# Sex differences in deleterious mutational effects in *D. melanogaster*: combining quantitative and population genetic insights

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## Abstract

Fitness effects of deleterious mutations can differ between females and males due to: (*i*) sex differences in the strength of purifying selection; and (*ii*) sex differences in ploidy. Although sex differences in fitness effects have important broader implications (*e.g.*, for the evolution of sex and lifespan), few studies have quantified their scope. Those that have belong to one of two distinct empirical traditions: (*i*) quantitative genetics, which focusses on multi-locus genetic variances in each sex, but is largely agnostic about their genetic basis; and (*ii*) molecular population genetics, which focusses on comparing autosomal and X-linked polymorphism, but is poorly suited for inferring contemporary sex differences. Here we combine both traditions to present a comprehensive analysis of female and male adult reproductive fitness among 202 outbred, laboratory-adapted, hemiclonal genomes of *Drosophila melanogaster*. While we find no clear evidence for sex differences in the strength of purifying selection, sex differences in ploidy generate multiple signals of enhanced purifying selection for X-linked loci. These signals are present in quantitative genetic metrics—*i.e.*, a disproportionate contribution of the X to male (but not female) fitness variation—and population genetic metrics—*i.e.*, steeper regressions of an allele's average fitness effect on its frequency, and proportionally less nonsynonymous polymorphism on the X than autosomes. Fitting our data to models for both sets of metrics, we infer that deleterious alleles are partially recessive. Given the often-large gap between quantitative and population genetic estimates of evolutionary parameters, our study showcases the benefits of combining genomic and fitness data when estimating such parameters.

## Introduction

Most new mutations affecting fitness are deleterious (Eyre-Walker and Keightley 2007) and segregating deleterious alleles contribute a large fraction of standing genetic variation for fitness (Charlesworth 2015). The evolutionary dynamics of deleterious alleles and their contributions to standing fitness variation depend on their "average effects" on fitness (*sensu* Fisher; see Theoretical background), which can differ between males and females. Such sex differences in the fitness effects of mutations have important implications for the evolutionary persistence of maladaptation (*e.g.*, genetic load; Whitlock and Agrawal 2009), the severity of inbreeding depression (Eanes *et al.* 1985; Mallet and Chippindale 2011), the genetic basis of fitness variation (Connallon 2010), and the evolution of sex (Agrawal 2001; Siller 2001; Roze and Otto 2012) and lifespan (Maklakov and Lummaa 2013).

Sex differences can influence the fitness effects of deleterious variation in two ways. First, the strength of purifying selection can differ between sexes (Bateman 1948; Trivers 1972; Whitlock and Agrawal 2009; Janicke *et al.* 2016; Singh and Punzalan 2018), owing to the divergent strategies females and males employ in achieving reproductive success (Darwin 1871; Andersson 1994; Arnqvist and Rowe 2005), or to sex differences in the fraction of the genome with sex-limited expression (and thus experiencing sex-limited selection; Connallon and Clark 2011; Allen *et al.* 2013, 2017). Second, the sexes show asymmetries in ploidy for sex-linked genes, with diploid X chromosomes in females and hemizygous (haploid) X chromosomes in males. Haploid expression is expected to enhance the expression of X-linked deleterious alleles in males (Reinhold and Engqvist 2013) and thereby strengthen purifying selection against them (Avery 1984), whether or not the sexes systematically differ in the strength of purifying selection. Quantifying and distinguishing these two sources of sexually dimorphic fitness effects is essential to our understanding of the genetic basis and evolutionary dynamics of deleterious variants.

Two distinct empirical traditions have investigated how sex differences mediate the fitness effects of deleterious variation. First, researchers have used classical quantitative genetic designs to estimate standing genetic variation for fitness (or fitness components) (Chippindale *et al.* 2001; Gibson *et al.* 2002; Long *et al.* 2009; Collet *et al.* 2016; Sultanova *et al.* 2018) or the effects of new mutations on fitness in each sex (*i.e.*, from mutation-accumulation experiments; Mallet and Chippindale 2011; Mallet *et al.* 2011; Sharp and Agrawal 2013, 2018; Grieshop *et al.* 2016; Allen *et al.* 2017; Prokop *et al.* 2017). The second empirical tradition—molecular population genetics—has addressed questions about sex differences through comparisons of genetic diversity between autosomal and X-linked genes (Vicoso and Charlesworth 2006; Ellegren 2009; Li *et al.* 2010; Leffler *et al.* 2012; Veeramah *et al.* 2014), which indirectly reflect sexually dimorphic fitness effects. For example, a disproportionate reduction in nonsynonymous X-linked polymorphism indicates stronger purifying selection on the X relative to autosomes, presumably as a consequence of male hemizygosity (see Theoretical background).

The two traditions differ in what they can, and cannot, tell us about sex differences in deleterious fitness effects. The quantitative genetic tradition is well suited for inferring broad-scale patterns of genetic variance and allows a straightforward assessment of multi-locus sex differences in fitness effects. However, quantitative genetic analyses cannot isolate the contributions of individual loci to fitness variance. Consequently, using the relationship between an allele's average fitness effect and its frequency (*e.g.*, Park *et al.* 2011; Josephs *et al.* 2015; Zeng *et al.* 2018), or comparing nonsynonymous and synonymous polymorphism (Li *et al.* 2010; Veeramah *et al.* 2014) to assess the strength of purifying selection, is out of reach with these data. Furthermore, quantitative genetic breeding designs rarely allow (or consider) partitioning fitness variances into X-linked and autosomal components (Simmons and Crow 1977; Eanes *et al.* 1985; Gibson *et al.* 2002; Brengdahl *et al.* 2018), despite the

differential contributions of sex-linked and autosomal loci to female and male variances (James 1973; Connallon 2010; Reinhold and Engqvist 2013). The population genetic tradition, on the other hand, provides variant-level resolution and is well-suited for detecting differences in the effectiveness of purifying selection between autosomal and X-linked sites (Vicoso and Charlesworth 2006). However, autosomal and X-linked polymorphism data do not reflect contemporary (and sex-specific) fitness effects but instead represent long-term averages over many thousands of generations, and across the two sexes. Sex-differential fitness effects can therefore only be indirectly inferred by autosomal and X-linked contrasts.

In this study, we combined: (*i*) replicated measurements of male and female outbred lifetime reproductive fitness from ~200 genotypes extracted from $LH_M$, a laboratory-adapted population of *Drosophila melanogaster* (Ruzicka *et al.* 2019; see Materials and Methods for further details); and (*ii*) whole-genome sequences from these same lines (Gilks *et al.* 2016). These data enabled us to perform a genome-wide association study (GWAS) of female and male fitness, and thereby study fitness variation at the level of individual loci. Our general approach was to estimate various metrics associated with deleterious variation—multi-locus additive genetic variation ($V_A$) for fitness, regressions of estimated fitness effects of alleles on their frequencies, and levels of nonsynonymous versus synonymous polymorphism—among sexes and chromosome "compartments" (*i.e.*, X and autosomes). By comparing these empirical estimates to theoretical models for each metric (see Theoretical background), we were able to comprehensively quantify sexually dimorphic fitness effects and dominance coefficients of deleterious variants. Given that estimates of evolutionary parameters (*e.g.*, selection and dominance coefficients) often differ markedly between quantitative and population genetic approaches (Manna *et al.* 2011; Charlesworth 2015), our study showcases the benefits of combining measurements of fitness (in the quantitative genetic tradition) and genomic data (in the population genetic tradition) when estimating such parameters.

## Theoretical background

We rely on three empirical metrics to make inferences about sex differences in the fitness effects of deleterious genetic variation: (A) multi-locus $V_A$ for fitness estimated from single nucleotide polymorphisms (SNPs); (B) regressions of estimated fitness effects of variants on their frequencies; and (C) allele frequency spectra for putatively deleterious (*i.e.*, nonsynonymous) alleles relative to neutral (*i.e.*, synonymous) alleles. Below, we use population genetic theory to briefly outline how these metrics can differ between the sexes or between the X and autosomes.

Our theoretical predictions focus on bi-allelic polymorphism maintained at an equilibrium between recurrent mutation, purifying selection and drift (*i.e.*, mutation-selection-drift balance; which should apply to most loci) in a randomly mating population. Genotypic fitness values for an arbitrary polymorphic locus *i*, with wild-type allele $A_i$ (at frequency $p_i$) and deleterious allele $a_i$ (at frequency $q_i$), are summarized in Table 1.

***A. Additive genetic variance for fitness ($V_A$).*** The contribution of the $i^{\text{th}}$ autosomal or X-linked locus to female $V_A$ for fitness is:

$$V_{f,i} = 2p_i q_i \alpha_{f,i}^2 \quad (1),$$

where $\alpha_{f,i} = s_{f,i}h_i + s_{f,i}q_i(1 - 2h_i)$ is the "average effect" of the deleterious allele on female fitness. The same expression applies to male $V_A$, with $\alpha_{m,i} = s_{m,i}h_i + s_{m,i}q_i(1 - 2h_i)$ in place of $\alpha_{f,i}$. The contribution of an X-linked locus to male $V_A$ is:

$$V_{m,i} = p_i q_i s_{m,i}^2 \quad (2),$$

with $\alpha_{m,i} = s_{m,i}$ representing the "average effect" of the hemizygous X-linked deleterious allele in males (both results follow from standard theory, *e.g.*: James 1973; Reinhold and Engqvist 2013). With no epistasis or LD, multilocus fitness variance for a given sex is the sum of variances contributed by individual loci (see Charlesworth 2015).

Each autosomal locus contributes more to variance of the sex that is subject to stronger purifying selection (Fig. 1A, where $V_{f,i}/V_{m,i} = \left(\alpha_{f,i}/\alpha_{m,i}\right)^2 = \left(s_{f,i}/s_{m,i}\right)^2$ for autosomal loci). Consequently, sex asymmetries in multi-locus fitness variance at autosomal loci can emerge from sex differences in the strength of purifying selection and/or differences in the number of loci with male- versus female-limited expression (*e.g.*, Sharp and Agrawal 2013). X-linked loci at mutation-selection balance contribute more to male than to female fitness variance for recessive or partially dominant mutations (Fig. 1A, where $V_{f,i}/V_{m,i} = 2\left(\alpha_{f,i}/s_{m,i}\right)^2 \approx 2\left(s_{f,i}h_i/s_{m,i}\right)^2$ for X-linked loci). X-linked loci also contribute disproportionately to fitness variance of males relative to autosomes (*i.e.*, owing to heightened expression of X-linked alleles through hemizygosity; Fig. 1A), whereas autosomal loci contribute disproportionately to variance of females (*i.e.*, owing to the lower deleterious allele frequencies on the X that results from hemizygous selection in males; Fig. 1A). These approximations (lines in Fig. 1A) are robust to effects of genetic drift (filled circles in Figs. 1A).

***B. Association between allele frequency and fitness effect.*** Assuming effectively strong selection (*i.e.*, $N_{eA}h_i(s_{f,i} + s_{m,i})$, $N_{eX}h_i(s_{f,i} + s_{m,i}) >> 1$, so that allele frequencies are close to deterministic mutation-selection balance), and holding $u$ (the per-locus mutation rate), $h$ and $s_m/s_f$ constant across loci, the slope of the regression of the "average effect" on the deleterious allele frequency will be:

$$\beta_{f,A} = \frac{\mathrm{cov}\left(\alpha_{f,A}, q_A\right)}{\mathrm{var}(q_A)} \approx \frac{h^2\left(1 + s_m/s_f\right)}{2u} \cdot \frac{\mathrm{cov}\left(s_f, 1/s_f\right)}{\mathrm{var}\left(1/s_f\right)} \quad (3),$$

for autosomal loci in females, and

$$\beta_{f,X} = \frac{\mathrm{cov}\left(\alpha_{f,X}, q_X\right)}{\mathrm{var}(q_X)} \approx \frac{h\left(2h + s_m/s_f\right)}{3u} \cdot \frac{\mathrm{cov}\left(s_f, 1/s_f\right)}{\mathrm{var}\left(1/s_f\right)} \quad (4),$$

for X-linked loci in females. Expressions for males are $\beta_{m,A} \approx \frac{s_m}{s_f}\beta_{f,A}$ and $\beta_{m,X} \approx \frac{s_m}{s_f h}\beta_{f,X}$. Regressions for autosomal loci are steeper for the sex that is subject to stronger purifying selection; regressions for X-linked loci tend to be steeper for males than females owing to hemizygosity of the former (lines in Fig. 1B). Regressions are steeper for X-linked than autosomal loci when $h < 1$, owing to enhanced purifying selection on the X (Fig. 1B). These predictions are robust to the effects of genetic drift (Figs. 1B; filled circles).

***C. Allele frequency distributions of deleterious alleles.*** Given the selection parameters from Table 1, the stationary allele frequency distribution for deleterious autosomal alleles is given by:

$$f(q_i) = C[q_i(1-q_i)]^{\theta_A - 1} e^{-\gamma_A} \quad (5),$$

where $\theta_A = 2N_{eA}u_i$, $\gamma_A = \frac{1}{2}N_{eA}\left(s_{m,i} + s_{f,i}\right)q_i\left(2h_i + q_i(1-2h_i)\right)$, $N_{eA}$ is the effective population size for autosomal loci (accounting for diploidy, such that $N_{eA}=2N_e$) and $C$ is a normalizing constant that ensures that the density function integrates to one. Eq. (5) can be used for the stationary distribution of X-linked loci by replacing $\theta_A$ and $\gamma_A$ with $\theta_X = 2N_{eX}u_i$ and $\gamma_X = \frac{2}{3}N_{eX}q_i\left(s_{f,i}\left(2h_i + q_i(1-2h_i)\right) + s_{m,i}\right)$, where $N_{eX}$ is the effective population size of X-linked loci. Where selection is much stronger than genetic drift (*i.e.*, $N_{eA}h_i(s_{f,i} + s_{m,i})$, $N_{eX}h_i(s_{f,i} + s_{m,i}) >> 1$), the expected frequencies of an autosomal and X-linked deleterious allele correspond to the deterministic mutation-selection balance equilibria: $\sim 2u_i/\left(s_{f,i}h_i + s_{m,i}h_i\right)$ and $\sim 3u_i/\left(2s_{f,i}h_i + s_{m,i}\right)$ at autosomal and X-linked loci, respectively (*e.g.*, Connallon 2010).

Among sites substantially affected by genetic drift, levels of diversity depend on the strength of purifying selection relative to drift (*i.e.*, the "efficacy of selection"), which is captured by the terms $\gamma_A$ and $\gamma_X$. Purifying selection is equally effective between the X and autosomes ($\gamma_X = \gamma_A$) with co-dominance and when $N_{eX}/N_{eA} = 3/4$, whereas combinations

of $N_{eX}/N_{eA} > 3/4$ and partial recessivity of deleterious alleles ($h$ < ½) enhance purifying selection on the X (see Sup. Fig. 2). These predictions are manifest in simulated nonsynonymous versus synonymous polymorphism data (Fig. 1C).

## Materials and Methods

### Existing genomic and fitness data from $LH_M$ hemiclones

Our study used genomic and fitness data from Gilks *et al.* (2016) and Ruzicka *et al.* (2019), respectively. Both studies employed the hemiclonal design, in which the unit of observation is a haploid chromosome set (a complement of chromosomes X, 2, and 3, representing ~99% of the *D. melanogaster* genome; the Y chromosome, fourth "dot" chromosome and mtDNA are allowed to vary among members of a given line). For phenotypic measurements, hemiclonal genomes are expressed alongside random sets of homologous chromosomes sampled from the base population to generate replicate "focal" females and males that express the same hemiclonal chromosome set in variable outbred genotypes (Abbott and Morrow 2011).

In Gilks *et al.* (2016), hemiclonal genomes were sampled from the $LH_M$ stock population using the crossing scheme depicted in Sup. Fig. 1. Briefly, each hemiclone line is initially derived from a single wild-type male, which is propagated by repeated crosses to "clone-generator" females. Hemiclone males can always be identified in crosses because clone-generator females carry fused autosomes and X chromosomes that are phenotypically marked; furthermore, the X-2-3 complement males carry is preserved intact owing to the absence of recombination in males. Focal individuals of a given line are obtained by crossing hemiclone males to a random set of wild-type females (generating focal hemiclone females), or to a random set of females carrying a fused X chromosome (generating focal hemiclone males).

In Ruzicka *et al.* (2019), sex-specific adult reproductive fitness was measured among 223 hemiclonal *D. melanogaster* genotypes from the $LH_M$ population (see Rice *et al.* (2005) for more details on the $LH_M$ population). Briefly, female and male fitness assays were performed so as to closely mimic the strictly controlled rearing regime of the $LH_M$ population, which had been laboratory-adapted for ~20 years (~500 generations) at the time assays were undertaken. Female fitness was measured as competitive fecundity (number of eggs laid) and male fitness as competitive fertilisation success (proportion of progeny sired), in competition with a stock homozygous for the recessive eye-colour mutation *brown* (*bw)* (the *bw* stock is a good competitor and has been used in similar *D. melanogaster* studies; *e.g.* Mallet and Chippindale 2011; Mallet *et al.* 2011; Sharp and Agrawal 2013). For each hemiclone line and sex, reproductive fitness was measured in a blocked design, among 25 replicate focal individuals across all blocks. In each sex, fitness measurements were normalised, scaled and centred within blocks, and averaged across blocks prior to subsequent analysis.

Gilks *et al.* (2016) generated whole-genome sequences for each hemiclonal line, while Ruzicka *et al.* (2019) called SNPs. Briefly, for each genotype, DNA was extracted from a female heterozygous for the hemiclonal genome and a complement derived from the sequenced reference stock. SNPs were called using the BWA-Picard-GATK pipeline and mapped to the *D. melanogaster* genome assembly (release 6). Indels, non-diallelic sites, sites with depth <10 and genotype quality <30, individuals with high missing rates (>15%) and an individual outlier from a PCA analysis were removed. Among the remaining hemiclonal genomes (n=202), sites with missing rates <5% and MAF >0.05 were retained, yielding a final set of 765,764 stringently quality-filtered SNPs.

**Genome-wide association studies**

We performed a GWAS separately in each sex using a linear mixed model, such that:

$$Y = \alpha X + g + e$$

where $Y$ is a vector of sex-specific fitness values, $\alpha$ the "average effect" of an allele on fitness (*sensu* Fisher; see Visscher and Goddard 2019), $X$ a vector of genotypic values (*i.e.*, either 0 or 1 in the hemiclonal design), $g$ the heritable component of random phenotypic variation, $e$ the non-heritable component of random phenotypic variation, with:

$$var(g) = N(0, V_A\mathbf{K})$$

$$var(e) = N(0, V_E\mathbf{I})$$

where $V_A$ is the additive genetic variance, **K** the kinship matrix derived from genome-wide SNPs, $V_E$ the residual variance and **I** an individual identity matrix. This GWAS approach has been shown to appropriately control false positives and increase power to detect true associations in samples with moderate degrees of population structure and close relatedness (Astle and Balding 2009; Price *et al.* 2010), such as $LH_M$ (Ruzicka *et al.* 2019).

Female and male GWAS were implemented in LDAK (Speed *et al.* 2012), which corrects for linkage between neighbouring SNPs when estimating kinships to avoid pseudo-replication among clusters of linked sites, and further allows SNPs to be weighted by their MAF when estimating kinships by specifying a scaling parameter ($\delta$), as $MAF(1 - MAF)^{1+\delta}$. We used a $\delta$ value of –0.25, which has been shown to provide a good fit to a range of quantitative trait data (Speed *et al.* 2017), though results from analyses using alternative $\delta$ values are also presented in the Supplementary Material (note that Speed *et al.* 2017 referred to this parameter as α; we use δ to distinguish it from the average effect parameter α). We applied a Wald $\chi^2$ test to generate p-values for each SNP, and corrected for multiple testing using Benjamini-Hochberg false discovery rates (Benjamini and Hochberg 1995), thereby converting p-values into FDR q-values. For each GWAS, we also estimated the genomic inflation factor ($\lambda_{median}$; calculated as median observed $\chi^2$ over median

257 expected $\chi^2$) to quantify the extent of p-value inflation, where a value close to 1 indicates
258 that relatedness and population structure have been well controlled.
259 We also performed gene-based association tests. Gene coordinates were obtained from
260 the UCSC genome browser and extended by 5kb up- and downstream to include potential
261 regulatory regions. LDAK's gene-based test estimates variance components for each gene by
262 fitting a linear mixed model, such that:
263 $$Y = N(0, V_A\mathbf{K} + V_E\mathbf{I})$$
264 with variables defined as previously and **K** corresponding to kinship matrix derived from
265 SNPs in each gene. To correct for genome-wide relatedness and population structure, the top
266 20 principal components derived from genome-wide kinships were also included as
267 covariates. Variance components were estimated using restricted maximum likelihood
268 (REML), with SNP heritability calculated as $V_A/(V_A + V_E)$, a likelihood ratio test performed
269 to generate a gene-based p-value, and FDR correction applied as above.
270
271 **Chromosomal distribution, biological functions and polygenicity of fitness-associated**
272 **loci**
273 We designated a set of 'candidate' loci associated with sex-specific fitness as loci with FDR
274 q-values<0.3. We further estimated the number of independently associated candidate SNPs
275 through LD clumping in PLINK (Purcell *et al.* 2007). LD clumping takes the candidate SNP
276 with the lowest association q-value as a 'lead' SNP, clusters neighbouring SNPs (*i.e.*, those
277 within a specified distance and LD threshold of the lead SNP), repeats this procedure for the
278 SNP with the next-lowest q-value, and so on, eventually forming clusters of candidate SNPs
279 that are approximately independent of one another. We specified a distance threshold of 10kb
280 and an LD ($r^2$) threshold of 0.4, reflecting typical LD decay in $LH_M$ (Ruzicka *et al.* 2019).

We assessed the functional effects of candidate SNP clusters using annotations based on the Variant Effect Predictor, and used $\chi^2$ tests to compare the observed number of candidate SNP clusters in a given functional category to the expected number among 35,726 LD-pruned SNPs (generated through LD clumping as above but choosing a random SNP as lead SNP). We also investigated the functional properties of candidate genes by performing a Gene Ontology analysis in PANTHER (Protein Analysis Through Evolutionary Relationships) v.13.1 (Mi *et al.* 2017), using the statistical overrepresentation test.

We tested whether the genetic basis of sex-specific fitness was polygenic. High trait polygenicity implies a diffuse scattering of causal loci with small (and difficult to identify with statistical confidence) effects across the genome, generating a positive relationship between the length of a genomic segment and its SNP heritability, since longer regions are expected to contain more causal SNPs (Yang *et al.* 2010). Because *D. melanogaster* harbours only five major chromosome arms of approximately equal length, we quantified polygenicity at the level of random genome partitions. Specifically, we divided each chromosome arm into 500 partitions (*i.e.*, 2,500 partitions across the five major arms) by randomly drawing 499 SNPs to represent "breakpoints" along a given arm. SNP heritability for a partition was then estimated using LDAK's gene-based association analysis but with partitions as the unit of interest. We then quantified the relationship between the number of SNPs in a given partition and that partition's SNP heritability using a Spearman's rank correlation, with 95% confidence intervals obtained by randomly sampling 2,500 new partitions (1,000 times, without replacement) and re-estimating the correlation coefficient on each set of random partitions. To complement partition-based analyses, we also performed analyses at the level of genes.

**Metrics associated with deleterious variation**

306 *A. $V_A$ estimates*

307 To obtain SNP-based estimates of $V_A$ for each sex and chromosome compartment, we used
308 LDAK to fit a linear mixed model, as:

309 $$Y = N(0, V_A\mathbf{K} + V_E\mathbf{I})$$

310 with variables as previously defined, **K** corresponding to kinship matrix derived from SNPs
311 in each chromosome compartment, and using REML to estimate variance components. We
312 adjusted $V_A$ estimates and their standard errors upwards by a factor of two (in both sexes for
313 autosomes; in females for the X) to account for the two-fold reduction in $V_A$ induced by the
314 hemiclonal design (Abbott and Morrow 2011).

315 To statistically compare $V_A$ between the sexes, we used female and male $V_A$ estimates
316 and their standard errors as inputs for Welch *t*-tests. To statistically compare $V_A$ among
317 chromosome compartments relative to expectations based on proportional genome content,
318 we used a permutation-based approach, in which SNPs were shifted to a random starting
319 point along a 'circular genome', thus breaking the relationship between each SNP and its
320 associated compartment while preserving the relative size of each compartment, the ordering
321 of SNPs along the genome and their LD structure (Cabrera *et al.* 2012). For each of 1,000
322 permutations, we estimated $V_A$ for each 'permuted X chromosome' and 'permuted autosome',
323 thereby generating a null distribution of $V_A$ for each compartment. An empirical p-value was
324 then obtained by comparing 1,000 permuted estimates of the fraction of total $V_A$ that is X-
325 linked to the observed fraction of total $V_A$ that is X-linked.

326

327 *B. Regressions of average allelic effect on allele frequency*

328 GWAS provide estimates of the average effect of each allele on sex-specific fitness, as
329 defined in the Theoretical background (*i.e.*: $\alpha_{f,i}$ and $\alpha_{m,i}$, above, can be estimated as the
330 regression of hemi-clone line fitness for a given sex on the allele count per line, which is zero

or one). Purifying selection generates a negative regression slope (*i.e.*, negative $\beta$) between $\alpha$ and minor allele frequency, with a steeper slope expected in the sex where selection is stronger, or the chromosomal compartment where selection is more effective (see Theoretical background; Fig. 1B). While sampling error alone can generate a negative $\beta$ (*e.g.*, because $\alpha$ estimates have higher sampling variances among rare SNPs), this artefact should affect each sex equally given that sample sizes are identical between sexes. Furthermore, we take this effect into consideration by using a permutation-based approach to obtain p-values (see below).

To compare $\beta$ estimates between sexes, we used the aforementioned set of 35,726 LD-independent SNPs. Then, for each chromosome compartment in turn, we modelled an allele's absolute average effect on fitness ($|\alpha|$) as a function of MAF, sex, and the sex-by-MAF interaction, fitting a generalised linear model (GLM) with Gamma (log link) error structure. This modelling choice was justified by the positive and right-skewed distribution of $|\alpha|$ and visual inspection of residuals from the fitted model. We then obtained p-values for each model term by running a GWAS on 1,000 permutations of male and female phenotypic values, and fitting the aforementioned GLM on permuted data, thereby obtaining a regression coefficient for each model term on each permutation run. The empirical p-value for the sex-by-MAF interaction term was obtained by comparing the observed coefficient to the null distribution of coefficients estimated in permuted data.

To compare $\beta$ between chromosome compartments, we repeated the procedure implemented for between-sex comparisons, with the following modifications: (*i*) the independent variables were MAF, chromosomal compartment, and the MAF-by-compartment interaction; (*ii*) a null distribution of model coefficients was generated through 1,000 circular permutations of genotypic values (as described in "$V_A$ estimates").

*C. Comparisons of nonsynonymous and synonymous polymorphism*

Purifying selection reduces the frequencies of deleterious nonsynonymous alleles relative to synonymous alleles, leading to fewer segregating nonsynonymous polymorphisms, as a fraction of all coding polymorphisms, in the chromosomal compartment under more effective purifying selection (see Theoretical background; Fig. 1A). To compare nonsynonymous and synonymous polymorphism on the autosomes and X, we excluded all non-coding polymorphisms from our data and LD-pruned the remaining loci (as described previously), yielding a set of 15,232 LD-independent coding loci. We then modelled the binary status of these loci (nonsynonymous or synonymous) as a function of chromosome compartment and MAF, using a logistic regression (binomial GLM) to generate regression coefficients and p-values.

**Quantitative inferences of evolutionary parameters**

Autosomal and X-linked patterns of $V_A$ and polymorphism allow us to make indirect inferences into the genetic properties (*e.g.*, dominance) and demographic parameters (*i.e.*, $N_{eX}$, $N_{eA}$) of autosomal and X-linked genetic variants. However, such inferences are qualitative rather than quantitative.

To make quantitative inferences, we took two approaches. First, we used estimates of the fraction of $V_A$ that is X-linked in each sex to estimate the average dominance coefficient of deleterious variants. Specifically, under a model of genetic variation maintained at mutation-selection balance, where dominance ($h$) is constant across loci, the ratio of the strength of purifying selection in each sex ($s_m/s_f$) is constant across loci, and the per-locus mutation-rate and sex-specific selection coefficients have the same distribution across X-linked and autosomal loci, we can approximate $h$ using female data as:

$$h \approx \frac{2\, s_m/s_f}{3\left(1 + s_m/s_f\right)\frac{P_X(1-F_X)}{F_X(1-P_X)} - 4}$$

where $P_X$ is the fraction of the genome that is X-linked and $F_X$ is the fraction of total female $V_A$ that is X-linked. We can approximate $h$ using male data as:

$$h = \frac{s_m}{4s_f}\sqrt{1 + \frac{6s_f}{s_m}\left(1 + \frac{s_f}{s_m}\right)\frac{P_X(1-M_X)}{M_X(1-P_X)}} - \frac{s_m}{4s_f}$$

where $M_X$ is the fraction of total male $V_A$ that is X-linked. We assumed that $P_X$=0.2, $s_m/s_f$=1, and we sampled autosomal and X-linked $V_A$ from a normal distribution with means and standard deviations as estimated in our data, thereby constructing confidence intervals for $F_X$ and $M_X$—and, ultimately, $h$—that take into account sampling error. We chose $s_m/s_f$=1 because of limited evidence for sex differences in purifying selection in this population, though we present $h$ estimates for $s_m/s_f$=2 and $s_m/s_f$=0.5 in the Supplementary Materials, along with full derivations for the above expression for $h$ (Supplementary Text 1).

Second, we performed random draws of allele frequencies at nonsynonymous and synonymous sites using X-linked and autosomal stationary distributions (see Theoretical background). We then used Approximate Bayesian Computation (ABC) to obtain posterior distributions of dominance ($h$), $N_{eX}/N_{eA}$ and other parameters (see below) that were consistent with our empirical polymorphism data. For each simulation run, we implemented the following algorithm:

1. $10^7$ autosomal "synonymous" loci and 2.5 x $10^7$ autosomal "nonsynonymous" loci were generated, reflecting the approximate 1:2.5 ratio of synonymous:nonsynonymous mutational opportunities in *D. melanogaster* (Huber *et al.* 2017; Kim *et al.* 2017). A smaller set of X-linked loci (0.177 x $10^7$ synonymous; 0.4425 x $10^7$ nonsynonymous) was also generated, reflecting the 1:0.177 ratio of autosomal:X-linked synonymous polymorphisms in our data.

2. Allele frequencies at autosomal and X-linked nonsynonymous sites were generated by randomly drawing from the stationary distribution for each locus, given its mutation rate ($\mu$), dominance coefficient ($h$), effective population size ($N_{eA}$ for autosomal loci, $N_{eX}$ for X-linked loci) and sex-specific selection coefficients ($s_m$ and $s_f$) ($s_m$ and $s_f$ were allowed to vary among loci; the remaining parameters were fixed across loci for each simulation run; see below for details of the prior distributions for each parameter). Because there is no built-in random number generator for a stationary distribution for non-neutral sites, sampling allele frequencies from stationary distributions was achieved using the rejection-sampling algorithm from Smith and Connallon (2017), based on the stationary distribution in eq. (1) and subsequent text, which assumes symmetric forward and backward mutation rates, per locus.
3. Allele frequencies at synonymous autosomal and X-linked sites were generated by sampling from a beta distribution with parameters $u = 2N_{eA}\mu$ and $v = 2N_{eA}\mu$ (for autosomal sites) and $u = 2N_{eX}\mu$, $v = 2N_{eX}\mu$ (for X-linked sites), as appropriate for neutral sites at mutation-drift equilibrium, where $\mu$ is the mutation rate per site and effects of ploidy are subsumed into $N_{eA}$ and $N_{eX}$ *(i.e.* $N_{eA}=2N_e$*)*.
4. Sample allele frequencies were obtained by binomial sampling from population allele frequencies obtained in Steps 2-3, with sample sizes matching the number of sequences in this dataset (n = 202), and further excluding sites with MAF<0.05 to match the filtering of our data.
5. From the simulated site frequency spectra, we fitted a binomial GLM of segregating site status (nonsynonymous or synonymous) as a function of compartment, MAF and their interaction, obtaining regression coefficients for each.
6. Finally, the regression coefficients obtained in Step 5 were compared to the equivalent coefficients in the observed data. We accepted simulation parameter sets if all three

simulated coefficients lay within a distance $\pm\varepsilon$ of the observed summary statistic, with $\varepsilon$ defined as the 95% confidence interval of the regression coefficient in the observed data.

Priors for input parameters were as follows: $N_{eA}$ = uniform [$10^2$,$10^4$] (because $N_{eA} = 2N_e$ in our models, our priors for $N_{eA}$ are consistent with the history of the population, which has been maintained at an adult census population size of $2N$ = 3,584 autosomal chromosomes; Rice *et al.* 2005); $N_{eX} = N_{eA} \times$ uniform [0.5,1] (*i.e.*, $N_{eX}$ may be roughly equal to or half of $N_{eA}$, as predicted by theory and as observed in natural populations of *D. melanogaster*; see Pool and Nielsen 2007; Langley *et al.* 2012; Mackay *et al.* 2012); $h$ = uniform [0,1] (estimates of $h$ in *D. melanogaster* are typically partially recessive but associated with large uncertainties; see Simmons and Crow 1977; Mallet *et al.* 2011); $\mu = 10^{-8}$ (based on Haag-Liautard *et al.* 2007). For a given simulation, we allowed selection coefficients to vary among loci. Specifically, we drew $s_m$ and $s_f$ from a symmetric bivariate Gamma, with shape parameter $k$ drawn from a uniform [0.25,0.4] (consistent with allele frequency-based estimates of the distribution of fitness effects (DFE) in *Drosophila*; see Loewe *et al.* 2006; Keightley and Eyre-Walker 2007; Haddrill *et al.* 2010; Huber *et al.* 2017), mean parameter $\bar{s}$ drawn from a uniform [$10^{-5}$,3.5 x $10^{-3}$] (the approximate range of values estimated in Loewe *et al.* 2006; Haddrill *et al.* 2010; Kousathanas and Keightley 2013; Huber *et al.* 2017) and $r$ (the correlation coefficient between $s_m$ and $s_f$) drawn from a uniform [0,1] (estimates of $r$ for new mutations are typically positive but vary widely in *Drosophila*; see Mallet *et al.* 2011; Sharp and Agrawal 2013; Allen *et al.* 2017).

**Statistical software**

All statistical analyses were performed in RStudio (RStudio Team 2015).

## Results

### A polygenic basis of female and male fitness

Figure 2A presents p-values from a GWAS of female and male fitness, respectively. The genomic inflation factors were close to 1 in both sexes (female $\lambda_{median}$=1.073; male $\lambda_{median}$=1.005), indicating that the mixed model appropriately controlled for relatedness and population structure in this sample (Sup. Fig. 3). For female fitness, the most significant individual SNP association p-value was 4.221 x $10^{-6}$ (the Bonferroni-corrected significance threshold was 6.529 x $10^{-8}$) and there were no SNPs with FDR q-values<0.3 (the minimum q-value value was 0.364). In a gene-wise analysis, we found 70 genes with q-values<0.3, representing candidate genes for the genetic basis of female fitness. For male fitness, the most significant individual SNP association p-value was 4.006 x $10^{-6}$ and there were 248 SNPs (31 LD-independent clusters) and 22 genes with q-values<0.3, representing candidates SNPs and genes, respectively, for the genetic basis of male fitness. A full list of genes associated with female and male fitness can be found in Sup. Tab. 1.

After LD-pruning, candidate SNPs for male fitness were significantly enriched on the X chromosome ($\chi_1^2$=28.809, observed=15, expected=4.745, odds ratio=5.917, p<0.001), as were candidate genes for male fitness ($\chi_1^2$=54.520, observed=16, expected=3.238, odds ratio=15.554, p<0.001). This pattern of X-enrichment was not observed for female candidate genes (N=70; $\chi_1^2$=0.063, observed=9, expected=10.239, odds ratio=0.861, p=0.802). Functional annotations of candidate SNPs and genes (predicted variant effects and GO terms) showed no significant over- or under-represented of terms after FDR correction, although the low number of candidates only provides modest power to these tests. Anecdotally, the leading SNPs from each male candidate cluster were found in functional regions (3'UTR, N=3; intronic, N=17; nonsynonymous, N=4) and none were intergenic.

The low number of individually significant candidate loci in both sexes, together with appreciable estimates of SNP-based additive genetic variance (see below), suggest that fitness is highly polygenic. If so, we expect to observe a positive relationship between the length of a chromosome region (*e.g.*, a gene, or random chromosome partition) and its SNP heritability (Yang *et al.* 2010), whereas a mono- or oligo-genic architecture predicts no such relationship. In line with polygenicity, we found a significant positive correlation between the length of random autosomal chromosome partitions and SNP heritability in both sexes (N=2,000 partitions; females – median $\rho$(±95CI)=0.066[0.025-0.104], empirical p=0.001; males – median $\rho$(±95CI)=0.068[0.027-0.108], empirical p=0.001), with a positive but non-significant correlation on the X (N=500 partitions; females – median $\rho$(±95CI)=0.048[–0.037-0.132], empirical p=0.126; males – median $\rho$(±95CI)=0.051[–0.035-0.131], empirical p=0.135; Fig. 2B). The relationship between gene length and SNP heritability provided similar results (Autosomes: females – $\rho$=0.101, p<0.001; males – $\rho$=0.062, p<0.001; X chromosome: females – $\rho$=0.068, p=0.001, males – $\rho$=0.005, p=0.807; Sup. Fig. 4). Overall, these analyses show that fitness is polygenic in both sexes.

**Mixed evidence for sex differences in the strength of purifying selection**

Multi-locus estimates of $V_A$ are informative about the relative strength of purifying selection in each sex, with the sex under stronger purifying selection expected to exhibit larger autosomal $V_A$ for fitness (because fitness effects are larger in that sex but allele frequencies are approximately equal between sexes; see Theoretical background; Fig. 1A). We found that autosomal female $V_A(\pm SE)$=0.437±0.133 and autosomal male $V_A(\pm SE)$=0.085±0.069 (Fig. 3A; Sup Fig. 5), corresponding to a statistically significant elevation in female $V_A$ on

autosomes (Welch's $t$=2.349, p=0.019), and suggesting that segregating variants tend to have larger fitness effects (and are therefore subject to stronger selection) in females.

Purifying selection also reduces the frequencies of alleles with large effects on fitness relative to alleles with small fitness effects, leading to a negative correlation between the fitness effects of deleterious mutations and their population frequencies (*e.g.*, Park *et al.* 2011; Zeng *et al.* 2018). The slope ($\beta$) of a linear regression of an allele's average fitness effect ($|\alpha|$) on its frequency is expected to be steeper in the sex under stronger purifying selection (see Theoretical background; Fig. 1B). We found that estimates of $\beta$ did not differ significantly between the sexes (Gamma GLM, sex-by-MAF interaction; Autosomes – empirical p=0.205; X chromosome – empirical p=0.207; Fig. 4A; Sup. Fig. 6), suggesting that—based on this metric—the sexes do not differ in the average strength of purifying selection.

**Multiple signals of enhanced purifying selection on the X chromosome**

$V_A$ and $\beta$ metrics also provide information about the expression of deleterious variation and the strength of purifying selection among chromosome compartments (*i.e.*, X and autosomes). The heightened expression of recessive or partially dominant X-linked alleles in hemizygous males is expected to elevate X-linked $V_A$ in males relative to females and generate stronger net purifying selection against X-linked deleterious alleles (see Theoretical Background; Fig. 1A). We found that SNP-based estimates of X-linked $V_A$ were roughly two-fold greater in males than females, with female $V_A(\pm SE)$=0.024±0.060 and male $V_A(\pm SE)$=0.052±0.031 (Fig. 3A), though the difference was not statistically significant (Welch's $t$=–0.418, p=0.676). The same theory also predicts that autosomal polymorphisms contribute disproportionately to total $V_A$ in females, while X-linked polymorphisms contribute disproportionately to total $V_A$ in males (Fig. 1A). We therefore performed a quantitative test

526 by comparing X-linked and autosomal $V_A$ estimates to 1,000 permuted estimates (random
527 shifts of SNPs along a circularised genome; see Materials and Methods), which reflect the
528 number of segregating sites in each compartment (~15% of sites are X-linked). Though the
529 deviations were not statistically significant (Females – empirical p=0.175; males – empirical
530 p=0.174), point estimates showed that the X accounted for 5.170% of total female $V_A$ and
531 38.128% of total male $V_A$ (Fig. 3B; Sup. Fig. 5), which is consistent with partially recessive
532 fitness effects of deleterious variants (see also Fig. 6A).

533 We also compared estimates of $\beta$ between chromosomal compartments. Consistent
534 with higher efficacy of selection on the X (see Theoretical background; Fig. 1B), we detected
535 a significantly steeper $\beta$ on the X chromosome than autosomes in females (Gamma GLM,
536 compartment-by-MAF interaction, empirical p=0.048; Fig. 4B; Sup. Fig. 6). Estimates of $\beta$
537 were also steeper on the X than autosomes in males, though the interaction term was not
538 statistically significant (Gamma GLM, compartment-by-MAF interaction, empirical p=0.140;
539 Fig. 4B; Sup. Fig. 6). Overall, statistical contrasts of fitness variation between chromosome
540 compartments do not reveal pronounced differences, but in each case, the direction of the
541 effect is consistent with more effective purifying selection on the X (Figs. 3B and 4B).

542 We can gain further information on the relative efficacy of purifying selection among
543 chromosome compartments by comparing levels of synonymous and nonsynonymous
544 polymorphism. Here, more effective X-linked purifying selection is predicted to reduce
545 nonsynonymous relative to synonymous polymorphism on the X (see Theoretical
546 Background; Fig. 1C). We found that a lower proportion of common alleles were
547 nonsynonymous rather than synonymous (Binomial GLM with probability of
548 nonsynonymous as response; MAF effect: odds ratio±SE=0.471±0.061, p<0.001; Fig. 5),
549 consistent with pervasive purifying selection against amino-acid changing mutations.
550 Furthermore, we detected proportionally fewer nonsynonymous polymorphisms on the X

chromosome than autosomes (X-chromosome effect: odds ratio±SE=0.801±0.041, p<0.001; Fig. 5), providing strong statistical support for more effective purifying selection against deleterious nonsynonymous variants on the X.

**Quantitative inferences of dominance and $N_{eX}/N_{eA}$**

The patterns observed in quantitative and population genetic metrics together suggest that purifying selection operates more efficiently at X-linked than autosomal loci, which implies that deleterious mutations tend to be partially recessive ($h < ½$) (see Theoretical background and Sup. Fig. 2). To make quantitative inferences about dominance, we first fit mutation-selection balance models for $V_A$ to our estimates of autosomal and X-linked $V_A$ in each sex, thereby estimating $h$ (while accounting for error in estimating genetic variances; see Materials and Methods). Second, we used approximate Bayesian computation (ABC) to infer distributions of $h$ and $N_{eX}/N_{eA}$ that are consistent with population genetic data (*i.e.*, common coding polymorphisms in the set of 202 experimental lines; see Materials and Methods).

We estimated $h$ [median±95% CI] to be 0.070 [−1.010-2.542] using estimates of the proportion of X-linked $V_A$ in females, and $h$ [median±95% CI] to be 0.364 [0-Inf.] using estimates of the proportion of X-linked $V_A$ in males. The point estimates suggest that partially recessive effects of deleterious variants fit our data well (Fig. 6A; Sup. Fig. 7), though confidence intervals are large because of estimation error. Using the ABC approach, we also found dominance estimates which were skewed towards partially recessive effects ($h$[±95% credible interval]=0.314[0.012-0.915]) and $N_{eX}/N_{eA}$ estimates skewed towards values greater than three-quarters ($N_{eX}/N_{eA}$=0.805[0.529-0.990]). Posterior distributions for both parameters differed markedly from their uniform prior distributions (Fig. 6B), and posterior estimates for both parameters were positively correlated (Spearman's $\rho$=0.067, p=0.033), implying that relatively small $N_{eX}/N_{eA}$ ratios and recessive fitness effects—or relatively large $N_{eX}/N_{eA}$ ratios

and dominant fitness effects—of nonsynonymous mutations provide a good fit to our data (Fig. 6B). In line with the observed excess of nonsynonymous polymorphisms among low-frequency sites (Fig. 5), and consistent with $LH_M$'s small $N_e$, we estimated that new nonsynonymous mutations are on average subject to weak purifying selection in this population (Autosomes: median $N_e\bar{s}$=2.238[0.347-7.719]; X chromosome: $N_e\bar{s}$=1.697[0.290-6.161]). The posterior distributions for all model parameters are presented in Sup. Fig. 8 and Sup. Tab. 2.

## Discussion

Our analyses of genome-wide variation in *D. melanogaster* combine two traditions (Charlesworth 2015): quantitative genetic analyses of phenotypic variation, in particular fitness variation, and molecular population genomic analyses of selection. Studies combining fitness measurements and genomic data are rare (Chenoweth *et al.* 2015; Chen *et al.* 2019; Dugand *et al.* 2019; Ruzicka *et al.* 2019) and allowed us to circumvent several limitations of previous research. First, our study focused on measurements of outbred reproductive fitness in a laboratory-adapted population (Ruzicka *et al.* 2019). These measurements should represent near-ideal proxies for fitness and are much more relevant to theories about deleterious variation than measurements of quantitative trait variation for components of fitness (*e.g.*, juvenile survival), or traits potentially covarying with fitness. Second, we estimated fitness among replicate individuals in a much larger array of genotypes than is typical for quantitative genetic studies of fitness, thereby increasing precision of our estimates. Third, whole-genome sequencing enabled us to quantify aspects of the genetic basis of fitness variation. We could therefore test and confirm that fitness is highly polygenic, and partition fitness variation between chromosomal contexts. Fourth, we estimated fitness variation in both sexes, allowing us to examine sexual dimorphism in deleterious fitness

601 effects. Finally, our quantitative genetic estimates are SNP-based and therefore directly
602 linked to sequence variability, which renders the connections between quantitative and
603 population genetic variability far more explicit than possible for most studies.
604
605 **Stronger X-linked purifying selection, but no clear evidence for sex differences in the**
606 **strength of selection**
607 Hemizygosity causes incompletely dominant X-linked alleles to exhibit heightened
608 expression in males relative to females (Fig. 1A). For example, several morphological and
609 life-history traits in *D. melanogaster* (Cowley *et al.* 1986; Cowley and Atchley 1988; Griffin
610 *et al.* 2016) and humans (Sidorenko *et al.* 2019) exhibit larger X-linked genetic variances in
611 males, and phenotypic variances for body size are typically higher in the heterogametic sex
612 (Reinhold and Engqvist 2013), consistent with the predicted effects of hemizygosity on
613 genetic variances. We found that estimates of $V_A$ based on X-linked SNPs were roughly
614 double in males compared to females, and X-linked SNPs contributed more to male $V_A$ than
615 expected based on the proportion of the *D. melanogaster* genome that is X-linked, though not
616 significantly so. Furthermore, we found that candidate loci for male fitness (SNPs and genes)
617 were over-represented on the X, whereas this was not the case for candidate loci for female
618 fitness. The outsized contribution of the X chromosome to male $V_A$ implies that selection is
619 more effective on the X (Avery 1984; see Theoretical background). In line with this, we
620 found a deficit of segregating nonsynonymous polymorphisms on the X relative to autosomes
621 (Fig. 5), as found previously in humans (Li *et al.* 2010; Veeramah *et al.* 2014). Furthermore,
622 X-linked $V_A$ estimates in females were less than the fraction of X-linked polymorphism,
623 consistent with more effective purifying selection on the X.
624 By contrast, our analyses did not suggest that selection is systematically stronger in
625 one sex than the other. While SNP-based estimates of $V_A$ revealed larger autosomal $V_A$ in

626 females than males, consistent with elevated female heritability in previous studies of this
627 population (Chippindale *et al.* 2001; Gibson *et al.* 2002; Long *et al.* 2009; Innocenti and
628 Morrow 2010; Collet *et al.* 2016) and potentially suggesting stronger selection in females,
629 regressions of average effects on allele frequencies revealed no sex differences. There are
630 additional reasons to doubt the hypothesis that females are, in general, under stronger
631 selection. First, we focus on common polymorphisms (MAF > 0.05) and therefore fail to
632 capture the rare deleterious variants with large effects on male fitness that are more easily
633 picked up by other experimental designs (*e.g.*, mutation-accumulation experiments),
634 potentially reducing our estimate of male $V_A$ relative to females. Second, some research
635 shows little evidence of male- or female-biased fitness effects of new deleterious mutations
636 (Grieshop *et al.* 2016; Prokop *et al.* 2017), while other studies show male-biased effects
637 (Mallet and Chippindale 2011; Mallet *et al.* 2011; Sharp and Agrawal 2013). Finally, it is
638 likely that our fitness assays do not capture the totality of fitness variation in each sex, despite
639 all attempts to mimic the laboratory rearing environment (Ruzicka *et al.* 2019). For example,
640 our assays, like others (*e.g.*, Sharp and Agrawal 2013), employ a *bw* competitor whose ability
641 to compete for matings (in male fitness assays) may differ from its ability to compete for
642 food resources (in female fitness assays), thus contributing to elevated autosomal fitness
643 variances in females.
644
645 **Combining sequence and fitness data to study the genetic basis of fitness variation: new**
646 **insights, limitations, and future directions**
647 Unlike previous studies (but see Chen *et al.* 2019; Dugand *et al.* 2019), including of the $LH_M$
648 population, our study can shed light on the specific genetic loci affecting fitness variation in
649 each sex. GWAS revealed that no common large-effect loci affect fitness in either sex,
650 despite appreciable multi-locus variances in both sexes. We also detected a positive genome-

651 wide correlation between the length of chromosome regions (*i.e.*, random partitions, or
652 genes) and the fitness variance a region explains. Both patterns are indicative of polygenicity
653 (Yang *et al.* 2010). Combining genomic and fitness data also allowed us to quantify
654 regressions of allelic effect on allele frequency—an indicator of the strength of purifying
655 selection that has not yet been applied to between-sex comparisons. The $\beta$ metric
656 corroborated inferences from $V_A$ and nonsynonymous/synonymous comparisons: specifically,
657 the X chromosome exhibited steeper $\beta$ than autosomes (in females, with a similar but non-
658 significant pattern in males), while $\beta$ did not differ between sexes—both patterns are
659 consistent with stronger purifying selection on the X chromosome but no sex differences in
660 the strength of purifying selection, as we have argued above.
661 Because quantitative and population genetic data often provide conflicting estimates
662 of evolutionary parameters (Charlesworth 2015), we were interested in fitting both types of
663 metric to models, and thereby quantifying the drivers of more effective purifying selection on
664 the X chromosome. Whether using simulations of nonsynonymous/synonymous
665 polymorphism or fitting mutation-selection balance models to the proportion of $V_A$ that is X-
666 linked in each sex, we inferred that deleterious mutations are partially recessive, though with
667 95% confidence/credible intervals overlapping 0.5. These results corroborate previous
668 estimates of dominance for new mildly deleterious mutations in *D. melanogaster* (h~0.1-0.3,
669 Simmons and Crow 1977; Eanes *et al.* 1985; Mallet and Chippindale 2011) and budding
670 yeast (Agrawal and Whitlock 2011), which were obtained using entirely different methods
671 (mutation-accumulation experiments and gene knock-outs, respectively).
672 It is important to note that our models rely on some simplifying assumptions. For
673 example, we follow previous research in assuming that fitness variation arises predominantly
674 from unconditionally deleterious variation under strong purifying selection (reviewed in
675 Charlesworth 2015). While aspects of our data support this inference—*e.g.*, nonsynonymous

676 sites are enriched among rare variants, as expected under purifying selection—this is unlikely
677 to be completely accurate. For example, fitness variation is highly polygenic, implying many
678 loci of small effect (Turelli and Barton 2004), and suggesting relatively weak purifying
679 selection at single loci. Nevertheless, our models for ratios of $V_A$ and $\beta$ (*i.e.*, Fig. 1) appear to
680 be robust to the effects of genetic drift, and our ABC simulations explicitly incorporate
681 genetic drift. Another potential issue is that previous studies of $LH_M$ (Ruzicka *et al.* 2019)
682 and other *D. melanogaster* populations (Bergland *et al.* 2014; Charlesworth 2015; Sharp and
683 Agrawal 2018) indicate that some fraction of fitness variance consists of loci under
684 antagonistic selection between environments, sexes or traits. Antagonistic loci can exhibit
685 proportionally different amounts of X-linked and autosomal $V_A$ than unconditionally
686 deleterious loci (*e.g.*, some types of balanced sexually antagonistic variation predict more $V_A$
687 on the X; Patten and Haig 2009; Fry 2010; Mullon *et al.* 2012; Ruzicka and Connallon 2020),
688 and may therefore cause deviations from the models outlined here.
689 Our ABC simulations also rely on some simplifying assumptions. For example, we
690 assumed a stationary population at demographic equilibrium, yet $LH_M$ underwent a
691 bottleneck of 400 individuals when it was brought into the laboratory (Rice *et al.* 2005) and
692 allele frequencies take on the order of 2-4$N_e$ generations (*i.e.*, ~2,800-5,600 generations for
693 $LH_M$ and much more than the ~500 generations of laboratory maintenance) to recover to
694 mutation-selection-drift equilibrium (Nei *et al.* 1975). Though we focus on interdigitated
695 nonsynonymous and synonymous sites to minimise the effects of non-equilibrium
696 demography, such effects cannot be ruled out completely (Sup. Fig. 9). We also made some
697 simplifying assumptions about the DFE, including that it is best described by a gamma
698 distribution (Eyre-Walker and Keightley 2007), that synonymous sites are neutral, and that it
699 is identical between X and autosomes. Such assumptions, though common, may not hold
700 entirely. For example, alternative distributions (*e.g.*, lognormal or various mixture

distributions; Loewe and Charlesworth 2006; Kousathanas and Keightley 2013; Kim *et al.* 2017) may fit the DFE better than a gamma, while some fraction of synonymous sites may be under weak purifying selection due to codon usage bias (Singh *et al.* 2008). Given that codon bias tends to be more pronounced on the X than autosomes (Singh *et al.* 2005), non-neutrality among a subset of synonymous sites will tend to downwardly bias our estimates of the nonsynonymous DFE (more so for the X), potentially requiring increased recessivity of deleterious nonsynonymous mutations to explain the deficit of nonsynonymous X-linked variants in our data. Finally, the *D. melanogaster* X chromosome harbours non-random sets of genes (Meisel *et al.* 2012), which may imply different DFEs for autosomal and X-linked sites (Perry *et al.* 2014; Fraïsse *et al.* 2019), though this eventuality remains, to our knowledge, untested.

Given current difficulties in estimating evolutionary parameters (Charlesworth 2015), such as average selection and dominance coefficients, how can future studies use genomic and fitness data to better estimate such parameters? First, it is clear that more precise estimates of deleterious mutational effects are needed. Our metrics of deleterious variation are associated with large uncertainties despite the relatively large sample of genomes in our study. One promising dataset is the UK Biobank, which contains genotype and fitness data for ~500,000 human males and females and can potentially be used to partition variances between sexes and chromosome compartments, compare $\beta$ between sexes, and compare autosomal X-linked polymorphism, though such an analysis remains to be undertaken (but see Sidorenko *et al.* 2019). Analysing a larger dataset such as the UK Biobank also permits rarer variants to be captured. Rare variants are likely to be enriched for deleterious effects and should thus be especially informative for parameter estimation. Second, current inferences about whether selection is stronger in one sex than the other come from a surprisingly narrow range of species—primarily *Drosophila* (Chippindale *et al.* 2001; Gibson

*et al.* 2002; Morrow *et al.* 2008; Mallet and Chippindale 2011; Mallet *et al.* 2011; Sharp and Agrawal 2018, 2013; Collet *et al.* 2016; Grieshop *et al.* 2016; Allen *et al.* 2017; Prokop *et al.* 2017; Sultanova *et al.* 2018). While selection gradients from a broader range of species suggest that selection is often male-biased (Janicke *et al.* 2016; Singh and Punzalan 2018), these studies suffer from two important limitations: (*i*) inferences are based on phenotypic rather than genetic variances, which can bias inferences about the relative strength of purifying selection between sexes when environmental variances also differ between sexes (see Wyman and Rowe 2014); (*ii*) such analyses do not account for the differential contributions of sex chromosomes to phenotypic variances in each sex, which can be sexually dimorphic even when the strength of purifying selection does not differ between sexes (see Theoretical background). Analyses in non-*Drosophila* systems where both limitations can be addressed are crucial to properly assess the relative strength of purifying selection between the sexes. Finally, there is scope for developing methods which further integrate both sets of metrics to estimate parameters (*e.g.*, estimating $h$ by jointly using data on nonsynonymous/synonymous polymorphism, $V_A$ and $\beta$ in a single analysis). This is not as easy as it first appears: for example, while one can reasonably neglect balanced polymorphisms when modelling polymorphism data, a few balanced polymorphisms can contribute substantially to multi-locus genetic variances. Nevertheless, developing such methods would likely help reduce uncertainty in parameter estimates.

## Data Availability

Fitness and genomic data is available at https://doi.org/10.5281/zenodo.571168 and https://zenodo.org/record/159472, respectively. All code for reproducing analyses in the manuscript is available at the following github respository: filipluca/GWAS_sex_specific_fitness_and_the_X_chromosome

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## Author contributions

F.R. and M.R. conceived the project; F.R. conducted analyses, wrote and edited the manuscript, with input from M.R. and T.C.; T.C. developed mathematical models.

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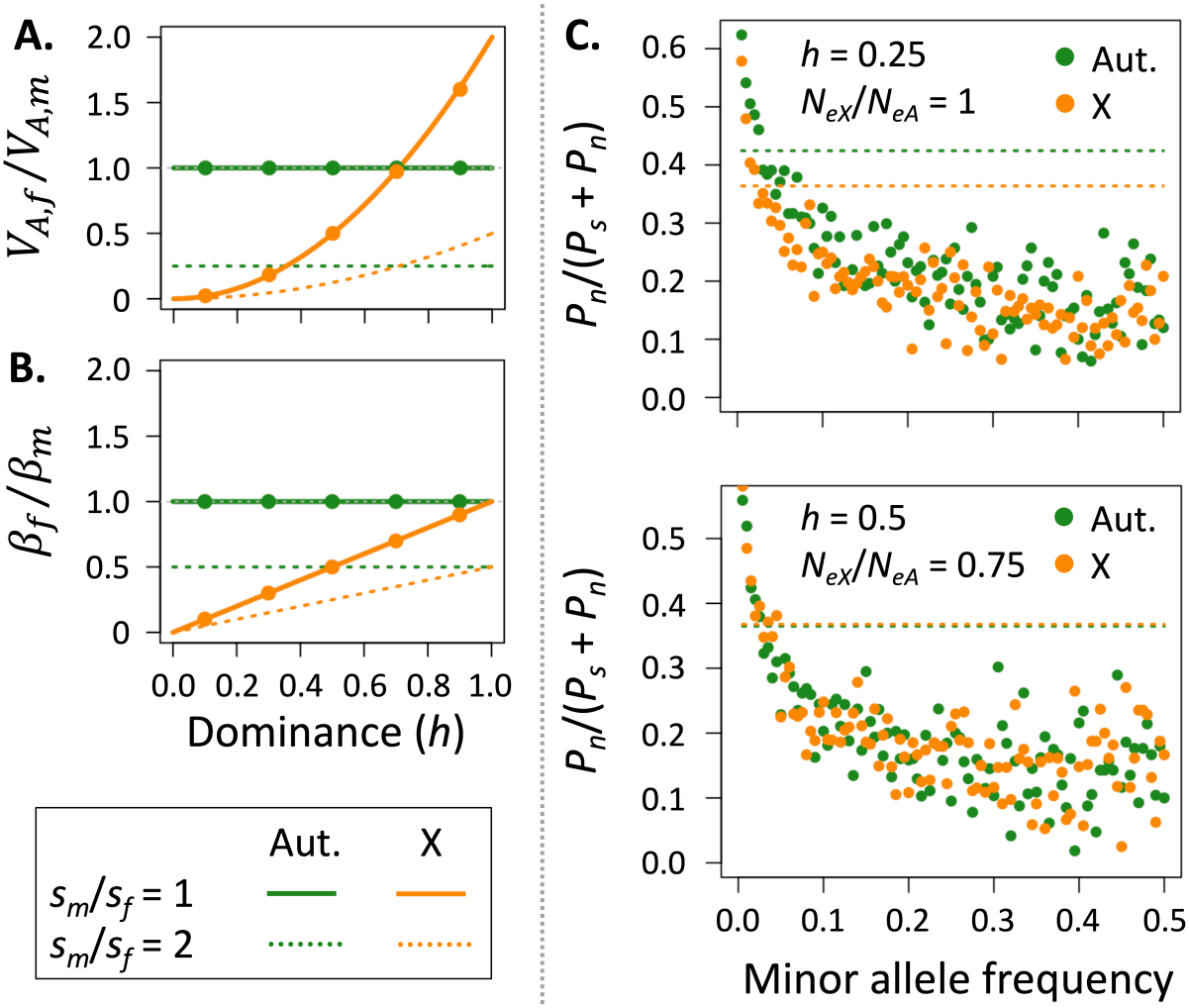


1031

1032 **Figure 1. Theoretical predictions outlining the effects of sex and chromosomal**

1033 **compartment (*i.e.*, autosomes vs. X) on three metrics of deleterious variation. A.**

1034 Contributions of autosomal and X-linked loci to sex-specific $V_A$ for fitness, illustrating that

1035 stronger purifying selection in a given sex increases autosomal $V_A$ for that sex, and that male

1036 $V_A$ is systematically elevated (and female $V_A$ systematically depleted) on the X chromosome,

1037 especially for partially recessive alleles. **B.** Contributions of autosomal and X-linked loci to

1038 sex-specific regressions of average fitness effect on deleterious allele frequency ($\beta$),

1039 illustrating that stronger purifying selection in a given sex increases $\beta$ for that sex, and male

1040 $\beta$ is systematically elevated (and female $\beta$ systematically depleted) on the X chromosome. **C.**

1041 The proportion of protein-coding variants that are nonsynonymous is a function of the

1042 dominance coefficient ($h$) and the ratio of X-linked to autosome effective population size

($N_{eX}/N_{eA}$), with a deficit of nonsynonymous variants on the X when $N_{eX}/N_{eA} > ¾$ and or $h < ½$ (see also Sup. Fig. 2). Simulations assume $N_{eA}\bar{s}$=400, $N_{eA}\mu$=$10^{-3}$, $s_m = s_f$, a gamma distributed DFE with shape parameter $k$ = 0.5. Datasets were simulated for a random sample of 200 haploid X chromosomes and 200 autosomes, with $10^7$ synonymous and 2.5 x $10^7$ nonsynonymous coding sites per chromosome, with population allele frequencies simulated using stationary distributions described in the main text. Broken lines show the results for $P_n/(P_s + P_n)$ for all segregating sites pooled across MAF. In panels A-B, filled circles represent stochastic simulations from the stationary distributions (assuming $N_{eX}/N_{eA}$=3/4 and otherwise following the simulation approach of panel A), while curves are based on deterministic mutation-selection balance approximations in the main text.

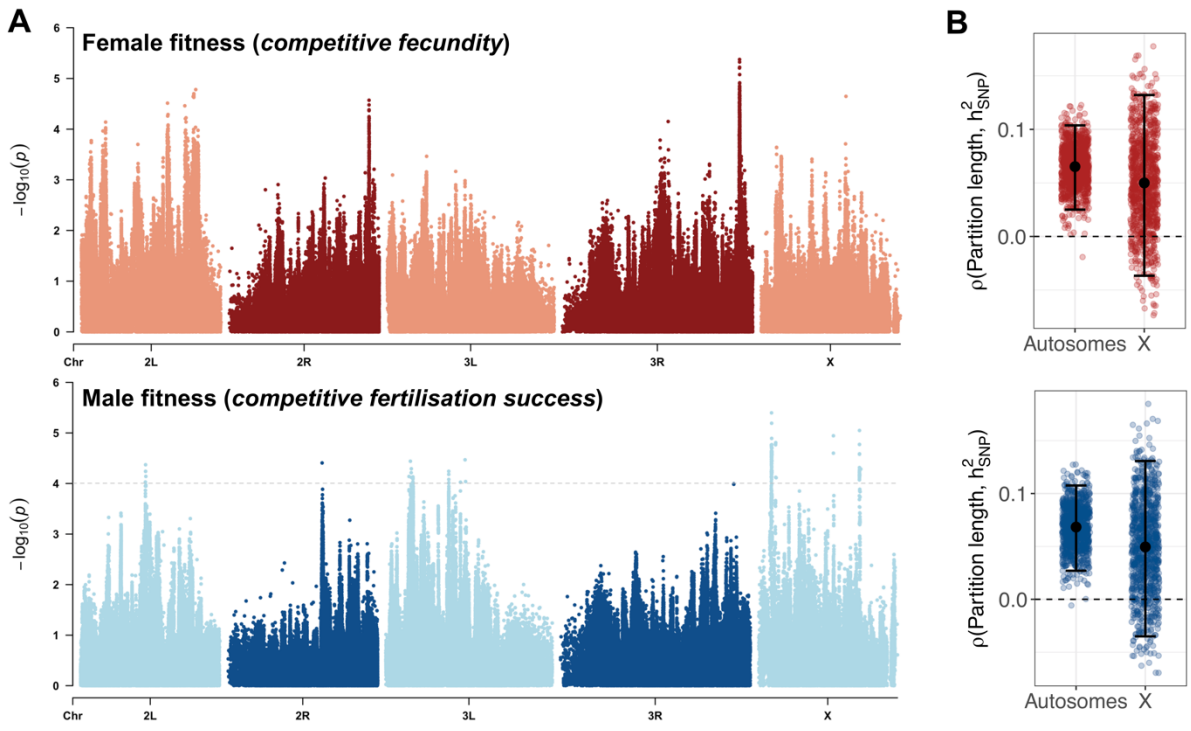


**Figure 2. A polygenic basis of sex-specific fitness. A.** $-log_{10}$(p) values from a Wald $\chi^2$ association test of each SNP variant against female fitness (top) and male fitness (bottom), presented as Manhattan plots along the five major chromosome arms of the *D. melanogaster* genome. Grey dashed line denotes a 30% FDR threshold (q-value=0.3) in males (no SNP reached the 30% FDR threshold in females, hence the absence of an equivalent line in females). **B.** Spearman's rank correlation coefficients between the length of a random chromosome partition and its SNP heritability, for females (red) and males (blue), on autosomes and X. Bars represent means and 95 confidence intervals across 1,000 partition sets.

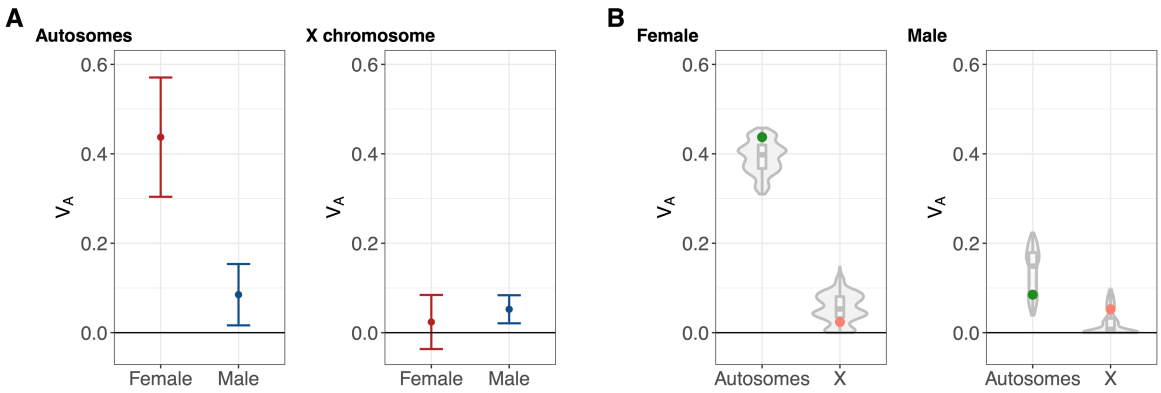


**Figure 3. The effects of sex and sex-linkage on estimates of $V_A$ for fitness. A.** $V_A$ (±SE) for fitness in females (red) and males (blue), on autosomes and the X chromosome, respectively. **B.** $V_A$ for fitness in observed data on autosomes (green dots) and the X chromosome (orange dots), along with 1,000 permuted estimates (grey boxplots and violin plots), in females and males, respectively.

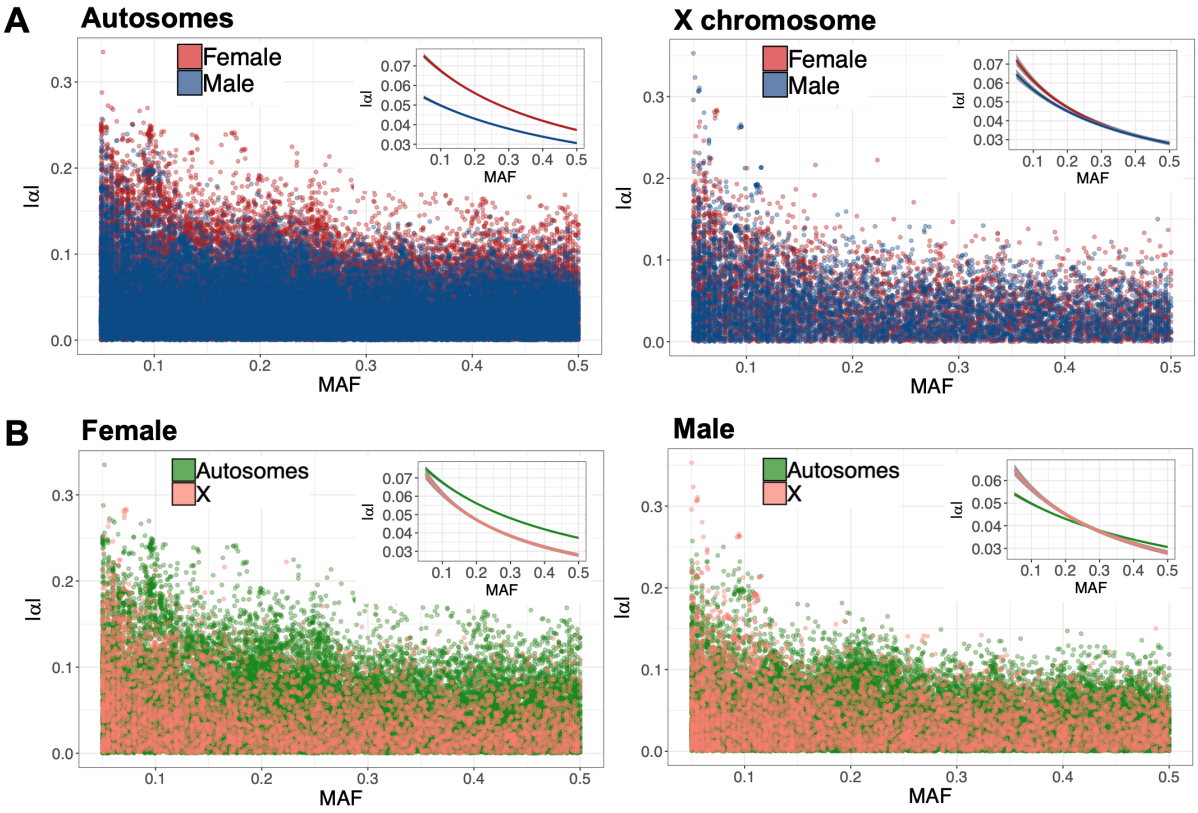


**Figure 4. The effects of sex and sex-linkage on the regression of an allele's average fitness effect on its frequency ($\beta$). A.** Scatter plot of an allele's absolute average effect on fitness ($|\alpha|$) and its MAF for females and males, on autosomes (left) and the X chromosome (right), respectively. The insets present fitted lines from a Gamma GLM of $|\alpha|$ as a function of MAF. **B.** Scatter plot of $|\alpha|$ and MAF for autosomes and X-linked loci, in females (left) and males (right), respectively. Inset as above.

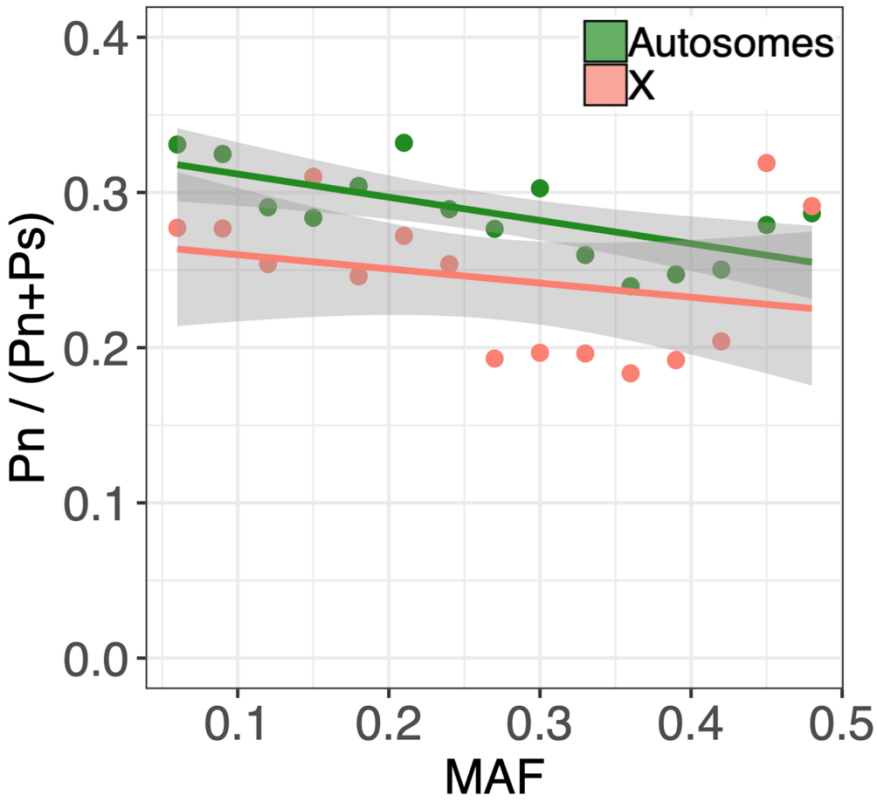


**Figure 5. The effect of sex-linkage on synonymous and nonsynonymous polymorphism.** Proportion of nonsynonymous sites among autosomal and X-linked LD-pruned protein-coding loci, for each of 15 MAF bins (MAF bin width=0.03; points are at MAF bin mid-point), with linear regression lines (±95% CIs) presented for visual emphasis. Regression coefficients from this analysis were used to make inferences about dominance and $N_{eX}/N_{eA}$ (*i.e.*, Fig. 6B).

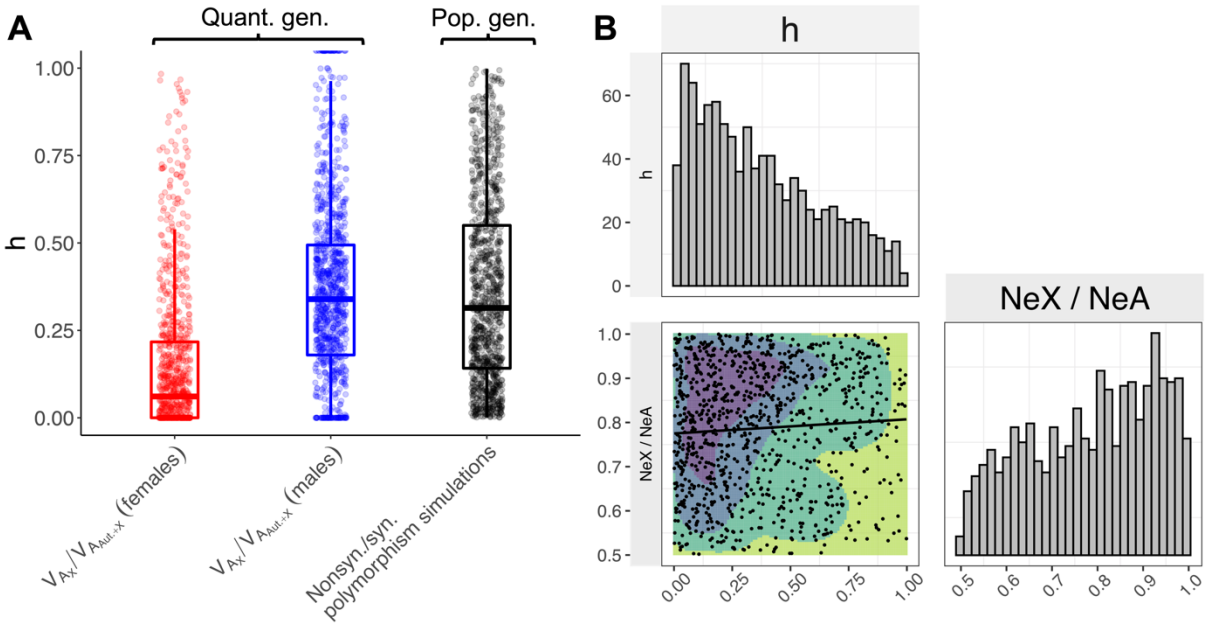


**Figure 6. Quantitative and population genetic inferences of dominance and $N_{eX}/N_{eA}$. A.** Boxplots of *h* estimates, based on quantitative genetic data (*i.e.*, the fraction of total $V_A$ that is X-linked in females, left; the fraction of total $V_A$ that is X-linked in males, middle) and population genetic data (simulated data fitted to logistic regression coefficients of nonsynonymous/synonymous status on MAF and chromosome compartment; right). For visualisation purposes, estimates of *h* greater than one and smaller than zero are not presented. **B.** Diagonal shows posterior distributions of dominance and $N_{eX}/N_{eA}$ (N=1,000 accepted simulations). Off-diagonal presents a scatter plot of both parameters, with contours and linear regression line for visual emphasis.

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**Table 1.** Fitness for each of three possible genotypes at the $i^{th}$ locus[1]

| | **Genotype (autosomal, X-linked)** | | |
|---|---|---|---|
| | $A_iA_i$ , $A_i$ | $A_ia_i$, – | $a_ia_i$ , $a_i$ |
| **Female fitness (autosomal or X-linked)** | 1 | $1 - s_{f,i}h_i$ | $1 - s_{f,i}$ |
| **Male fitness (autosomal)** | 1 | $1 - s_{m,i}h_i$ | $1 - s_{m,i}$ |
| **Male fitness (X-linked)** | 1 | – | $1 - s_{m,i}$ |

[1]Selection and dominance coefficients are subject to the constraints: $0 < s_{f,i}, s_{m,i}, h_i < 1$.

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