X and Sex: Role of the X chromosome in inter and intra-locus sexual conflict in *Drosophila melanogaster*

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Certificate of Examination

This is to certify that the dissertation titled "X and Sex: Role of the X chromosome in inter and intra-locus sexual conflict in *Drosophila melanogaster*" submitted by Ms. Jigisha (MS15124) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

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(Supervisor)

Date

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. N. G. Prasad at the Indian Institute of Science Education and Research Mohali. This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bona fide record of original work done by me and all sources listed within have been detailed in the bibliography.

Jigisha

Dated: May 4, 2020

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. N. G. Prasad

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"Help"- I'll be using this word quite frequently in the coming few sentences because it took a great amount of help from many people to get this thesis done. This is also why I have written this thesis in the first-person plural. I thank TJ and Manas for being so approachable and for their help at all stages of the project- from experimental design and population maintenance to monotonous progeny count and stimulating discussions. A huge thank you to Mayank and his 'awesome' aspiration skills for helping me out literally every other week. I thank Amisha for her help with everything- working out crosses, managing cooks, maintaining populations and being a great friend through it all. Thanks to JJ, Paresh, Abhishek, Santy, Sohit, Aaditya, Imran, Neetika and Komal for always being ready to help and making it possible for me to meet deadlines. I thank Broti, Poddar, Vivek, Akshay and all the undergrads who showed up to help me with progeny counting- Rakesh, Soumya, Cheshta, Kimaya, Britny, Shubhangi and Akanksha. I must also thank Neetika for 'adopting' me in 2017 and teaching me fly handling, and Kapila for his words of wisdom and being my partner-in-crime. I thank Nagender bhaiya and Negi bhaiya for their constant assistance. I thank all EBL members, past (special shoutout to Zeeshan and Bhatti) and present, for making lab the fun, happy and safe place it was.

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Abstract

Males and females often have different fitness optima for many shared traits but are unable to achieve these optima because of the constraint of a shared genome. This leads to the accumulation of sexually antagonistic variation (SAV) in a population that favours one sex and is detrimental to the other. Theory predicts that such sexually antagonistic variation is most likely to be present on the X chromosome (in an XY mating system). Various empirical studies have tested this prediction in many systems and have produced mixed results. In this study, we first investigated if there was any X linked SAV in a laboratory-adapted baseline population of *Drosophila melanogaster*. We then explored how the degree of sexual antagonism varied in populations evolved under increased and decreased levels of sexual conflict. For this, we used a population of *D. melanogaster* that is subjected to the selection pressure of a male-biased or female-biased sex ratio every generation. We isolated 25 random X chromosomes from males of all three replicates of each selection regime and expressed them in random autosomal backgrounds. We then performed fitness assays on males and females expressing these X chromosomes. We looked at the genetic correlation of fitness between the sexes to comment on the degree of sexual antagonism. We did not find evidence of X-linked sexual antagonism in our populations. We also found that the X chromosome did not contribute significantly to the fitness differences between individuals from the two selection regimes. Our results add to a growing body of work that suggests that the X chromosome may not be a hot-spot for sexually antagonistic variation and it may be worthwhile to explore other parts of the genome and their interactions.

1. Introduction

The evolutionary interests of the two sexes are divergent in most sexually reproducing species, and this causes the sexes to often be at conflict with each other (Parker, 1979). Stemming from differential investment in gametes (Trivers, 1972), examples of such 'sexual conflict' are almost ubiquitous (Arnqvist & Rowe, 2005). In many species, including the fruit fly *Drosophila melanogaster*, the optimal number of matings for males is higher than that for females (Bateman, 1948). This may lead to a conflict over remating rates, with males and females potentially co-evolving antagonistic persistence and resistance strategies, respectively (Holland & Rice, 1998; Rice & Holland, 1997). In the above-stated scenario, if different loci were responsible for mating rate in the two sexes, the resulting conflict would be between different loci or inter-locus (reviewed in Chapman et al., 2003) .

The other kind of sexual conflict, intra-locus sexual conflict, occurs when the two sexes experience different selection pressures as a result of having different fitness optima for the *same* trait. Examples of such traits include adult locomotory activity in *Drosophila melanogaster* (Long & Rice, 2007), immune defence in lizards *Uta stansburiana* (Svensson et al., 2009), wing length in great reed warblers *Acrocephalus arundinaceus* (Tarka et al., 2014). The sexes are, however, prevented from attaining their respective optima due to the constraint of a shared genome. As a result, sexually antagonistic variation accumulates in a population (Rice & Chippindale, 2001). Such alleles, when expressed in one sex, enhance fitness but have a detrimental effect on fitness when expressed in the other sex. If mechanisms of sex-specific or sex-limited expression of traits under sexual conflict can evolve via modifiers or gene duplication, the conflict over that trait can potentially get resolved and eventually lead to sexual dimorphism (Bonduriansky & Chenoweth, 2009; but see Harano et al., 2010). This way, each sex can be allowed to achieve its optimum trait value. Quantitatively, intra-locus sexual conflict in a population is characterized by negative values of the intersexual genetic correlation for fitness (r_{mf}). $\mathbf{r_{mf}}$ is defined as the ratio of the additive genetic covariance between the sexes (COV_{Amf}) to the geometric mean of additive

genetic variance of males (V_{Am}) and females (V_{Af}) for the trait (here, fitness) (Bonduriansky & Chenoweth, 2009).

$$r_{mf} = \frac{COV_{Amf}}{\sqrt{V_{Am} \times V_{Af}}}$$

Rice (1984) theorized that sexually antagonistic polymorphisms in a population are more likely to be X-linked than autosomal. He used a one-locus two-allele population-genetic model to derive the equilibrium frequency of a sexually antagonistic allele that conferred a cost T upon one sex and a benefit S upon the other, relative to an allele with equal fitness effects in both sexes. He showed that the frequency gain of this allele depended on the S/T ratio, and polymorphism would be expected over a wide range of S/T values if the locus was X-linked and not autosomal, in an XY system. Empirical evidence for this prediction comes from both field and laboratory-based studies. A study on a natural population of the red deer *Cervus elaphus* showed a negative correlation between father-daughter fitness and no correlation between father-son fitness, suggesting X-linked sexual antagonism (Foerster et al., 2007). A series of studies on *Drosophila melanogaster* further strengthened this theory. Gibson et al. (2002) randomly sampled 20 X chromosomes from a large lab-adapted population and expressed them in random autosomal backgrounds using cytogenetic cloning techniques. They compared the fitness variation present on these X chromosomes to the genome-wide levels using data from a previous study (Chippindale et al., 2001). Their results showed that around 97% of the genome-wide sexually antagonistic variation was present on the X chromosome. A subsequent study on the same population identified candidate genes under sexually antagonistic selection and found them to be overrepresented on the X chromosome (Innocenti & Morrow, 2010). Other laboratory studies on *Drosophila* have also shown evidence of X-linked sexually antagonistic variation (Connallon & Jakubowski, 2009; Oneal et al., 2007; Pischedda & Chippindale, 2006).

However, this theory is not unchallenged. Fry (2010) argued that if Rice's assumption of equal dominance in both sexes was relaxed, it could be mathematically shown that the autosomes provide more flexible conditions for sexually antagonistic variation (SAV) to be present, compared to the X chromosome. There also exists experimental evidence showing that SAV is present mainly on autosomes in different species like side-blotched

lizards(Calsbeek & Sinervo, 2004), the cricket *Allonemobius socius* (Fedorka & Mousseau, 2004) and *Drosophila serrata* (Delcourt et al., 2009). Further, the studies mentioned earlier in support of the X-linked hypothesis have been critiqued to have small or non-random samples (Ruzicka, 2018). Thus, further investigation is needed to establish the generality of this theory.

Inter-locus and intra-locus sexual conflict differ fundamentally in the level of biological organisation they take place over. While the former occurs over the outcome of reproductive interactions between males and females, the latter arises out of genetic constraints of shared loci (Schenkel et al., 2018; Van Doorn, 2009). However, since both are likely to affect the evolution of reproductive traits, it would be interesting to investigate how the two interact. For instance, we would expect genes under sexual selection to also show a bias in terms of residing on the X chromosome (Gibson et al., 2002; Van Doorn, 2009). A few theoretical studies have addressed this and have found interesting evolutionary dynamics arising from this interaction (Pennell et al., 2016; Pennell & Morrow, 2013), but empirical studies in this field remain lacking.

Here, we attempt to address a few of the missing links mentioned earlier using laboratory-adapted populations of *Drosophila melanogaster*. *D. melanogaster* has four pairs of chromosomes- 1 is the sex chromosome (X/Y), 2 and 3 are large autosomes and 4 is the dot chromosome (Kaufman, 2017). Sex determination in this species depends on several X-encoded signal elements that depend on the number of X chromosomes (Erickson & Quintero, 2007). Generally, males are of the type XY and females XX. In this study, we first investigated if there was X-linked sexually antagonistic variation in our baseline population using cloning techniques similar to those described in Gibson et al., 2002. Next, we explored the contribution of the X chromosome in fitness variation in populations evolved under increased and decreased levels of inter-locus sexual conflict. For this, we used two populations subjected to selection pressures of male-biased (M) and female-biased (F) sex ratios. As a result of this selection imposed every generation,

- M males had evolved to court more, harm their mates more, have increased sperm competitive abilities and locomotory activity, and sire more offspring compared to F males (Nandy, Chakraborty, et al., 2013; Nandy, Gupta, et al., 2013)

- M females had evolved higher mate harm resistance abilities than F females (Nandy et al., 2014)

We randomly sampled X chromosomes from males of these populations, cloned and amplified them, and expressed them in males and females. We performed fitness assays for both sexes and compared results between M and F males and females. Finally, we asked how the degree of X-linked sexual antagonism changed in populations evolved under varying intensities of inter-locus sexual conflict.

2. Materials and Methods

Study System

We used laboratory-adapted populations of *Drosophila melanogaster* selected for increased and reduced levels of sexual conflict for this study. These selection lines were derived from the LHst baseline population, a derivative of the LH population. LH is a large, outbred, long term laboratory adapted population of *Drosophila melanogaster* maintained in a 14 day discrete generation cycle at 25◦C, 12 h light/12 h dark, 60%-80% relative humidity conditions on standard cornmeal yeast molasses media (Appendix A) in standard vials (90 mm length X 30 mm diameter) (Chippindale et al., 2001). LHst has the same genetic background as LH but possesses a benign, autosomal recessive scarlet eye colour marker (Prasad et al., 2007). Three replicate populations (C_{1-3}) were derived from LHst and maintained at equal sex ratios. After five generations, two sex ratio regimes were derived from all three C populations. The male biased regime (M_{1-3}) had 3 adult males for every adult female while the female biased regime (F_{1-3}) had 3 adult females for every adult male (Nandy, Chakraborty, et al., 2013). The populations with the same numerical subscript were more closely related to each other than to those with different subscripts. All populations (M, C, F) with the same numerical subscript were handled together during population maintenance and were treated as one statistical 'block'. These populations were reared at a controlled larval density of 140-160 per 8-10 ml food under environmental conditions identical to those experienced by their ancestors, LHst and LH. Every generation, adults were collected as virgins and housed in single sex vials until the 12^{th} day post egg laying (of the previous generation) when they were combined in their respective sex ratios. These adult competition vials also contained 0.47mg of live yeast per adult female (given in the form of yeast suspension in water). 48 hours later, all flies were transferred to fresh food vials where females were allowed to oviposit. After 18 hours, all adults were discarded, and excess eggs were culled to maintain larval density at approximately 150 per vial, marking the beginning of a new generation.

Sampling and Cytogenetic Cloning of X chromosomes

In this study, we sampled and subsequently cloned X chromosomes from the LH, M and F populations to investigate how selection for increased or reduced sexual conflict affected the degree of X-linked sexual antagonism. This involved setting up a series of crosses to obtain the target X chromosomes in random, ancestral autosomal backgrounds (Figure 2.1). 25 X chromosomes were sampled from LH, the ancestral baseline population, and also from each block of the M and F populations (total = 175). This was done by randomly selecting 25 males from LH, and M and F (generation 243), and allowing them to mate with special clone generator (CG) *D. melanogaster* females. These females had a compound X chromosome (DX) comprising two X chromosomes fused together at the centromere, a Y chromosome (from LH base population), and a homozygous-viable translocation of the two major autosomes [T(2;3) *rdgC st in ri* p^P *bw*] (Prasad et al., 2007). This setup, along with the absence of intrachromosomal recombination in males of *Drosophila melanogaster,* allowed us to clone the target X chromosomes cytogenetically. The sex chromosomes of the clone generator females were of the kind XXY where the two Xs represent the compound X chromosome. When mated to the males containing the target X chromosomes, these females passed on the Y chromosome to their male offspring. As a result, their sons inherited the X chromosome from the fathers. Each male with the target X chromosome was housed with 5 clone generator females in a vial for 48 hours, following which the adults were removed, and their offspring were allowed to develop. All male offspring had the target X chromosome along with the Y chromosome from their CG mothers. Additionally, they had one set of autosomes from LH, M or F and the other set was the one with the translocation. 2 sons from each X chromosome 'family' were combined with 5 DXLH females. These females had a compound X chromosome and normal LH autosomes. Male progeny from this cross that had one set of translocated autosomes and the other from DXLH were selected using the phenotypic markers present on the translocated autosomes. 2 such males from each family were combined with 5 virgin DXLH females to get sons with the target X chromosome and random ancestral (LH) Y chromosome and autosomes. 4-6 such males from each family were combined with 4-6 DXLH virgin females every generation for 48 hours to maintain the stock for each family. 48 hours later, all adults were discarded and the egg density per vial was

controlled at about 120-150. These eggs were allowed to hatch and adult males from these vials were used to start the next generation.

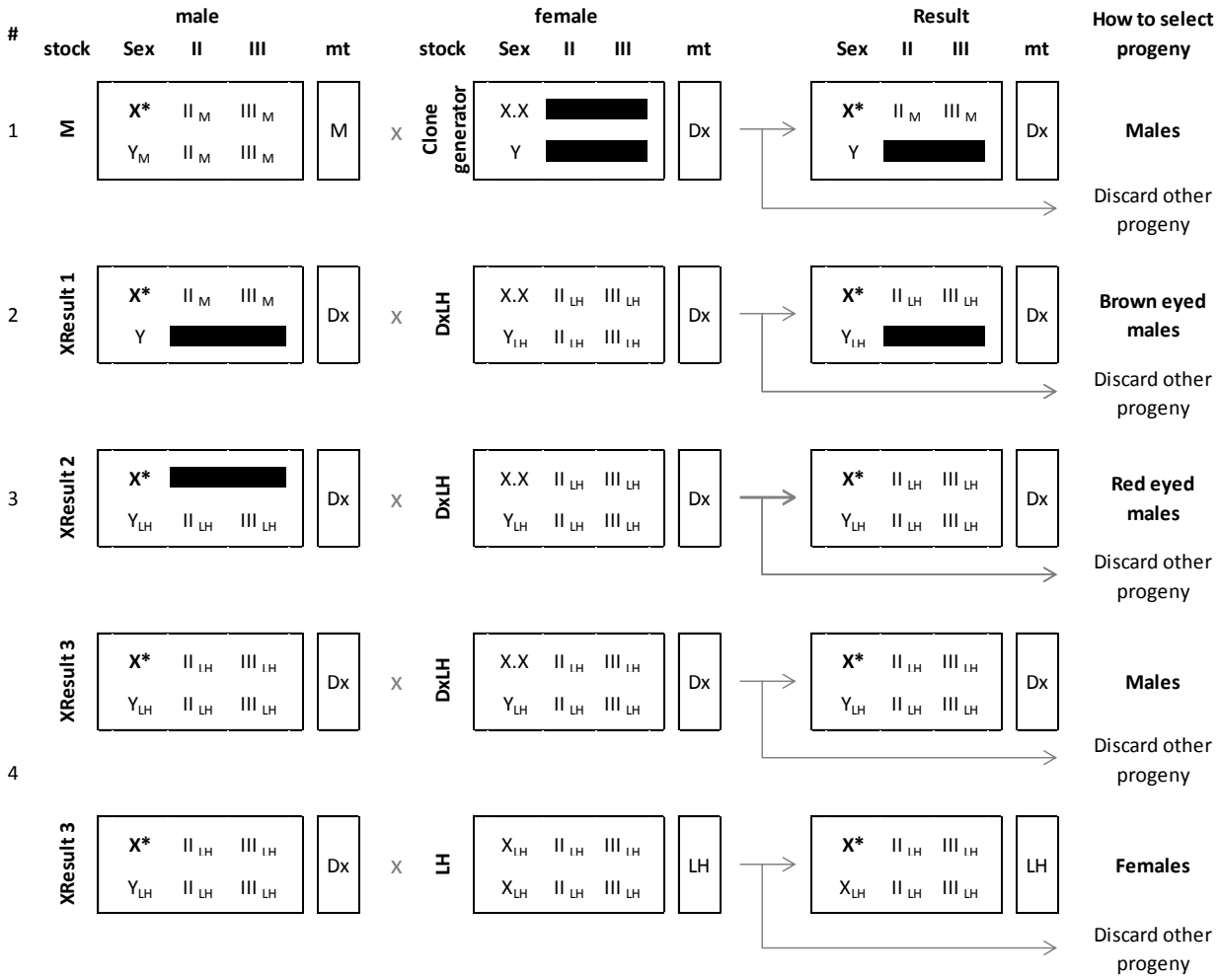

Figure 2.1: Schematic representation of the cytogenetic cloning of X chromosomes from M males. The target X chromosome is marked with a '*'. Credits: Amisha Agarwala

Generation of experimental flies

Male fitness assay

8 males from the stock vials were combined with 8 virgin DXLH females for 48 hours in standard food vials along with excess live yeast. 48 hours later, all adults were transferred to fresh oviposition vials for 18 hours after which, all adults were discarded. The egg density in

these vials was trimmed to approximately 150 per vial. The eggs laid during these 18 hours were allowed to hatch and develop as adults over 9 days, following which 5 males from each family were collected as virgins for the experiment. Eggs from LHst were also collected (density- 150 eggs/vial) on the day when the focal male vials were trimmed to ensure that all adults were of the same age on the day of the experiment. LHst males were collected as virgins and housed in densities of 10 and 15 per vial respectively until the 12^{th} day when the experiment was performed.

Female fitness assay

To obtain females that had one target X chromosome expressed in a random ancestral autosomal background, we combined 6 males from each family's stock with 6 virgin LH females for 48 hours in food vials in the presence of excess yeast. The adults were then transferred into fresh oviposition vials for 18 hours. The eggs laid during this 18-hour window were allowed to hatch and develop into adults. On the 12^{th} day post egg collection, 5 females from each family were collected for the experiment. Eggs from LHst were also collected on the day when the focal female vials were trimmed to ensure that all adults were of the same age on the day of the experiment. On the 12^{th} day post egg collection, LHst adults (15 males and 10 females per family) were sorted using light CO_2 anaesthesia into experimental vials.

Experimental Procedure

Male fitness assay

In order to get a measure of fitness of males with the target X chromosomes, we performed a competitive fertilization success (CFS) assay. On the 12^{th} day post egg collection, for each X family, 5 virgin focal males, 10 virgin competitor LHst males and 15 virgin LHst females were combined in a single food vial with excess live yeast for 48 hours. These conditions closely resembled the usual maintenance conditions of the LH and the C populations. After 48 hours, using light CO2 anaesthesia, 7 females were randomly sampled and singly housed in fresh food vials for oviposition. After 18 hours, all adults were discarded, and the eggs were allowed to develop into adults. 11 days later, these vials were frozen, and the number of

red eyed and scarlet eyed adult offspring in each vial was counted. The proportion of red eyed progeny was taken as the measure of male fitness.

Female Fitness Assay

We measured the fitness of the target X chromosomes in females using a competitive oviposition assay. On the 12^{th} day post egg collection, we sorted 10 LHst females and 15 LHst males into fresh food vials using light CO2 anesthesia. We also transferred 5 virgin focal females to these vials. All food vials contained 0.47 mg live yeast per female. All adults were housed in these vials for 48 hours, after which, the 5 focal (red eyed females) were singly transferred into fresh oviposition vials for 18 hours. Following oviposition, all adult females were discarded and the eggs were allowed to develop into adults. 11 days later, these vials were frozen and the number of adult offspring in each vial was counted and recorded as the measure of female fitness.

Statistical Analysis

Data from three replicates of female fitness assay and two replicates of male fitness assay were analysed using R v.3.6.3 (R Core Team, 2020) on RStudio (RStudio Team, 2016). All analyses were performed separately on the three blocks.

Mean male and female fitness values were obtained for each family by taking averages across all sampled individuals and then across replicates. These values were then divided by the average fitness of the fittest family in that sex to obtain relative fitness values. Intersexual genetic correlations were then calculated by using Pearson's product moment correlation.

The effects of selection and family on fitness variation were analysed using Bayesian mixed models in the R package "MCMCglmm" (Hadfield, 2010). Selection was treated as a fixed effect while family, as random. A random effects model was run on scaled fitness values to test for fitness variation on the X chromosome. A mixed effects model was run on absolute fitness values to determine the difference between LH, M and F. Flat priors were used in these models, similar to those used by Ruzicka et al. (2019). All models ran for 100,000 iterations with a burn-in phase of 25000 and a thinning interval of 100. Convergence of the models was tested by checking whether the chains mixed properly.

3. Results

Fitness variation on the X chromosome

A random effects model (MCMCglmm) was run to estimate the contribution of 'family' in fitness variation in both sexes of all populations independently. Distributions of the variance estimates were plotted as histograms to check significance of the effect. All distributions were 'pushed up against zero', suggesting that family did not have a significant effect on fitness variation of either sex in all blocks (Figs. 3.1-3.5, Tables B1 and B2).

Intersexual correlation for fitness

Pearson's product moment correlation between male and female relative fitness was calculated for LH and each block of M and F. The correlation values were not significantly different from zero in any of the seven cases (alpha = 0.05) (Table 3.1, Figures 3.6-3.8).

Effect of selection on fitness differences

Mixed effects models (MCMCglmm), with selection as a fixed factor and family as a random factor, were run to see how the X chromosome contributed to fitness differences between LH, M and F. The results of the models show that there are no fitness differences between the X chromosomes of LH, M and F in males as well as females. There was, however, one exception. Fitness of M females was significantly greater than that of F females in block 3 (post.mean = 2.5074, pMCMC = 0.0422). (Tables B3-B8)

Table 3.1: Pearson's product moment correlation between male and female relative fitness

Selection	Block	df	t	p	cor
LH		20	1.369	0.1862	0.292713
M	1	19	0.53306	0.6002	0.121387
M	2	20	1.2949	0.2101	0.278129
M	3	22	-0.11865	0.9066	-0.02529
F	1	20	0.21284	0.8336	0.047539
F	2	22	-0.02967	0.9766	-0.00633
F	3	19	-0.30749	0.7618	-0.07037

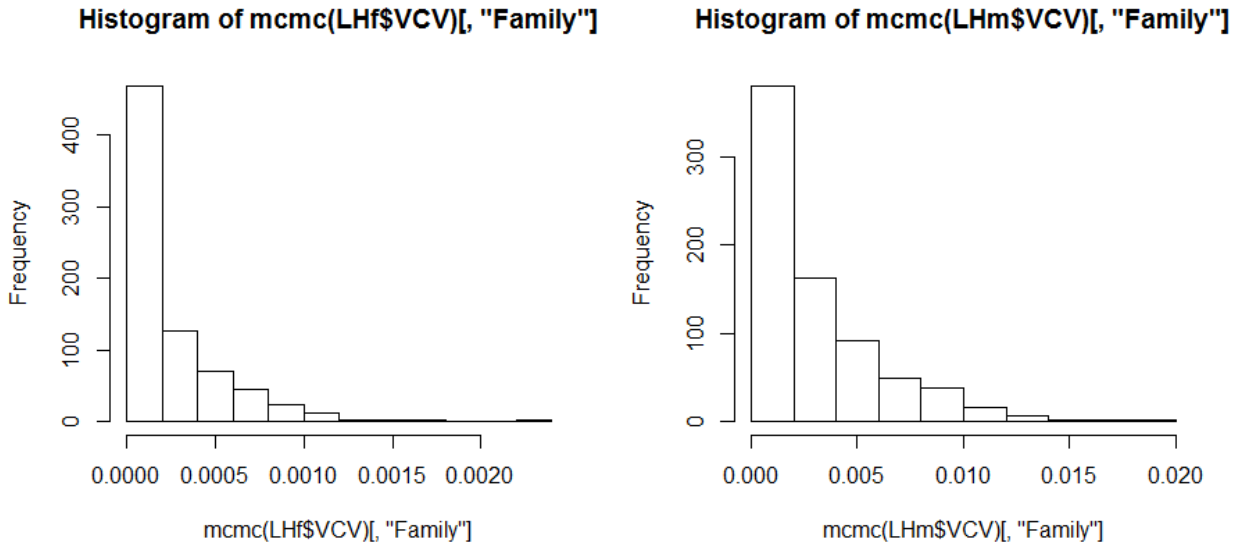

Figure 3.1: Distribution of variance estimate of female and male fitness attributed to 'family' in LH

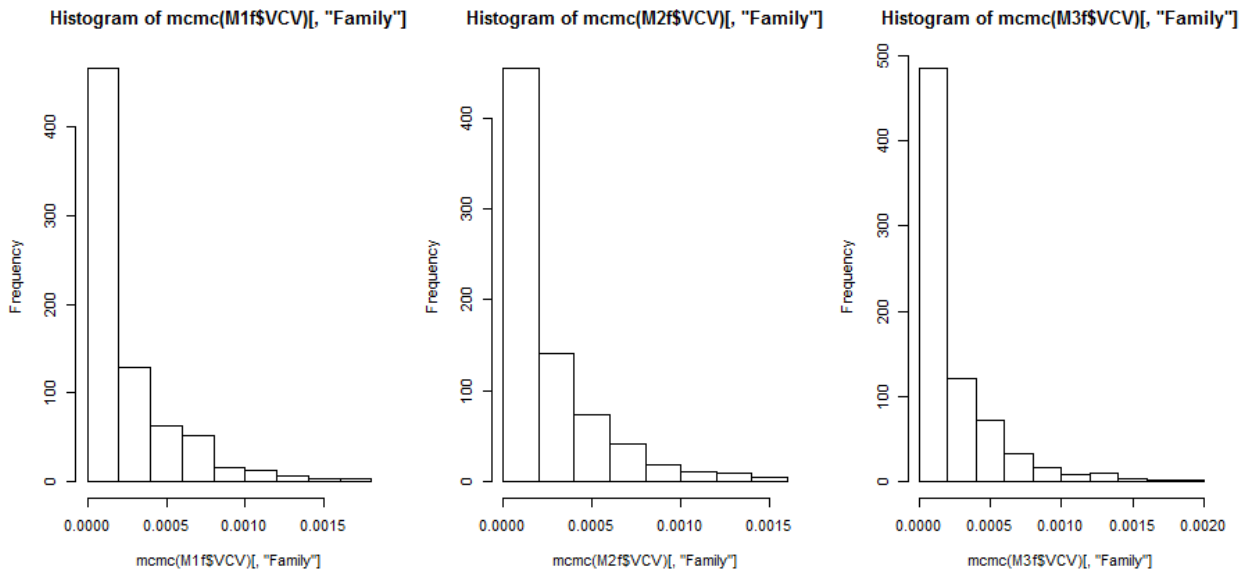

Figure 3.2: Distribution of variance estimate of female fitness attributed to 'family' in M1-3

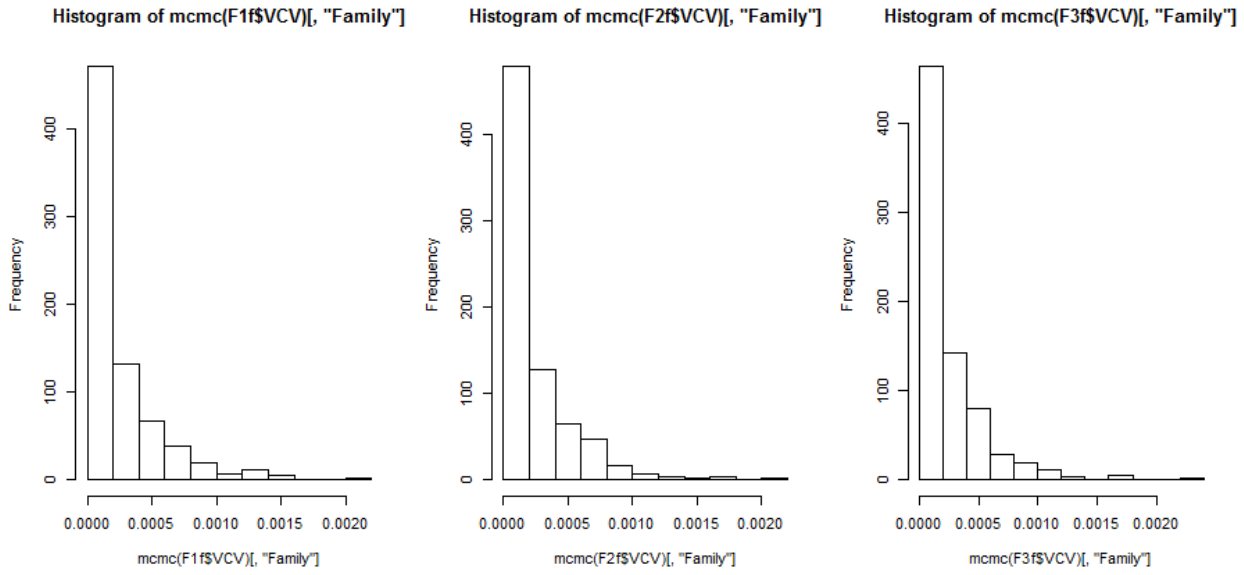

Figure 3.3: Distribution of variance estimate of female fitness attributed to 'family' in F1-3

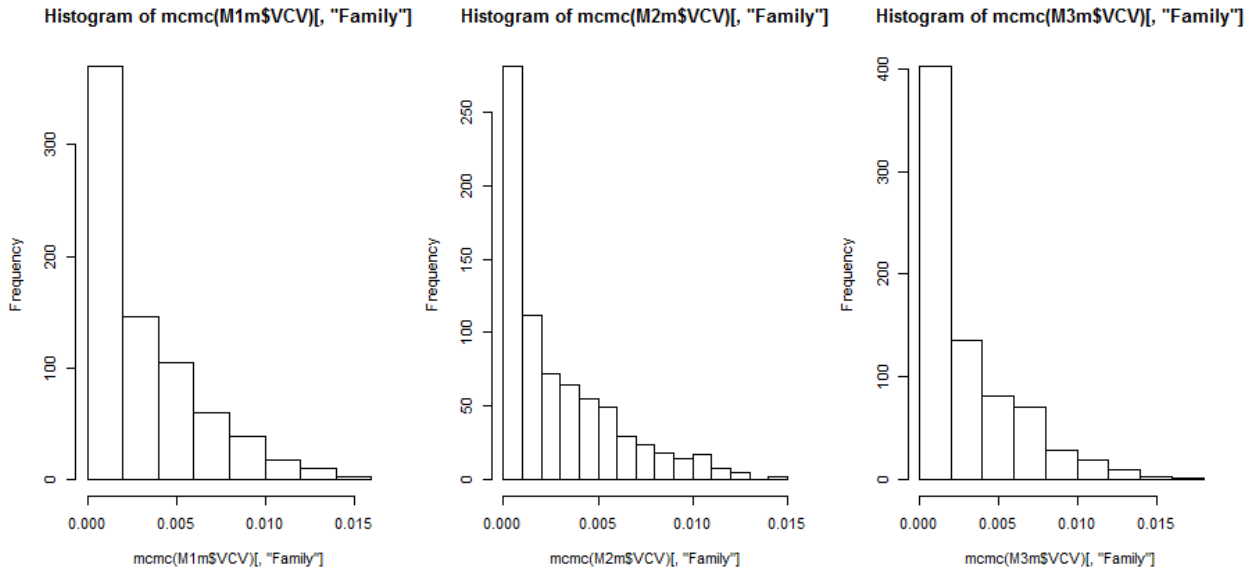

Figure 3.4: Distribution of variance estimate of male fitness attributed to 'family' in M1-3

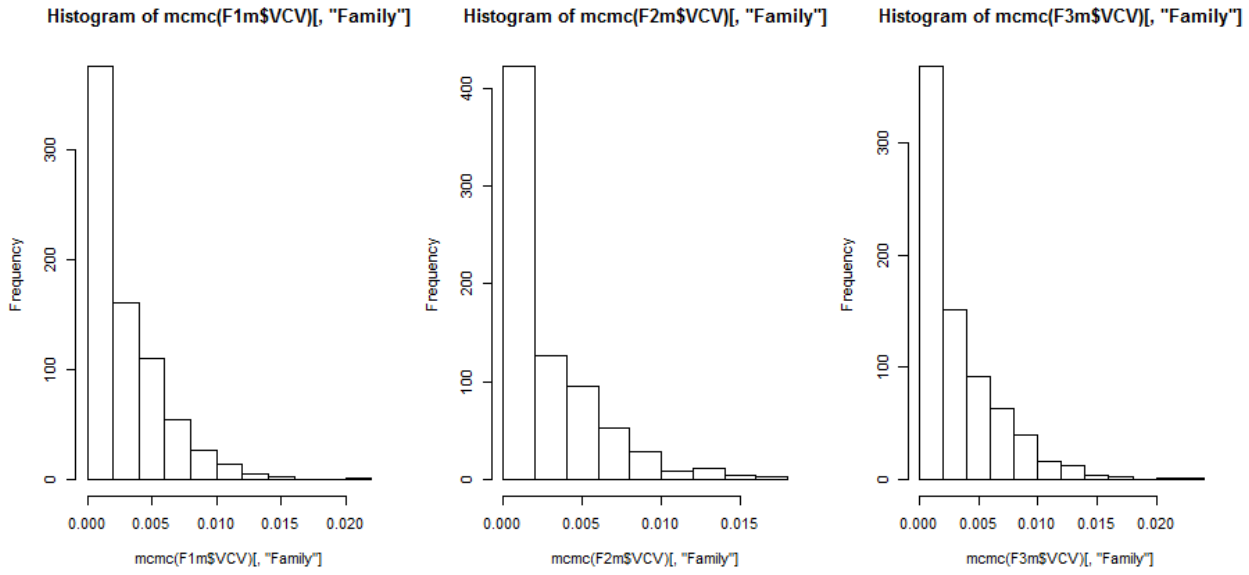

Figure 3.5: Distribution of variance estimate of male fitness attributed to 'family' in F1-3

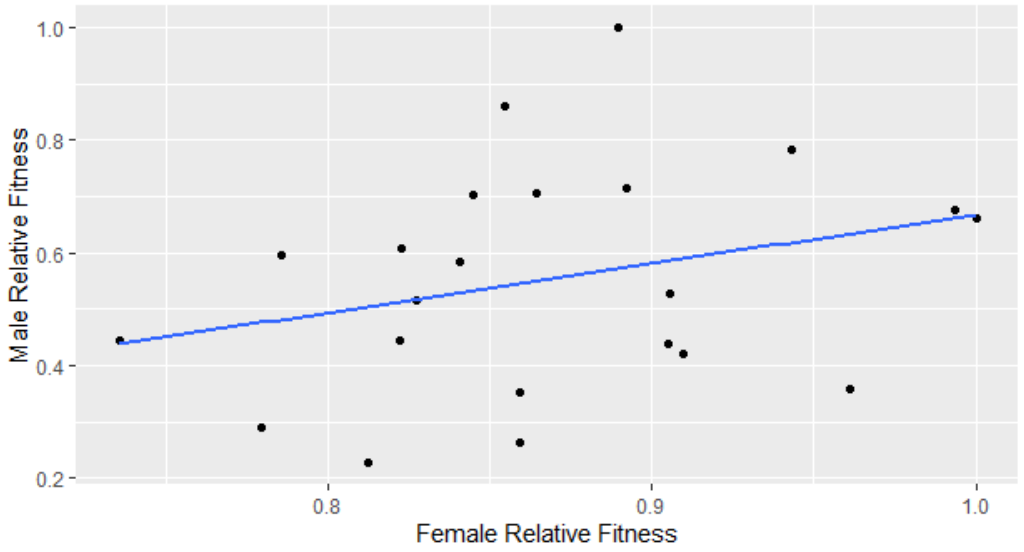

Figure 3.6: Intersexual regression for fitness in the LH population

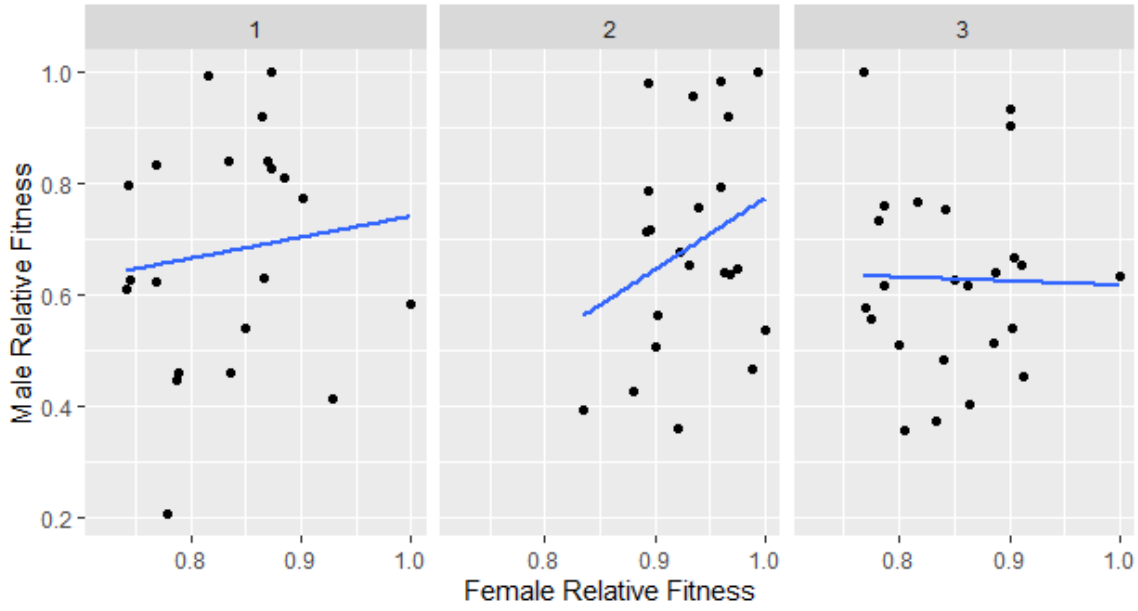

Figure 3.7: Intersexual regression for fitness in M1-3

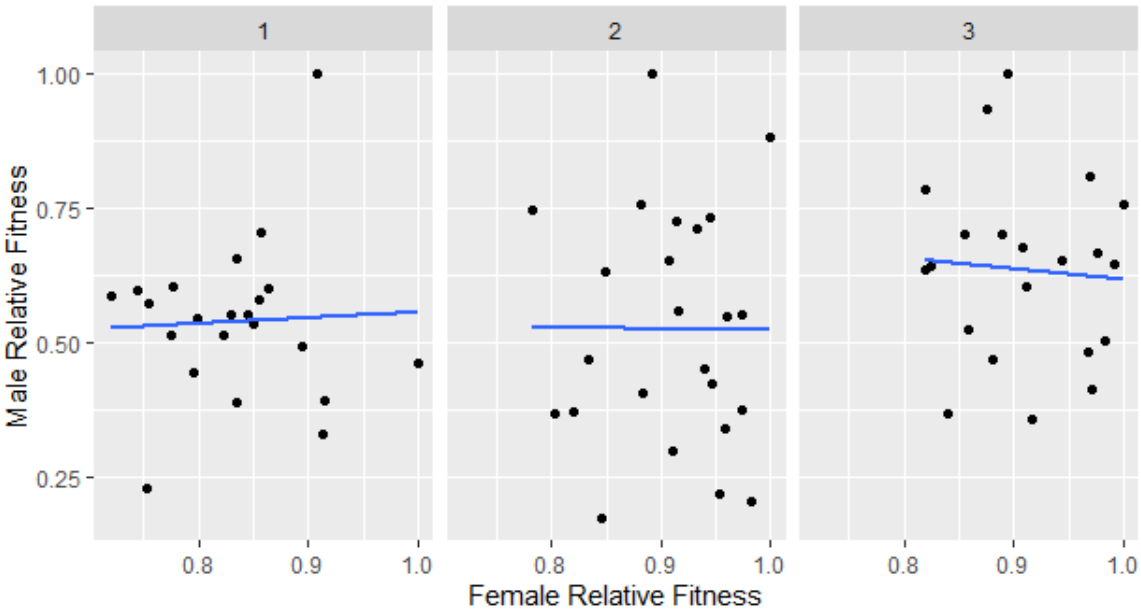

Figure 3.8: Intersexual regression for fitness in F1-3

4. Discussion

Theory predicts that the X chromosome is likely to house a large proportion of sexually antagonistic genes, as well as genes under sexual selection (Rice, 1984). Experimental evidence for these predictions, however, remains equivocal (Delcourt et al., 2009; Foerster et al., 2007). Through this study, we investigate if the X chromosome in lab populations of *Drosophila melanogaster* contributes towards sexual antagonism. We also explore the role of the X chromosome in fitness differences between populations evolved under increased and decreased levels of sexual conflict.

In this study, we sampled X chromosomes from a baseline population (LH), and from six independent replicate populations evolved under male-biased (M1-3) or female-biased (F1-3) sex ratios. These X chromosomes were made to express in random autosomal backgrounds in males and females, and the fitness of each X chromosome was measured in both sexes. We found no variation for fitness among families in all blocks and both sexes. This suggests that the X chromosomes we sampled did not house significant additive genetic variation for fitness, despite the X chromosome making up about 20% of the *D. melanogaster* genome. Sexually concordant fitness variation is expected to be overrepresented on autosomes (Haldane, 1937) while sexually antagonistic variation, on the X chromosome (Gibson et al., 2002; Rice, 1984). Our results do not conform to this hypothesis. They are, however, consistent with the theory proposed by Fry (2010), which suggests SA variation is likely to be autosomal in some cases when the dominance assumptions of Rice's model are relaxed. Though Chippindale et al. (2001) and Gibson et al. (2002) showed evidence for X-linked SA using the same baseline population as ours in their study, more recent studies on this population (for example, Long et al., 2009, Ruzicka et al., 2019) have failed to do so. As Ruzicka et al. (2019) suggest, this result may be a consequence of genetic drift excessively affecting the X chromosome because of its smaller effective population size, compared to autosomes (Caballero, 1995). Since the ancestral population (LH) does not show X-linked sexually antagonistic fitness variation, it is not surprising that the derived M and F populations behave similarly. These populations have evolved under different levels of sexual selection and have been shown to evolve mate choice (Chechi et al., unpublished

data). Recent theoretical work by Arnqvist (2011) suggests that assortative mating by fitness can lead to an increase in autosomal sexually antagonistic fitness variation. It would thus be interesting to explore how SA patterns on the autosomes have evolved in M and F.

We found the correlation between male and female fitness, for the same X chromosome, to be zero in all seven populations. Evidence for sexually concordant selection would be rmf = 1 while that for sexually antagonistic selection would be rmf<0. Our results indicate that we cannot infer the presence or absence of sexually antagonistic traits on the X chromosome conclusively (Bonduriansky & Chenoweth, 2009). This contrasts the results of previous studies on *Drosophila melanogaster* (Dean et al., 2012), including the one performed on LH (Gibson et al., 2002). However, this finding follows from our previous result showing the lack of sexually antagonistic variation on the X chromosome. Other possible reasons for no correlation between male and female fitness include the X chromosome housing alleles with sex-limited effects. Gene expression studies in these populations may help us test the role of sex-limited alleles in driving intralocus sexual conflict (Connallon & Knowles, 2005). Another probable reason could be the masking of recessive alleles in females, as the fitness assays were performed on females heterozygous for the target X chromosome.

Finally, we observed no effect of selection on fitness differences in males, as well as females, between the LH, M and F populations. Previous competitive fitness assays on these populations (Nandy, 2012) revealed that M males had evolved higher fitness compared to F males. Female fitness showed a block interaction, with M females evolving greater fitness in one block and F females evolving to have lower fitness in another. These assays were performed after 8-12 generations of selection. In the present study, the X chromosomes were sampled from generation 243 of the same populations. Since the maintenance regime of the populations has remained constant over time, with selection imposed every generation, and due to their large effective population sizes, we expect fitness differences between M and F to have persisted. Sexually selected traits like increased sperm competitive abilities (Nandy, Chakraborty, et al., 2013) and higher courtship frequency (Nandy, Gupta, et al., 2013) also evolved in the M males, relative to F. Our study found 'selection' to have no significant effect on X-linked fitness, except for female fitness in one block. Since all blocks are maintained as independently evolving populations, it is likely that there is variation in results

between blocks. However, we suggest that this anomalous result is investigated further through genomic studies, and we restrict our present discussion to the overall results. Previous studies have identified genes that are sexually selected and are involved in sexual antagonism in *Drosophila melanogaster.* Innocenti & Morrow (2010) found such genes to be overrepresented on the X chromosome, while Fitzpatrick (2004) found no such pattern. However, we must highlight that many of the genes identified by Innocenti & Morrow were associated with tissues of reproductive glands. Chechi et al. (2017) have shown that M males have not evolved larger testes or accessory glands, as compared to F males. This result, combined with our findings, suggests that the X chromosome has had little role to play in these populations evolving fitness differences in response to different levels of sexual selection. As suggested by Engqvist & Reinhold (2005), populations subjected to altered sex ratios, such as ours, may evolve to invest more in traits under pre-copulatory sexual selection.

Intra-locus sexual conflict has been demonstrated to be widespread in *Drosophila melanogaster* (Chippindale et al., 2001; Long & Rice, 2007). We tested theoretical predictions of disproportionate representation of X-linked sexually antagonistic variation. We also asked how inter-locus and intra-locus sexual conflict interacted, vis-à-vis the X chromosome. Overall, our results show no contribution of the X chromosome in sexual conflict in *Drosophila melanogaster*. As discussed above, these results are consistent with recent theory and other empirical studies. A growing body of literature suggests that apart from the autosomes and X chromosomes, there may be other significant sources of fitness variation such as epistatic effects of the Y chromosome (Chippindale & Rice, 2001; Kutch & Fedorka, 2017; & Fedorka, 2018), X-cytoplasm (Rand et al., 2001) and other mito-nuclear interactions (Dowling et al., 2007), and X-autosomal interactions (Frank & Crespi, 2011). Future studies exploring these areas may help us understand our system better. Additionally, we suggest combining a diverse range of approaches like analysis of gene expression studies, genomic analyses, and other quantitative genetic techniques such as QTL mapping to dissect out our present results further.

Appendix A

Composition of the standard corn meal-molasses food

The following ingredients are used to prepare 1 litre of the food:

1. 1100 ml water
2. 14.8 g agar powder
3. 100 ml molasses
4. 100 g corn meal
5. 41.2 g yeast
6. 8 ml propionic acid
7. 2.25 g p-hydroxymethyl benzoate
8. 22.5 ml ethanol

(1-5) are mixed and boiled to form a thick suspension. After a little cooling, (6) and a solution of (7) in (8) are added to the suspension. This food is then poured into vials and allowed to solidify before use.

Appendix B

Table B1: Effect of 'family' on fitness variation in females. "post.mean" gives the variance estimate attributed to family in each population

Selection	Block	post.mean	l-95% CI	u-95% CI
LH		0.0002277	1.49E-09	0.000846
M	1	0.0002234	1.86E-10	0.000835
M	2	0.0002351	5.78E-09	0.000852
M	3	0.000232	4.70E-10	0.000858
F	1	0.0002347	8.58E-13	0.000815
F	2	0.0002379	2.62E-09	0.000835
F	3	0.0002356	2.23E-11	0.000862

Table B2: Effect of 'family' on fitness variation in males. "post.mean" gives the variance estimate attributed to family in each population

Selection	Block	post.mean	l-95% CI	u-95% CI
LH		0.003063	1.22E-09	0.009969
M	1	0.002968	7.24E-09	0.009633
M	2	0.003057	1.05E-08	0.009747
M	3	0.002997	1.19E-09	0.01036
F	1	0.002957	4.05E-08	0.008976
F	2	0.00304	4.87E-08	0.009742
F	3	0.003035	1.28E-09	0.009654

Table B3: Model summary for effect of selection on female fitness in Block 1

	post.mean	l-95%CI	u-95%CI	eff.samp	pMCMC
(Intercept)	49.5925	48.1055	51.0967	805.1	<0.001
SelectionLH	-0.3958	-2.751	1.7493	900	0.7444
SelectionM	-1.8664	-4.0309	0.412	900	0.0889

Table B4: Model summary for effect of selection on female fitness in Block 2

	post.mean	l-95%CI	u-95%CI	eff.samp	pMCMC
(Intercept)	51.0497	49.5877	52.6681	1089	<0.001
SelectionLH	-1.8328	-3.863	0.7405	900	0.131
SelectionM	-0.1077	-2.5601	1.8892	900	0.958

Table B5: Model summary for effect of selection on female fitness in Block 3

	post.mean	l-95%CI	u-95%CI	eff.samp	pMCMC
(Intercept)	46.9845	45.4841	48.6226	900	<0.001
SelectionLH	2.2456	-0.1089	4.4371	900	0.06
SelectionM	2.5074	0.5123	5.0573	900	0.0422*

Table B6: Model summary for effect of selection on male fitness in Block 1

	post.mean	l-95%CI	u-95%CI	eff.samp	pMCMC
(Intercept)	0.437345	0.3843	0.495237	735.8	<0.001
SelectionLH	-0.006033	-0.07972	0.071408	999.8	0.891
SelectionM	-0.029075	-0.10535	0.04887	900	0.478

Table B7: Model summary for effect of selection on male fitness in Block 2

	post.mean	l-95%CI	u-95%CI	eff.samp	pMCMC
(Intercept)	0.39706	0.34059	0.45154	900	<0.001
SelectionLH	0.0336	-0.05258	0.11111	900	0.407
SelectionM	0.04626	-0.03664	0.12542	799.7	0.267

Table B8: Model summary for effect of selection on male fitness in Block 3

	post.mean	l-95%CI	u-95%CI	eff.samp	pMCMC
(Intercept)	0.41225	0.35346	0.46471	900	<0.001
SelectionLH	0.01977	-0.05749	0.09775	900	0.633
SelectionM	0.02319	-0.04751	0.10178	900	0.567

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