Selection in males purges the standing genetic load on female fitness

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Abstract

Theory predicts that the ability of selection and recombination to purge mutation load is enhanced if selection against deleterious genetic variants operates more strongly in males than females. However, direct empirical support for this tenet is limited, perhaps because traditional quantitative genetic approaches allow dominance and intermediate-frequency polymorphisms to obscure the effects of rare and partially recessive deleterious alleles that make up the main part of a population's mutation load. Here, we exposed the mutation load of a population of *Callosobruchus maculatus* seed beetles via successive generations of inbreeding, and quantified its effects by measuring heterosis – the increase in fitness upon the masking of deleterious alleles by heterozygosity – in a fully factorial sex-specific diallel cross among 16 inbred strains. Competitive lifetime reproductive success (i.e. fitness) was measured in male and female outcrossed F_1s as well as inbred parental 'selfs', and we estimated the 4×4 male-female inbred-outbred genetic covariance matrix (**G**) for fitness using Bayesian Markov chain Monte Carlo simulations of a custom-made general linear mixed effects model. We found that heterosis estimated in males and females was highly correlated among strains, and that heterosis was strongly negatively correlated to strains' outcrossed breeding values for male fitness, but not female fitness. This suggests that the additive genetic variation for fitness in the males, but not females, of this population reflect the amount of (partially) recessive deleterious alleles segregating at mutation-selection balance, and that the population's mutation load therefore has greater potential to be purged via selection in males. These findings contribute to our understanding of the prevalence of sexual reproduction in nature and the maintenance of genetic variation in fitness-related traits.

Impact statement

A mainstay evolutionary question has been: why do the large majority of eukaryotic species reproduce sexually if such females must spend half of their reproductive effort producing sons, which produce no offspring themselves? In principle, a lineage of a mutant asexual female that simply clones herself into daughters would grow at twice the rate of her sexual competitors (all else equal). What prevents this from being the predominant mode of reproduction throughout eukaryotes? One category of hypotheses regards the role of males in facilitating the purging of deleterious mutations from the population's genome since very strong selection in males, unlike females, can occur in many species without direct consequence to population offspring numbers. Due to the inherent difficulties of detecting selection on segregating genetic variation, empirical evidence for this theory is limited to indirect evidence from manipulative experiments and experimental evolution studies. Here we demonstrate that the standing deleterious allelic variation in a population of the seed beetle, *Callosobruchus maculatus*, is selected against strongly in males but not females. Using a fully factorial diallel cross among 16 inbred strains, we measured the degree to which fitness in the outbred offspring of those crosses improved relative to their inbred parents. This measure is known as heterosis and offers an estimate of the relative number of deleterious alleles carried among strains. We then analyzed the relationship between strains' heterosis values and their sex-specific additive genetic breeding values for fitness, revealing the extent to which those segregating deleterious alleles are selected against in males and females. We found that strains heterosis values were strongly correlated with male fitness, but not female fitness. This demonstrates that the population's deleterious mutations can be efficiently selected against (i.e. purged) via selection in males. This process would offer a benefit to sexual reproduction that may outweigh its costs, and therefore yields insight to the prevalence of sex in nature.

Introduction

Sexual reproduction is paradoxically prevalent when considering the cost of producing male offspring, which produce zero offspring themselves (Maynard Smith 1971, Lehtonen et al. 2012, Gibson et al. 2017). Counterintuitively, this same male feature may offer long-term benefits to producing sons, as the capacity for sexual recombination to facilitate the purging of mutation load (Haldane 1927, Muller 1964, Felsenstein 1974, Kondrashov 1982,1988, Otto and Lenormand 2002, Hartfield and Keightley 2012) can be enhanced via strong selection in males without appreciable costs to population growth (Manning 1984, Kodric-Brown and Brown 1987, Agrawal 2001, Siller 2001, Whitlock 2002, Lorch et al. 2003, Whitlock and Agrawal 2009). For this process to advantage sexual lineages over mutant asexual competitors, and thereby account for the maintenance and prevalence of sex in eukaryotes, the segregating load of alleles with deleterious effects on female offspring production (and hence, population mean fitness) must (1) be likewise selected against in males (i.e. selection needs to be sexually concordant (SC) across the sexes), and (2) be selected more strongly in males than in females (Agrawal 2001, Siller 2001, Whitlock and Agrawal 2009).

Empirical support for males facilitating the purging of mutation load comes mostly from studies of induced, accumulated, or known mutations (e.g. Radwan 2004, Sharp & Agrawal 2008, 2013, Hollis et al. 2009, Grieshop et al. 2016) or extreme experimental evolution treatments (e.g. Firman and Simmons 2010, 2012, Lumley et al. 2015, Dugand et al. 2018, 2019, Yun et al. 2018, Buzatto and Clark 2020; reviewed in Cally et al. 2019), but the process and/or the ability to detect it seems limited when looking at a snapshot of the standing genetic variation captured from natural populations (e.g. Chenoweth et al. 2015). This, however, could itself be a result of strong SC selection continually purging the many rare variants that make up a population's mutation load, causing a population at mutation-selection balance to have disproportionate amounts of sexually antagonistic (SA) genetic variance in fitness (Chippindale et al. 2001, Bonduriansky and Chenoweth 2009, van Doorn 2009, Long et al. 2012, Berger et al. 2014) owing

to the remaining polymorphic sites whose alleles pose opposite fitness effects between the sexes (Connallon et al. 2010, Connallon and Clark 2012). It therefore remains unclear whether sexual selection acts primarily on SA genetic variation, as some studies suggest (e.g. Rice 1992, Chippindale et al. 2001, Pischedda and Chippindale 2006, Prokop et al. 2012, Berger et al. 2014), or on SC genetic variation, which many theoretical models on the benefits of sex assume (e.g. Manning 1984, Kodric-Brown and Brown 1987, Agrawal 2001, Siller 2001, Lorch et al. 2003, Martinossi-Allibert et al. 2019a).

Here we increased homozygosity by successive multigenerational inbreeding of seed beetle strains to expose the rare, deleterious, partially recessive alleles that comprise a population's mutation load (Lande 1975, Zhang X.S. et al. 2004). We then measured heterosis – the increase in fitness between inbred strains and their outcrossed progeny due to the masking of rare partially recessive deleterious alleles by heterozygosity (Charlesworth and Willis 2009). The data are from a fully factorial diallel cross (Lynch and Walsh 1998) among 16 inbred strains (see Grieshop and Arnqvist 2018) and are measures of competitive lifetime reproductive success (hereafter: fitness) in the outcrossed male and female F_1 offspring and the within-strain F_1 'selfs'. We assessed the extent to which sex-/strain-specific estimates of heterosis are reflected in corresponding male and female additive breeding values for fitness, and therefore, the extent to which this population's mutation load is exposed to selection in each sex. A genotype's additive genetic breeding value for fitness should be negatively correlated to its relative share of the population's mutation load. Thus, we predicted that estimates of additive genetic breeding values for fitness would be negatively correlated to estimates of heterosis among strains. Further, if mutation load is purged more efficiently via males, then this relationship should be stronger in males than females.

Methods

Study population and inbred strains

Callosobruchus maculatus (Coleoptera: Bruchidae) is a pest of leguminous crops that has colonized most of the tropical and subtropical regions of the world (Southgate 1979). Laboratory conditions and fitness assays closely resemble the grain storage facilities and crop fields to which they are adapted. Females lay eggs on the surface of dry beans and hatched larvae bore into the beans, where they complete their life cycle, emerging from the beans as reproductively mature adults (Southgate 1979). This species is facultatively aphagous (requiring neither food nor water to reproduce successfully), exhibits a generation time of ~3 weeks (Southgate 1979), and exhibits a polyandrous mating system (Miyatake and Matsumura 2004).

The origins of this population's isofemale lines and inbred lines have been described thoroughly by Berger et al. (2014), Grieshop et al. (2017), and Grieshop and Arnqvist (2018). Briefly, 41 isofemale lines were constructed from a wild population of *C. maculatus* that was isolated from *Vigna unguiculata* seed pods collected at a small-scale agricultural field close to Lomé, Togo (06°10'N 01°13'E) during October and November 2010 (Berger et al. 2014; Figure S1A). Each isofemale line stemmed from a single virgin male/female pair whose offspring were expanded into small sub-populations (Figure S1A). These 41 isofemale lines have an inbreeding coefficient of 0.25 (Falconer and Mackay 1996), and were cultured for 12 generations prior to the fitness assays of Berger et al. (2014). Then, from January 2013 to January 2014, 20 replicate lineages of each isofemale line (totaling >800 lineages) were subjected to single-pair full-sibling inbreeding (i.e. "close" inbreeding; Falconer and Mackay 1996) for 10 consecutive generations or until extinction (Grieshop et al. 2017; Figure S1A). For the 16 inbred strains chosen for the present diallel cross, this was followed by one generation of expansion into small populations, comprising a total of 12 generations of full-sibling mating (1 full-sibling isofemale line expansion + 10 generations of close inbreeding + 1 full-sibling inbred line expansion), which corresponds to an inbreeding coefficient of 0.926

(Falconer and Mackay 1996). During the inbreeding regime, inbred lineages stemming from isofemale lines that were enriched for male-benefit/female-detriment SA genetic variation tended to go extinct prior to completing the full inbreeding program (Grieshop et al. 2017), making it impossible to retrieve a representative inbred line from four of the most male-benefit/female-detriment isofemale lines. The present 16 inbred strains were chosen with the aim of countering that biased representation (Figure S2).

Sex-specific fitness assay

The present study used data from a fully factorial diallel cross (Lynch and Walsh 1998) among the 16 inbred strains, where sex-specific competitive lifetime reproductive success (hereafter: fitness) was measured in F_1 males and females separately. The partitioning of genetic variance in fitness is reported in detail by Grieshop and Arnqvist (2018), and the aspects that bear relevance to the present study are given in the *Statistical methods* and *Discussion* sections. The experiment was conducted in two replicate 'blocks' for a total of 3278 individual fitness estimates performed in 237/240 possible outbred crosses and all 16 parental selfs (Figure S1B). Male and female fitness, as well as inbred and outbred fitness, were measured independently (Figure S1B,C), and all were approximately Gaussian distributed. There are 1616 outbred male (o_M), 1450 outbred female (o_F), 115 inbred male (i_M), and 97 inbred female (i_F) individual fitness estimates. Each observation of the fitness assay consisted of a 90 mm ø petri dish containing ca. 100 *V. unguiculate* seeds, a focal individual from a given outbred cross or inbred self, a sterilized same-sex competitor from the outbred base population, and two opposite-sex individuals from the base population (Figure S1C). Same-sex competitors were sterilized with a 100 Gy dose of ionizing radiation from a Cs^{137} source (Figure S1C), which does not notably reduce lifespan or reproductive competitiveness in either sex, but does cause zygote lethality, accrediting all emerging offspring to focal individuals (Grieshop et al. 2016). Thus, counts of F_2 offspring emerging in the petri dishes are the measures of focal individuals' fitness (Figure S1C), the differences among them being attributable to

focal F_1 individuals' pre- and post-copulatory reproductive success and their offspring's larval viability. Our fitness assays therefore enable, but are not limited to, the following mechanisms of selection in adults: mate searching and oviposition site selection, mating success, mating resistance, sexual conflict over remating rate, sperm competition, cryptic female choice, fertility, fecundity, and lifespan (discussed further by Grieshop et al. 2016, Grieshop and Arnqvist 2018). Despite lacking many of the elements of natural selection that might apply to these beetles in nature (as would any laboratory fitness assay), this method (or similar) has been effective in revealing sex-specific genetic variance in fitness (Berger et al. 2014, 2016a,b, Grieshop et al. 2016, Martinossi-Allibert et al. 2019b), perhaps owing its relatively greater resemblance to the beetles' natural ecology compared with other model systems and/or the inherent three-dimensional physical complexity provided by the beans, which may play an important role in achieving balance between SC and SA mating interactions in the laboratory (Singh et al. 2017, Yun et al. 2017).

Statistical Methods

Here, we modelled the male-female-inbred-outbred additive genetic covariance matrix, **G**, for fitness, which can be thought of as four submatrices:

$$\mathbf{G} = \begin{bmatrix} \mathbf{G}_F & \mathbf{B} \\ \mathbf{B}^T & \mathbf{G}_M \end{bmatrix} = \begin{bmatrix} i_F & i_F, o_F & i_F, i_M & i_F, o_M \\ o_F, i_F & o_F & o_F, i_M & o_F, o_M \\ i_M, i_F & i_M, o_F & i_M & i_M, o_M \\ o_M, i_F & o_M, o_F & o_M, i_M & o_M \end{bmatrix}, \quad (1)$$

where $\mathbf{G}_F$ and $\mathbf{G}_M$ respectively represent the female- and male-specific covariance matrices between the inbred and outbred states, **B** represents the cross-sex cross-state covariance matrix, and $\mathbf{B}^T$ its transpose (Lande 1980). Variances were estimated for parameters listed along the diagonal of the expanded matrix, and covariances were estimated for pairs of parameters listed in the off-diagonal. Cross-sex cross-trait **G**

matrices are typically modeled with the aim of assessing the sex-specific genetic architecture of multiple traits and whether it constrains or enables (sex-specific) adaptation (Lande 1980, Gosden and Chenoweth 2014, Ingleby et al. 2014, McGlothlin et al. 2019), however, our **G** matrix is unique in two important ways: (1) it is a cross-sex cross-*state* (rather than cross-trait) **G** matrix, which models inbred/homozygous effects versus outbred/heterozygous effects for the same "trait", and (2) that "trait" is fitness. Together, these enable an interpretation of our results that is not typically enabled in studies of sex-specific **G** matrices.

The **G** matrix was modeled as a random effect in a general linear mixed-effects model (GLMM) using Bayesian Markov chain Monte Carlo (MCMC) simulations in the 'MCMCglmm' package (v. 2.25; Hadfield 2010) for R (v. 3.6.0; R Core Team 2019). To attain proper estimates of additive genetic variance, two additional variance components (random effects) were designed to estimate symmetrical epistasis (*v*) and sex-specific symmetrical epistasis (*v*×*S*) (i.e. (sex-specific) strain-strain interaction variance among outcrossed families only), as these effects are known to be present in these data (Grieshop and Arnqvist 2018). Fixed factors in this model were sex (*S*, male or female), inbred (*I*, inbred self or outbred cross), block (*B*, first or second replicate of the full diallel cross), and the interactions *S*×*I* and *B*×*I*, making the full GLMM:

$$y = \mu + S + B + I + S{\times}I + B{\times}I + \mathbf{G} + v + v{\times}S + \varepsilon, \qquad (2)$$

where y is fitness, μ is the intercept, and ϵ is the residual/unexplained error, normally distributed as $\epsilon \sim N(0, \sigma^2)$ with variance σ^2. We enabled (co)variance estimation to differ among elements of the **G** matrix, and used minimally informative parameter-expanded priors (Hadfield 2012). The model was run for 2,000,000 iterations after a burn-in of 200,000, with a thinning interval of 2000, which provided 1000 uncorrelated posterior estimates of each sex-/strain-specific effect to be stored and used for resampling the relationships described below. Posteriors were unimodally distributed and their trend stable over the

duration of the simulations after the burn-in period. The model that was fit for the purpose of estimating the **G** matrix and resampling the stored posteriors (see below) was fit to relative fitness, i.e. fitness standardized by the sex-specific outbred mean, whereas the model that was fit for the purpose of plotting results was fit to untransformed/raw data.

Heterosis (outbred (*o*) fitness – inbred (*i*) fitness) is of central interest to our study but is not explicitly modeled in our **G** matrix. Relationships involving heterosis were therefore assessed by resampling the sex-/state-specific breeding values from the 1000 stored posteriors. This approach incorporates the uncertainty around those breeding values into the estimated relationships and their credibility intervals, hence enabling the quantification of statistical significance in a way that accounts for breeding values being otherwise anticonservative when used to assess relationships that are not accounted for by the model (Postma 2006, Hadfield et al. 2010). Of specific interest is the comparison of male and female covariances between sex-averaged heterosis (*o*-*i*) and fitness (i.e. o_M or o_F) – that is, $COV(o_M, o-i)$ versus $COV(o_F, o-i)$, where $o-i = \left(\frac{o_M+o_F}{2}\right) - \left(\frac{i_M+i_F}{2}\right)$, as the genetic covariance between outbred fitness and heterosis represents an estimate of the efficacy of selection in each sex to purge mutation load. However, retrieving unbiased estimates of the two covariances is problematic due to measurement error in outbred fitness also featuring in the sex-averaged heterosis term, which could drive spurious correlations and false positive discoveries (Postma 2011, Berger and Postma 2014). To address this, we explored whether sex-specific estimates of heterosis (o_M-i_M and o_F-i_F) might be so highly correlated that they effectively convey the same information, such that the covariance between the outbred breeding values of one sex and heterosis in the other (i.e. $COV(o_M, o_F - i_F)$ and $COV(o_F, o_M - i_M)$) may offer an assessment that cannot share measurement error between fitness and heterosis (since male and female fitness were measured separately; see *Sex-specific fitness assay*, and Figure S1B). The correlation between male and female heterosis is also informative as to the degree of SA versus SC genetic variation underlying heterosis. Thus, the following Pearson's correlation was

resampled 1000 times from the stored posteriors:

$$r_{oM-iM,oF-iF} = \frac{COV(o_M - i_M, o_F - i_F)}{\sqrt{V(o_M - i_M) \cdot V(o_F - i_F)}}, \quad (3)$$

where *V* refers to the variance of the variable immediately following it, and *COV*(,) refers to the covariance between the variables separated by the comma. However, even if o_M-i_M and o_F-i_F were highly correlated, $COV(o_M, o_F - i_F)$ and $COV(o_F, o_M - i_M)$ would not be directly comparable. By contrast, the resampled Pearson's correlations between outbred breeding values in one sex and heterosis in the other are directly comparable to one another since their estimates are standardized in the denominators, and they likewise do not share measurement error between fitness and heterosis:

$$r_{oM,oF-iF} = \frac{COV(o_M, o_F - i_F)}{\sqrt{Vo_M \cdot V(o_F - i_F)}}, \text{ and} \quad (4)$$

$$r_{oF,oM-iM} = \frac{COV(o_F, o_M - i_M)}{\sqrt{Vo_F \cdot V(o_M - i_M)}}. \quad (5)$$

Thus, our main interpretations are based on these resampled Pearson's correlations (equations 4 and 5). We also present the resampled estimates of $COV(o_M, o - i)$ and $COV(o_F, o - i)$, despite each of them being potentially biased by shared measurement error, because they offer a like-to-like comparison in which the fold difference between those covariances is our best estimate of the difference in the efficacy of selection at purging mutation load via males versus females. Note that $COV(o_M, o - i)$ and $COV(o_F, o - i)$ would not only feature shared measurement error (see above) but potentially also shared MCMC sampling error between fitness and heterosis upon resampling those estimates from the

posteriors. To avoid the latter of those issues, each of the 1000 resampled estimates of $COV(o_M, o - i)$ and $COV(o_F, o - i)$ drew their outbred fitness values from different simulations when estimating o_M and o_F than when estimating sex-averaged heterosis, $\left(\frac{o_M+o_F}{2}\right) - \left(\frac{i_M+i_F}{2}\right)$.

The point estimates of these correlations and covariances are the posterior mode of the 1000 resampled estimates, which were unimodal in all cases. The (95%) credibility intervals (CIs) around those point estimates are given by the highest posterior density (HPD) intervals. The two tailed *P* values for these correlations and covariances were calculated as the proportion of times that those 1000 estimates fell on the opposite side of zero relative to the posterior mode point estimate (or overlapped the point estimate of the other sex in the case of assessing the difference between $COV(o_M, o - i)$ and $COV(o_F, o - i)$), multiplied by two. The plotted breeding values, heterosis estimates, and 95% confidence ellipses are for visual purposes only, and are based on the HPD means of the model fit of untransformed/raw fitness; they do not depict the uncertainty in those breeding values that was incorporated into the resampled estimates of the covariances, correlations, their CIs, and their *P* values. Because these HPD means are zero-centered in the 'MCMCglmm' output (even for models fit to untransformed data), they were rescaled to the more intuitive original scale before plotting.

The potential for non-genetic parental effects and sex-chromosome inheritance to explain our findings was thoroughly addressed. In short, we statistically removed these effects from our data and re-ran our analyses to confirm that our findings stand in the absence of those effects (Supplementary Material S1). See *Data availability* regarding the R code for reproducing all analyses, procedures, and tables.

Results

The genetic variance for inbred males (i_M) was 1.85x that of inbred females (i_F), and the genetic variance for outbred males (o_M) was 1.24x that of outbred females (o_F) (Table 1). Males also exhibited 3.37x and 5.58x the residual variance in fitness relative to females when inbred and outbred, respectively (Table 1). Genetic variance for inbred fitness was 10.92x and 7.35x that of outbred fitness in males and females, respectively (Table 1).

Table 1. Estimated **G** matrix displaying genetic variances (with credibility intervals (CIs) and residual variances) on the diagonal, their covariances in the lower triangle, and their Pearson's correlation coefficients (*r*) in the upper triangle. Covariances and correlations with 95% credibility intervals excluding zero are bolded.

	i_F	o_F	i_M	o_M
i_F	0.0108 (0.004, 0.021) (0.0256)	0.43	**0.85**	0.46
o_F	0.001	00015 (<0.001, 0.003) (0.0150)	0.20	0.03
i_M	**0.0106**	0.0002	0.0199 (0.006, 0038) (0.0863)	**0.67**
o_M	0.0016	-2.5×10^{-5}	**0.0030**	0.0018 (<0.001, 0.004) (0.0837)

Whereas the outbred breeding values for fitness in males and females were uncorrelated (r_{o_M, o_F} = 0.03, (95% CIs: -0.56, 0.54); Figure 1A, Figure S3), their inbred breeding values were highly correlated (r_{i_M, i_F} = 0.85 (0.38, 0.97); Figure 1A, Figure S4) – these correlations are explicitly estimated in the **G** matrix (Table 1). The cumulative effects of the recessive variants that were masked by heterozygosity upon outcrossing are undoubtedly deleterious, as evidenced by the globally improved fitness of outcrossed observations relative to the inbred parental selfs (i.e. the fixed effect of *I*, mean reduction in relative fitness of inbreds versus outbreds = 0.294 (0.19, 0.41), $P_{\text{MCMC}} < 0.001$; Figure 1A).

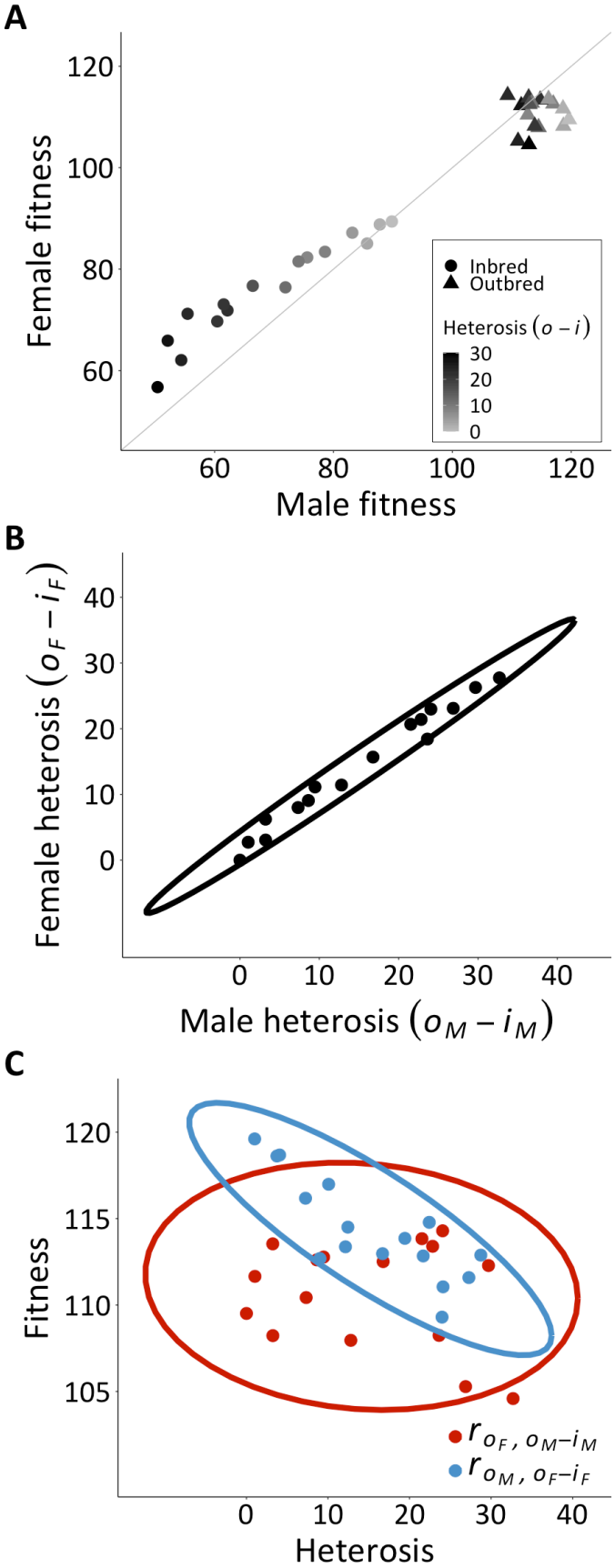

Figure 1. Breeding values from the MCMC model of untransformed (raw) fitness (for plotting purposes only). (A) A summary of the data and main result (y=x line for reference), showing each strain's male (x-axis) and female (y-axis) fitness in the inbred (circles: r_{i_M, i_F} = 0.85 (0.38, 0.97)) and outbred (triangles: r_{o_M, o_F} = 0.03, (-0.56, 0.54)) state, shaded by sex-averaged heterosis (i.e. $((o_M\text{-}i_M)+(o_F\text{-}i_F))/2$). Variation in heterosis is clearly distributed along the population's outbred male, but not female, breeding values. (B) Depiction of the correlation between heterosis in male and female fitness across strains, showing that these sex-specific measures are conveying essentially the same information ($r_{o_M - i_M, o_F - i_F}$ = 0.86, (0.66, 0.95), P < 0.001). (C) Depiction of main finding: the statistically significant resampled correlation between the outbred male breeding values and heterosis in female fitness (blue: $r_{o_M, o_F - i_F}$ = -0.59, (-0.81, -0.11), P = 0.008) would enable the mutation load on population mean fitness to be purged via selection in males. In contrast, the outbred female breeding values do not reflect this mutation load (red: $r_{o_F, o_M - i_M}$ = -0.14, (-0.40, 0.28), P = 0.672). Ellipses are 95% confidence ellipses fit to the breeding values, and therefore do not depict the uncertainty that was included in the resampled estimates of statistical significance (see Methods).

Heterosis in male and female fitness were highly correlated (resampled $r_{o_M - i_M,\, o_F - i_F}$ = 0.86, (0.66, 0.95), *P* < 0.001; Figure 1B) with narrower credibility intervals than the inbred intersexual genetic correlation, $r_{i_M,\, i_F}$ (see above). Strains' outbred breeding values for male fitness were significantly negatively correlated to heterosis in female fitness (resampled $r_{o_M,\, o_F - i_F}$ = -0.59, (-0.81, -0.11), *P* = 0.008; Figure 1C). Female outbred breeding values, by contrast, were not correlated to male heterosis (resampled $r_{o_F,\, o_M - i_M}$ = -0.14, (-0.40, 0.28), *P* = 0.672; Figure 1C). The point estimates of the covariance between outbred fitness and sex-averaged heterosis in males ($COV(o_M, o - i)$ = -0.0012, (-0.0029, -0.0004), *P* = 0.008) was 6.75x greater than that in females ($COV(o_F, o - i)$ = -0.0002, (-0.0011, 0.0007), *P* = 0.558). While neither the sex-difference in covariance (proportion of 1000 estimates of $COV(o_M, o - i)$ > $COV(o_F, o - i)$ times two: *P* = 0.104) nor the sex-difference in correlation (proportion of 1000 estimates of $r_{o_M,\, o_F - i_F}$ > $r_{o_F,\, o_M - i_M}$ times two: *P* = 0.132) were statistically significant, both approaches are directly comparable between the sexes and showed that the relationship between outbred breeding values and heterosis was strong and significant in males but absent in females.

Discussion

Our findings show that the mutation load of the population is more effectively purged via selection in males than females (Figure 1C). With heterosis being so highly SC (Figure 1B), we were able to circumvent the potential bias posed by shared measurement error between fitness and heterosis by assessing the correlations between the outbred breeding values in one sex and heterosis in the other (i.e. $r_{o_M,\, o_F - i_F}$ or $r_{o_F,\, o_M - i_M}$; see Methods, equations 3 and 4). Moreover, the former of those two relationships, $r_{o_M,\, o_F - i_F}$, is much more central to the question of whether selection via males can purge a population's mutation load, since population productivity in most taxa is a female-limited process and

this correlation reveals the relationship between outbred male breeding values and female heterosis. That the female equivalent of this assessment, $r_{o_F,\, o_M - i_M}$, was found to be indistinguishable from zero indicates that the genetic variation in fitness among females of this population does not strongly reflect the deleterious mutations that they carry in their genomes.

Our findings are pertinent to the longstanding question of why sexual reproduction is so prevalent in nature. Since, all else equal, the production of sons would halve the exponential growth rate of a sexual female's lineage relative to an asexual competitor (Maynard Smith 1971, Lehtonen et al. 2012, Gibson et al. 2017), there must be some mechanism(s) that compensate for this two-fold cost of sex. One explanation is that the efficacy of selection against the mutation load on a population's offspring production is greater in males relative to females, which would allow that load to be purged without the demographic costs that would ensue given that same strength of selection acting on the population's females (Manning 1984, Kodric-Brown and Brown 1987, Agrawal 2001, Siller 2001, Whitlock 2002, Lorch et al. 2003, Whitlock and Agrawal 2009). As for whether the difference between the sexes of our population in this respect, our best estimate is given by the fold difference between $COV(o_M, o - i)$ and $COV(o_F, o - i)$, which are both potentially biased by shared measurement error between fitness and heterosis (unlike the correlations described above), and which are not statistically significantly different from one another. Nevertheless, as a rough guide, that estimate indicates that selection against load is several times greater in males than in females, in concordance with a previous estimate of selection against induced deleterious mutations in *C. maculatus* suggesting ~3x stronger selection in males (Grieshop et al. 2016). We note that other costs of sex such as sexual conflict could further increase the total cost of sex (Lehtonen et al. 2012) and hence would require an even greater sex-difference in selection to compensate for these costs. Indeed, all else is not equal between the sexes of our population: both intra- and inter-locus sexual conflict – i.e. SA selection on sex-homologous and sex-heterologous traits, respectively – pose costs to our population's offspring production (Berger et al.

2016a), which most likely drives the costs of sex to be >> two-fold. Nevertheless, at the very least, our findings indicate that the ability of selection to purge the population's mutation load is detectable and strong via males, but absent in females, representing a striking difference between the sexes that may at least partially compensate for the cost of sex.

We use heterosis as a measure of the relative share of the population's mutation load that is captured within each of our strains. While fitness variance and inbreeding depression can be attributable to genetic variation with either low or intermediate minor-allele frequencies (Charlesworth and Charlesworth 1987, 1999, Lynch and Walsh 1998, Charlesworth and Hughes 2000, Kelly and Willis 2001, Barton and Keightley 2002, Kelly 2003, Charlesworth et al. 2007, Mitchell-Olds et al. 2007, Charlesworth 2015, Sharp and Agrawal 2018), heterosis in crosses among inbred strains of a given population should be predominantly due to the masking of rare, partially recessive deleterious alleles by heterozygosity (Charlesworth and Willis 2009). That is, heterosis should predominantly reflect the same genetic variation that is expected to constitute a population's mutation load – rare, partially recessive deleterious alleles (Haldane 1927, Lande 1975, Zhang X.S. et al. 2004, Charlesworth and Willis 2009) – as opposed to alleles maintained by some form of balancing selection at (typically) intermediate frequencies. If variance in fitness is underlain by rare, partially recessive deleterious alleles, then genetic variance in fitness should be substantially greater, and mean fitness substantially lower, in the inbred versus outbred state (Robertson 1952; Kelly 1999; Charlesworth and Hughes 2000, Kelly and Tourtellot 2006), both of which are indeed upheld in the present findings (Table 1, Figure 1A). We note that while a substantial fraction of this population's genetic variance in fitness is likely owing to intermediate allele frequency polymorphisms under SA balancing selection (Berger et al. 2014, 2016a, Grieshop and Arnqvist 2018), the present findings show heterosis to be highly SC (Figure 1B), suggesting little if any role for the population's SA genetic variation in our measures of heterosis. Further, a previous estimate of dominance variance in this population, which to large degree is based on the heterosis term (Hayman

1954, Lynch and Walsh 1998, Lenarcic et al. 2012, Maurizio et al. 2018, Shorter et al. 2019), was likewise found to describe SC effects (Grieshop and Arnqvist 2018). Reciprocally, the sex-reversed dominance effects in the present data, which are characteristic of SA balancing selection (Kidwell et al. 1977, Fry 2010, Barson et al. 2015, Spencer and Priest 2016, Connallon and Chenoweth 2019), were detected via methods that are not based on heterosis (Grieshop and Arnqvist 2018). Thus, variation in heterosis in this population seems exclusively attributable to rare, partially recessive deleterious alleles, and its relationship to sex-specific outbred breeding values for fitness therefore indicates how selection would act to purge those alleles in males and females. Supplementary material S2 contains further discussion/exclusion of the role of SA genetic variation in this population's heterosis estimates.

Mechanistic understanding

One explanation for why male fitness exhibits greater sensitivity to mutation load is that (1) the fitness consequences of genetic variation in traits under selection are greater (i.e. phenotypic selection is stronger) in males, and/or (2) phenotypic variance in fitness-related traits is more sensitive to mutational input in males. That is, rare partially recessive deleterious mutations may not manifest via female fitness components strongly enough, and/or those female fitness components may not vary enough, to expose those deleterious alleles to selection. Although we cannot distinguish between these two processes with the present data, our findings support both concepts: outbred males exhibited 1.24x the genetic variance in fitness relative to outbred females (Table 1), and our males appear to have suffered moderately greater detriments than females from having their partially recessive mutation load revealed by inbreeding/homozygosity (see inbred points above the y=x line in Figure 1A and Figure S4).

These broader characteristics of our population may hold across other animal taxa. Laboratory estimates from insects suggest that males are more sensitive to mutation load than females, as revealed

by inbreeding (Mallet and Chippindale 2011, and findings herein), mutation accumulation (Mallet et al. 2011, 2012, Sharp & Agrawal 2013) and induced or known mutations (Sharp & Agrawal 2008, Almbro and Simmons 2014, Grieshop et al. 2016). Further, meta-analyses suggest that the opportunity for, and strength of, selection tend to be greater in males than females (Janicke et al. 2016, Singh and Punzalan 2018).

Studies of sex-biased genes (those with sexually dimorphic expression) provide broad mechanistic support for selection in males tending to purge alleles with SC fitness detriments. Unsurprisingly, male fitness components in *Drosophila melanogaster* are, at least to some extent, determined by the expression levels of genes that typically show male-biased expression (Dean et al. 2018). Further, the male-biased genes of *D. serrata* exhibit greater heritability (H^2) than female-biased genes, suggesting selection could act more efficiently to purge the deleterious alleles of male-biased genes (Allen et al. 2018). As for how this might affect female fitness, that same study found higher intersexual genetic correlations (r_{MF}) for the expression levels of those male-biased than for female-biased genes (Allen et al. 2018). High r_{MF} for gene expression or other traits is often interpreted as genetic constraints to sexual dimorphism, possibly posing SA fitness consequences (Bonduriansky and Chenoweth 2009, van Doorn 2009, Cox and Calsbeek 2009, Connallon et al. 2010, Stewart et al. 2010, Griffin et al. 2013, Ingleby et al. 2014, McGlothlin et al. 2019). However, it is certainly still possible for such male-biased genes to pose SC fitness effects, a core prediction of the "condition dependence" theory for sexually selected traits (Rowe and Houle 1996). Indeed, while mutations in *D. melanogaster*'s male- and female-biased genes pose greater detriments to male and female fitness components, respectively, the direction of these effects nevertheless tends to be SC – i.e. detrimental in both sexes (Connallon and Clark 2011). As another example, five naturally occurring evolutionary transitions to obligate asexuality in *Timema* stick insects resulted in those asexual females upregulating the male-biased genes of their sexual sister taxa, suggesting that at least some of those male-biased genes pose SC

fitness effects (Parker et al. 2019). Of particular relevance to the present study, the large majority of male-biased genes in *C. maculatus* – the present study species – that are expressed in females are actually upregulated in females after mating (Immonen et al. 2017). Thus, much of the mutation load on female fitness in *C. maculatus* could manifest via the expression of male-biased genes, which the present findings show would be purged more effectively via males. Fittingly, *C. maculatus* male-biased genes show a clear pattern of purifying selection – low rates of nonsynonymous sequence divergence (Sayadi et al. 2019).

The bulk of evidence in *Drosophila* and other organisms shows male-biased genes exhibiting *high* rates of nonsynonymous sequence and expression divergence among species (Meiklejohn et al. 2003, Ranz et al. 2003, Zhang Z. et al. 2004, Zhang Z. and Parsch 2005, Metta et al. 2006, Pröschel et al. 2006, Zhang Y. et al. 2007, Grath et al. 2009, Assis et al. 2012, Grath and Parsch 2012, Müller et al. 2012, Harrison et al. 2015, Dutoit et al. 2018, Whittle and Extavour 2019). At first, this may not obviously accord with our findings/explanation (above) since these patterns can either be interpreted as stronger directional selection in males relative to females and/or *relaxed* purifying selection relative to female- and un-biased genes (Ellegren and Parsch 2007, Meisel 2011, Parsch and Ellegren 2013, Dapper and Wade 2016, Grath and Parsch 2016) – the latter seemingly incongruent. However, elevated nonsynonymous sequence divergence certainly does not rule out purifying selection from having ensued simultaneously alongside adaptive sequence divergence (Ellegren and Parsch 2007, Parsch and Ellegren 2013, Dapper and Wade 2016, Grath and Parsch 2016). For example, in the male-biased genes (but not female- or un-biased genes) of *Drosophila* Zhang Z. and Parsch (2005) found a positive correlation between interspecific nonsynonymous divergence and local recombination rates; high levels of the former would be consistent with both interpretations (see above), whereas high recombination rates should facilitate purifying selection. Their findings suggest that male-biased genes (at least in *Drosophila*) may exhibit a less detectable history of enhanced purifying selection acting simultaneously alongside

their commonly observed fast rates of adaptive sequence divergence. Thus, perhaps intraspecific variation is a more relevant scale for contrasting genomic signatures of purifying selection among male-/female-/un-biased genes than the usual interspecific comparisons. Indeed, the finding of purifying selection in male-biased *C. maculatus* genes discussed above is based on them having relatively low non-synonymous sequence divergence among populations (Sayadi et al. 2019). Likewise, *D. melanogaster* male-biased genes show comparatively limited sequence and expression divergence within and between populations relative to between *D. melanogaster* and *D. ananassae* (Müller et al. 2012). More intra-specific studies of sequence divergence are clearly needed in order to establish whether male-biased genes tend to show a stronger signature of purifying selection than female- and un-biased genes, which could reflect the payoff to females/populations in return for the cost of producing males.

Our findings also serve as evidence of the prerequisite conditions for "good genes" sexual selection (Zahavi 1975, Lande 1981, Kirkpatrick and Ryan 1991, Kirkpatrick 1996) wherein female choosiness for male traits reflects the "genetic quality" of their barer (i.e. males' breeding values for fitness; Hunt et al. 2004) that will be passed on to both sons and daughters, and where "genic capture" (Rowe and Houle 1996, Tomkins et al. 2004) prevents that variation in genetic quality from being depleted. Empirical evidence for good genes sexual selection remains scant (Prokop et al. 2012). To this end, our findings provide a piece of the puzzle lacking from many other studies, insofar as female choice determines male fitness in *C. maculatus*. While male mating success in this species does seem more to do with male competition than female choice (Savalli and Fox 1999), the kicking behavior that females exhibit may still serves as a baseline level of resistance that enables females to choose the males that are capable of overcoming it (Maklakov and Arnqvist 2009). Further, post-copulatory cryptic female choice (Thornhill 1983, Eberhard 1996, Pitnick et al. 2009, Arnqvist 2014) apparently comprises a large fraction of male fitness variance in this species (Hotzy et al. 2012, Bayram et al. 2019). Thus, while the exact target(s) of selection and relative roles of male competition versus female choice may be unclear, the

present findings nevertheless demonstrate that the genetic architecture underlying a sexually selected male trait in *C. maculatus* could very well confer "good genes" to choosy females.

In order for selection via males to pose net benefits to females/populations, thereby contributing to the maintenance of sexual reproduction and/or female trait preferences via "good genes", selection should necessarily act primarily on SC genetic variation (Whitlock and Agrawal 2009). Our study population is known to originally consist of primarily SA genetic variance in fitness (Berger et al. 2014, 2016a), and as discussed above, the present data apparently still do consist of some SA genetic variation (Grieshop and Arnqvist 2018). The present findings are a testament to the fact that the SC mutations that would enable selection via males to contribute to the maintenance of sex (Whitlock and Agrawal 2009) can still be present and act simultaneously alongside SA genetic variation.

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Authors' contributions

KG, DB and GA conceived of and designed the experiment. KG conducted the experiment. KG, DB, and PLM conducted the statistical analyses. KG wrote the first draft of the manuscript. All authors contributed to editing the manuscript.

Data availability

Data and a single R script containing all analyses and procedures are available upon request.

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Supplementary Materials for:

Selection in males purges the standing genetic load on female fitness

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S1. Regarding the role of parental-effects and sex-chromosomes

Rationale

There is the potential that non-genetic parental effects of each strain could partly confound our additive genetic interpretation of the o_M and o_F estimates. That is, the o_M and o_F estimates of each point on Figure 1C stem from replicate observations of the same genetic crosses, meaning that, in one extreme limit, differences among them could be attributable to the inheritance/transfer of phenotypic condition from those same recurrent strains to their F_1 offspring rather than the inheritance of the genetic makeup of those strains, *per se*. Such "condition transfer" via parent-of-origin effects (e.g. epigenetics) may be a common adaptive feature of many organisms (Bonduriansky and Crean 2018). Further, genes with male-biased gene expression, which we discuss as possibly mediating our findings (see *Mechanistic understanding*), may be likewise particularly relevant to condition transfer via parent-of-origin effects, as they have been shown to exhibit elevated condition-dependent expression in *Drosophila melanogaster* (Wyman et al. 2010). In addition to non-genetic parental effects, our main finding of sex differences in the relationship between heterosis and outbred breeding values for fitness could be partly attributable to the mutation load carried only by males on the Y-chromosome, and/or that revealed only in males on their unmasked hemizygous X-chromosome, whereas X-chromosome heterozygosity might mask these effects in females.

We sought to address these potential concerns. Diallel data lend themselves particularly well to identifying these effects via contrasts of reciprocal full siblings – pairs of crosses that are autosomally identical but have inherited their sex-chromosomes, mitochondria, cytoplasm, and other epigenetic information from opposite strains (e.g. the F_1 offspring of a strain-1 father and strain-2 mother are reciprocal full siblings with the F_1s of a strain-2 father and strain-1 mother, see Figure S1B). These parent-of-origin effects were identified via diallel variance partitioning (after Shorter et al. 2019) and removed

from the fitness data, yielding a new "Y-adjusted" fitness variable that was subject to the same analyses reported in the *Statistical methods* section of the Methods.

Methods

To estimate and remove the contribution of strain-specific parental sex effects (m and ϕ^m) and asymmetric epistatic effects (w and ϕ^w) from our fitness phenotype, y, resulting in y^{adjusted}, we implemented an updated version of the linear mixed model previously used in Shorter et al. (2019), using the R software package MCMCglmm (Hadfield 2010). We used the BayesDiallel 'fulls' (full, sexed, 'BSabmvw') model to estimate the contribution of parental strains and their various effects as described in (Lenarcic et al., 2012), where for an individual *i* the phenotype is modeled as:

$$y_i = \mu^{\text{d}} + \alpha_{\text{block}[i]} + \boldsymbol{d}^T\boldsymbol{\beta} + \epsilon_i$$

with $\epsilon_i \sim N(0, \sigma^2)$. Here, the overall mean and block effects are modeled by μ^{d} and α_{block}, respectively. For dam *j*, sire *k,* and sex *s*, with indicator functions ψ, the diallel effects, $\boldsymbol{d}^T\boldsymbol{\beta}$, are estimated by

$$\boldsymbol{d}^T{}_{(jks)}\boldsymbol{\beta} = a_j + a_k + m_j - m_k + \psi_{\{j=k\}}\left(\beta_{\text{inbred}} + b_j\right) + \psi_{\{j\neq k\}}\left(v^d_{jk} + \psi_{j<k}\cdot w_{jk}\right) + \psi_{\{s=\text{female}\}}\cdot\left[\phi + \phi^a_j + \phi^a_k + \phi^m_j - \phi^m_k + \psi_{\{j=k\}}\left(\phi_{\text{inbred}} + \phi^b_j\right) + \psi_{\{j\neq k\}}(\phi^v_{jk} + \psi_{j<k}\cdot\phi^w_{jk})\right]$$

where for all *j* strains, the strain-specific effects are modeled marginally for additive effects as $a_1, a_2, \ldots, a_J \sim N(0, \tau^2_a)$, parental sex effects as $m_1, m_2, \ldots, m_J \sim N(0, \tau^2_m)$, inbred effects $b_1, b_2, \ldots, b_J \sim N(0, \tau^2_b)$ and similarly for sex-specific versions of these effects classes (ϕ^a, ϕ^m, ϕ^b). The strainpair-specific effects for all unique *j*-by-*k* non-inbred combinations, are modeled similarly for symmetric epistatic (v^d), asymmetric epistatic (w), and sex specific versions (ϕ^v and ϕ^w, respectively), e.g. as marginally $w_{jk} \sim N(0, \tau^2_w)$ for asymmetric epistatic effects.

The priors for the five fixed effects ($\mu^{\mathrm{d}}, a_{\mathrm{block}}, \beta_{\mathrm{inbred}}, \phi$ (overall female), and ϕ_{inbred}) are each set to a vague normal distribution, $\mathrm{fixed.effect} \sim N(0{,}10^3)$, and the priors for variance of the residuals (σ^2) and for the variance for each class of effects ($\tau_a^2, \tau_m^2, \tau_b^2, \tau_v^2, \tau_w^2$ and sex-specific versions), are set as a weakly informative Inverse-Wishart with V=2, and nu=0.002, equivalent to an inverse gamma prior with shape and scale of 0.001. The estimates are based on models that were run for 17,000 iterations after 2,000 iterations of burn-in.

After obtaining stable estimates, e.g. $\widehat{m}$ for parental effects, for all strains and strainpairs, the original fitness phenotypes are adjusted using the following relationship for each *j*, *k*, and *s*:

$$y_{jk,s=\mathrm{male}}^{\mathrm{adjusted}} = y_{jks} - \left(\widehat{m_j} - \widehat{m_k}\right) - \widehat{w_{jk}}$$

$$y_{jk,s=\mathrm{female}}^{\mathrm{adjusted}} = y_{jks} - \left(\widehat{m_j} - \widehat{m_k}\right) - \widehat{w_{jk}} - \left(\widehat{\phi_j^m} - \widehat{\phi_k^m}\right) - \widehat{\phi_{jk}^w}$$

These adjusted Y fitness values are then modeled in the same way as the original fitness values in the main text (see *Statistical methods*).

Results

The results we obtained using the Y_adjusted fitness values were highly consistent with those reported in the main text. The model fits (see equation 2 of *Statistical methods*, Methods) before and after removing the parental effects were negligibly different (DIC = -1219.80 and DIC = -1222.275, respectively). The standardized **G** matrix estimated after removing the parental effects from the data (Table S1) was qualitatively similar to before (Table 1). Resampling this model revealed that heterosis in males and females was still highly positively correlated ($r_{o_M - i_M,\, o_F - i_F}$ = 0.84, (0.67, 0.96), P < 0.001). Likewise, male outbred breeding values were highly negatively correlated with heterosis in females ($r_{o_M,\, o_F - i_F}$ = -0.53, (-0.84, -0.14), P = 0.014), whereas female outbred breeding values were not

correlated to heterosis in males ($r_{o_F,\, o_M - i_M}$ = -0.09, (-0.44, 0.25), *P* = 0.674). Thus, our analysis suggests that non-genetic parental effects, sex-chromosome effects, and their epistatic interactions with autosomal genetic variation do not contribute to our main findings. For any parental effects variance to still be present in the Y-adjusted fitness variable, and hence still clouding our interpretation, the pattern of strain-specific non-genetic inheritance would need to be identical between reciprocal full-sibling contrasts (as well as between the sex-specific (i.e. son-daughter) contrasts of those reciprocal full-sibling differences). We argue that this is highly unlikely considering that parental effect variance manifests in fathers and mothers via fundamentally different and highly asymmetric pathways (e.g. cytoplasmic, mitochondrial, and sex-chromosome inheritance).

S2: Regarding SA variants

In principle, the present synthetic diallel population may be largely fixed throughout all 16 strains for female-benefit SA alleles at many genetic loci due to excess lineage extinction among inbred lines that originated from isofemale lines that were enriched for male-benefit/female-detriment SA genetic variants. Yet, some remaining SA polymorphisms may still segregate among the inbred strains. That is, some strains could be completely fixed for the female-benefit alleles across all SA loci, while other strains may be fixed for most, but not all, female benefit alleles, and thus fixed for male-benefit alleles at the remining sites. Under diminishing returns epistasis (Whitlock et al. 1995, Martin et al. 2007, Berger and Postma 2014), which is theoretically predicted among polymorphic SA sites (Arnqvist et al. 2014), strains fixed for female-benefit alleles throughout all SA loci would tend to have relatively low male fitness (o_M) and relatively high measures of male heterosis (o_M-i_M) owing to the recruitment of male-benefit alleles (upon outcrossing with strains that are fixed for some male-benefit alleles) in an otherwise male-detriment genetic background. By contrast, strains that have some male-benefit alleles fixed would have relatively high male fitness and relatively low measures of male heterosis, as they have less to gain than strains that are fixed for female-benefit alleles across all SA loci (Arnqvist et al. 2014). Female fitness and heterosis would be relatively unaffected in this context because they are either completely or mostly saturated for alleles that benefit their fitness, making the recruitment of additional female-benefit alleles in an otherwise female-benefit background relatively ineffectual (Arnqvist et al. 2014). At first glance, this explanation seems to match our results in that fitness would be negatively correlated to heterosis in males but not females. However, our main finding is specifically between male fitness (o_M) and *female* heterosis (o_F–i_F) (Figure 1C). Further, o_M-$i_M \approx o_F$-i_F (Figure 1B). Neither of those findings are compatible with an explanation based on SA genetic variation (and SA diminishing returns epistasis) underlying heterosis.

Supplemental tables:

Table S1. G matrix (with CIs and residual variances) estimated for relative Y-adjusted fitness (i.e. relative fitness after removing parental effects; see Methods and S2). Genetic variances (and residual variances) on the diagonal, their covariances in the lower triangle, and their Pearson's correlation coefficients (*r*) in the upper triangle. Covariances and correlations with 95% credibility intervals excluding zero are bolded.

	i_F	o_F	i_M	o_M
i_F	0.0109 (0.004, 0.021) (0.0257)	0.35	**0.82**	0.48
o_F	0.0011	0.0015 (<0.001, 0.003) (0.0149)	0.16	-0.02
i_M	**0.0106**	0.0002	0.0195 (0.006, 0.038) (0.0857)	**0.70**
o_M	0.0017	-1.8×10^{-5}	0.0030	0.0019 (<0.001, 0.004) (0.0836)

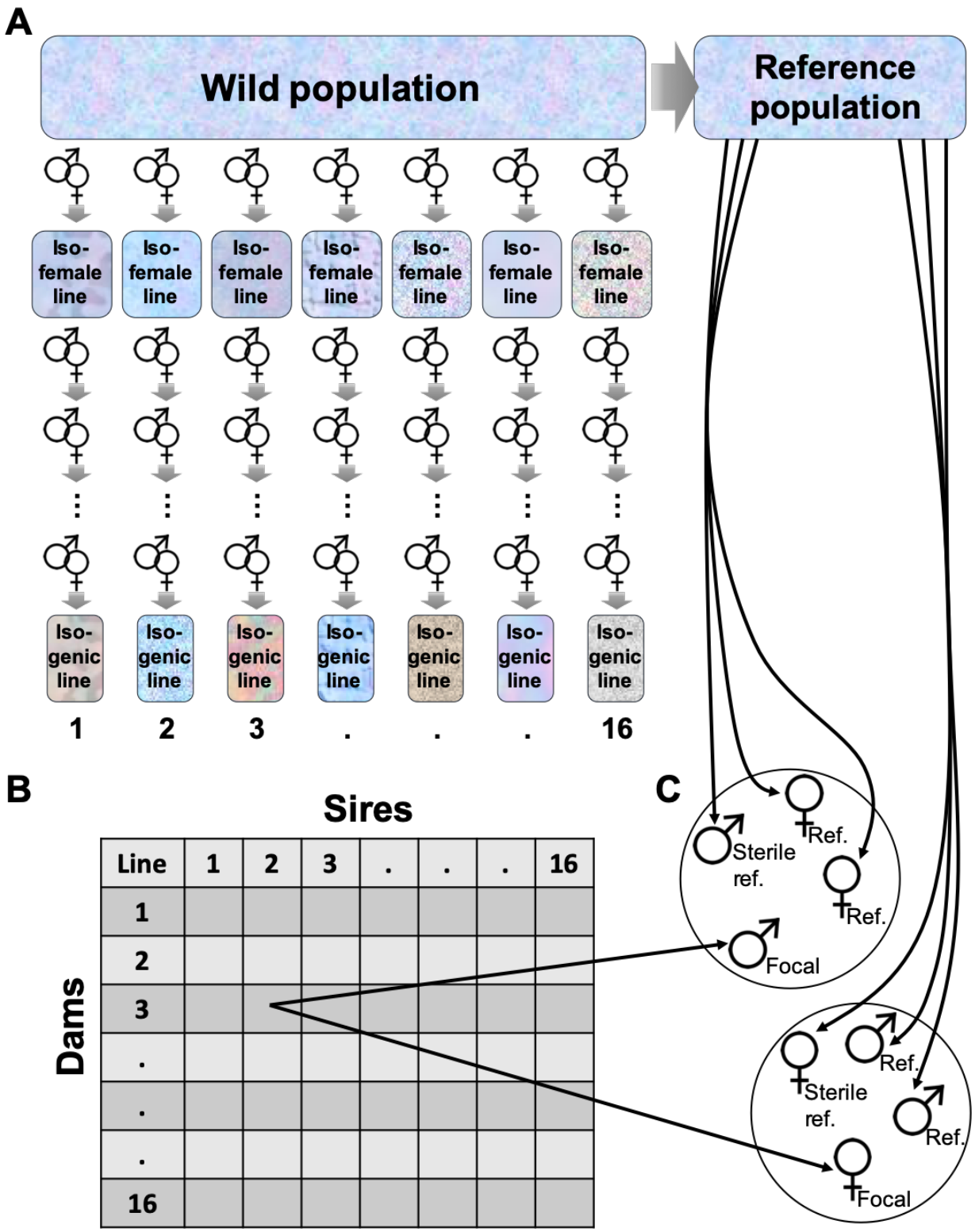

Figure S1: Lome population history and experimental design. (A) A wild population was divided into an outbred laboratory reference population and 41 isofemale lines (Berger et al. 2014). The latter were further inbred (Grieshop et al. 2017) to obtain the 16 inbred/isogenic lines used presently (Grieshop and Arnqvist 2018). Genetic diversity is depicted by color and texture. (B) A full diallel cross (Lynch and Walsh 1998) among the 16 inbred strains, where F_1 inbred parental selfs (*i*) are on the diagonal and outcrossed F_1s (*o*) are on the off-diagonal. (C) Replicate F_1 male and female fitness estimates included a focal F_1 individual (e.g. outbred (*o*) F_1s from a strain-2 sire and strain-3 dam), a sterile same-sex competitor from the reference population, two (fertile) opposite-sex competitors from the reference population, and ca. 100 *V. unguiculate* seeds in a 90 mm ø petri dish. These beetles were left to interact/mate/oviposit for the duration of their lifetime, and F_2 offspring counts = focal individuals' fitness.

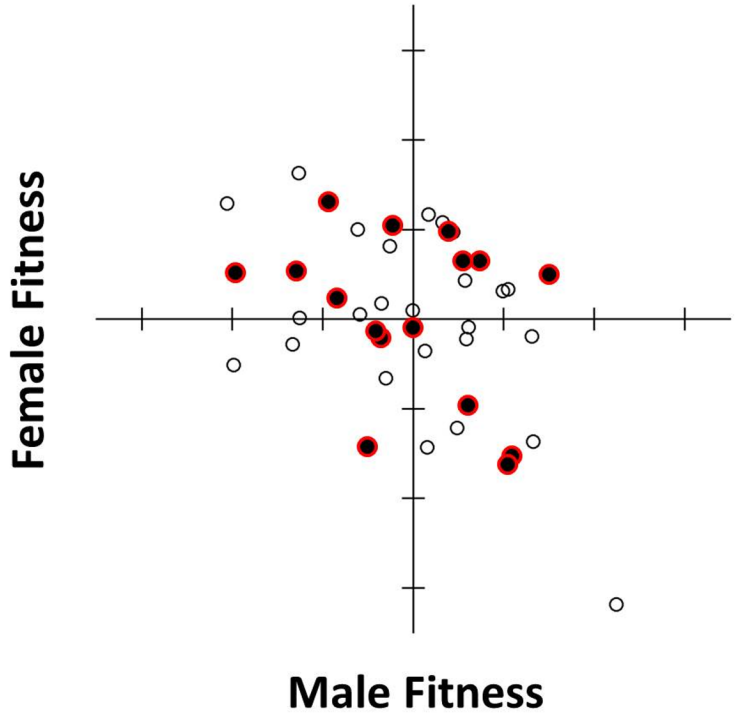

Figure S2: The raw-means r_{MF} of the isofemale lines (see Figure S1; Berger et al. 2014). Those from whence the present 16 inbred strains stem are outlined in red and filled in black. Because all 20 replicate inbreeding lineages stemming from some of the most male-benefit/female-detriment isofemale lines went extinct prior to completing the full inbreeding program (Grieshop et al. 2017), the present 16 inbred strains were chosen from throughout the isofemale line r_{MF} with the aim of countering that bias.

Supplemental figures:

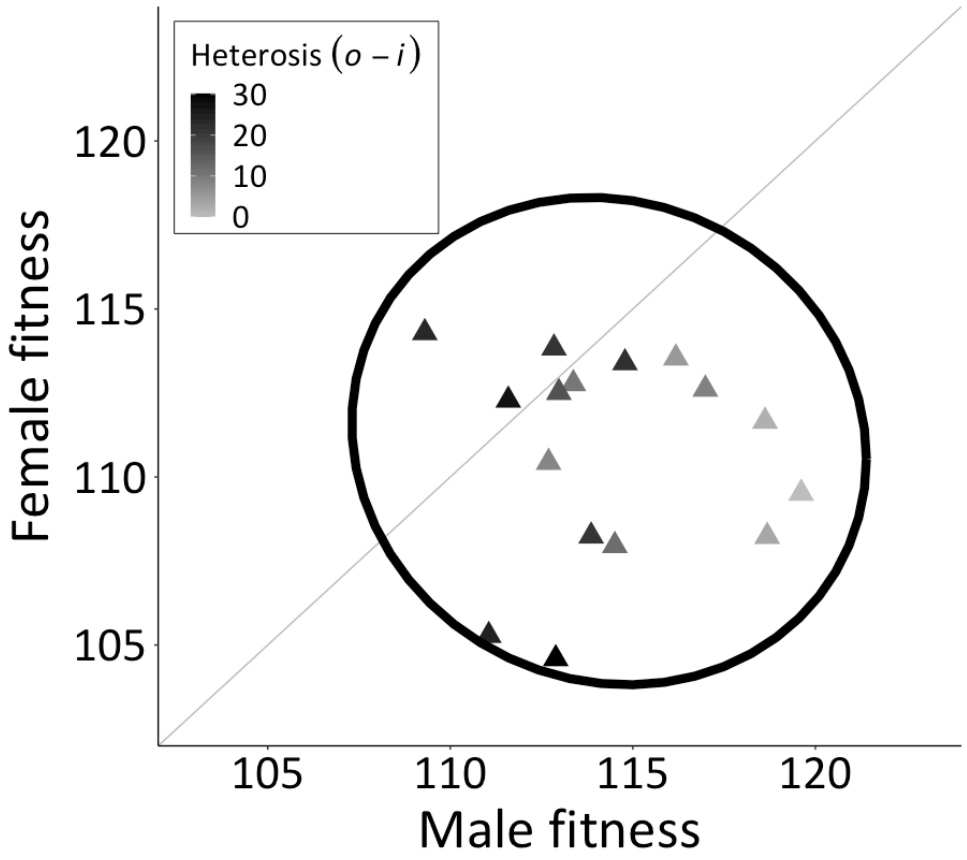

Figure S3: Outbred (*o*) breeding values from Figure 1A shaded by sex-averaged heterosis. An informal depiction of our main finding is apparent in that heterosis is clearly distributed along a horizontal gradient, according to male breeding values for fitness, but is clearly not distributed along the vertical dimension. Thus, sex-/strain-specific heterosis, the degree to which inbred strains benefit from having their rare partially recessive deleterious mutations covered up by heterozygosity, is reflected in those strains' outcrossed males, but not their outcrossed females.

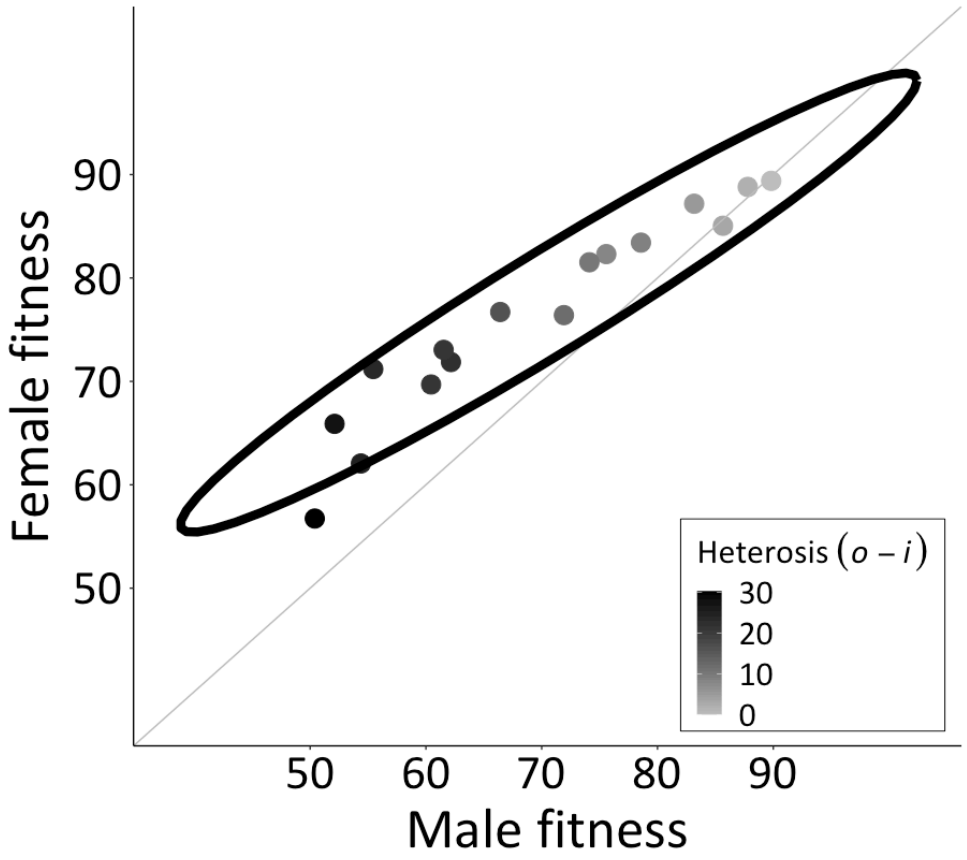

Figure S4: Inbred (*i*) breeding values from Figure 1A shaded by sex-averaged heterosis. By definition, strains with greater inbred fitness experience less heterosis. Male fitness is more negatively impacted by inbreeding/homozygosity than female fitness, as the large majority of these strains' inbred breeding values lie above the y=x line.

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