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Strong sexual selection in males against a mutation load that reduces offspring production in seed beetles

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Abstract

Theory predicts that sexual reproduction can increase population viability relative to asexual reproduction by allowing sexual selection in males to remove deleterious mutations from the population without large demographic costs. This requires that selection acts more strongly in males than females and that mutations affecting male reproductive success have pleiotropic effects on population productivity, but empirical support for these assumptions is mixed. We used the seed beetle *Callosobruchus maculatus* to implement a three-generation breeding design where we induced mutations via ionizing radiation (IR) in the F_0 generation and measured mutational effects (relative to nonirradiated controls) on an estimate of population productivity in the F_1 and effects on sex-specific competitive lifetime reproductive success (LRS) in the F_2. Regardless of whether mutations were induced via F_0 males or females, they had strong negative effects on male LRS, but a nonsignificant influence on female LRS, suggesting that selection is more efficient in removing deleterious alleles in males. Moreover, mutations had seemingly shared effects on population productivity and competitive LRS in both sexes. Thus, our results lend support to the hypothesis that strong sexual selection on males can act to remove the mutation load on population viability, thereby offering a benefit to sexual reproduction.

Introduction

Sexual selection can act as a purifying force removing alleles with deleterious effects on population mean fitness if the mutations that render individuals less successful in competition over fertilizations are also those that detriment offspring production (Zahavi, 1975; Rowe & Houle, 1996; Tomkins *et al.*, 2004). This mutational pleiotropy can allow sexual selection to, at least partly, compensate for the two-fold cost of sexual reproduction (Whitlock & Agrawal, 2009). By acting more strongly in males than females, sexual selection can remove inferior males of low genetic quality from the mating pool, thereby reducing the population's mutation load without discernible demographic costs (Manning, 1984; Agrawal, 2001; Siller, 2001; Lorch *et al.*, 2003). Whereas studies in *Drosophila* indicate that selection against new mutations is stronger in males, little is known about such sex biases in selection intensities in other organisms (reviewed in: Whitlock & Agrawal, 2009).

If mutations instead have sex-limited, or even opposing (i.e. sexually antagonistic), fitness effects in the sexes, sexual selection on males would be inefficient at reducing mutation load and could even increase the frequency of mutations that reduce female fecundity, imposing a severe gender load on the population (Brooks, 2000; Chippindale *et al.*, 2001; Pischedda & Chippendale, 2006; Arnqvist & Tuda, 2010). The expected impact of sexual selection on adaptive rates is therefore highly contingent upon the fitness effects of allelic variation at loci experiencing sexually concordant versus sexually antagonistic selection (Bonduriansky & Chenoweth, 2009; Whitlock & Agrawal, 2009). Recent theoretical approximations (e.g. Connallon *et al.*, 2010; Stewart *et al.*, 2010; Connallon & Clark, 2014) and empirical estimates based on standing genetic variation in laboratory (e.g. Chippindale *et al.*, 2001; Fedorka &

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Mousseau, 2004; Pischedda & Chippendale, 2006; Bilde *et al*., 2009; Berger *et al*., 2014a,b) and wild populations (e.g. Brommer *et al*., 2007; Foerster *et al*., 2007; Mainguy *et al*., 2009; Svensson *et al*., 2009; Tarka *et al*., 2014; Barson *et al*., 2015) alike suggest that natural populations harbour variable, but potentially abundant, amounts of sexually antagonistic genetic variance for fitness. In accordance, effects of sexual selection on rates of adaptation from standing genetic variation are idiosyncratic and inconclusive (reviewed in: Candolin & Heuschele, 2008; Whitlock & Agrawal, 2009).

Furthermore, mutations with sexually concordant fitness effects should be efficiently eliminated (or fixed) by selection, whereas those with sexually antagonistic effects may not be (Kidwell *et al*., 1977; Connallon & Clark, 2012). Thus, allelic variation at sexually antagonistic loci should contribute disproportionately to standing genetic variation for fitness (Connallon & Clark, 2012, 2014; Long *et al*., 2012; Berger *et al*., 2014b). Inferences based on standing genetic variation, therefore, likely underestimate the potential for sexual selection to purge the genome of deleterious mutations. Methods inducing de novo mutations may therefore be more informative regarding the capacity for sexual selection to purge a population's mutation load.

As mentioned above, several studies in *Drosophila* support the notion that selection against new mutations is stronger in adult males than females (e.g. Sharp & Agrawal, 2008, 2013; MacLellan *et al*., 2009; Mallet *et al*., 2011, 2012; Clark *et al*., 2012). However, sexual selection is surprisingly inconsistent across studies and mutations in its effect on population-level fitness, reported as being positive (e.g. Hollis *et al*., 2009), ineffectual (e.g. McGuigan *et al*., 2011; Arbuthnott & Rundle, 2012), or even negative (e.g. Hollis & Houle, 2011; Arbuthnott & Rundle, 2012). Thus, while the sexes may share much of their developmental genes, sexual selection in adult males could mostly target male-limited genes (see: Chippindale *et al*., 2001), weakening the potential for strong purifying sexual selection to remove mutations with deleterious effects on female fecundity and juvenile viability.

Here, we measured the strength of sex-specific selection on novel mutations, and their shared effect on population productivity and competitive adult reproductive success, in another model organism, the seed beetle *Callosobruchus maculatus*. We induced a mutation load by exposing individuals to ionizing (gamma) radiation (IR) and subsequently implemented a middle-class neighbourhood (MCN) breeding design (Shabalina *et al*., 1997) to minimize selection on the induced mutations, allowing them to be passed through three subsequent experimental generations. To estimate the strength of selection on induced mutations, we compared competitive lifetime reproductive success (LRS) of F_2 adults originating from irradiated grandparents relative to that of F_2 controls originating from nonirradiated grandparents. The estimated strength of selection was then compared across the sexes. Finally, we estimated the shared effect of mutations on a measure of population productivity (measured in F_1 adults) and male competitive LRS (measured in F_2 adults) by correlating family means of the two measures across generations. Our results show that selection operates against new mutations in adult males and that these induced mutations have shared effects on male LRS and population productivity.

Materials and methods

Study system

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). Males and females are sexually mature upon adult eclosion and exhibit a polyandrous mating system (Miyatake & Matsumura, 2004). The eggs are glued onto the surface of dry beans and hatched larvae bore into the beans, where they complete their life cycle.

The study population 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. Isofemale lines were created by mating a single male and female emerging from the collected seeds. After establishment, isofemale lines were expanded to a population size of approximately 200–300 adults and then kept on ca. 600 *V. unguiculata* seeds at 29 °C, 55% RH and a 12 : 12 h L : D photoperiod. They were cultured under this regime for ~30 generations prior to the start of this experiment (see further: Berger *et al*., 2014b). Four isofemale lines were randomly selected (from the 41 available for use) as the focal genetic backgrounds in which we either induced mutations (in the case of treated beetles) or did not (in the case of controls). In addition, a mixture of all the 41 isofemale lines was set up to create a reference population, initiated six generations prior to the start of the experiment, against which focal individuals from our experiment competed in the assays of competitive LRS.

Inducing mutations in the F_0 generation

We induced mutations using ionizing (gamma) radiation (IR) from a Cs^{137} source. IR causes double-strand breaks (DSB) to DNA, which occur naturally during recombination, and can produce point mutations and deletions as a consequence of mistakes during their repair (Evans & DeMarini, 1999; Sudprasert *et al*., 2006; Shrivastav *et al*., 2008; Shee *et al*., 2013). It has been used to induce mutation loads and infer selection in a range of study systems (e.g. bulb mites: Radwan, 2004; *Drosophila*: Agrawal & Wang, 2008; Maklakov *et al*.,

2013; dung beetles: Almbro & Simmons, 2014; seed beetles: Power & Holman, 2015).

A pilot study was conducted to generate dose-response curves for F_0 productivity (i.e. the number of offspring produced by the irradiated individuals) upon sex-specific exposure to IR (see supplementary material, Fig. S1). These dose-response curves indicated that 20 Gy was a suitable dosage for this experiment, inducing a quantifiable mutation load while still allowing irradiated individuals to produce enough F_1 offspring with which to conduct experiments.

Egg-laden *V. unguiculata* seeds from each of the four isofemale lines were isolated to collect virgin adults as they emerged. Zero-day-old virgins from each isofemale line were separated by sex and held in 90 mm ∅ Petri dishes ($n \approx 20$ per container) and then assigned randomly to one of four treatment categories: female-irradiated, male-irradiated, female-control and male-control (Fig. 1). Males and females assigned to the male- and female-irradiated categories, respectively, were exposed to 20 Gy of IR, whereas males and females assigned to the male- and female-control categories, respectively, were not exposed IR, but were otherwise treated exactly the same in terms of collection, handling and holding container density (Fig. 1). Two hours following the irradiation treatment, the individuals from each of these four treatment categories were paired with a zero-day-old virgin individual of the opposite sex from their respective isofemale line in a Petri dish (90 mm ∅) containing ca. 100 *V. unguiculata* seeds (Fig. 1). The pairs were kept together for their entire lifetime under the same abiotic conditions stated above. The number of F_1 offspring emerging from each F_0 pair was counted; this formed our measure of F_0 productivity, which was used only to generate the dose-response curves (Fig. S1). This procedure was repeated over two consecutive days, generating two different cohorts from which families were derived – this structure was maintained over generations throughout the experiment, and cohort was included as a fixed effect when analysing the results (see Statistical analysis). In total, we set up 4–8 F_0 pairs per treatment, sex-treated category and genetic background.

F_1 productivity

From each F_0 pair, we created two F_1 pairs by pairing randomly selected virgin male and female offspring (Fig. 1). This middle-class neighbourhood (MCN) breeding design prevents selection from operating on all but the unconditionally lethal mutations by allowing high- and low-fitness individuals to contribute an equal number of offspring (in this case four) to the next generation (Shabalina *et al.*, 1997; Morrow *et al.*, 2008). This was important as we aimed to measure and relate the effects of mutations (induced in the F_0) in the F_1 and F_2 generations and therefore could not allow selection to remove induced mutations over generations. The mating pairs

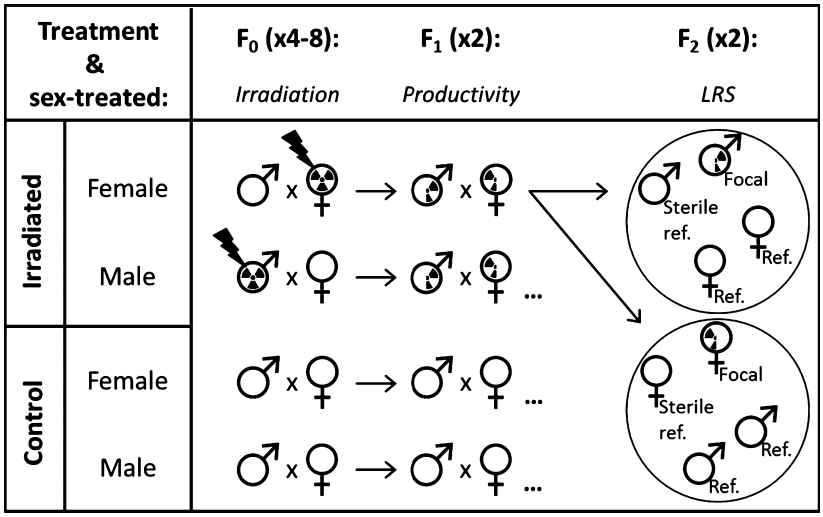

Fig. 1 Methodological schematic followed for each of four genetic backgrounds. Each treatment (irradiated or control) contained male and female 'sex-treated' categories. F_0 individuals indicated by a lightning bolt had their whole genomes exposed to 20 Gy of IR (indicated by IR symbols). They passed half their genomes to their F_1 offspring (indicated by half IR symbols). F_1 pairs from the same F_0 parents produced F_2 offspring (the number of which was each F_1 pairs' productivity) with half their genomes consisting of grandparental DNA exposed to IR (also indicated by half IR symbol). F_2 individuals were used to estimate each F_1 family's sex-specific competitive lifetime reproductive success (LRS). In parentheses are the number of replicate pairs for each treatment and sex-treated category of each genetic background in the F_0, for each F_0 pair in the F_1 and for each F_1 pair in the F_2.

were kept under the same abiotic conditions stated above, and the F_2 offspring that emerged from these F_1 pairs were counted to estimate each F_1 pair's productivity and used to assay F_2 male and female LRS (Fig. 1).

We chose to construct the F_1 pairs from within-family mating pairs (i.e. via full-sib mating). This way, our breeding design preserved mutations induced in F_0 such that F_1 and F_2 individuals from irradiated treatments had, on average, half of their genome exposed to IR, and F_1 and F_2 individuals from the same family were more likely to share mutations induced in their F_0 ancestors. Consequently, individuals were inbred one additional generation beyond the one generation of inbreeding inherent in the establishment of the genetic backgrounds (isofemale lines). We note that the offspring production of inbred F_2 control individuals was not lower than what is usually observed for this species in our laboratory, consistent with *C. maculatus* being resistant to multiple generations of inbreeding (e.g. Tran & Credland, 1995). Thus, this extra generation of inbreeding is in itself unlikely to have affected our results.

F_2 competitive lifetime reproductive success

Two randomly selected virgin F_2 males and females from each F_1 pair were used for estimating each F_1 pair's male and female F_2 competitive LRS (Fig. 1). Competitive LRS assays consisted of a single focal individual placed in a Petri dish (90 mm ∅) containing *ad libitum V. unguiculata* seeds together with a sterile virgin standard competitor of the same sex from the reference population and two opposite-sex individuals from the reference population (a 1:1 sex ratio; Fig. 1). Competitor individuals were sterilized with a 100 Gy dose of IR, which does not notably reduce lifespan in either sex (Boshra, 1994). In males, this allows their sperm to function and fertilize eggs with a negligibly slight reduction in sperm competitiveness (Eady, 1991; Edvardsson & Canal, 2006) such that there is no net reduction in reproductive competitiveness (Ahmed *et al*., 1977), but their zygotes die, revealing paternity. This is a standard protocol in seed beetles (Hotzy & Arnqvist, 2009; Maklakov & Arnqvist, 2009; Hotzy *et al*., 2012; Berger *et al*., 2014b) and other insects (Simmons, 2001) for assaying sperm competition and competitive LRS. The fertilized eggs of females receiving a 100 Gy dose of IR do not hatch. Thus, both male and female competitive LRS assays included mating competition, male assays also included sperm competition, and female assays included competition for available oviposition sites. As these assays represent an environment that these beetles experience naturally in grain storage facilities (Southgate, 1979; Fox, 1993), they also incorporate naturally occurring selection pressures, including but not limited to mate searching, female mating resistance, competition over matings, sexual conflict over remating rate, and female competition for oviposition sites. At the same time, these assays exclude potentially ecologically relevant factors such as predation, adult food resources and fluctuations in population size and adult sex ratio. However, some of these aspects are likely excluded from the natural habitat of these beetles as well (e.g. adult food availability is very low on arid crop fields as well as in grain storage facilities). These assays were placed in the same abiotic conditions stated above, where individuals competed for matings/fertilizations and laid eggs for their entire lifespan. The number of individuals emerging from these assays was counted to estimate sex-specific F_2 competitive LRS (Fig. 1).

Statistical analysis

All analyses were conducted in R v.3.2.3 (R core team 2015). Productivity and competitive LRS were analysed using maximum likelihood (ML) estimation in generalized linear mixed effects models with a Poisson error structure and log-link function, implemented in the lme4 package V. 1.1-10 (Bates *et al*., 2015). When analysing productivity, fixed effects included treatment (irradiated vs. control), sex-treated (male vs. female) and their interaction (Fig. 1). Genetic background was included as a random effect crossed by treatment and sex-treated, assuring the correct level of replication for the main effects. We also blocked out possible differences between cohorts by adding it as a main effect. These same terms were used in a model with a binomial error structure to analyse the difference in the number of males and females emerging from productivity assays – testing for sex differences in juvenile survival. When modelling competitive LRS, we included sex-assayed (male or female LRS) as an additional fixed effect crossed with treatment and sex-treated. Genetic background was included as a random effect crossed by treatment, sex-treated and sex-assayed.

In the models on productivity and LRS, we included each observation as a random effect (i.e. 'observation-level random effects'). This estimates the true residual variance in the model rather than setting it equal to the mean of the response (which is only true for a perfectly Poisson distributed variable) and thus accounts for overdispersion, providing a more conservative analysis. Statistical significance was evaluated by likelihood ratio tests of models with and without the effects of interest using type II sums of squares in the car package V. 2.1-1 (Fox & Weisberg, 2011).

To estimate selection coefficients along with their 95% credible intervals, we ran Bayesian Markov chain Monte Carlo simulations using the MCMCglmm package V. 2.22 for R (Hadfield, 2010) on data where the response variable (number of offspring produced) had been standardized for each genetic background and sex by dividing all observations by the mean number of off-

spring produced by each respective groups' controls. Thus, the selection coefficients were calculated as: $s = 1\text{-}LRS_{IRR}/LRS_{CON}$ (i.e. in terms of relative fitness), and we calculated credible intervals and *P*-values for selection coefficients (i.e. we tested if they were significantly different from 0) in males and females based on the resampled Bayesian posterior estimates. Except for modeling relative fitness as a normally distributed response variable (and therefore not including 'observation-level random effects'), the model was identical to the one specified for the ML estimation using lme4. We used weak ($nu = 10^{-6}$) gamma priors for our random effects where the variances were set as [total variance in data/number of variance components] for each random effect term. Simulations started with a burn-in phase (100 000 iterations) followed by 1 000 000 iterations during which posterior estimates were sampled. The models ran with large sampling intervals (thin = 500) to minimize autocorrelation ($r < 0.05$ for all parameters) of the stored posterior estimates. This generated an effective sample size of 2000 uncorrelated posteriors of male and female selection coefficients against the induced mutations (see Fig. 2a). In addition, we also ran models for each genetic background and sex independently (i.e. in eight separate models) to estimate sex-specific selection coefficients on each genetic background (see Fig. 2b).

Finally, we calculated means for each F_1 pair's male and female competitive LRS (measured in the F_2) to estimate their (Pearson's) correlation coefficients with productivity (measured in the F_1). To minimize the effect of standing genetic variation on the correlations, we blocked out main effects of genetic background. Thus, if there is positive mutational pleiotropy between population productivity and male competitive LRS, we expect more positive correlations across families in the irradiated treatments (carrying mutations with variable fitness effects) relative to families of the control treatments.

Results

F_1 productivity

Offspring of irradiated parents had significantly lower productivity than controls overall ($\chi^2 = 7.41$, d.f. = 1, $P = 0.0065$). However, the effect of treatment was clearly detectable via irradiated fathers, but not mothers, as shown by a significant interaction between treatment and sex irradiated ($\chi^2 = 4.09$, d.f. = 1, $P = 0.043$) (Fig. S2; Table S1). There was no overall significant sex difference in mutational effects on juvenile survival ($\chi^2 = 0.98$, d.f. = 1, $P = 0.322$; Table S2).

F_2 competitive LRS: sex-specific strengths of selection on induced mutations

Overall, male and female individuals of irradiated grandparents had significantly lower competitive LRS compared to control individuals ($\chi^2 = 4.99$, d.f. = 1, $P = 0.026$). There was, however, a tendency for an interaction between treatment and sex-assayed ($\chi^2 = 2.71$, d.f. = 1, $P = 0.0997$). Investigating this further by analysing the sexes separately showed that male LRS was strongly decreased by novel mutations ($\chi^2 = 8.43$, d.f. = 1, $P = 0.0037$), whereas this effect was much weaker and non-significant in females ($\chi^2 = 2.38$, d.f. = 1, $P = 0.123$). These effects were independent of the (grandparental) sex-treated, as indicated by a nonsignificant interaction between treatment and sex-treated (full summary: Fig. S3, Table S3).

The Bayesian MCMC posterior estimates of selection coefficients (*s*) corroborated the results from the analyses based on ML. Selection on the induced mutations was consistently stronger in males relative to females both across sex-treated categories (Fig. 2a) and genetic backgrounds (Fig. 2b). Again, there was no statistically significant sex difference in the strength of selection ($s_M - s_F = 0.10$, CI: −0.03–0.26, $P_{MCMC} = 0.15$), but selec-

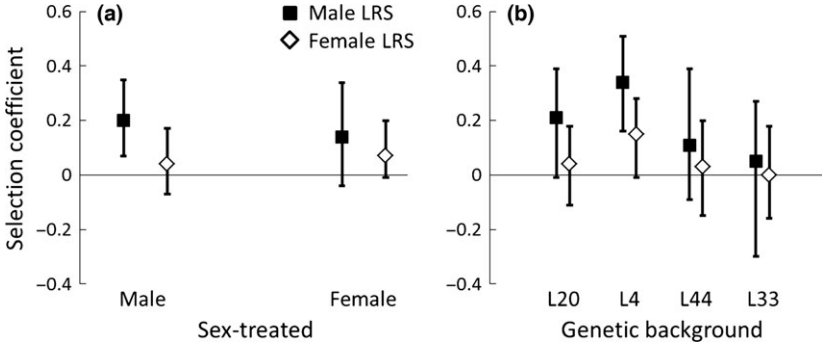

Fig. 2 Bayesian estimates (posterior modes ±95% credible intervals) of selection coefficients against genome-wide induced mutations in males and females of *C. maculatus*. Selection on new mutations tended to be stronger in males relative to females, depicted (a) across the two sex-treated categories in which either male or female grandparents were irradiated and (b) for each of the four genetic backgrounds pooled across sex-treated categories.

tion was overall significant and strong in males (s_M = 0.20, CI: 0.04; 0.32, P_{MCMC} = 0.010), whereas it was weak and nonsignificant in females (s_F = 0.07, CI: −0.01; 0.14, P_{MCMC} = 0.08).

Correlations between F_1 productivity and F_2 competitive LRS

Within the irradiated treatment, pooled over sex-treated categories, productivity was positively correlated to competitive LRS of both females (r = 0.34, n = 80, P = 0.002) and males (r = 0.26, n = 74, P = 0.024; Fig. 3). This was not the case among control individuals (with regard to male or female LRS: r = 0.10, n = 82, P = 0.39; and r = 0.02, n = 87, P = 0.84, respectively), indicating that novel mutations had shared effects on competitive LRS and productivity. There were no significant differences in correlations depending on which sex was irradiated (Table S4).

Discussion

This study aimed to assess whether sexual selection can, at a relatively small demographic cost, act to remove mutations that are detrimental to population mean fitness. For this to be the case, mutations must firstly be selected against more strongly in males than females and secondly detriment both male reproductive success and overall population productivity. We found (i) that induced mutations had strong fitness effects in adult males but not adult females, and (ii) a positive correlation between male reproductive success and productivity in irradiated treatments, but not in control treatments, indicating that novel mutations may generally have shared effects on male reproductive success and population productivity in seed beetles. Taken together, our results offer support for the theoretical prediction that sexual selection in males can offer an evolutionary benefit to sexual reproduction by reducing mutation load at a small demographic cost (Manning, 1984; Agrawal, 2001; Siller, 2001).

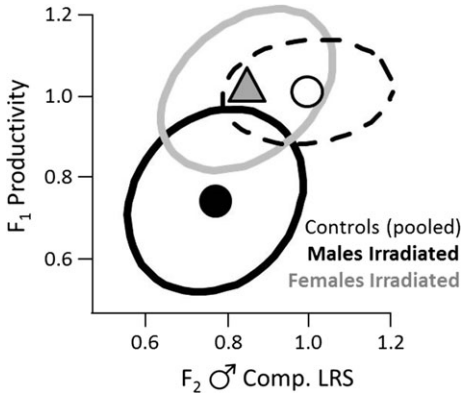

Fig. 3 Family-level correlation between F_1 family productivity and F_2 male competitive lifetime reproductive success (LRS). Data ellipses depict 50% bivariate probability distributions around treatment means. Families formed by control males and females are pooled for clarity and depicted by the hatched ellipse and white circle (mean = 1). Families in which F_0 females were irradiated are depicted by the grey ellipse and triangle, and families in which F_0 males were irradiated are depicted by the black ellipse and circle.

We induced mutations either via males or females in the F_0 generation, and in both cases, point estimates of selection against the mutations were greater in males (Fig. 2a). Thus, potential male bias in the strength of sexual selection against new mutations seems unlikely to be due to mutations induced on the Y chromosome. Positive mutational pleiotropy between male fitness and population productivity can alone compensate for the two-fold cost of reproducing sexually if the intensity of selection on males is greater than on females and the genome-wide deleterious mutation rate is sufficiently high (Agrawal, 2001; Siller, 2001). Indeed, despite the overall strength of selection against novel mutations varying across genetic backgrounds, point estimates of selection coefficients were consistently two to three times greater in males relative to females within each genetic background (Fig. 2b).

Importantly, as our assays measured effects on adult competitive LRS, they do not give a complete picture of the sex bias in selection acting across the entire life cycle. For example, including ecological factors and life stages that invoke the same intensity of selection in males and females could reduce the overall sex bias in selection against a novel mutation with male-biased effects on competitive LRS. Indeed, our analysis of juvenile survival indicated no significant difference in selection between the sexes (Table S2). Additionally, other ecological aspects of these beetles that were not included in our selection estimates, such as more extensive mate searching in males and host searching in females, could affect sex differences in selection against novel mutations.

Previous studies investigating the effect of sexual selection on adaptation have reached mixed results (reviewed in Whitlock & Agrawal, 2009), which likely reflects the wide variety of techniques, mating systems and evolutionary histories of the experimental populations studied. Recent examples highlight some of this complexity. For example, Lumley *et al.* (2015) subjected treatments of flour beetles to ~50 generations of experimental evolution at different intensities of sexual selection and then subjected replicated lineages from these treatments to single-pair full-sib inbreeding. Lineages from populations evolving under intense sexual selection on males tolerated sustained inbreeding for a greater number of generations relative to those from populations evolving under enforced monogamy or intense sexual selection on *females*. Tolerance to inbreeding is indicative of the level of mutation load

(Charlesworth & Charlesworth, 1999; Charlesworth & Willis, 2009). Thus, Lumley *et al.* (2015) demonstrated that enhanced sexual selection on males reduced populations' accumulating mutation load.

In contrast, Chenoweth *et al.* (2015) studied the fixation of single nucleotide polymorphisms (SNPs) across populations maintained over 13 generations under experimental evolution treatments varying in the strength of both natural and sexual selection. Whereas as many as 80 SNPs showed statistically significant differences among the selection treatments, only six SNPs showed aligned responses across the sexual selection and natural selection treatment. Moreover, for 43 of the 80 SNPs, the effect of sexual selection when applied simultaneously with natural selection was to oppose the response observed when natural selection was applied in isolation. This last result implies that sexual selection impeded adaptation and the authors provided additional evidence showing that males directed courtship and harassment disproportionally towards high-quality females (a form of interlocus sexual conflict), thereby offering a relative benefit to smaller females with lower fecundity (Chenoweth *et al.*, 2015).

The discrepancy between these two recent landmark studies may serve to illustrate the opposing outcomes of sexual selection that can be expected when selection is either allowed to act over longer periods of time to target ongoing mutational input like in the study of Lumley *et al.* (2015), or when it acts on standing genetic variation over shorter periods of time like in the study of Chenoweth *et al.* (2015), for which purifying selection has already ensued, and the remaining sexually antagonistic genetic variation in combination with interlocus sexual conflict is likely to swamp the beneficial effects of purifying sexual selection (Whitlock & Agrawal, 2009).

Turning the focus to two recent studies that employed similar methods to ours, Power & Holman (2015), found results that they interpret as opposite to ours despite using the same system (*C. maculatus*). Using (X-ray) IR, they created mutated populations with significantly reduced egg-to-adult survivorship, but no difference in the number of offspring produced, relative to control populations. Then, looking within their mutated populations only, they compared females that had been mated via enforced monogamy to females that were mated by the winner of three competing males (allowing precopulatory sexual selection). Perhaps understandably, they found that females produced the same number of offspring regardless of whether or not precopulatory sexual selection was allowed. They conclude that sexual selection did not benefit female productivity, but their results are difficult to interpret considering the dosage of IR they used did not elicit a reduction in female productivity, relative to controls, from the start, and considering that precopulatory sexual selection is typically weak relative to post-copulatory sexual selection in this species (Fox *et al.*, 2007; Fritzsche & Arnqvist, 2013).

In contrast, Almbro & Simmons (2014) recently argued that sexual selection was effective at increasing population fitness by purging a mutation load induced by (gamma) IR in the dung beetle *Onthophagus taurus*. However, the induced mutations had no discernible effects on female fecundity and only affected the measured male traits. Not surprisingly, the implemented sexual selection treatment improved some of the male performance traits in the following generations, but had no measurable effect on how the induced mutation load affected female fecundity, suggesting pronounced sex-specificity of mutational effects.

The significant positive correlation between male reproductive success and productivity we report here is consistent with the induced mutations having shared effects on these two measures in our seed beetle population. The fact that this correlation was ≈ 0 in the control treatment, as well as in the base population from which the four genetic backgrounds originate (D. Berger *et al.* 2016, *in revision*), further reiterates the difference in sex-specificity of fitness effects expected for novel mutations versus standing genetic variation.

Nevertheless, two points deserve specific consideration. First, when estimated over multiple mutations induced across the entire genome, the correlation between male LRS and population productivity provides a quantitative estimate of the directionality of mutational effects on the two variables averaged over all mutations. In our study, this correlation ranged between 0.21 (males-irradiated) and 0.34 (females-irradiated), indicating that far from all mutations had shared effects on the two variables. As our estimates of F_1 pair means from which we calculated correlations were based on low sample sizes, measurement error may have caused our correlations to fall below unity. However, this is unlikely to fully explain the low correlations because, as expected, the corresponding correlations between *female* LRS and productivity for both male- and female-irradiated categories were stronger (0.29 and 0.42, respectively) than that for *male* LRS (see Results and Table S4). This implies that sexual selection on males has the potential to purge only a fraction of those mutations with negative effects on population productivity in *C. maculatus*. Indeed, in the extreme case, the underlying reason for observing stronger selection in males could be due to sexual selection acting with particular efficacy on those mutations with largely male-limited effects, which would greatly reduce the population-level benefits of sexual selection. Characterizing selection intensities on alleles with sex-limited versus sexually concordant fitness effects therefore remains an important challenge for understanding the role of sexual selection in promoting population mean fitness, which has only just begun with the study

of selection on single mutations in isolation in *Drosophila* (see Introduction).

Second, as we induced mutations in lineages kept isolated throughout the three generations of the experiment, it is possible that a positive correlation between F_1 productivity and F_2 LRS may have been generated by variation among families in the *number* of mutations rather than variation in the effect sizes of mutations with shared effects on the two traits, a caveat that applies generally to studies inducing mutation loads to study sexual selection (Whitlock & Agrawal, 2009). The two alternative explanations are not mutually exclusive, and we cannot rule out that this second mechanism may be partly responsible for the observed positive correlation. If so, however, it would imply that our F_0 individuals varied substantially in their ability to repair DNA damage within each genetic background, since the number of DSB in cells exposed to a given dosage of a given type of IR appears to be relatively constant (Daly, 2012), and we blocked out overall differences among genetic backgrounds when estimating correlations.

One final detail of our study design worth addressing is that our F_1 productivity measures were significantly lower than controls when it was F_0 males that were irradiated, but not when F_0 females were irradiated (Figs 3 and S2). This could indicate a lower threshold for the number of mutations tolerated/passed on by female gametes relative to male gametes (in line with the sex differences in response to our 20 Gy dosage, Fig. S1), such that more detrimental mutations were filtered out in the F_0 generation when coming in through females, whereas more detrimental mutations coming in through males were filtered out in the F_1 generation. Nevertheless, our F_2 LRS estimates did not differ significantly between sex-treated categories, rendering this detail of our findings inconsequential to our interpretations.

In summary, we have provided empirical support for the hypothesis that sexual selection has the potential to remove mutations that reduce population viability at a low demographic cost, by generating strong selection in males against mutations with shared effects on male reproductive success and population productivity. This finding is congruent with theoretical expectations and contributes to a growing body of literature aiming to evaluate the ability of sexual selection to counterbalance the two-fold cost of sex across a wide variety of study organisms.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:
Figure S1 Dose response curve for irradiated F_0 individuals. 20 Gy was used to induce mutations.
Figure S2 F_1 family productivity upon F_0 male (black circles) and female (grey triangles) parents being irradiated, for each of the four genetic backgrounds. There were detrimental mutational effects on F_1 productivity only when F_0 males were irradiated.
Figure S3 Female (left) and male (right) F_2 competitive LRS upon F_0 male (black circles) and female (grey triangles) grandparents being irradiated, for each of the four genetic backgrounds. Male LRS was negatively affected by novel mutations.
Table S1 Effects of irradiation on F_1 productivity.
Table S2 Bayesian MCMC estimates (mean ± 95% credible interval) of the ratio of the number of males or females respectively, emerging from irradiated relative to control families in the F_1 productivity assays, on each of the four genetic backgrounds. Parameter estimates were derived from Bayesian models that were run on each of the four genetic backgrounds separately.
Table S3 Effects of irradiation on F_2 competitive lifetime reproductive success (LRS).
Table S4 Family-mean correlations.

Data deposited at Dryad: doi: 10.5061/dryad.8dt7r

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