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# The genetic architecture of sexual conflict: male harm and female resistance in *Callosobruchus maculatus*

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## Abstract

Males harm females during mating in a range of species. This harm is thought to evolve because it is directly or indirectly beneficial to the male, despite being costly to his mate. The resulting sexually antagonistic selection can cause sexual arms races. For sexually antagonistic co-evolution to occur, there must be genetic variation for traits involved in female harming and susceptibility to harm, but even then intersexual genetic correlations could facilitate or impede sexual co-evolution. Male *Callosobruchus maculatus* harm their mates during copulation by damaging the female's reproductive tract. However, there have been no investigations of the genetic variation in damage or in female susceptibility to damage, nor has the genetic covariance between these characters been assessed. Here, we use a full-sib/half-sib breeding design to show that male damage is heritable, whereas female susceptibility to damage is much less so. There is also a substantial positive genetic correlation between the two, suggesting that selection favouring damaging males will increase the prevalence of susceptible females. We also provide evidence consistent with intralocus sexual conflict in this species.

## Introduction

Male harming of females during mating occurs across a broad range of taxa (Clutton-Brock & Parker, 1995). Harm can be inflicted as a form of sexual coercion that requires damage to the female, or it may simply be inadvertent damage resulting from other activity. Examples include yellow dung flies and elephant seals where fights between males or females for territories can result in the injury or death of females (females drowned in dung or crushed) (Parker, 1978; Leboeuf & Mesnick, 1991). Males can also harm females during the act of copulation itself. For example, in the fly *Sepsis cynipsea*, the male aedeagus damages females during intromission. The more a female copulates, the more damage she sustains and the greater the risk of death (Blanckenhorn *et al.*, 2002; Hosken *et al.*, 2003). Copulatory harm has been taken to extremes in traumatically inseminating species where males bypass the female reproductive tract altogether and inject their sperm through the female body wall using a hypodermic-style intromittent organ (Siva-Jothy, 2006). In some cases, this has led to the evolution of a 'secondary' female reproductive tract, presumably to offset the costs of body wall piercing (Morrow & Arnqvist, 2003; Tatarnic & Cassis, 2010). Two adaptive hypotheses have been proposed to explain copulatory harm (Johnstone & Keller, 2000; Lessells, 2005): the pleiotropic (or collateral) harm hypothesis suggests that harm is a side effect of adaptations beneficial in male–male competition, whereas the adaptive harm hypothesis posits that harm benefits males directly, by deterring females from subsequently remating and/or altering female perceptions of their health status resulting in resource reallocation to reproduction. However, the support for either hypothesis is limited (Hosken *et al.*, 2003; Morrow *et al.*, 2003; Tregenza *et al.*, 2006; Hotzy & Arnqvist, 2009).

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Male harming of females generates sexual conflict when inflicting damage is beneficial for males, either directly (adaptive harm) or indirectly (collateral harm), but reduces female fitness, and therefore has the potential to generate sexual arms races (Parker, 1979; Rowe *et al.*, 2005). This requires genetic variation for harm and susceptibility to harm. When these conditions are fulfilled, selection for harm can cause harmful traits to spread through a population. This in turn generates opposing selection on female susceptibility to harm (resistance), and if female resistance subsequently evolves, it can generate selection for more harmful males and so on (Parker, 1979; Holland & Rice, 1998; and see Rowe *et al.*, 2005). There are a handful of experimental evolution studies that provide evidence partly or wholly consistent with sexually antagonistic co-evolution (Rice, 1996; Holland & Rice, 1999; Hosken *et al.*, 2001; Pitnick *et al.*, 2001; Martin & Hosken, 2003; Martin *et al.*, 2004; Wigby & Chapman, 2004; Gay *et al.*, 2010), but direct documentation of genetic variation for harm and female susceptibility to harm is largely limited to *Drosophila melanogaster* (Civetta & Clark, 2000; Sawby & Hughes, 2001; Linder & Rice, 2005). Additionally, Lew *et al.* (2006) found low but significant genetic variation for female resistance to male harm in this fly, and this accounted for more than half of the standing genetic variation for net female fitness.

The genetic covariance between harm and susceptibility/resistance also has the potential to influence sexually antagonistic co-evolution, retarding or facilitating evolution (Agrawal & Stinchcombe, 2009). If, for example, harm and resistance to harm are positively genetically correlated, then selection for harm can increase the prevalence of resistant females, even if there is no additive genetic variation for resistance, which could in turn select for more harming males (see e.g. Moore & Pizzari, 2005). In some ways, this is analogous to classical sexual selection where positive genetic covariances can generate the rapid co-evolution of male attractiveness and female preference (Lande, 1981). Alternatively, if male harm and female resistance are negatively genetically correlated, selection for harm could increase the frequency of less resistant females, which could represent an additional indirect cost of harm when harmful males reach an appreciable frequency in the population. Costs of harm are usually assessed as direct costs to females (e.g. Chapman *et al.*, 1995; Civetta & Clark, 2000; Crudgington & Siva-Jothy, 2000; Martin *et al.*, 2004; Rönn *et al.*, 2006). If harm generates this indirect cost, then predicting trait evolution becomes more complicated (Moore & Pizzari, 2005). However, whereas intersexual genetic correlations have been investigated in a classical sexual selection context (Bakker, 1993), the covariance between harm and resistance has not been assessed (to the best of our knowledge). It is also usually absent from mathematical models (e.g. Gavrilets & Hayashi, 2005), in spite of calls for the studies of the genetic architecture of traits involved in sexual conflict (Moore & Pizzari, 2005).

Understanding the sign of intersexual genetic correlations for fitness is also helpful in assessing intralocus sexual conflict (Rice & Chippindale, 2001; Bonduriansky & Chenoweth, 2009). Intralocus sexual conflict arises when antagonistic selection on a shared trait prevents one or both sexes from achieving their fitness optima (Arnqvist & Rowe, 2005). Thus, genotypes that have high male fitness have low female fitness, as revealed by experimental studies in *Drosophila melanogaster*. Using this fly as a model, Chippindale *et al.* (2001) expressed hemiclonal genomes in males and females and measured lifetime fitness. They found substantial crossing over of the adult fitness rank order of each genotype and an overall negative association between male and female fitness of each genotype. Similarly, intralocus sexually antagonistic selection has been directly documented in a number of other species (e.g. Merila *et al.*, 1997; reviewed in Bonduriansky & Chenoweth, 2009), and sexual dimorphism, which is extremely common, may be the ghost of conflict past (Fairbairn *et al.*, 2007; Harano *et al.*, 2010). Negative intersexual genetic correlations for fitness are the unmistakable signature of intralocus sexual conflict. However, even when one sex does not express a trait (i.e. there is sex limitation and the intersexual genetic correlation for the trait is zero), intralocus conflict can still occur because mutations that are deleterious for the character can accumulate because of their neutrality in nonexpressing sex (Day & Bonduriansky, 2004; Harano *et al.*, 2010).

The seed beetle, *Callosobruchus maculatus*, has become an iconic example of male harm. Males have a complex aedeagus that is covered with spines, and these puncture the female genital tract during copulation (Crudgington & Siva-Jothy, 2000). Male spine length is positively correlated with the number of scars in the female genital tract (Hotzy & Arnqvist, 2009). Therefore, scaring can be regarded as an indicator of female harm (Crudgington & Siva-Jothy, 2000; Hotzy & Arnqvist, 2009). There is some evidence that more spiny penises provide an advantage in sperm competition (Hotzy & Arnqvist, 2009). This would suggest that female damage is a collateral effect of this male–male competition. Copulations artificially engineered to be longer also result in more damage and reduce female lifespan (Crudgington & Siva-Jothy, 2000). Moreover, multiple mating also reduces female longevity (Savalli & Fox, 1999). However, these costs of copulation can confound the cost of damage with the trade-off between fecundity and longevity (Rönn *et al.*, 2006; Eady *et al.*, 2007) and are not consistent across studies (Fox, 1993). Consequently, the direct link between damage and female fitness remains unclear, but there seems to be some evidence of sexually antagonistic selection for damage. Here, we used a full-sib/half-sib design to assess whether male genotypes differ in the damage they inflict on their mates, while

simultaneously assessing genetic variation in female susceptibility to damage. We also estimated the intersexual genetic correlation between male damage and female susceptibility to damage to understand how sexually antagonistic co-evolution could be influenced by genetic architecture. We finally examined the genetic architecture of several other characters (longevity, copulation duration and offspring production) to assess a potential intralocus sexual conflict in these seed beetles, for which there is some evidence (Rankin & Arnqvist, 2008).

## Materials and methods

### Study species

*Callosobruchus maculatus* is a widely distributed pest of legumes. Females attach their eggs to the surface of beans and the larvae develop inside. The beetles used in this experiment were derived from Niamey, Niger, and maintained at the University of Lincoln for hundreds of generations at 27 °C (32% RH and LD 16 : 8 h photoperiod) at a population size of approximately 500 individuals on ca. 2000 black-eyed beans (*Vigna unguiculata*) per generation. Twenty generations before the experiment, the population size was increased to ca. 2500 individuals (on 250 g of black-eyed beans).

### Full-sib/half-sib design

Approximately 200 females from the mass culture were allowed to oviposit for 6 h on ca. 3000 black-eyed beans. At this density, we expect females to lay one egg per bean (Horng, 1997). Before emergence, randomly chosen beans were removed and isolated in 48-well cell culture plates (VWR International Ltd., Lutterworth, UK). Virgin males (sires) and females (dams) emerging from these beans (all 1–2 days old) were placed in individual 30-mm-diameter Petri dishes for 10 min or until they mated. After mating, dams were transferred to Petri dishes containing approximately 120 black-eyed beans and remained there for the rest of their lives. Sires were transferred to Eppendorf tubes for 24 h before being placed into a new 30-mm Petri dish together with a new virgin female (dam). This process was repeated so that all sires mated with three dams over a period of 3 days. In total, 100 sires were mated to 281 dams (18 sires failed to remate with the third dam) and we collected 1650 offspring. Virgin sons subsequently emerging from these families were isolated, and the date of emergence was recorded. The first three sons to emerge from each family were then placed with a random, unrelated, virgin female (also collected from the sire-dam matings earlier) until they mated. Randomly crossing sons and daughters instead of using mates from another unrelated population enables us to estimate indirect genetic effects (see Statistical analyses below). As earlier, matings were staged in Petri dishes with 30 mm diameter, but with each son mated to only one female. Once paired, the beetles were observed continuously until they finished mating.

Once copulation ceased, males and females were separated. Sons were placed in individual perforated Eppendorf tubes and checked daily until death to calculate their longevity. Mated females were moved onto 40 beans for 24 h and then moved to another 60 beans for the remainder of their lifespan. Longevity was estimated by recording any female mortality every 24 h. After their natural death, females were dissected and we counted the number of damage points in their genital tract caused by the spines on the male's aedeagus that perforate the genital tract during copulation. Female elytra length was also measured to use as a proxy for body size. After offspring emergence, we counted the number of offspring produced during the first 24 h after mating and over females' entire lifespan. The total represents the lifetime reproductive success (LRS) of a pair. For females, this seems to be an accurate proxy of fitness. For males on the other hand, LRS will be limited by access to a single female (only one mating). However, even though we acknowledge this is far from an ideal measure of male fitness, it is a direct constraint from pairing sons and daughters, which was essential to dissociate male damage (harm) from female susceptibility (resistance to male harm).

### Statistical analyses

In our experiment, some traits were measured independently in males and females (longevity, body size) and others were measured for each pair (copulation duration, LRS, scarring in the female). Our experimental design allows us to estimate indirect effects for traits measured in each sex (longevity) and differentiate the contribution of females and males to additive genetic variance for shared traits (copulation duration, LRS and damage). Indeed, because these traits result from an interaction within the pair, we can consider them from either the male or the female point of view. For example, the damage sustained by a female results from the susceptibility of the female [i.e. the thickness of her genital wall, (Rönn *et al.*, 2007)] and the damaging effect of a male [i.e. spine length on the male's aedeagus (Hotzy & Arnqvist, 2009)]. We use 'susceptibility' for the female component of damage (when looking at the pedigree of daughters) and 'damage' for the male component (male pedigree, i.e. indirect effect of female's mate).

Son's damage and daughter's susceptibility, longevity, LRS and elytra length were transformed to *z*-scores prior to genetic analysis. Our genetic design was unbalanced because 18 sires failed to remate with the third dam, several dams produced too few sons and a few daughters were damaged during dissection. To account for this unbalanced design, we fitted the following nested model using REML implemented in ASReml (version 2.0; VSN International Ltd.) (Gilmour *et al.*, 2006) to estimate the

genetic (co)variances: $z_{ijk} = \mu + s_i + d_{ij} + e_{ijk}$, where $z_{ijk}$ is the phenotype of the $k$th offspring from the family of the $i$th sire and the $j$th dam, $s_i$ is the effect of the $i$th sire, $d_{ij}$ is the effect of the $j$th dam mated to the $i$th sire, and $e_{ijk}$ is the residual variance (Lynch & Walsh, 1998). REML approximation is more reliable than conventional least squares ANOVA when the breeding design is unbalanced (Lynch & Walsh, 1998). We estimated the additive genetic variance and heritability for male damage and female susceptibility, as well as longevity and LRS for males and females. Coefficients of additive genetic variation may be a more appropriate measure for comparing potential responses to selection because the additive genetic variance is scaled by the trait mean (Price & Schluter, 1991). They were calculated following Houle (1992) using untransformed values as recommended. Some of these values (for longevity and copulation duration) were incorrectly calculated in a previous publication (Brown *et al.*, 2009). Furthermore, we also calculated the within-sex and intersexual genetic correlations between these traits. We determined the significance of our heritability estimates using a log-likelihood ratio test by comparing the full nested model to one where the sire term was removed. We determined the significance of $r_A$ using a log-likelihood ratio test by comparing the nested model with an unfixed covariance structure to one with a fixed covariance structure. In both instances, the test statistic ($D$) was calculated as twice the difference between the two negative log-likelihood values, and the probability distribution of $D$ can be approximated by a $\chi^2$ distribution with 1 degree of freedom (Sokal & Rohlf, 1994). The errors on our genetic estimates should be large, as they represent the variance of a variance component, but nevertheless the sign of these associations is still informative (Lynch & Walsh, 1998) and this is what we are primarily interested in here. As a result, our interpretation and discussion is based on the sign of the covariances rather than their significance value *per se*.

## Results

### Sons

We found substantial additive genetic variance in how damaging sons were and this trait was significantly heritable ($h^2 = 0.32 \pm 0.12$, Table 1). Sons' longevity, LRS and copulation duration were also significantly heritable (Table 2). Son's ability to damage was positively genetically associated with their longevity, but negatively associated with their LRS, as may be expected when using a single noncompetitive mating to assess male LRS (Table 2, Fig. 1). This means more damaging male genotypes were long lived but tended to have lower LRS from their single mate. We also found that copulation duration was negatively associated with longevity as previously reported (Brown *et al.*, 2009).

**Table 1** Samples size ($N$), phenotypic mean (± standard error) and genetic estimates for the traits measured: additive genetic variance ($V_A$) and its coefficient of variation ($CV_A$).

| | $N$ | Mean ± SE | $V_A$ | $CV_A$ |
|---|---|---|---|---|
| ♂ Longevity | 745 | 18.3 ± 0.2 | 24.5 | 27.1 |
| ♂ Damage | 737 | 15.4 ± 0.4 | 39.6 | 40.8 |
| ♂ Copulation duration | 833 | 9.9 ± 0.1 | 5.7 | 24.2 |
| ♂ LRS | 794 | 79.1 ± 0.6 | 97.7 | 12.5 |
| ♀ Longevity | 844 | 11.7 ± 0.1 | 6.1 | 21.1 |
| ♀ Size | 819 | 2.0 ± 0.003 | 0.001 | 1.8 |
| ♀ Susceptibility | 737 | 15.4 ± 0.4 | 18.8 | 28.1 |
| ♀ Copulation duration | 833 | 9.9 ± 0.1 | 3.9 | 19.9 |
| ♀ LRS | 794 | 79.1 ± 0.6 | 136.2 | 14.8 |

LRS, lifetime reproductive success.

**Table 2** Heritabilities (along the diagonal), genetic correlations (beneath the diagonal) and phenotypic correlations (above the diagonal) for males. Estimates in bold are significant at $P < 0.05$. All results are given ± standard error.

| | ♂ Longevity | Damage | ♂ Copulation duration | ♂ LRS |
|---|---|---|---|---|
| ♂ Longevity | **0.58 ± 0.17** | −0.01 ± 0.04 | **−0.17 ± 0.04** | −0.05 ± 0.04 |
| Damage | **0.35 ± 0.17** | **0.32 ± 0.12** | **0.15 ± 0.04** | −0.04 ± 0.04 |
| ♂ Copulation duration | −0.26 ± 0.21 | 0.22 ± 0.25 | **0.41 ± 0.15** | 0.02 ± 0.04 |
| ♂ LRS | 0.25 ± 0.21 | −0.19 ± 0.25 | −0.32 ± 0.24 | **0.38 ± 0.12** |

LRS, lifetime reproductive success.

### Daughters

Daughters' susceptibility to damage (analysed using daughter's pedigree) had a lower heritability than sons' ability to damage and was not statistically significant ($h^2 = 0.15 \pm 0.13$). Longevity and LRS were significantly heritable for daughters, however, but size was not (Table 3). Heritability estimates were broadly similar to those of males. Daughter's susceptibility to damage was negatively genetically associated with longevity and LRS (Table 3, Fig. 1). Thus, female genotypes more susceptible to damage tended to live shorter lives and to produce fewer offspring. The genetic correlation between longevity and LRS was positive, providing no evidence for this life-history trade-off. Copulation duration was negatively associated with longevity and LRS. The positive association between copulation duration and damage levels was much stronger in females than in males, and the sign of the covariance between longevity and damage was reversed compared to males (negative). Even though the covariance between LRS and damage was negative for both sexes, it was stronger in males.

### Intersexual genetic covariance

Sons' ability to damage and daughter susceptibility to damage had a positive genetic correlation (Table 4), and

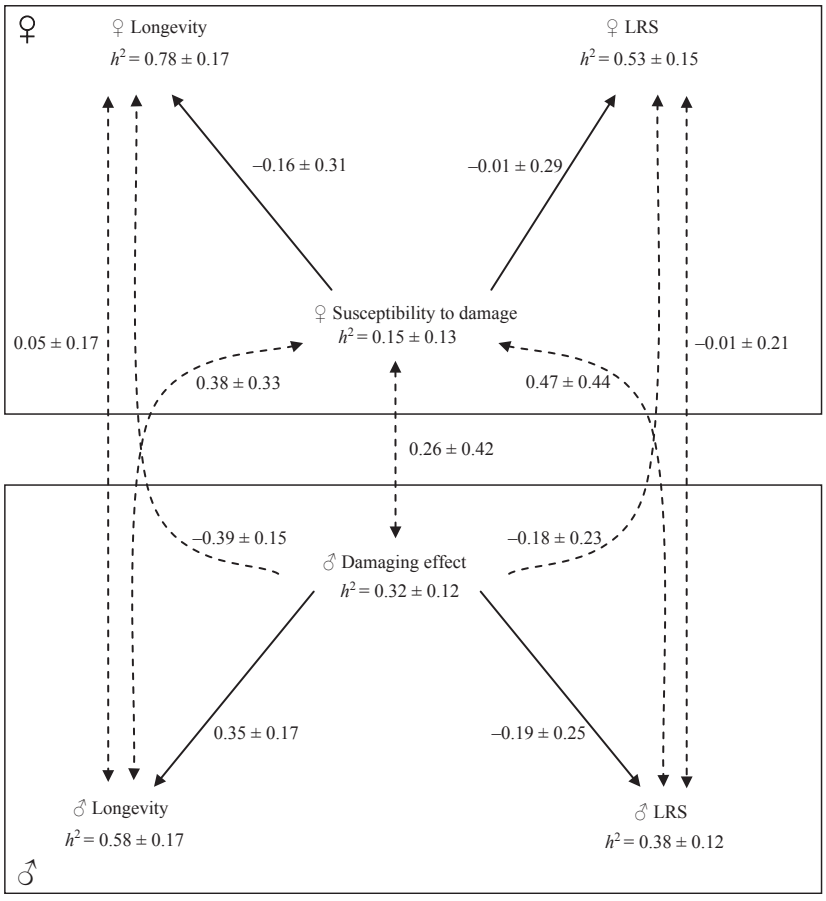


**Fig. 1** Heritabilities and genetic correlations within and between sexes, indicated ± standard error.

**Table 3** Heritabilities (along the diagonal), genetic correlations (beneath the diagonal) and phenotypic correlations (above the diagonal) for females. Estimates in bold are significant at $P < 0.05$. All results are given ± standard error.

| | ♀ Longevity | ♀ Size | Susceptibility | ♀ Copulation duration | ♀ LRS |
|---|---|---|---|---|---|
| ♀ Longevity | **0.78 ± 0.17** | **0.18 ± 0.04** | −0.01 ± 0.04 | **−0.14 ± 0.04** | **−0.11 ± 0.04** |
| ♀ Size | 0.08 ± 0.28 | 0.18 ± 0.13 | −0.04 ± 0.04 | −0.04 ± 0.04 | 0.20 ± 0.04 |
| Susceptibility | −0.16 ± 0.31 | 1.18 ± 0.79 | 0.15 ± 0.13 | **0.15 ± 0.04** | −0.04 ± 0.04 |
| ♀ Copulation duration | **−0.68 ± 0.20** | −0.11 ± 0.40 | **0.93 ± 0.42** | **0.28 ± 0.13** | 0.02 ± 0.04 |
| ♀ LRS | 0.23 ± 0.17 | 0.15 ± 0.30 | −0.01 ± 0.29 | −0.34 ± 0.26 | **0.53 ± 0.15** |

LRS, lifetime reproductive success.

sons' LRS was also positively associated with daughters' susceptibility to damage. This implies that any positive selection for damaging males, or males able to induce their mates to produce offspring, will indirectly select for more susceptible females. However, sons' ability to damage was negatively associated with daughters' longevity and LRS, which could inhibit the spread of susceptibility genes. Sons' LRS was also negatively associated with daughters' size, and there was effectively no association between sons' and daughters' LRS (Table 4). Sons' longevity was positively associated with all of the female characters measured, including susceptibility of daughters to damage, but the association between sons' and daughters' longevity was very weak (Table 4). The only positive associations between male and female fitness components were between male longevity and female LRS, and male LRS and female longevity (i.e. only two of nine fitness measures indicate good males genotype = good female genotypes). Therefore, the overall picture to emerge from these associations is that genotypes producing good males – long lived, more damaging, high LRS – tend to produce poorer females – more susceptible to damage (positive correlations with all male traits), lower LRS (negative/no association with male damage/LRS) and lower longevity (negative/no association with male damage/longevity) (Fig. 1).

**Table 4** Intersexual genetic correlations ± standard errors. Estimates in bold are significant at $P < 0.05$.

| | ♂ Longevity | Damage | ♂ Copulation duration | ♂ LRS |
|---|---|---|---|---|
| ♀ Longevity | 0.05 ± 0.17 | **−0.39 ± 0.15** | **−0.84 ± 0.14** | **0.39 ± 0.18** |
| ♀ Size | **0.62 ± 0.30** | 0.01 ± 0.36 | −0.26 ± 0.35 | −0.33 ± 0.34 |
| Susceptibility | 0.38 ± 0.33 | 0.26 ± 0.42 | 0.22 ± 0.38 | 0.47 ± 0.44 |
| ♀ Copulation duration | **−0.45 ± 0.21** | 0.08 ± 0.30 | **0.78 ± 0.25** | −0.26 ± 0.27 |
| ♀ LRS | **0.33 ± 0.15** | −0.18 ± 0.23 | −0.28 ± 0.22 | −0.01 ± 0.21 |

LRS, lifetime reproductive success.

## Discussion

Although genetic variances and covariances play critical roles in the dynamics of trait evolution (Falconer, 1981; Lynch & Walsh, 1998; Fox & Wolf, 2006), there has been little investigation of the underlying genetics of traits putatively involved in sexual conflict (Moore & Pizzari, 2005). Here, we employed a paternal half-sib design to quantify genetic variation and covariation in characters that have been implicated in sexual conflict in seed beetles. We found substantial genetic variation in most of the traits measured, and intersexual genetic correlations were often substantial. There was also some evidence of intralocus sexual conflict.

Within our population, we found substantial heritable variation in males' ability to damage females. This implies that if there is a male fitness benefit to harm, the additive genetic variation available is sufficient for selection to drive its evolution. Recent evidence in seed beetles suggests that the degree of male damage inflicted on females is dependent on the length of spines on the aedeagus and that longer spines are advantageous during sperm competition (Hotzy & Arnqvist, 2009), although a causal relationship remains to be demonstrated. Furthermore, when levels of sperm competition are experimentally altered, male damage evolves in the expected direction (Gay *et al.*, 2010). Taken together, these studies (Hotzy & Arnqvist, 2009; Gay *et al.*, 2010; current study) suggest that male damage is an evolvable trait. We also found that male damage was negatively, genetically (and phenotypically, although the association was very weak) associated with male LRS. This means that damaging genotypes were also those gaining the lowest reproductive output from their single mates. However, because focal males (sons) only mated monogamously, we have no information on the genetic architecture of sperm competitiveness, which has been suggested to be the underlying explanation for the benefits to males of damage (Hotzy & Arnqvist, 2009). More generally, harming males are expected to have lower reproductive success in each mating, but this is compensated for by their increased mating success (Holland & Rice, 1998). Consequently, assessing male fitness in single females after monogamous mating probably does not accurately reflect their LRS in the presence of post-copulatory male–male competition. Hence, our results showing that more damaging genotypes were genotypes with a lower return from single matings are consistent with sexual conflict over damage.

Female susceptibility to damage was measured as scarring in the female genital tract. In stark contrast to male damage, female susceptibility could be constrained by a relative lack of genetic variation, as this character was not significantly heritable. However, we found a positive genetic correlation between male damage and female susceptibility to damage. This implies that any selection for more damaging males will also select for more susceptible females. Therefore, even though there is little heritable variation for susceptibility, it could still evolve via indirect selection on males. In spite of this, susceptibility to harm (scarring) did not evolve in experimental populations where sexual conflict was manipulated over many generations (Gay *et al.*, 2010), but across species there is evidence of male damage and female resistance co-evolution (Rönn *et al.*, 2006).

In any case, neither the across species data nor standard arguments of chase-away sexual selection (Holland & Rice, 1998) suggest that selection for more male harm could indirectly select for more susceptible females, which is what we found here. Additionally, in mathematical models of sexual conflict (reviewed in Gavrilets & Hayashi, 2005; and see Moore & Pizzari, 2005), the covariance between male and female traits is typically ignored and explicitly including these details in evolutionary models would be interesting. If the covariance between male damage and female susceptibility increases the prevalence of susceptible females, it may either accelerate the spread of damage or slow it by increasing the fitness loss per mating for harmful males, for example (c.f. Moore & Pizzari, 2005). Consequently, how the covariance we document influences sexually antagonistic co-evolution remains unclear, but it is likely to slow the spread of female resistance, even if there was significant heritability for this trait (see equation 7b in Moore & Pizzari, 2005). In agreement with this, we only found weak phenotypic associations between female susceptibility and longevity or LRS, albeit in the predicted direction, suggesting that resistance to damage was not selected in females. However, previous work has shown that female longevity is negatively associated with damage levels (Crudgington & Siva-Jothy, 2000; Gay *et al.*, 2010; but see Eady *et al.*, 2007). Additionally, even though female susceptibility to damage is constrained by the genetic correlation with male damage and the lack of additive genetic variation, female resistance could still evolve via reducing the cost of the scars in terms of female fitness. For example, the threshold or the rate at which genital damage impacts longevity or fecundity could be reduced. Evidence consistent with this was found in a previous experimental evolution study. There, the reintroduction of sexual conflict did not result in the evolution of female resistance to damage (scaring), but females that evolved with damaging males lived longer

than nonconflict females at any given level of damage (Gay *et al.*, 2010), which suggests that the consequences of this scaring did evolve. The cost of damage is therefore likely to be a key parameter in understanding the evolutionary dynamics of male genitalia/damage and female resistance to damage.

Male's ability to damage females was also negatively genetically correlated with female LRS and lifespan. Therefore, damage genes will be lost via short-lived, low-output sisters. This could slightly offset any direct selection for damage. It is important to note, however, that direct effects will generally be stronger than indirect effects (Kirkpatrick & Barton, 1997), and some evidence suggests that damage is directly favoured in males (Hotzy & Arnqvist, 2009). Again, the LRS association needs to be treated with some caution as this was female reproductive output after a single copulation.

In addition to sexual conflict over damaging male genitalia, there is also potential intralocus conflict between the sexes. If this were occurring, we expect high fitness male genotypes to produce low fitness female genotypes and *vice versa* (Rice & Chippindale, 2001). We found negative genetic correlations between male damage and female longevity and LRS. If more damaging males are more successful males (Hotzy & Arnqvist, 2009), this is consistent with intralocus sexual conflict in these beetles, as is our finding that male LRS is not genetically correlated with female LRS (Day & Bonduriansky, 2004). However, as explained elsewhere, the male LRS measure we employed is unlikely to be a good proxy for male fitness because the number of offspring gained after one mating is unlikely to correlate with lifetime offspring sired under polygynous conditions (Holland & Rice, 1998). Consequently, male longevity is probably a better surrogate for male fitness than LRS in our study. Using this as a measure of male fitness, both female longevity and LRS are positively associated with male longevity, although the former only very weakly, with no correlation probably a more accurate summation. As female LRS was also measured after only one copulation, the longevity result is arguably more revealing and the lack of a significant intersexual correlation is again consistent with intralocus conflict (Day & Bonduriansky, 2004). Overall, the evidence is indicative of some intralocus conflict in these beetles, as per previous reports (Rankin & Arnqvist, 2008), because most of the parameters that most accurately reflect fitness are negatively or unassociated genetically across the sexes.

In conclusion, we found that the damage inflicted by males to females *C. maculatus* is heritable and positively genetically correlated with female susceptibility, although susceptibility was not significantly heritable. This suggests that selection for more damaging males will generate more susceptible females as a correlated response, but the net impact of this on the evolution of male damage is not entirely clear. We also found evidence indicative for antagonistic fitness effects across the sexes adding to a growing body of evidence suggesting such intralocus conflicts may be common.

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