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# Assessing the alignment of sexual and natural selection using radiomutagenized seed beetles

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sperm competition.

## Abstract

A major unsolved question in evolutionary biology concerns the relationship between natural and sexual selection. Sexual selection might augment natural selection, for example if mutations that harm female fecundity also reduce male mating success. Conversely, sexual selection might favour traits that impair naturally selected fitness components. We induced detrimental mutations in *Callosobruchus maculatus* beetles using X-ray irradiation and then experimentally measured the effect of precopulatory sexual selection on offspring number and survival rate. Sexual selection treatment had a negative effect on egg-to-adult survivorship, although the number of progeny reaching adulthood was unaffected, perhaps because eggs and juveniles that failed to develop lessened competition on the survivors. We hypothesize that the negative effect of sexual selection arose because sexually competitive males transmitted a smaller nuptial gift or carried alleles that conferred reduced survival. Although we found no evidence that sexual selection on males can purge alleles that are detrimental to naturally selected fitness components, such benefits might exist in other environmental or genetic contexts.

## Introduction

Sexual selection can be defined as selection arising from competition for mates or their gametes. Sexual selection has long been hypothesized to complement natural selection and assist in the clearance of mutations that harm naturally selected fitness components (e.g. Darwin, 1859; Agrawal, 2001; Siller, 2001; Whitlock & Agrawal, 2009). Sexual selection allows females to benefit from a genome that has been purged of mutations as a result of competition among males, potentially increasing population mean fitness (Whitlock & Agrawal, 2009; Holman & Kokko, 2013) and elevating the rate of adaptation (Lorch *et al.*, 2003; Fricke & Arnqvist, 2007; Candolin & Heuschele, 2008; Plesnar-Bielak *et al.*, 2012). A key assumption of this argument is that there is a net positive correlation between alleles' fitness effects in the context of natural and sexual selection; for example, mutations that reduce survival or female fecundity also tend to decrease success in competition for mates or their gametes.

There is substantial support for this idea, although most comes from studies of *Drosophila* (reviewed in Whitlock & Agrawal, 2009; Holman & Kokko, 2013). For example, eight *D. melanogaster* mutations were selected against by both natural and sexual selection, with sexual selection being stronger than natural selection (Sharp & Agrawal, 2008). Studies of *Drosophila* mutation accumulation lines suggest that many mutations that negatively affect male mating success also negatively affect female fecundity, consistent with the idea that natural selection and sexual selection augment one another (McGuigan *et al.*, 2011; Sharp & Agrawal, 2012). However, the evidence is not unanimous: in other *Drosophila* experimental evolution studies, sexual selection had no effect, or a negative effect, on naturally selected fitness components (e.g. Holland & Rice, 1999; Rundle *et al.*, 2006; Hollis & Houle, 2011; Arbuthnott & Rundle, 2012), and local adaptation did not improve success in sexual selection (Arbuthnott & Rundle, 2014).

Such discrepancies may be partly explained by the various concurrent costs of sexual selection to naturally

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selected fitness components. Males of many species improve their reproductive success at the expense of female survival and fecundity, for example via harmful courtship behaviour, or infanticide of offspring sired by other males (*inter*locus sexual conflict; e.g. Arnqvist & Rowe, 2005; Parker, 2006; Harano *et al.*, 2010; Rankin *et al.*, 2011; Holman & Kokko, 2013). Individuals (especially males) sometimes also invest in traits that increase their mating and fertilization success (e.g. aggression, large testes) at the expense of traits mediating survival, fecundity or parental care (e.g. vigilant or cryptic behaviour, immunity, parental care, high-fidelity DNA copying in gametes) (e.g. Rolff, 2002; Kokko & Brooks, 2003; Møller & Cuervo, 2003; Skorping & Jensen, 2004; Holman & Kokko, 2013; Maklakov *et al.*, 2013), and the 'wastage' of resources that occurs in the competition for mates can reduce population mean fitness (see, e.g. Rankin & Kokko, 2007; Kokko & Jennions, 2008; Holman & Kokko, 2013). *Intra*locus sexual conflict is another fitness cost that might depend on sexual selection. There is substantial evidence that many traits cannot be simultaneously optimized in both sexes because males and females share a genome, resulting in a suboptimal compromise that prevents both sexes from reaching their respective selective optima (e.g. Bonduriansky & Chenoweth, 2009; Innocenti & Morrow, 2010; Lewis *et al.*, 2011; Connallon & Clark, 2014). Strong sexual selection may increase the difference between the sexes' optimum phenotypes, for example by selecting for adaptations that only benefit competition for mates in one sex (e.g. horns, bright feathers), and could thereby exacerbate intralocus sexual conflict.

Given that sexual selection can have multiple positive and negative effects on fitness, further studies of its net effect on naturally selected fitness components are warranted. In particular, few studies have investigated whether sexual selection is able to clear deleterious mutations in species other than *Drosophila*. An exception is a series of studies of bulb mites (*Rhizoglyphus robini*) testing the effects of sexual selection on fitness (e.g. Radwan, 2004; Radwan *et al.*, 2004; Jarzebowska & Radwan, 2010). In an intriguing experiment, Radwan (2004) exposed mites to ionizing radiation, producing many heritable deleterious mutations. Their descendants were then bred after experimentally permitting or disallowing sexual selection (by setting up promiscuous mating groups or randomly assigned monogamous pairs, respectively), and sexual selection was found to improve embryo viability. Plesnar *et al.* (2011) performed a similar experiment, but found no effect of sexual selection on embryo viability. Studies in insects by Pekkala *et al.* (2009) and Almbro & Simmons (2013) showed that radiomutagenesis worsened male sexually selected traits, consistent with the idea that male competitive trait expression is correlated with mutation load, and the latter study also found that sexual selection elevated female fitness.

Here, we empirically measure the effects of sexual selection on fecundity and survival in a radiomutagenized population of the seed beetle *Callosobruchus maculatus*. Adults are short-lived, facilitating measurement of lifetime reproductive success, and sexual interactions have been found to have both positive and negative direct effects on female fitness (Arnqvist *et al.*, 2005; Rönn *et al.*, 2006). For example, males transfer a nutritious ejaculate that can aid progeny production (Edvardsson, 2007; Ursprung *et al.*, 2009), although mating and the species' persistent male courtship behaviour are thought to be harmful for females (Siva-Jothy & Crudgington, 2000; Edvardsson & Tregenza, 2005; Gay *et al.*, 2009). Quantitative genetic studies found some evidence for intralocus sexual conflict, although several male and female fitness components were positively genetically correlated and the degree of conflict is sensitive to environmental conditions, confounding predictions of the net effect of sexual selection on males on female fitness (Gay *et al.*, 2011; Berger *et al.*, 2014).

We hypothesized that sexual selection might help to clear mutations that also affect survival and female fecundity, and thereby augment natural selection. Alternatively, sexual selection may predominantly promote different alleles, leading to the prediction that sexual selection would depress survival and fecundity, or leave them unaffected. To evaluate these possibilities, we permitted or prevented the operation of sexual selection and took two measures of female fitness. A third possibility is that systematic nongenetic differences between sexual selection treatments, such as the amount of male-induced harm or nuptial gift size, might swamp any genetic effects of sexual selection on fitness. As we were interested in genetic effects, we standardized the degree of male harm as much as possible and statistically controlled for copulation duration (a reliable indicator of the amount of nuptial gift provided; Edvardsson & Canal, 2006).

## Materials and methods

### Stock population and general procedures

Beetles were obtained from a large outbred stock population derived from a culture held at the Stored Grain Laboratory at CSIRO (Canberra, ACT, Australia) and maintained for at least 25 generations at ANU in multiple 15 × 10 × 7 cm plastic boxes on dried, organic black-eyed beans (*Vigna unguiculata*) in a controlled temperature room (30 °C, 12:12 h light cycle). We collected virgin beetles of known age by isolating beans containing larvae in 1.5 mL Eppendorf tubes (pierced for ventilation) and checking them daily for eclosing adults.

### Production of the mutated population by X-ray irradiation

To elevate mutation load, we subjected beetles to X-ray radiation using an X-RAD 320 irradiator (Precision X-Ray, Inc., North Branford, CT, USA). Mutations induced by low doses of ionizing radiation are typically single-base substitutions or deletions (Evans & DeMarini, 1999; Sudprasert *et al.*, 2006), and natural radioactivity is thought to contribute to mutagenesis in the wild (Møller & Mousseau, 2013). We only irradiated males, because previous work has found that maternal condition can have large effects on offspring traits (e.g. Fox *et al.*, 2003), and we wished to minimize any nongenetic transgenerational effects of irradiation (e.g. irradiation might stress mothers into producing lower quality eggs).

As to our knowledge, this is the first study to use irradiation to induce mutation in *C. maculatus*, we were unsure what dose to use. We therefore created offspring by mating males exposed to 0.6, 6 or 12 Gy of X-ray radiation with one female each ($n = 33$ pairs per dose: males and females were virgins derived from the stock population); we did the same with control males (not exposed to radiation, but collected simultaneously and handed identically). Beetles were randomly assigned to the control group and three radiation treatments. Males were irradiated by placing them in groups of 33 in a 9-cm plastic Petri dish in the irradiation chamber, which was set to 320 kV with a sample-to-source distance of 54 cm. Mated females were placed individually in 9-cm Petri dishes and allowed to oviposit on black-eyed beans provided *ad libitum*.

Females mated to 6 Gy- or 12 Gy-irradiated males produced too few offspring to establish a sizeable irradiated stock population (6 Gy: mean ± SE progeny per fertile female = 7 ± 1.2, $n = 33$; 12 Gy: 12.3 ± 5.0, $n = 8$: the remaining 25 matings yielded no progeny; difference to control stock assessed by quasi-Poisson GLM: $P < 0.001$). Females mated to 0.6 Gy-irradiated males and females mated to control (nonirradiated) males produced similarly high numbers of offspring (control: mean = 29.1 ± 2.9, $n = 33$; 0.6 Gy: mean = 28.2 ± 3.5, $n = 33$; $P = 0.25$), so we established the irradiated stock population (M1) and the control population (C1) from the offspring of these pairings (Fig. 1).

### Experimental design

*Fitness assays*

We assayed female fitness using two metrics: number of adult progeny and egg-to-adult survivorship. To assess the number of adult progeny, we simply counted the total number of offspring that eclosed into adults. Egg-to-adult survivorship was estimated by isolating beans containing approximately 30 eggs per female (mean = 29.6 ± 0.2) and counting the number of adult offspring that subsequently eclosed. We also recorded the sex of all eclosing adults, giving an estimate of the adult sex ratio. Fitness assays were performed blind to treatment.

In the eventual data set, egg-to-adult survivorship was strongly correlated with offspring number (binomial GLM: $z = 6.0$, $n = 78$, $P < 0.0001$), and both measures were highly variable (Fig. S1; offspring number range: 1–73; egg-to-adult survivorship range: 25–90%). There was substantial residual variation in a simple linear regression of progeny number on egg-to-adult survivorship (Fig. S1; $R^2 = 0.23$ in a simple linear regression), implying that the two fitness measures

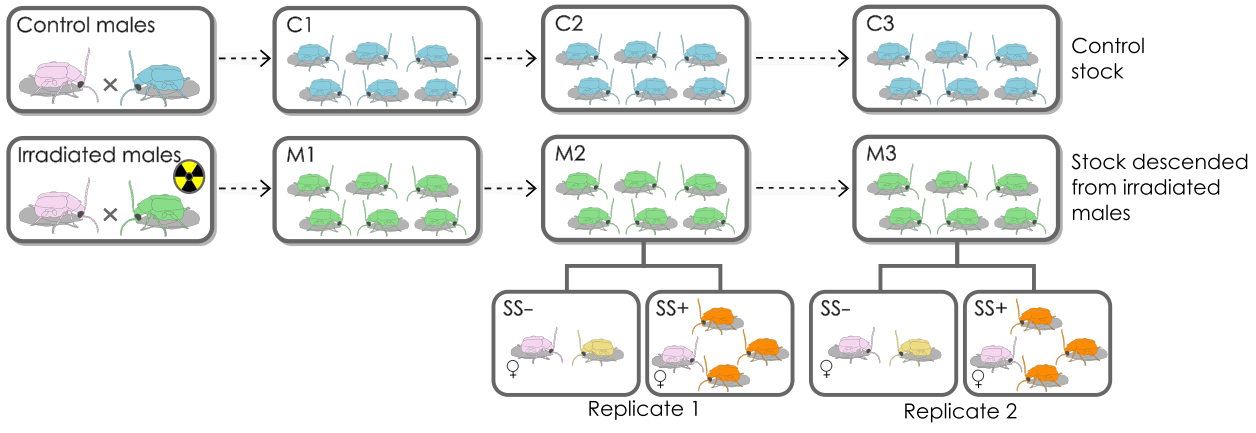


**Fig. 1** Scheme showing creation of two large stocks from irradiated males (three generations of descendants, termed M1–M3) and control males (C1–C3), as well as replicates 1 and 2 (which derived from the M2 and M3, respectively) of Experiment 2. The M and C stocks were propagated using a middle-class neighbourhood design to minimize natural selection. Fitness comparisons of C1–C3 and M1–M3, termed Experiment 1 in the text, are shown in Fig. 2. In each replicate, we collected M2 or M3 virgins of both sexes and then placed one female with either one male (no sexual selection: SS-) or three males (sexual selection: SS+); mating groups were formed at random. We then assayed the number of adult progeny as well as egg-to-adult survivorship in these matings.

were not completely correlated and, hence, that it is worthwhile analysing them both.

*Experiment 1: Assaying the control and mutated stocks*
The control and mutated stocks were propagated for three generations (Fig. 1). To breed the control and mutated stocks, we mated pairs of virgins in individual Petri dishes of beans and took one male and one female offspring from each pair to start the next generation. These offspring were randomly paired to form the next generation. This 'middle-class neighbourhood' breeding protocol is expected to preserve all but the most deleterious mutations by ensuring that low-fitness pairs contributed just as many offspring to the next generation as high-fitness pairs (e.g. Shabalina *et al.*, 1997; Morrow *et al.*, 2008). We term the first, second and third generations of the control and mutated stocks C1–3 and M1–3, respectively (Fig. 1); for all of these, we performed fitness assays as described above.

*Experiment 2: Manipulation of opportunity for sexual selection*
Beetles from the M2 and M3 stocks were subjected to two treatments: one in which sexual selection was allowed to act (SS+) and one in which we removed it by creating monogamous pairs at random (SS−) (Fig. 1). In the SS+ treatment, we placed three virgin males and one virgin female in a 5-cm Petri dish. As soon as mating occurred, the two unsuccessful males were immediately removed. We recorded the duration of copulation (in seconds) and removed the male after copulation was complete. In the SS− treatment, we similarly mated pairs of virgins, but females were only provided with a single male (who was again removed after the cessation of copulation). Thus, precopulatory sexual selection was present in the SS+ treatment but not the SS− treatment, and post-copulatory sexual selection was absent in both. This protocol ensured that effective population size was equal and all offspring were full siblings in the SS+ and SS− treatments. Females had only slightly more contact with males in the SS+ treatment, as males were removed from the Petri dishes at the earliest opportunity. This protocol should minimize the difference in the amount of harmful courtship experienced by females in the two treatments and thus give the experiment the best possible chance of detecting any fitness benefits of sexual selection. Mated SS+ and SS− females were allowed to oviposit in individual 9-cm Petri dishes with *ad libitum* beans, and we assayed their fitness by counting the number and egg-to-adult survival of their progeny.

Experiment 2 was performed in two replicates: once using beetles from the M2 and once using beetles from the M3. In all cases, the sample size was 20 families per treatment (SS+ and SS−), so we assayed the fitness of a total of 78 families across replicates 1 and 2 (2/80 females died before laying eggs).

### Statistical analyses

We used generalized linear models (GLMs) to test for the effects of the fixed factors 'sexual selection treatment' (SS+ or SS−) and 'replicate' (M2 and M3) on each component of fitness. We assumed Poisson-distributed errors for count data (progeny counts) and binomial errors for proportion data (egg-to-adult survivorship and sex ratio). As overdispersion was present, we used quasi-likelihood estimation.

We analysed the sexual selection experiments using model averaging. Data were first mean-centred and divided by two standard deviations using the *standardize* function in the *arm* package for R 3.0.1 (Gelman, 2008; Grueber *et al.*, 2011). We then ranked all possible models, from the null model to our specified full model (which contained the treatment × replicate interaction and the associated main effects, as well as the main effect copulation duration), by their QAIC scores (as we were using quasi-likelihood estimation) using the *dredge* function in the *MuMIn* package. We then calculated model-averaged estimates for each parameter (plus the associated 95% confidence intervals) from all the models using the *model.avg* function in the *MuMIn* package (following Grueber *et al.*, 2011). Model averaging avoids the limitation of relying on any particular model, which is advantageous for our data set because multiple models were similarly well supported (i.e. their QAIC scores were similar). We also calculated the importance of each predictor by summing the Akaike weights (which are equivalent to the probability that the focal model is the best one in the set) of all the models in which the focal predictor appears. Importance values close to the maximum value of one suggest that the predictor is commonly found in the best-supported models in the set being evaluated, whereas low importance values suggest that the predictor is predominantly found in poorly supported models (Symonds & Moussalli, 2011).

## Results

### Experiment 1: Radiomutagenesis reduced fitness

We first tested whether irradiation produced heritable deleterious mutations by comparing the fitness of the F1, F2 and F3 progeny of irradiated and control males (termed the M1–M3 and C1–C3; Fig. 1). For egg-to-adult survivorship, there was a significant interaction between generation and radiation treatment, showing that radiation treatment had a more deleterious effect when comparing the M1 and C1, relative to M2 vs. C2 (contrast from quasi-binomial GLM: $z_{104} = 3.88$, $P = 0.0002$) or M3 vs. C3 ($z_{104} = 2.93$, $P = 0.004$) (Fig. 2). Despite this reduced egg-to-adult survival rate, there was no significant effect of radiation treatment (likelihood ratio test: $P > 0.85$), generation ($P = 0.065$)

or their interaction ($P = 0.64$) on the number of progeny produced per female.

These results suggest that irradiation produced mutations that increased the frequency of egg hatching failure or pre-adult mortality. This added mortality nevertheless had no detectable effect on the number of offspring per female that eclosed into adults, perhaps due to the concomitant reduction in competition on the survivors. An alternative explanation is that female descendants of irradiated males laid larger numbers of eggs, each of which had a lower chance of surviving, resulting in comparable number of adults (although this seems less likely).

The persistence of the negative effect of radiation treatment on survival across multiple generations (Fig. 2) is consistent with the production of heritable, deleterious changes to the irradiated beetles' genes. The decline in the effect of irradiation treatment on egg-to-adult survival over successive generations implies that some of the new deleterious mutations were lost from the population, in spite of our 'middle-class neighbourhood' protocol, which weakens selection.

### Experiment 2: Sexual selection reduced egg-to-adult survival

Sexual selection had a negative effect on egg-to-adult survivorship (Fig. 3; Table 1; mean ± SE survival rate in SS− treatment: 63.3 ± 2.2%; SS+ treatment: 53.3 ± 2.1%). There was also an evidence for a negative correlation between copulation duration and egg-to-adult survivorship, although this relationship depended on a large outlier (one pair of beetles mated for 26 min, and the average was 7.8 ± 0.4). There was no effect of experimental replicate on egg-to-adult survivorship and no replicate × treatment interaction.

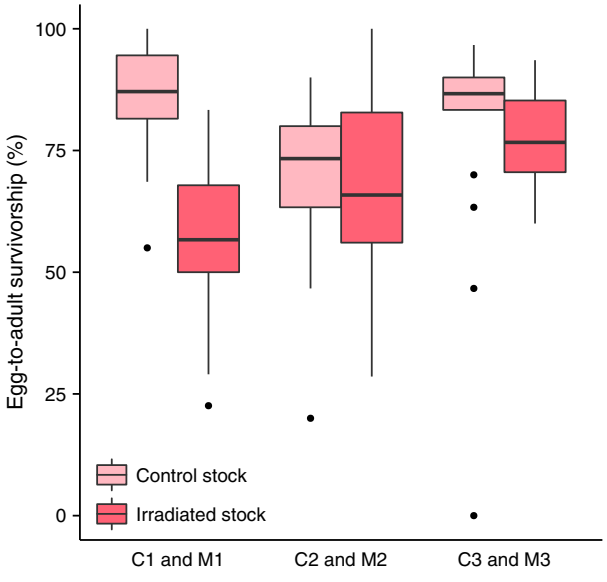


**Fig. 2** The egg-to-adult survivorship of the progeny of the F1, F2 and F3 descendants of irradiated males was lower than that of the corresponding descendants of nonirradiated males. The boxes illustrate the 1st, 2nd and 3rd quartiles; the whiskers show the farthest point from the 1st or 3rd quartile that is within 1.5× the interquartile range, and the points denote outliers outside this distance ($n = 105$).

By contrast, sexual selection did not significantly affect the number of progeny eclosing as adults (Fig. 3; Table 2; mean ± SE progeny number in SS− treatment: 44.10 ± 2.14; SS+ treatment: 44.05 ± 2.21). Although productivity was somewhat lower in the SS+ treatment in Replicate 1 (Fig. 3), overall the data suggested no effect of sexual selection treatment. Productivity was significantly lower in Replicate 2 than Replicate 1, although there was no effect of copulation duration or the replicate × treatment interaction.

The contrasting results regarding the effects of sexual selection on egg-to-adult survival and number of eclosing adult data imply either (i) SS+ females laid more eggs, although their eggs had poorer survival, or (ii) the elevated death rate of juveniles in the SS+ treatment lessened competition on the remaining juveniles, improving their survival. In either case, we found that sexual selection was associated with elevated juvenile mortality, but that this did not translate into reduced offspring numbers for females in the SS+ treatment.

Copulation duration was not significantly different in the SS+ and SS− treatments (SS+: 471 ± 34s; SS−: 463 ± 30s; Mann–Whitney $U$ test: W = 815, $P = 0.59$), further suggesting that a difference in copulation duration (and associated differences in nuptial gift size or other direct effects of copulation on females) did not explain the observed treatment effect.

Offspring sex ratio was unrelated to any variables we measured. The top-ranked model was the null model ($\Delta$QAIC = 1.21), and the model-averaged estimates of all parameters had confidence intervals overlapping zero (Table S1).

## Discussion

Experiment 1 strongly suggested that irradiation induced heritable deleterious mutations, which persisted into the M2 and M3. Experiment 2 (which used beetles from the M2 and M3) found no evidence that sexual selection helps to purge mutations that harm naturally selected components of fitness, namely egg-to-adult survival and progeny production. Instead, females exposed briefly to three males (the SS+ treatment) produced eggs with a lower probability of survival relative to females exposed to a single male (SS−). The number of offspring reaching adulthood was nevertheless not significantly affected by sexual selection treatment, perhaps because offspring that die early in development lessen competition on their surviving siblings.

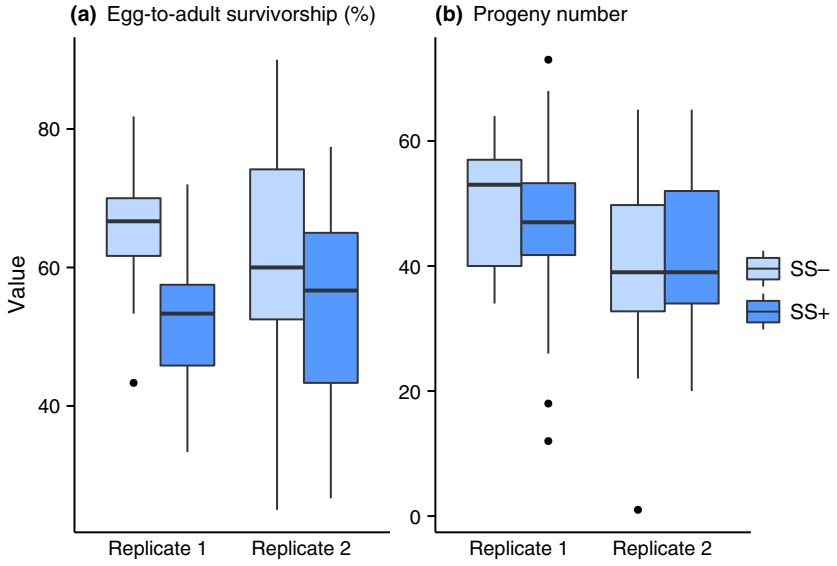


**Fig. 3** Sexual selection treatment negatively affected fitness as measured by egg-to-adult survivorship(a), but did not significantly affect the number of progeny reaching adulthood(b). The sample size was 78 (39 females per treatment, summed across replicates), and box plots depict the same summary statistics as in Fig. 2.

**Table 1** Effects of each predictor on fitness as measured by egg-to-adult survival probability. The table shows the model-averaged estimates for each predictor after averaging all models in the model set. The models were quasi-binomial GLMs with egg-to-adult survival probability as the response variable (coded as a two-column matrix of the number of eggs surviving to adulthood and the number not surviving, in our sample of eggs – typically 30 per female; $n = 78$ fitness measurements). Sexual selection treatment had a statistically significant effect (shown in bold; $\alpha = 0.05$) and was over-represented in the top-ranked models ('Importance' column).

| Predictor | Estimate | SE | 95% confidence limits | Importance |
|---|---|---|---|---|
| Intercept | **0.36** | **0.060** | **0.23 to 0.48** | |
| Treatment | **−0.45** | **0.12** | **−0.69 to −0.21** | **> 0.99** |
| Replicate | 0.006 | 0.12 | −0.24 to 0.25 | 0.39 |
| Copulation duration | −0.25 | 0.12 | −0.49 to −0.0010 | 0.74 |
| Treatment × Replicate | 0.27 | 0.25 | −0.21 to 0.76 | 0.16 |

**Table 2** Effects of each predictor on fitness as measured by progeny number. The table shows the model-averaged estimates for each predictor after averaging all models in the model set. The models were quasi-Poisson GLMs with progeny number as the response variable ($n = 78$ fitness measurements). Because we standardized the variables prior to running the model, the estimates are in unit of one-half standard deviation. Replicate had a statistically significant effect (shown in bold; $\alpha = 0.05$) and was over-represented in the top-ranked models ('Importance' column).

| Predictor | Estimate | SE | 95% confidence limits | Importance |
|---|---|---|---|---|
| Intercept | **3.78** | **0.034** | **3.71 to 3.85** | |
| Treatment | −0.0033 | 0.069 | −0.14 to 0.13 | 0.35 |
| Replicate | **−0.16** | **0.069** | **−0.30 to −0.023** | **0.85** |
| Copulation duration | −0.0056 | 0.071 | −0.15 to 0.14 | 0.27 |
| Treatment × Replicate | 0.11 | 0.14 | −0.16 to 0.39 | 0.1 |

The harmful effects of sexual selection on offspring survival are interesting. It is possible, but perhaps unlikely, that the effect of treatment on egg-to-adult survival could have resulted from contact with three males (as in the SS+ treatment), rather than contact with one male (SS−). However, contact with males was brief: in the SS+ treatment, we removed the two unsuccessful males as soon as the female began to mate with the third male, and copulations typically occurred within seconds or minutes of introducing the males and females. Thus, females in the SS+ treatment had scarcely more contact with males than did SS− females, so

the amount of male harassment (see, e.g., Gay *et al.*, 2008; den Hollander & Gwynne, 2009) they experienced was presumably similar. Another possibility is that males that are particularly successful at attaining a mating under competitive conditions also tend to induce more copulation-induced harm (although there seems to be no evidence for this; Ronn & Hotzy, 2012), or provide smaller nuptial gifts in their seminal fluid (although we detected no difference in copulation duration, which counts against this hypothesis). A third possibility is that males facultatively adjust their mating behaviour in response to the presence of rival males (Bretman *et al.*, 2011), although we observed no difference in copulation duration. A previous study found that males transferred their ejaculate more quickly when mating in the presence of rivals, although the overall mass of the ejaculate was the same, hinting at differences in mating behaviour based on social context (Wilson *et al.*, 2014). Finally, sexually competitive males might on average transmit genes that negatively affect egg-to-adult survival, for example because of antagonistic pleiotropy. There is an evidence from *C. maculatus* that sexually competitive males tend to carry genes that result in lower female fitness (at least in benign conditions), implying the existence of genetic trade-offs between some naturally and sexually selected traits (Berger *et al.*, 2014), which provides some indirect support for this hypothesis.

A previous study of *Drosophila montana* flies found that the sons of irradiated males had reduced mating success and altered mating behaviour, although the effects were weak (Pekkala *et al.*, 2009). The authors hypothesized that because the radiation-induced mutations were generally inherited in single copy, as matings were outbred as in the present study, mutations had little effect on fitness because of recessivity. Thus, even if sexual selection did purge some mutations, the fitness effects might be subtle and undetectable, at least across a single mating episode. In dung beetles, Almbro & Simmons (2013) found no differences in male sexually selected traits between the sons of irradiated males and control males, although males in subsequent generations (in which some mutant homozygotes were likely present) were stronger and had nonsignificantly higher testis mass, again implying that the recessivity of mutations may reduce the fitness benefits of sexual selection in outbred matings (i.e. in matings where partners tend to carry different recessive mutations).

In the light of these results, we hypothesize that sexual selection might have stronger benefits when the mutations carried by prospective mates are often identical by decent to those carried by the focal individual. That is, there may be only a weak benefit to avoiding a mutation-laden mate whose mutations are all different to one's own (as in our experiment), because most deleterious mutations are recessive (e.g. Houle *et al.*, 1997; Lynch *et al.*, 1998). A small number of studies have tested whether the effects of sexual selection on males change with the level of genetic similarity between mates. Duffy *et al.* (2014) measured the intersexual genetic correlation ($r_{mf}$) for fitness, where male fitness was scored as success in pre- and post-copulatory sexual selection and female fitness as productivity, under three different inbreeding levels. $r_{mf}$ was positive under low inbreeding and significantly declined to become flat or negative under high inbreeding. These results illustrate that homozygosity and the nature of current genetic variation (e.g. the relative frequency, polymorphism and fitness effects of loci with sexually antagonistic vs. concordant fitness effects) can both affect $r_{mf}$, although they do not provide a direct measurement of the effect of inbreeding on the net effect of sexual selection on fitness. Similarly, female multiple mating (which potentially strengthens sexual selection) provided fitness benefits in inbred populations of *Tribolium* beetles but not in outbred ones (Michalczyk *et al.*, 2011); this is consistent with the hypothesis that sexual selection is more beneficial when the available males tend to be genetically similar to the female.

The fitness consequences of sexual selection at varying levels of inbreeding thus seem ripe for study, especially in the light of evidence that the fitness consequences of sexual selection depend on the environment and the adaptive history of the population (Long *et al.*, 2012; Berger *et al.*, 2014; Punzalan *et al.*, 2014). It is also unclear to what extent sexual selection contributes to genetic purging (but see Jarzebowska & Radwan, 2010), in which extended bouts of inbreeding remove deleterious recessive alleles (Crnokrak & Barrett, 2002). Future models of the effects of sexual selection on mutation load may benefit from considering dominance, homozygosity and inbreeding, and models of genetic purging could incorporate sexual selection. Additionally, empiricists seeking to measure 'good genes' benefits of sexual selection should keep inbreeding in mind when designing experiments. For example, estimates of the genetic benefits of sexual selection might be biased downwards if experimental matings are restricted to nonrelatives, as inbreeding is often common in nature (Crnokrak & Roff, 1999; Walling *et al.*, 2011).

A potential criticism of this and similar studies using mutagenesis to test for pleiotropy between naturally and sexually selected fitness components (e.g. Radwan, 2004; Hollis & Houle, 2011; Plesnar *et al.*, 2011; Almbro & Simmons, 2013) is that the mutagenesis procedure may create linkage disequilibrium between mutations that separately affect the focal traits (see Whitlock & Agrawal, 2009). Therefore, a correlated response in one trait to selection on the other may be explained by linkage disequilibrium between separate mutations affecting the two traits, rather than by pleiotropy. Specifically, if some individuals by chance receive a larger dose of radiation/mutagen and thus carry many mutations, their offspring might have a low breeding value

for naturally and sexually selected fitness components even if none of the new mutations simultaneously affect both traits. Linkage disequilibrium between radiation-induced mutations is expected to decay over successive generations (Whitlock & Agrawal, 2009), so we attempted to mitigate this effect by propagating the mutated stock (under relaxed selection) for one generation prior to beginning the experiment. We also replicated our experiment, first using the grand-offspring (Replicate 1) and then the great-grand-offspring (Replicate 2) of the irradiated males. Linkage disequilibrium between radiation-induced mutations would have been stronger in Replicate 1, so the strength of the statistical interaction between treatment and replicate gives an indication of the role of linkage disequilibrium in generating our results (namely that sexual selection reduced survival). This interaction was weak and non-significant, implying that linkage disequilibrium between mutations separately affecting naturally and sexually selected fitness components was minor. If anything, the detrimental effect of sexual selection was weaker in Replicate 2, which is opposite to the prediction of the linkage disequilibrium hypothesis. We nevertheless recommend that studies using mutagenesis to examine pleiotropy keep Whitlock & Agrawal (2009)'s caution in mind.

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## References

Agrawal, A.F. 2001. Sexual selection and the maintenance of sexual reproduction. *Nature* **411**: 692–695.

Almbro, M. & Simmons, L.W. 2013. Sexual selection can remove an experimentally induced mutation load. *Evolution* **68**: 295–300.

Arbuthnott, D. & Rundle, H.D. 2012. Sexual selection is ineffectual or inhibits the purging of deleterious mutations in *Drosophila melanogaster*. *Evolution* **66–7**: 2127–2136.

Arbuthnott, D. & Rundle, H.D. 2014. Misalignment of natural and sexual selection among divergently adapted *Drosophila melanogaster* populations. *Anim. Behav.* **87**: 45–51.

Arnqvist, G. & Rowe, L. 2005. *Sexual Conflict*. Princeton University Press, Princeton.

Arnqvist, G., Nilsson, T. & Katvala, M. 2005. Mating rate and fitness in female bean weevils. *Behav. Ecol.* **16**: 123–127.

Berger, D., Grieshop, K., Lind, M.I., Goenaga, J., Maklakov, A.A. & Arnqvist, G. 2014. Intralocus sexual conflict and environmental stress. *Evolution* **68**: 2184–2196.

Bonduriansky, R. & Chenoweth, S.F. 2009. Intralocus sexual conflict. *Trends Ecol. Evol.* **24**: 280–288.

Bretman, A., Gage, M.J.G. & Chapman, T. 2011. Quick-change artists: male plastic behavioural responses to rivals. *Trends Ecol. Evol.* **26**: 467–473.

Candolin, U. & Heuschele, J. 2008. Is sexual selection beneficial during adaptation to environmental change? *Trends Ecol. Evol.* **23**: 446–452.

Connallon, T. & Clark, A.G. 2014. Evolutionary inevitability of sexual antagonism. *Proc. Roy. Soc. B* **281**: 20132123.

Crnokrak, P. & Barrett, S.C.H. 2002. Purging the genetic load: a review of the experimental evidence. *Evolution* **56**: 2347–2358.

Crnokrak, P. & Roff, D.A. 1999. Inbreeding depression in the wild. *Heredity* **83**: 260–270.

Darwin, C. 1859. *On the Origin of Species*. John Murray, London, UK.

Duffy, E., Joag, R., Radwan, J., Wedell, N. & Hosken, D.J. 2014. Inbreeding alters intersexual fitness correlations in *Drosophila simulans*. *Ecol. Evol.* **4**: 3330–3338.

Edvardsson, M. 2007. Female *Callosobruchus maculatus* mate when they are thirsty: resource-rich ejaculates as mating effort in a beetle. *Anim. Behav.* **74**: 183–188.

Edvardsson, M. & Canal, D. 2006. The effects of copulation duration in the bruchid beetle *Callosobruchus maculatus*. *Behav. Ecol.* **17**: 430–434.

Edvardsson, M. & Tregenza, T. 2005. Why do male *Callosobruchus maculatus* harm their mates? *Behav. Ecol.* **16**: 788–793.

Evans, H.H. & DeMarini, D.M. 1999. Ionizing radiation-induced mutagenesis: radiation studies in *Neurospora* predictive for results in mammalian cells. *Mutat. Res.* **437**: 135–150.

Fox, C.W., Bush, M.L. & Wallin, W.G. 2003. Maternal age affects offspring lifespan of the seed beetle, *Callosobruchus maculatus*. *Funct. Ecol.* **17**: 811–820.

Fricke, C. & Arnqvist, G. 2007. Rapid adaptation to a novel host in a seed beetle (*Callosobruchus maculatus*): the role of sexual selection. *Evolution* **61**: 440–454.

Gay, L., Eady, P.E., Vasudev, R., Hosken, D.J. & Tregenza, T. 2008. Costly sexual harassment in a beetle. *Physiol. Entomol.* **34**: 86–92.

Gay, L., Hosken, D.J., Vasudev, R., Tregenza, T. & Eady, P.E. 2009. Sperm competition and maternal effects differentially influence testis and sperm size in *Callosobruchus maculatus*. *J. Evol. Biol.* **22**: 1143–1150.

Gay, L., Brown, E., Tregenza, T., Pincheira-Donoso, D., Eady, P.E., Vasudev, R. *et al.* 2011. The genetic architecture of sexual conflict: male harm and female resistance in *Callosobruchus maculatus*. *J. Evol. Biol.* **24**: 449–456.

Gelman, A. 2008. Scaling regression inputs by dividing by two standard deviations. *Stat. Med.* **27**: 2865–2873.

Grueber, C.E., Nakagawa, S., Laws, R.J. & Jamieson, I.G. 2011. Multimodel inference in ecology and evolution: challenges and solutions. *J. Evol. Biol.* **24**: 699–711.

Harano, T., Okada, K., Nakayama, S., Miyatake, T. & Hosken, D.J. 2010. Intralocus sexual conflict unresolved by sex-limited trait expression. *Curr. Biol.* **20**: 2036–2039.

Holland, B. & Rice, W.R. 1999. Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *PNAS* **96**: 5083–5088.

den Hollander, M. & Gwynne, D.T. 2009. Female fitness consequences of male harassment and copulation in seed beetles, *Callosobruchus maculatus*. *Anim. Behav.* **78**: 1061–1070.

Hollis, B. & Houle, D. 2011. Populations with elevated mutation load do not benefit from the operation of sexual selection. *J. Evol. Biol.* **24**: 1918–1926.

Holman, L. & Kokko, H. 2013. The consequences of polyandry for population viability, extinction risk and conservation. *Phil. Trans. Roy. Soc. B* **368**: 20120053.

Houle, D., Hughes, K.A., Assimacopoulos, S. & Charlesworth, B. 1997. The effects of spontaneous mutation on quantitative traits. II. Dominance of mutations with effects on life-history traits. *Genet. Res.* **70**: 27–34.

Innocenti, P. & Morrow, E.H. 2010. The sexually antagonistic genes of *Drosophila melanogaster*. *PLoS Biol.* **8**: e1000335.

Jarzebowska, M. & Radwan, J. 2010. Sexual selection counteracts extinction of small populations of the bulb mites. *Evolution* **64**: 1283–1289.

Kokko, H. & Brooks, R. 2003. Sexy to die for? Sexual selection and the risk of extinction. *Ann. Zool. Fenn.* **40**: 207–219.

Kokko, H. & Jennions, M.D. 2008. Parental investment, sexual selection and sex ratios. *J. Evol. Biol.* **21**: 919–948.

Lewis, Z., Wedell, N. & Hunt, J. 2011. Evidence for strong intralocus sexual conflict in the Indian meal moth, *Plodia interpunctella*. *Evolution* **65**: 2085–2097.

Long, T.A.F., Agrawal, A.F. & Rowe, L. 2012. The effect of sexual selection on offspring fitness depends on the nature of genetic variation. *Curr. Biol.* **22**: 204–208.

Lorch, P.D., Proulx, S., Rowe, L. & Day, T. 2003. Condition-dependent sexual selection can accelerate adaptation. *Evol. Ecol. Res.* **5**: 867–881.

Lynch, M., Latta, L., Hicks, J. & Giorgianni, M. 1998. Mutation, selection, and the maintenance of life-history variation in a natural population. *Evolution* **52**: 727.

Maklakov, A.A., Immler, S., Løvlie, H., Flis, I. & Friberg, U. 2013. The effect of sexual harassment on lethal mutation rate in female *Drosophila melanogaster*. *Proc. Roy. Soc. B* **280**: 20121874.

McGuigan, K., Petfield, D. & Blows, M.W. 2011. Reducing mutation load through sexual selection on males. *Evolution* **65**: 2816–2829.

Michalczyk, Ł., Millard, A.L., Martin, O.Y., Lumley, A.J., Emerson, B.C., Chapman, T. *et al.* 2011. Inbreeding promotes female promiscuity. *Science* **333**: 1739–1742.

Møller, A.P. & Cuervo, J.J. 2003. Sexual selection, germline mutation rate and sperm competition. *BMC Evol. Biol.* **3**: 6.

Møller, A.P. & Mousseau, T.A. 2013. The effects of natural variation in background radioactivity on humans, animals and other organisms. *Biol. Rev. Camb. Philos. Soc.* **88**: 226–254.

Morrow, E.H., Stewart, A.D. & Rice, W.R. 2008. Assessing the extent of genome-wide intralocus sexual conflict via experimentally enforced gender-limited selection. *J. Evol. Biol.* **21**: 1046–1054.

Parker, G.A. 2006. Sexual conflict over mating and fertilization: an overview. *Phil. Trans. Roy. Soc. B* **361**: 235–259.

Pekkala, N., Puurtinen, M. & Kotiaho, J.S. 2009. Sexual selection for genetic quality: disentangling the roles of male and female behaviour. *Anim. Behav.* **78**: 1357–1363.

Plesnar, A., Konior, M. & Radwan, J. 2011. The role of sexual selection in purging the genome of induced mutations in the bulb mite (*Rizoglyphus robini*). *Evol. Ecol. Res.* **13**: 209–216.

Plesnar-Bielak, A., Skrzynecka, A.M., Prokop, Z.M. & Radwan, J. 2012. Mating system affects population performance and extinction risk under environmental challenge. *Proc. Roy. Soc. B* **279**: 4661–4667.

Punzalan, D., Delcourt, M. & Rundle, H.D. 2014. Comparing the intersex genetic correlation for fitness across novel environments in the fruit fly, *Drosophila serrata*. *Heredity* **112**: 143–148.

Radwan, J. 2004. Effectiveness of sexual selection in removing mutations induced with ionizing radiation. *Ecol. Lett.* **7**: 1149–1154.

Radwan, J., Unrug, J., Śnigórska, K. & Gawrońska, K. 2004. Effectiveness of sexual selection in preventing fitness deterioration in bulb mite populations under relaxed natural selection. *J. Evol. Biol.* **17**: 94–99.

Rankin, D.J. & Kokko, H. 2007. Do males matter? The role of males in population dynamics. *Oikos* **116**: 335–348.

Rankin, D.J., Dieckmann, U. & Kokko, H. 2011. Sexual conflict and the tragedy of the commons. *Am. Nat.* **177**: 780–791.

Rolff, J. 2002. Bateman's principle and immunity. *Proc. Roy. Soc. B* **269**: 867–872.

Ronn, J.L. & Hotzy, C. 2012. Do longer genital spines in male seed beetles function as better anchors during mating? *Anim. Behav.* **83**: 75–79.

Rönn, J., Katvala, M. & Arnqvist, G. 2006. The costs of mating and egg production in *Callosobruchus* seed beetles. *Anim. Behav.* **72**: 335–342.

Rundle, H.D., Chenoweth, S.F. & Blows, M.W. 2006. The roles of natural and sexual selection during adaptation to a novel environment. *Evolution* **60**: 2218–2225.

Shabalina, S.A., Yampolsky, L.Yu. & Kondrashov, A.S. 1997. Rapid decline of fitness in panmictic populations of *Drosophila melanogaster* maintained under relaxed natural selection. *PNAS* **94**: 13034–13039.

Sharp, N.P. & Agrawal, A.F. 2008. Mating density and the strength of sexual selection against deleterious alleles in *Drosophila melanogaster*. *Evolution* **62**: 857–867.

Sharp, N.P. & Agrawal, A.F. 2012. Male-biased fitness effects of spontaneous mutations in *Drosophila melanogaster*. *Evolution* **67**: 1189–1195.

Siller, S. 2001. Sexual selection and the maintenance of sex. *Nature* **411**: 689–692.

Siva-Jothy, M.T. & Crudgington, H.S. 2000. Genital damage, kicking and early death. *Nature* **407**: 855–856.

Skorping, A. & Jensen, K.H. 2004. Disease dynamics: all caused by males? *Trends Ecol. Evol.* **19**: 219–220.

Sudprasert, W., Navasumrit, P. & Ruchirawat, M. 2006. Effects of low-dose gamma radiation on DNA damage, chromosomal aberration and expression of repair genes in human blood cells. *Int. J. Hyg. Environ. Health* **209**: 503–511.

Symonds, M. & Moussalli, A. 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. *Behav. Ecol. Sociobiol.* **65**: 13–21.

Ursprung, C., den Hollander, M. & Gwynne, D.T. 2009. Female seed beetles, *Callosobruchus maculatus*, remate for male-supplied water rather than ejaculate nutrition. *Behav. Ecol. Sociobiol.* **63**: 781–788.

Walling, C.A., Nussey, D.H., Morris, A., Clutton-Brock, T.H., Kruuk, L.E.B. & Pemberton, J.M. 2011. Inbreeding depression in red deer calves. *BMC Evol. Biol.* **11**: 318.

Whitlock, M.C. & Agrawal, A.F. 2009. Purging the genome with sexual selection: reducing mutation load through selection on males. *Evolution* **63**: 569–582.

Wilson, C.J., Buzatto, B.A., Robinson, S.P. & Tomkins, J.L. 2014. Sociosexual environment influences patterns of ejaculate transfer and female kicking in *Callosobruchus maculatus*. *Anim. Behav.* **94**: 37–43.

## Supporting information

Additional Supporting Information may be found in the online version of this article:

**Figure S1** Our two fitness measures, namely the number of progeny surviving to adulthood and the egg-to-adult survivorship of the progeny, were correlated.

**Table S1** Effects of each predictor on offspring sex ratio (proportion sons).

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