, and reported that both males and females from the male biased populations evolved to become more active than their counterparts evolving under equal and female biased sex ratios. This suggests that at male biased sex ratio, where levels of IeSC are the highest, IaSC over locomotory activity seems to be weakened, if not removed entirely, so as to permit the evolution of increased locomotory activity levels in both males and females.

Second, increasing the strength of IeSC could ameliorate IaSC if male and female traits (unfortunately called "persistence" and "resistance" traits, respectively) involved in IeSC are positively genetically correlated. If the most "resistant" females preferentially mate with the most "persistent" males a positive linkage disequilibrium between "resistance" and "persistence" could build up in the population. As the strength of IeSC increases, by definition, the strength of selection on "persistence" and "resistance" traits increases. If the two sets of traits are positively genetically correlated, this would result in an increase in the strength of sexually concordant selection; all else being equal, this would yield a weakened IaSC signal. Rice et al. [73] could not find a significant correlation between male and female remating rates in a laboratory population of *D. melanogaster*. However, they did not explicitly observe mating, but measured mating rates in terms of the proportion of females in a vial that remated after their first mating. There are several alternate ways of measuring proxies of persistence and resistance including measuring the latency between the first and the second mating, explicit observations to record matings or measuring courtship related behaviours in males and females. It remains to be explored if these traits are genetically correlated in our panel of hemigenomes.

Our study is also relevant in the context of the "evolutionary inevitability of sexual antagonism" [74]. Connallon and Clark [74] used a variant of Fisher's geometric model to show that as populations adapt to their environments the degree of sexual antagonism in the populations should increase. Consequently, if a population that is well-adapted to its environment is exposed to a novel environment, the degree of sexually antagonistic selection experienced by the population should be lower [75]. This idea has been tested in insects by numerous studies, with some studies finding evidence in support of the idea [62, 76], while others either failed to detect any effect of change of environment on the degree of sexual antagonism [77, 78] or reported an increase in sexual antagonism in novel environments [79, 80]. In our case the LH population has been maintained in the laboratory for > 500 generations at equal sex ratio. Therefore, male biased and female biased sex ratios represent novel environments to which the population is not expected to have adapted. Our results do not provide any evidence in favour of the idea that maladapted populations should exhibit weaker IaSC. We found that compared to

equal sex ratio, male biased sex ratio exhibited a comparable intensity of IaSC, while the female biased sex ratio resulted in a statistically non-significant *increase* in the strength of IaSC (lower $r_{w,g,mf}$ and higher proportion of sexually antagonistic fitness variation). One of the reasons why we could not detect a clear increase in the strength of IaSC in our novel environments (male biased and female biased sex ratio) could be the fact that our sex ratio treatments were applied only for 2 days in the adult stage of the flies. This duration is fairly short, compared to the life cycle of the LH population (14 days). Therefore, it could be argued that the novel environments (male biased and female biased sex ratio) were not sufficiently novel. However, this explanation is unlikely for two reasons. First, while two days is indeed a short period compared to the entire life cycle of the LH population, the period between day 12 and day 14, when sex ratio treatments were applied in our experiments, is a crucial phase for the reproductive fitness of LH flies. Eggs laid in the 18 h post day 14 contribute to the next generation (see "Methods" section). Additionally, there is strong last male sperm precedence in *D. melanogaster* [81]. Therefore, male–female interactions from day 12 through day 14 are crucial determinants of both male and female fitness, and also, potentially, mediate IeSC in the LH population [73]. The LH population has been maintained using the current protocol for more than 500 generations. Therefore, the period between day 12 and day 14 in the LH life cycle is, perhaps, the most ecologically relevant phase to perform adult-stage experimental manipulations. Second, we found a strong three-way interaction between sex, sex ratio, and hemigenome line ($p = 0.0002$) for reproductive fitness. This clearly suggests that the three sex ratio environments are different in terms of how sex-specific selection operates in them.

At each of the three sex ratios our estimates of $r_{w,g,mf}$ were strongly positive. This is in sharp contrast to Chippindale and Rice [44] who had reported a negative $r_{w,g,mf}$ in the ancestral population of the LH population used by us. In fact, several studies have attempted to estimate $r_{w,g,mf}$ in replicates of the original LH_M population with different outcomes. Innocenti and Morrow [82] reported a negative $r_{w,g,mf}$. Collet et al. [45] compared $r_{w,g,mf}$ across two replicates of the LH_M population and reported that one of the replicates had a negative $r_{w,g,mf}$ while the other had an $r_{w,g,mf}$ indistinguishable from 0. Ruzicka et al. [46] sampled 200 hemogenomes from a replicate of the LH_M population and found a positive but non-significant $r_{w,g,mf}$. Ours is the first study to report an $r_{w,g,mf}$ significantly greater than 0. While it is tempting to interpret this as evidence indicating resolution of IaSC through the traditional pathway of sex-specific expression, it might well be a byproduct of strengthening of IeSC driven by an escalating arms race between males and females in the LH population. As sexually antagonistic coevolution in the LH population resulted in an increase in the intensity of IeSC, the signals of IaSC could also evolve to be lower, assuming higher intensities of IeSC correspond to weaker signals of IaSC as suggested by our statistically non-significant results. Therefore, further experimental work aimed at understanding the genetic relationships between traits involved in IaSC and IeSC, as well as their selection gradients under various environments is required.

Using our experimental design, we were also able to obtain estimates of sex-specific heritabilities at the three sex ratios. Consistent with previous studies with similar experimental populations, female heritabilities for adult fitness were higher than male heritabilities [45, 46]. Both male and female heritabilities at the female biased sex ratio were considerably lower than male biased or equal sex ratios, suggesting that the rate of adaptation ought to be lower at female biased sex ratio. This is consistent with the findings that experimental evolution at male biased sex ratio leads to rapid sex specific adaptations in reproduction related traits, compared to populations evolving at female biased sex ratio [11, 41, 53]. An intriguing aspect of our heritability estimates was that they were fairly large. This is likely to be a result of various components of residual fitness variance (that is, fitness variance other than additive genetic variance) being mis-attributed to between-line variance due to some of the shortcomings of our experimental design. In our fitness assays, we expressed entire haploid genomes (barring the "dot" chromosome), in a large number of randomly sampled complementary chromosomes sampled from the LH population. This had two consequences. First, variance due to epistatic interactions between loci also contributed to between-hemigenome line fitness variance. Second, sampling error in the complementary background in which various hemigenomes of interest were expressed would also inflate the between-hemigenome line fitness variance. Therefore, our estimates of heritabilities likely represent upper bounds for the actual additive genetic variance, rather than heritabilities per se.

An important caveat of our study is that it measures the consequences of altering the intensity of IeSC for one generation to the intensity of IaSC *in the same generation*. This is quite distinct from how signals of IaSC are expected to *evolve* over several generations under either intense IeSC (male biased sex ratio) or weak IeSC (female biased sex ratio). A tractable experimental approach to investigate how signals of IaSC evolve under either high or low intensities of IeSC could involve sampling hemigenomes from populations experimentally evolving at

either male biased or female biased sex ratio, and measuring sex-specific fitness for those hemigenomes.

Conclusions

In conclusion, the key findings of our study are as follows:

1. Strengthening the intensity of inter-locus sexual conflict led to a statistically non-significant decrease in the strength of intra-locus sexual conflict.
2. In contrast with previous studies, we report significantly positive intersexual genetic correlation for fitness.
3. Both males and females exhibited higher heritabilities for reproductive fitness in male biased and equal sex ratio environments as compared to the female biased sex ratio.

Methods

In order to investigate the interaction between IaSC and IeSC, we performed hemiclonal analysis on a laboratory adapted population of *D. melanogaster* called LH. We sampled a panel of 39 hemigenomes from the LH population, and measured the contribution of each hemigenome towards male and female fitness at three different adult sex ratios (3:1 male biased, equal, and 1:3 female biased).

Fly populations

LH

LH is a large laboratory adapted population of *D. melanogaster*. It is a direct descendent of the LH_M population used to measure $r_{w,g,mf}$ by Chippindale and Rice [44], and is related to the populations used by other similar studies [45, 46]. The detailed maintenance protocol of LH has been described elsewhere [83]. Briefly, it is maintained on a 14 day discrete generation cycle on a standard cornmeal-molasses diet at 25 °C, 50% relative humidity, and a 12 h: 12 h light–dark cycle. The population consists of a total of 60 vials each containing about 150 eggs in 8–10 ml food. On the 12th day post egg collection, by which time all individuals develop into adult flies, the population is randomly divided into 6 groups of 10 vials each. Flies from each group are mixed together in a flask and subsequently, using light CO_2 anesthesia, are sorted into 10 food-vials, each containing 16 males and 16 females. Thus, the total population size is 960 females and 960 males spread over 60 vials. Males and females are then allowed to interact for two days in presence of limiting amounts of live yeast. On the 14th day post egg-collection, flies are transferred to fresh food-vials, where they are allowed to lay eggs for 18 h. The adult flies are then discarded and the eggs are trimmed to a density of 150 per vial. These eggs then start the next generation.

In our experiments, we used the LH population to sample a panel of 39 hemigenomes (see below).

LHst

LHst was established by introgressing an autosomal, recessive and benign scarlet eye-colour marker in the LH population. Its maintenance protocol is similar to that of LH, except that the population size is half the population size of LH. LHst is regularly back-crossed to LH to replenish any genetic variation lost due to drift.

DxLH

The DxLH population was created by back-crossing the DxIV population (provided to us by Prof. Adam Chippindale) to the LH population for ten generations. DxLH males have a normal X chromosome and a normal Y chromosome. DxLH females have a normal Y chromosome and a compound X chromosome [C(1)DX yf]. This ensures that sons inherit their X chromosome from their father and their Y-chromosome from their mother. Both DxLH males and females have autosomes derived from LH.

Clone generators (CG)

CG males and females have a translocation between the two major autosomes [T(2;3) rdgCst in ripPbwD] [39]. CG females have a compound X chromosome [C(1)DX yf] and a Y chromosome. Males have a Y chromosome and an X [snsu(b)] chromosome. CG females enabled us to sample entire haploid genomes (barring the "dot" chromosome 4) and maintain them indefinitely without being damaged by recombination.

Sampling and maintaining hemigenomes

We followed a protocol of sampling and maintaining hemigenomes that was similar to the one described by Abbott and Morrow [40]. We chose forty-three males from the LH population randomly. We housed them in separate food-vials with 3 virgin CG females each. From each of the forty-three crosses, we selected one brown-eyed male offspring. Each of these brown-eyed male offspring had a unique haploid "hemigenome" from LH. We then crossed them with 3 virgin CG females each. Absence of molecular recombination in male *D. melanogaster* and the unique features of CG females ensure that the sampled hemigenome gets passed on faithfully from sire to son without being recombined (with the exception of the "dot chromosome"). Each of these 43 crosses represents a unique hemigenome line. We maintained each hemigenome line subsequently by crossing 10 brown-eyed males with 20 CG females every generation. The brown-dominant and scarlet-recessive eye-colour markers on the translocation of the CG females

enabled us to distinguish between males that carried the sampled hemigenomes (which were brown-eyed as they were heterozygous for the translocation) and males that were homozygous for the translocation (which were white-eyed). (See Box 2 of [40] for a detailed schematic.) Four hemigenome lines were lost in an accident. Therefore, we present data from 39 lines.

Fitness assays

We expressed each hemigenome in males and females carrying the rest of the genome from the LH population and measured their adult fitness at male biased (8 females: 24 males), equal (16 females: 16 males) and female biased (24 females: 8 males) sex ratios. Barring the sex ratios, we tried to ensure that the environment of the fitness assays mimicked the typical LH environment as closely as our experiments could permit.

Female fitness assay

Generating experimental flies In order to express hemigenomes from each line in females containing a random background from the LH population, we crossed brown-eyed males (heterozygous, carrying the target hemigenome and the translocation) with virgin LH females. To that end, first we collected 30 vials containing 150 eggs each from the LH population. The females emerging from these vials were collected as virgins (within 6 h of their eclosion) with the help of mild CO_2 anesthesia by sorting them into vials containing 10 females each. These females were then combined with brown-eyed males from each hemigenome line. For every hemigenome line we set up three to four vials, each containing 5 males from that line and 10 virgin LH females. We allowed these males and females to interact for two days in presence of ad-libitum live yeast (to boost fecundity) and then transferred them to fresh food vials for oviposition for around 18 h. After discarding the adults, we trimmed the egg-density in each vial to around 250, so that the expected number of larvae surviving in each vial would be around 125. Half the eggs were expected to be unviable. This was a consequence of the fact that the males used for this cross were heterozygous for the translocation between chromosome 2 and chromosome 3. This meant that the progeny that inherited a translocated autosome along with a normal chromosome 2 or chromosome 3 from their father (expected to be 50% of the total progeny) were unviable, as they either carried an extra portion of chromosome 3, while missing a portion of chromosome 2, or the other way around. We kept the expected larval density lower than the normal density in the LH population (around 150 per vial) in order to avoid overcrowding in vials that had higher than expected levels of survivorships. Red-eyed females emerging from these vials would be females carrying the target hemigenomes expressed in a random LH background. We refer to these as "focal females". Brown eyed females (which were heterozygous for the translocation) were discarded. In order to generate males and competitor females for the assay, we also collected 100 vials of 150 eggs each from the LHst population on the day the eggs from the crosses were trimmed. This ensured that on the day of the experiment all experimental flies were of the same age.

Fitness assay We collected focal females (red-eyed female progeny emerging from the crosses described above) as virgins using light CO_2 anesthesia and held them in food-vials at a density of 8 females per vial. On the 12th day post egg collection, when all experimental flies were 2–3 day old as adults, we set up adult competition food-vials supplemented by 100 µL of live- yeast suspension in water. The concentration of the yeast suspension was adjusted according to the sex ratio treatment such that the per-female yeast availability in the vial was always 0.47 mg. In these adult competition vials, we combined the focal females with competitor LHst females and LHst males at appropriate numbers depending on the sex ratio treatment. Regardless of the sex ratio treatment, the total number of flies (males + females) in a vial was always 32, and the ratio of focal females to competitor females was always 1:3. For the male biased sex ratio, each vial had 24 LHst males, 2 focal females and 6 LHst competitor females. The equal sex ratio had 16 LHst males, 4 focal females and 12 LHst competitor females in each vial. The female biased sex ratio had 8 LHst males, 6 focal females and 18 LHst competitor females. We allowed males and females to interact in the adult competition vials for two days. Subsequently, from each vial (regardless of the sex ratio) we transferred two focal females to a fresh food-vial for egg-laying. We discarded these females after 18 h and counted the eggs laid in that period, which was used as a measure of the fitness of the focal females in that vial. We performed two replicate assays for each of the sex ratios, all on separate days. For each replicate assay of each sex ratio we set up 7 adult competition vials for every hemigenome family. However, due to experimental contingencies, in some cases we had to set up fewer than 7 adult competition vials for some hemigenome lines. See Additional file 1 for more details. Overall, we assayed the fecundity of nearly 3276 females (39 lines × 3 sex ratios × 2 replicate assays × 7 adult competition vials × 2 females from each adult competition vial).

Male fitness assay

Generating experimental flies The protocol for generating flies for the male fitness assay was similar to the female fitness assay, except that instead of crossing brown-eyed males from each hemigenome line to LH females, we crossed them to virgin DxLH females. This ensured that the red-eyed male progeny emerging from these crosses (the "focal males") had the target hemigenomes expressed in a random background from the LH population. The eggs laid in the crosses between brown-eyed males from each line and DxLH females were trimmed to a density of around 500 so as to ensure the larval density would be around 125. Note that among all the zygotes from the crosses described above, half the zygotes were expected to be unviable as they either carried two Y chromosomes, or had an X chromosome in addition to a compound X chromosome. Among the remaining zygotes, half were expected to be unviable as they either carried an extra portion of chromosome 3, while missing a portion of chromosome 2, or the other way around, which was a consequence of the sires being heterozygous for the translocation between chromosome 2 and chromosome 3. Therefore, among all the eggs laid, only about a quarter were expected to survive. We also collected 100 vials of 150 eggs each from the LHst population to generate competitor males and females for the fitness assay.

Fitness assay The design of the male fitness assay mirrored that of the female fitness assay. We collected focal males (red-eyed male progeny emerging from the crosses described above) as virgins in food-vials in groups of 8. We also collected as virgins LHst females in groups of 8 per food-vial and competitor LHst males in groups of 6 per vial. On the 12th day post egg collection, when all experimental flies were 2–3 day old as adults, we set up adult competition vials as described for the female-fitness experiment. We then combined focal males, competitor LHst males and LHst females in the adult competition vials in appropriate numbers based on the sex ratio (Male biased: 6 focal males, 18 LHst competitor males, 8 LHst females; Equal: 4 focal males, 12 LHst competitor males, 16 LHst females; Female biased: 2 focal males, 6 LHst competitor males, 24 LHst females). We let the flies interact in the adult competition vials for two days. On the 14th day post egg collection, from each vial we transferred 7 randomly chosen LHst females individually into separate test-tubes containing food for oviposition. After 18 h, we discarded the females and incubated the test tubes in standard maintenance conditions. Twelve days later, when all progeny in the test tubes had developed into adults we froze the test-tubes at − 20 °C. We scored the progeny from each test-tube for their eye colour. The proportion of red-eyed progeny among all the progeny from the 7 test tubes corresponding to a vial was used as the measure of the fitness of focal males from that vial. For males too, we performed two replicate assays for each of the sex ratio-treatments, with all six assays being set up separately. Within each assay, for every sex ratio treatment, we set up 5 adult competition vials for every hemigenome family. In some cases, there were fewer than 5 adult competition vials. See Additional file 1 for details. Thus, in total, we scored the progeny for eye colour from nearly 8190 females (39 lines × 3 sex ratios × 2 replicate assays × 5 adult competition vials × 7 females from each adult competition vial).

Statistical analysis

All analyses were performed in R version 4.0.2.

In order to examine if there was a significant effect of hemigenome line and its interaction with sex and sex ratio, we used the R packages "lme4" [84] and "lmerTest" [85] to fit the following linear mixed effects model on male and female fitness data scaled and centred separately for each day of the experiment:

$$\begin{aligned}\text{Standardised Fitness} \sim\ & \text{Sex} + \text{Sex.Ratio} + \text{Sex} : \text{Sex.Ratio} \\ & + \left(1|\text{Hemigenome line}\right) + \left(1|\text{Hemigenome line} : \text{Sex}\right) \\ & + \left(1|\text{Hemigenome line} : \text{Sex.Ratio}\right) + \left(1|\text{Hemigenome line} : \text{Sex} : \text{Sex.Ratio}\right).\end{aligned}$$

In order to calculate the $r_{w,g,mf}$ we calculated the mean fitness associated with hemigenome line in both males and females. To that end first we arcsin-square-root transformed the male fitness data for each adult competition vial. We divided the data for each day by the mean fitness of that day. Since, we had performed two replicate fitness assays for each sex ratio with multiple measurements on each day, we calculated the average fitness for hemigenome lines for each sex ratio in two steps. For both males and females, for each sex ratio, we first calculated the average fitness for each hemigenome line on each of the two replicate days and then calculated the average of the two averages. We then scaled and centered the data for each sex × sex ratio combination separately. First, we used this data to calculate genetic correlations for sex-specific fitness across sex ratios. We then calculated the intersexual genetic correlation for fitness ($r_{w,g,mf}$) for each sex ratio. Following [46, 62], we also

calculated the proportion of fitness variation along the sexually antagonistic axis by rotating our original coordinate system represented by a female fitness axis (X-axis) and a male fitness axis (Y-axis) by 45° in the anti-clockwise direction. As a result of this transformation the new X-axis is the axis of sexually concordant fitness variation, while the new Y-axis is the axis of sexually antagonistic fitness variation. We used the following matrix operation separately for the scaled and centered data for each sex ratio: $\begin{pmatrix} \overline{W}_{C,i} \\ \overline{W}_{A,i} \end{pmatrix} = \begin{pmatrix} 1/\sqrt{2} & 1/\sqrt{2} \\ -1/\sqrt{2} & 1/\sqrt{2} \end{pmatrix} \begin{pmatrix} \overline{W}_{F,i} \\ \overline{W}_{M,i} \end{pmatrix}$, where $\overline{W}_{C,i}$ and $\overline{W}_{A,i}$ are the sexually concordant and sexual antagonistic fitness components, respectively for the hemigenome line i for that sex ratio, and $\overline{W}_{F,i}$ and $\overline{W}_{M,i}$ are the average female and male fitnesses, respectively for the hemigenome line i for that sex ratio. We then calculated the proportion of variance in fitness lying along the sexually antagonistic axis for each sex ratio.

In order to calculate 95% confidence intervals around our estimates of across sex ratio correlations for sex-specific fitness, $r_{w,g,mf}$ and proportion of sexually antagonistic fitness variation we used a stratified bootstrap approach using the R package "boot" [86]. For each sex ratio, we created 10000 data-sets by sampling with replacement within each sex × hemigenome line × day combination. This procedure ensured that each of the bootstrapped data-sets had representation from each sex × hemigenome line × day combination in the same proportions as the original data-set. We also calculated 95% confidence intervals for differences between $r_{w,g,mf}$ and proportion of sexually antagonistic fitness variation estimates of male biased and female biased sex ratios to test if they included 0.

Following [46], we used the R package "MCMCglmm" [87] to fit a Bayesian linear mixed effects model using Monte Carlo sampling methods to estimate across sex ratio correlations for sex-specific fitness, $r_{w,g,mf}$ and male and female heritabilities for each sex ratio separately. We first scaled and centered arcsin-squareroot transformed male fitness data and female fitness data separately for each day. We fit the following model for each sex ratio: $W_{ijkmn} \sim S_i + R_j + S.R_{ij} + L_{ijk} + D.L_{km} + \varepsilon_{ijkmn}$, where W_{ijkmn} is the scaled and centered fitness of adult-competition vial n of sex i, sex ratio j, and hemigenome line k on day m. S_i, R_j and $S.R_{ij}$ represent the fixed effects of sex, sex ratio and their interaction. L_{ijk} represents a term corresponding to the sex-specific random effect of each hemogenome line for sex ratio j. $D.L_{km}$ represents a scalar corresponding to the random interaction of day and hemigenome line. L_{ijk} is modeled to follow a multivariate normal distribution with a mean 0, and whose variance–covariance matrix is given by the additive genetic variance in female fitness ($\sigma^2_{w,g,f}$) and male fitness ($\sigma^2_{w,g,m}$) in each of the three sex ratios; the intersexual genetic covariance for fitness ($Cov_{w,g,mf}$) for each of the three sex ratios; as well as sex-specific genetic covariances for fitness between male biased and female biased sex ratio ($\sigma^2_{w,g,mb-fb}$), between male biased and equal sex ratio ($\sigma^2_{w,g,mb-e}$), and between female biased and equal sex ratio ($\sigma^2_{w,g,e-fb}$); along with other terms corresponding to genetic covariances for fitness across sex and sex ratios both. ε_{ijkmn} represents the sex and sex ratio specific residuals. ε_{ijkmn} is modeled to follow a normal distribution with a mean 0 and variance given by the sex and sex ratio specific residual fitness variance ($\sigma^2_{w,r,m}$ for males and $\sigma^2_{w,r,f}$ for females for each of the three sex ratios). We used these estimates to calculate the following sex- or sex ratio-specific quantitative genetic parameters:

1. Genetic covariance for fitness between male biased and female biased sex ratio in sex i, $r_{w,g,mb-fb,i} = \frac{Cov_{w,g,mb-fb,i}}{\sqrt{\sigma^2_{w,g,fb,i}}\sqrt{\sigma^2_{w,g,m,i}}}$.
2. Genetic covariance for fitness between male biased and equal sex ratio in sex i, $r_{w,g,mb-e,i} = \frac{Cov_{w,g,mb-e,i}}{\sqrt{\sigma^2_{w,g,mb,i}}\sqrt{\sigma^2_{w,g,e,i}}}$.
3. Genetic covariance for fitness between equal and female biased sex ratio in sex i, $r_{w,g,e-fb,i} = \frac{Cov_{w,g,e-fb,i}}{\sqrt{\sigma^2_{w,g,e,i}}\sqrt{\sigma^2_{w,g,fb,i}}}$.
4. Heritability for female fitness in sex ratio j, $h^2_{w,f,j} = \frac{\sigma^2_{w,g,f,j} \times 2}{\sigma^2_{w,r,f,j} + \sigma^2_{w,g,f,j}}$.
5. Heritability for male fitness in sex ratio j, $h^2_{w,m,j} = \frac{\sigma^2_{w,g,m,j} \times 2}{\sigma^2_{w,r,m,j} + \sigma^2_{w,g,m,j}}$.
6. Intersexual genetic correlation for fitness in sex ratio j, $r_{w,g,mf,j} = \frac{Cov_{w,g,mf,j}}{\sqrt{\sigma^2_{w,g,f,j}}\sqrt{\sigma^2_{w,g,m,j}}}$.

Abbreviations

IaSC: Intra-locus sexual conflict; IeSC: Inter-locus sexual conflict; $r_{w,g,mf}$: Intersexual genetic correlation for fitness.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12862-022-01992-0.

Additional file 1. Details of the sample sizes.

Additional file 2. Data file.

Additional file 3. Metadata (readme file) for the data file.

Acknowledgements
MGA thanks CSIR, Govt. of India for Senior Research Fellowship. TSC thanks IISER Mohali for Senior Research Fellowship. SDB thanks IISER Mohali for Summer Research Fellowship. S thanks DST, Govt. of India for INSPIRE Scholarship. We would like to thank Naginder Kumar, Bhupender Negi, Rohit Kapila, Neetika Ahlawat, Komal Maggu, Aparajita, Aabeer Kumar Basu, Amisha Agarwala, Jigisha, Aaditya Narasimhan, Syed Imran, Broti Biswas, Mayank Kashyap for their help in lab-work. We would like to thank Prof. Adam Chippindale for providing us with the clone-generator system.

Authors' contributions
MGA and NGP designed the experiments. MGA, TSC, RM, SDB and S carried out the experiments. MGA, TSC and NGP interpreted the results. MGA wrote the first draft of the manuscript with the help of discussions with RM, SDB and S. All authors reviewed the manuscript and contributed to subsequent revisions. All authors read and approved the final manuscript.

Funding
This research was funded by the Indian Institute of Science Education and Research Mohali.

Availability of data and materials
The data used in this study, along with the corresponding metadata, has been included as Additional files 2 and 3, respectively.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publications
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 9 December 2021 Accepted: 15 March 2022
Published online: 28 March 2022

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