Female fitness consequences of male harassment and copulation in seed beetles, *Callosobruchus maculatus*

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Despite widespread evidence for the benefits of polyandry, there are costs associated with each mating for females, and for many species, it is unknown whether the costs of extra matings outweigh the benefits. In the seed beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae: Bruchinae), costs might come from male harassment during mating attempts or from injuries that females sustain during copulation. Benefits of mating might come from nutrients or water transferred in the ejaculate. If mating is costly overall, male presence (sexual harassment) and multiple mating in *C. maculatus* is expected to reduce female fitness. Females were housed with differing numbers of males (1–4) and differing opportunities for copulation. When females were both harassed by and could remate with more than one male, they had lower lifetime reproductive rates and reduced life span relative to monandrous females. These results indicate that when females are continually exposed to multiple males, the direct benefits of multiple mating do not compensate for the costs.

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There are well-known costs for females associated with mating. The direct costs of copulation have been quantified in a number of systems, and can include time away from other activities, reduced life span, increased risk of predation and physical damage to the female (Daly 1978; Thornhill & Alcock 1983; Magnhagen 1991; Krupa & Sih 1993; Rowe 1994; Chapman et al. 1995; Hurst et al. 1995; Crudgington & Siva-Jothy 2000). Additionally, the act of rejecting a copulation attempt can have negative fitness consequences for females. Sexual coercion, where males use force, harassment or sensory exploitation to obtain copulations from reluctant females is thought to be a powerful factor influencing sexual selection in animal systems (Clutton-Brock & Parker 1995; Holland & Rice 1998).

One form of sexual coercion is male harassment, where repeated copulation attempts by males are costly to females (Clutton-Brock & Parker 1995). These costs can be severe for females, where physical injury and even death can occur as a result of male harassment (McKinney et al. 1983; Mesnick & Le Boeuf 1991; Reale et al. 1996). Other, less obvious costs of male harassment include increased predation risk (Shine et al. 2000), reduced feeding (Rowe 1992) or oviposition opportunities (Sakurai & Kasuya 2008), and decreased longevity and/or fecundity (Mühlhäuser & Blanckenhorn 2002; Bateman et al. 2006; Rönn et al. 2006). Female response to male harassment is varied in form and probably depends on the economics of rejection, avoidance and acceptance of superfluous matings. Females may also concede to additional matings in response to harassment; this is convenience polyandry, where the costs of continuous rejection are higher than the costs of superfluous matings (Thornhill & Alcock 1983; Rowe 1992).

While male-imposed costs of harassment indicate that the optimum female mating rate is generally lower than that for males, females are still widely observed to mate more than the requisite once (Ridley 1988; Arnqvist & Nilsson 2000; Jennions & Petrie 2000; Tregenza & Wedell 2000; Simmons 2005). Adaptive explanations of female polyandry typically invoke the acquisition of benefits to the female (direct benefits: Waage 1979; Gwynne 1984; Andersson 1994; indirect benefits: Loman et al. 1988; Zeh & Zeh 1996, 1997; Jennions & Petrie 2000) rather than the avoidance of direct costs as is the case with convenience polyandry. Often when a general lack of benefits is found in a polyandrous species, the conclusion is that polyandry is occurring in order to avoid the direct costs of male harassment (i.e. convenience polyandry: Lee & Hays 2004; Blyth & Gilburn 2006).
Sexual harassment probably occurs in many species and can have serious consequences in terms of the realized female mating rate (Härdling & Kaitala 2005), but it is often neglected in laboratory studies. For example, in wild populations, females will avoid areas where males congregate, and thus avoid superfluous rematings (Stone 1995; Chivers et al. 2005). In contrast, during laboratory experiments, females are often confined with a male in a small space to encourage mating (Torres-Vila et al. 2004; Sakurai & Kasuya 2008); however, this may eliminate the natural avoidance response that females may use to reduce harassment costs. The converse situation is also possible; high densities in natural populations may favor convenience polyandry (Blyth & Gilburn 2006).

To understand fully the economics of mating in polyandrous systems, it is important to assess the cost of male harassment and how it interacts with the costs and benefits of multiple mating.

The lifetime fitness costs to females of male sexual harassment has received very little empirical attention in laboratory or field studies. Studies of seed beetles, Callosobruchus maculatus have shown that females suffer fitness costs when they are confined continuously with males (Fox 1993; Crudgington 2001; Rönn et al. 2006; Edvardsson 2007); however, none of these studies examined the direct costs of copulation (e.g. genital wounding; Crudgington & Siva-Jothy 2000) and potentially toxic ejaculates (Yamane et al. 2008) versus the costs of male harassment.

Here, we examine the lifetime offspring production (fitness) of females that received different levels of exposure to males and opportunities to copulate. The costs and benefits of polyandry in C. maculatus have been studied extensively but with contradictory findings. The sclerotized intromittant organ of the male causes internal damage to the female during copulation, reducing life span in a cumulative fashion (Crudgington & Siva-Jothy 2000). Male C. maculatus may also transfer toxic compounds in their ejaculate that further reduce female propensity to remate (Yamane et al. 2008). Despite the obvious costs of injury during mating, females are polyandrous, and studies of C. maculatus have shown fecundity and/or life span benefits of multiple mating (Fox 1993; Eady et al. 2000; Arnvist et al. 2005; Edvardsson 2007; but see Frick & Maklakov 2007). These benefits are hypothesized to accrue through nutrients, water (Fox 1993; Arnvist et al. 2005; Edvardsson 2007; Ursprung et al. 2009), or oviposition stimuli (Chen 1984; Guedes & Smith 2008) transferred in the ejaculate. The conflicting nature of the studies thus far indicates that the costs and benefits of polyandry in C. maculatus are complex (Eady et al. 2007). It is important to investigate how the costs of exposure to males may alter the overall fitness outcome for females.

The main adult activity of female C. maculatus is oviposition, as they do not typically feed or drink as adults (capital breeders). Therefore, we predicted a negative effect of exposure to males on females, which would be manifested either through time/energy costs or by male disruption of oviposition behaviour. Callosobruchus maculatus are polyandrous (Rup 1986), and female C. maculatus increase their mating frequency under arid conditions (reflective of their natural dry habitat) compared to when they are given additional sources of water, indicating that water is transferred in the ejaculate (Edvardsson 2007; Ursprung et al. 2009). Therefore, if females are given the opportunity to remate, this may positively affect fitness, perhaps even to the extent of compensating for the costs. However, the extent to which female fitness is enhanced will depend on the female's optimum remating rate, which is influenced by the number of previous matings (and thus, hydrating ejaculates that she has received), her condition, genotype (Eady et al. 2004; Harano & Miyatake 2009) and life-history strategy (Guedes & Smith 2008). In this experiment, females were maintained in their species-typical arid conditions, where polyandry is highest (Ursprung et al. 2009), and they had varying exposure to either mating males or nonmating males. Specifically, we predicted that (1) cohabitating with males would be costly for females, and the degree to which females were negatively affected would increase with the number of males with which they were housed and (2) given the hydration benefits of mating (Arnvist et al. 2005; Ursprung et al. 2009), females that were allowed to remate would have higher fitness than females that were exposed to males that could attempt copulations but were unable to do so.

METHODS

Study Organism and Rearing

Callosobruchus species are pests of stored legumes, and they inhabit tropical and subtropical regions pan-globally (Southgate 1978, 1979). The life cycle of C. maculatus begins when females firmly attach individual eggs to host seeds, and within 5 days, the larva hatches and burrows into the seed. The larva remains in a single seed until emergence as an adult, 3–4 weeks later. Within 24 h postemergence, adults are fully sexually mature and begin mating. Adults are facultatively aphagous, relying solely on nutrients gathered in the larval stage (i.e. capital breeders; Stearns 1992, Messina & Slade 1999). Populations living in grain storage environments have little to no access to food and water, and there is scant evidence that adults feed outside of this environment (Fox et al. 2006). Laboratory adult populations are not typically fed, and adults live approximately 8–12 days when the primary activity is reproduction.

The population used in this study was derived from individuals collected from infested stored cowpeas (Vigna unguiculata) in 1989 in Ouagadougou, Burkina Faso (Messina 1993). Since then, they have been cultured in the Messina laboratory with each generation starting from 1500–2500 individuals. Therefore, this population has a long history (>100 generations) of laboratory rearing at relatively high densities, where females frequently encounter sexually receptive males and are thus vulnerable to harassing behaviour (Messina 1993).

This laboratory population was started in November 2006, keeping prior culturing conditions relatively similar: 25 °C, constant light and ambient humidity averaging 50% RH. These conditions are a reasonable approximation of the stored legume environment. We started each generation by adding ~1300 individuals to a 1 litre glass jar with approximately 300 g of cowpea seeds (V. unguiculata). To minimize directional selection for development time, we gathered individuals from the midpoint of the emergence period to start the new generation (Møller et al. 1990). To avoid genetic drift within each culture container, we continually exchanged individuals (while in the larval stage) between isolated containers to produce a single interbreeding population.

Experiment

We obtained virgin beetles by isolating infested beans from the main cultures and removing adults as they emerged. To reduce the effects of larval competition (Vamosi 2005), most eggs were scraped off the beans before they hatched. We weighed all females (Mettler AE 50) soon after emergence. Individuals were given a minimum of 24 h to attain sexual maturity before placing them into the experimental treatments (Fox et al. 1995). We haphazardly paired virgin females (4–36 h old) with a virgin male, placed each pair into an Eppendorf tube (1.5 ml) and allowed them to mate. If a female refused to mate within 20 min, we replaced the male with another male. If the female again refused to mate, she was
discarded from the experiment. The occurrence of virgin females that refused to mate was very low (<1%). Females were then placed into small petri dishes (35 mm), each containing 10 cowpea seeds.

Treatments differed according to the sex, type and number of cohabitating conspecifics. We randomly assigned females to one of six treatments. In the control solo-female treatment \((N = 26)\), a one-mated female was left to oviposit in isolation; in the second control, two-female treatment \((N = 26)\), the female was paired with a virgin female to control for effects of the presence of a conspecific. Virgin females of *C. maculatus* will occasionally dump unfertilized eggs on host seeds (Wang & Horgan 2004), but these eggs are distinguishable from fertilized eggs; in the latter case, the small dark mandibles of the larva are visible. The solo-female treatment can be compared to the two-female treatment to evaluate potential effects generated by having a conspecific of the same sex present during oviposition.

In two treatments, females experienced potential costs of harassment by males in the absence of costs of copulation per se (e.g. genital injury) by emasculating males (details below). We placed either one emasculated male \((1\text{-emasc treatment}, N = 27)\) or four emasculated males \((4\text{-emasc treatment}, N = 27)\) in a petri dish with a single female corresponding to exposure \((\text{without mating})\) to one male and four males, respectively. The 1-emasc treatment can be compared to the two-female control to evaluate whether the presence of a conspecific is sex specific in its effects. In another two treatments, females were again continually exposed to either one male or four males; however, males were not emasculated and were able to copulate. Treatments 1-intact \((N = 26)\) and 4-intact \((N = 23)\), corresponding to exposure to and potential copulation with one male and four males, respectively. These two treatments assessed the effects of remating with either one or many males, in addition to the effects of potential male harassment. Although the two mating treatments were expected to maintain the same levels of harassment as corresponding treatments 1-emasc and 4-emasc (see tests of assumptions below), they also included direct costs of copulation, so these two treatments could not be separated experimentally.

We transferred females and their cohabitants every 24 h to a new petri dish containing 10 fresh cowpea seeds for 5 consecutive days. This number of seeds ensured that females were not limited by oviposition sites. Deaths were noted daily, and if a male died, we replaced him with a new virgin male of the appropriate type. The exception was in the 1-intact treatment, where dead males were not replaced, as this would create differences in whether or not females had the opportunity to mate with more than one male. In cases where this occurred before the end of the 5 days, the female was removed from the analysis. Females remained in the fifth petri dish with the same cowpea seeds until death, and cohabitating males were removed at the end of day 5. We tabulated daily fecundity by visually inspecting each bean for eggs. Offspring were allowed to develop and emerge in the petri dishes where the eggs were laid under the same environmental conditions as described above. For each female we recorded both daily and lifetime offspring production.

### Male Emasculation

Males in treatments 1-emasc and 4-emasc were emasculated within 24 h of emergence before being placed into treatments. We haphazardly assigned virgin males to either be emasculated or remain intact. Emasculated males were created by gently squeezing the male’s abdomen to cause extrusion of the aedeagus, approximately half of which was removed using microscissors. Males used in treatments 1-intact and 4-intact were treated in the same manner, except instead of cutting the aedeagus, it was gently touched with ends of the microscissors. None of the males were anaesthetized during manipulation because of the potential negative effects of anaesthetizing agents on reproductive behaviour in *Callosobruchus* species (Mbata et al. 1998). Emasculated males are unable to fertilize females, because virgin females that are kept with them do not produce fertilized eggs (personal observation).

### Statistical Analysis

All statistical analysis was conducted with SYSTAT, version 12 (SPSS, Inc., Chicago, IL, U.S.A.). Not all replicates were started the same day, because there was a lack of experimental animals of the same age available at one time. Therefore ‘cohort’ is used to identify individuals that started treatment on the same day; all treatments are represented equally in all cohorts. Several cases were removed from the analysis; these included cases where females escaped or died within the first 2 days, or where males from treatment 1-intact died during the experimental period.

### Tests of Assumptions

We tested several assumptions before the main experiment. First, that there are sexual differences in remating rate, where males have a higher optimal remating rate and thus sexually harass reluctant females for copulations. Second, that emasculations of males does not change their rate of sexual harassment compared to intact males. The third assumption relates to potential differences in fecundity and fertility of females. Fecundity refers to the number of eggs laid, while fertility is used in reference to the number adult offspring that are produced. It was assumed that fecundity and fertility would be highly correlated in all treatments, and that there would not be a treatment effect on egg-to-adult survival. Thus, a lack of difference would justify analysis of number of offspring only.

#### Assumption 1: differences in remating frequency between the sexes

For sexual harassment to occur, there must be differences between the sexes in their probability of remating within a given interval. *Callosobruchus maculatus* males can attempt to copulate minutes after disengaging from a previous partner (personal observation); however, female remating interval varies between strains of this species from 6 h (Edwardsson et al. 2008) to every few days (Shu et al. 1996) in a laboratory setting. To estimate how soon a female would remate, we exposed once-mated females to a second mate 2, 4, 6, 8 and 24 h after the initial copulation, and females in the 24 h treatment were given three opportunities to mate at 24 h intervals. We chose these time intervals to obtain a conservative estimate of the frequency with which females would remate. All females, regardless of whether they had copulated the day before, were offered a virgin male daily, so the percentage of females that remated was independent of the outcome of the previous day’s trial. We placed a once-mated female (approximately 24 h old) in an Eppendorf tube at room temperature, with a virgin male and observed them for 20 min for a successful copulation. If a female refused to copulate, we introduced a replacement virgin male, and again observed them for 20 min. If a female rejected two virgin males, she was scored as refusing to mate.

#### Assumption 2: harassment rates of intact and emasculated males

To compare harassment rates in emasculated and intact males, virgin males (26–28 h old) were haphazardly assigned to be either emasculated or intact. Emasculation was performed as described above in the main experiment. Virgin females (26–28 h old) were
mated once to a virgin male approximately 2 h before the experiment to reduce their motivation to mate. We placed one male and one female in an Eppendorf tube and recorded the number of failed mating attempts made by the male for 20 min. An individual mating attempt was scored when the male mounted and antennated the female.

**Assumption 3: Egg-to-adult survivorship**

Both the total number of eggs and total number of offspring per day were recorded for each female in the main experiment. The egg-to-adult survivorship was calculated as the percentage of eggs that resulted in successful emergence of an adult beetle.

**RESULTS**

**Tests of Assumptions**

**Differences in remating frequency between the sexes**

All females mated as virgins (25 females in each treatment). None of the females remated after 2, 4, 6 or 8 h after their first copulation. When females were offered a virgin male 24 h post-copulation, 60% (15) of them remated. When the same females were again offered a virgin male 24 h later, 76% (19) of them mated again. On the third day, 72% (18) of females remated. This finding supports the assumption that females are unwilling to mate for much longer following a mating than males (see also Ursprung et al. 2009 showing similar mating rates for this strain of *C. maculatus*).

**Harassment rates of intact and emasculated males**

There was no significant difference in the rate of copulation attempts between emasculated and intact males (Mann–Whitney U test: \(U = 138, N_1 = N_3 = 19, P = 0.21\); \(X\) failed copulation attempts: intact males: 15.53; emasculated males: 13.89). A confidence interval for the effect size was calculated using a Monte Carlo simulation for the average difference between harassment rates in the two groups. The observed effect size (harassment rate of intact minus emasculated) was –3.3 with a 95% confidence interval of –9.0–2.7. The confidence interval spans zero and the range of values is consistent with the null hypothesis of no effect. This supports our assumption that emasculation did not alter the males’ ability to sexually harass females.

**Egg-to-adult survivorship**

All treatments had high rates of adult emergence (\(\bar{X} + SE = 88.8 \pm 1.61\%\)). There were no significant differences between treatments (\(F_{143} = 0.712, P = 0.615\)), indicating that there was no effect of treatment on egg-to-adult survival. Therefore, the assumption that egg-to-adult survivorship did not differ between treatments was upheld and the remainder of the analysis deals only with number of offspring produced.

**Main Experiment**

**Effects of treatment on female offspring production**

There were no significant differences in female weight between treatments (\(F_{140} = 0.70, P = 0.624\); \(\bar{X} + SE = 0.0585 \pm 0.00057\) g). Therefore, we excluded body weight as a covariate in all further analyses. A full model ANOVA revealed no significant effect of cohort and no cohort by treatment interaction on female lifetime offspring production. Therefore, the second-order term (cohort \(\times\) treatment) was removed from the analysis. The ANOVA revealed significant treatment effects on total offspring production (\(F_{143} = 4.44, P = 0.001\)), but no significant effect of cohort (\(F_{6,143} = 0.976, P = 0.44\); Fig. 1, Table 1).

The first prediction, that there would be costs associated with increasing numbers of cohabitating males, was not supported. If these costs increase with the number of emasculated males present with the female, treatment 4-emasc should have had lower offspring production than treatment 1-emasc, which was not the case (Tukey’s HSD test: \(P = 0.98\)). These treatments did not differ from either control treatment (solo-female and two-female treatments). The second prediction was that the presence of intact males (allowing for remating) would be beneficial for females, assuming that additional nutrients/water or ovipositional stimulants contained in the ejaculate would more than compensate for costs of copulation and harassment. On the contrary, multiple mating appears to be costly; both in the 4-intact treatment, where there were opportunities for females to mate with many males, and in 1-intact treatment, where there were opportunities for females to remate with the same male, females had reduced lifetime offspring production. Although there was no significant difference between 4-intact and 4-emasc, there was a tendency for lifetime offspring production of females in the 4-intact treatment to be lower, and, in post hoc comparisons of all treatments (Tukey’s HSD), females in the 4-intact treatment had significantly lower offspring production than females in either the 1-intact (\(P = 0.004\)) or the control solo-female (\(P = 0.001\)) treatment (Fig. 2). This finding suggests that high levels of harassment and matings with many males combined (4-intact) were detrimental to female offspring production.

The presence of a female conspecific did not negatively affect female fitness, as females in the 2-female treatment did not have lower reproductive success than solo-females (\(P = 0.258\)). Thus, the presence of a single nonmating conspecific (2-female) did not affect offspring production. Moreover, solo-females and 1-emasc treatment females did not differ in lifetime reproduction (\(P = 0.99\)). Thus, the presence of a potentially harassing nonmating male (1-emasc) did not lower fitness, supporting the conclusion that the presence of a male, and thus, increased harassment (e.g. sexual) did not reduce female reproduction.

Offspring production was measured in each of the first 5 days, and we examined treatment effects on the pattern of offspring production over time. A repeated measures analysis of variance revealed a significant within-subjects effect of ‘day’ (corrected Huynh–Feldt: \(F_{1,552} = 560.30, P < 0.001\)). This simply indicates that females have reduced reproductive output over time (Fig. 1). The interaction between day and treatment was not significant (\(F_{1,552} = 1.45, P = 0.120\)). Therefore, treatment did not affect the pattern of offspring production through time. However, Fig. 1 shows a strong pattern of reduced reproductive output in the 4-intact treatment beginning on day 3.

**Effects of level of male exposure and mating opportunities on offspring production**

Treatments 1- and 4-emasc and 1- and 4-intact were designed to test the independent effects of the level of nonmating male presence (i.e. harassment) and the effects of the number mating males. ANOVA revealed significant effects of male presence (\(F_{1,99} = 4.25, P = 0.042\)), but not of number of mating males (\(F_{1,99} = 0.001, P = 0.969\)) on lifetime offspring production. However, there was a significant interaction between level of male presence and number of mating males (\(F_{1,99} = 9.09, P = 0.003\)). This was due to the large difference in offspring production between treatments 1-intact and 4-intact and between 1-emasc and 1-intact (Table 2, Fig. 3). Thus, the presence of males that are able to mate negatively affects female fitness only when females receive potential harassment and/or copulatory damage from more than one male.

Differences in total offspring production between females could be due to differences in life span or reproductive rate. Increased rate...
of reproduction may even compensate for a shorter life span. Both rate of reproduction and life span were analysed to determine the cause of the difference in total offspring production between treatments.

**Female life span.** Female life span included the 24 h postemergence before the experiment began. Any females that died within the first 2 days of the experiment were removed from the analysis, as they did not experience the full effects of the treatment, and the cause of death was assumed to be from factors other than the treatment. The mean ± SE life span for females was 8.6 ± 0.15 days. A three-factor ANOVA revealed significant effects of male presence on survivorship (\(F_{1,75} = 14.0, P < 0.001\)), but no significant effects of the number of mating males (\(F_{1,75} = 0.27, P = 0.604\)), cohort (\(F_{6,75} = 1.1, P = 0.387\)) or higher-order interactions. Post hoc comparisons of all the treatments showed that when females were exposed to four males (treatments 4-emasc and 4-intact), they had significantly lower survival compared to all other treatments. There was a strong trend for 4-intact females to have shorter life spans than 4-emasc females (\(P = 0.0574\)) (Table 3, Fig. 4). There was also a nonsignificant tendency for solo females to live the longest. This finding indicates that when there is a high level of male exposure (4-intact and 4-emasc), females suffer reduced life span, and that this cost is amplified when there is the potential to mate with several males (4-intact). The short female life span in the 4-intact treatment may explain the lowered reproductive output in this treatment.

**Female reproductive rate.** Reproductive rate was calculated for each female by dividing the total number of offspring produced by life span. Neither exposure to males nor opportunity for copulation significantly affected reproductive rate (Table 4). However, a highly significant interaction term (male presence × opportunity to copulate: \(F_{1,99} = 7.14, P = 0.009\)) revealed that when females were exposed to four males (4-intact and 4-emasc), their reproductive rate decreased when the males could copulate, but when females were exposed to only one male (1-intact and 1-emasc), their reproductive rate was elevated when the male was able to copulate (Fig. 5). Despite the strong interaction between opportunity to copulate and male presence, when reproductive rate was compared among treatments, there were no significant differences (\(F_{5,149} = 1.83, P = 0.111\)). Therefore, differences in reproductive rate did not appear to cause the observed treatment differences in total offspring production. It appears that the difference in life span was the main mechanism by which treatment differences in offspring production were manifest.

**DISCUSSION**

A key prediction of this study was that male harassment would be costly to females in terms of reduced fitness. This prediction was not upheld; an increase in nonmating male presence and thus harassment alone (1-emasc versus 4-emasc) did not affect lifetime offspring production. Furthermore, the harassment treatments did not differ from either control, indicating that the presence of a conspecific of either sex had no significant effect on female offspring production. Although there were no significant effects of male presence alone on offspring production, females housed with...
more than one male (4-emasc and 4-intact treatments) had significantly shorter life spans, approximately 1.5 days less than females housed with just one male (Fig. 4). Thus, there is a life span cost associated with male cohabitation at higher levels that appears to come from the harassment common to both 4-emasc and 4-intact treatments. Costs of male harassment in *C. maculatus* were also reported by Gay et al. (2009) in an experiment housing (non-receptive) singly mated females with five virgin males for 6 h postcopulation, thereby isolating the effects of harassment from copulation. High levels of sexual harassment during this time reduced early lifetime fecundity in females. Thus, in contrast to our results, male harassment alone appeared to negatively affect early lifetime fitness of females in Gay et al.’s (2009) study, perhaps because females were exposed to very high levels of harassment for a shorter period than in our study.

Our results also revealed a cost of copulation for females in the 4-intact treatment, where females tended to have a shorter life span; this cost resulted in fewer offspring only in 4-intact females. The decreased reproductive output of females in the 4-intact treatment began on day 3 and continued to the end of the experiment. Females in the 4-intact treatment not only lived, on average, half a day less than females in the 4-emasc treatment, but they also had the fewest offspring of all treatments.

Female *C. maculatus* receive hydration benefits (Edvardsson 2007; Ursprung et al. 2009) but no nutritional benefits (Ursprung et al. 2009) from rematings. Thus, we also predicted that the potential to mate frequently with one or more males (1-intact and 4-intact) would increase offspring production. This prediction was refuted. In fact, females that cohabitated with four sexually active males had significantly fewer offspring than females in all other treatments. Therefore, the cost of copulation and harassment may be an important factor in determining female fitness in this species.
males (4-intact) had significantly fewer offspring than all other treatment combinations, averaging 20 fewer offspring than females that had mated once and were allowed to oviposit in isolation (solo-females). This cost appeared to occur after day 3, when the reproductive costs for females in the 4-intact treatment tended to increase relative to that for females in the other treatments. It is possible that cohabitating with four intact males resulted in a female mating rate that was too high for the hydration benefits to outweigh the costs associated with each mating. This did not appear to be the case when females were housed with a single intact male as these females had a much higher total offspring production than females housed with one emasculated male.

Although number of matings were not directly manipulated or observed in this experiment, females of this strain would be expected to mate at least two or three times given the duration of this experiment (see Ursprung et al. 2009), and *Callosobruchus* species in general are expected to mate more than three times in their lifetime (Katvala et al. 2008). Thus, the negative effect of the presence of four intact males on female fitness probably results from both additional harassment and/or copulation damage. Multiple mating in this species can be costly, as males not only physically damage females during copulation with spines at the distal tip of the aedeagus (Mukerji & Bhuya 1937; Crudgington & Siva-Jothy 2000), but male ejaculates may also have direct toxic effects that reduce female life span (Yamane et al. 2008). Harassing males may increase the costs of rejection so that it is more economical for females to concede to superfluous matings than to continue rejecting (convenience polyandry).

An alternative possibility is that despite the direct costs of mating, females are motivated to remate at high rates in order to

Figure 4. Mean ± SE life span for female *Callosobruchus maculatus* in each treatment. Significant differences are indicated by different letters and were calculated using Tukey HSD tests.

Figure 5. Least squares mean ± SE reproductive rate for female *Callosobruchus maculatus* that cohabitated with (a) emasculated males and (b) intact males, grouped by the number of males (either 1 or 4) that were cohabitating.

<table>
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<th>Table 3</th>
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<td>Summary of log-rank comparisons of life span for female <em>Callosobruchus maculatus</em> in each treatment</td>
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Females were housed alone (1F), with another virgin female (2F), with one or four emasculated males (1-emasc, 4-emasc), or with one or four intact males (1-intact, 4-intact). Significant *P* values are shown in bold.

Table 4

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<th>Analysis of variance of the effects of exposure to males (cohabitation with either one or four males) and opportunities for copulation (presence/absence of an aedeagus) on reproductive rate in female <em>Callosobruchus maculatus</em></th>
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<td>Source</td>
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<td>Opportunity for copulation</td>
</tr>
<tr>
<td>Exposure-opportunity for copulation</td>
</tr>
<tr>
<td>Error</td>
</tr>
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</table>

(a) Emasculated males

(b) Intact males

10 8.5 7 5.5 4 1-f 2-f 1-e 4-e 1-i 4-i Number of males

10.5 9.5 8.5 7.5 6.5 5.5 4.5 Female life span (days)
obtain indirect benefits such as increased genetic diversity of their offspring or to increase genetic compatibility. This would require that indirect benefits of polyandry outweigh the direct costs, a situation that has little theoretical support (Cameron et al. 2003; but see Head et al. 2005) or empirical support in C. maculatus (Edvardsson et al. 2008). In any case, in the 4-intact treatment, female mating rate may have exceeded the optimum mating frequency for females, causing the observed decrease in fitness (Arnqvist & Nilsson 2000). This result is consistent with other studies that did not manipulate mating rate, but instead varied the number of males placed with the female, and found that polyandrous females had reduced fecundity (Lan & Horng 1999; Kołodziejczyk & Radwan 2003; Pai & Yan 2003; Bybee et al. 2005).

As female life spans in both treatments with multiple males were lower than those in the other treatments, the cause of reduced fitness is probably persistent male copulation attempts despite initial rejection by the female (personal observation). An unreceptive female typically kicks her metathoracic legs at a mounting male, or will move away from her pursuer. Energetic costs of behaviours such as these may be substantial for capital breeders, such as C. maculatus, as resources that are acquired in the larval stage are not replenished through adult feeding. Males also disturb ovipositing females (which may also disorient reproductive strategies in the short-lived C. maculatus). In another study on the same species (but a different strain), Gay et al. (2009) estimated that females may be subjected to four harassment events/h when housed with a single male and up to 18 events/h when housed with five males. In summary, male sexual harassment may involve both time and energy costs for C. maculatus resulting in the observed reduction in life span. The life span cost from harassment was only apparent when the sex ratio was strongly male biased (1:4 in this study).

Manipulating the operational sex ratio is a common technique to study the effects of male harassment (Le Galliard et al. 2005; Head & Brooks 2006; Shuker et al. 2006). The costs probably accrue faster for females when there is more than one male attempting to copulate (Clutton-Brock & Parker 1995). However, these costs did not translate into reduced offspring production when females were harassed but were unable to remate.

Recently, the idea that males may transfer harmful seminal substances in ejaculate or even in nuptial gifts has been suggested for several insect species (Simmons 2001; Sakaluk et al. 2006; Wedell et al. 2008; see also Gwynne 2008), but few have examined the overall fitness effects of polyandry on females. As in C. maculatus (see Ursprung et al. 2009), female katydids (Requena verticalis) that have been deprived of material benefits (i.e. nuptial gifts of food) remate in order to obtain those benefits (Gwynne 1990). Although Wedell et al. (2008) concluded that the material benefits obtained by polyandrous katydid females do not compensate for their shortened life span, they did not measure mating success and offspring growth rate. In the present study, harassment and copulation costs as well as the potential material benefits of copulating with differing numbers of sexually active males were measured by a lifetime fitness measure: number of emerging adult offspring. Using these fitness measurements, we found that the costs were not offset by the material benefits of mating.

Despite the costs of multiple mating, past studies examining polyandry in C. maculatus have come to different conclusions about overall fitness effects of polyandry (Fox 1993; Savalli & Fox 1999; Arnqvist et al. 2005; Eady et al. 2007). Some studies have found that polyandrous female C. maculatus live longer (Fox 1993; Savalli & Fox 1999; but see Crudgington & Siva-Jothy 2000) and have increased fecundity (Fox 1993; Savalli & Fox 1999; Eady et al. 2000; Ursprung et al. 2009; M. den Hollander, unpublished data). Callosobruchus maculatus males lose up to 8% of their mass during copulation (Fox et al. 1995; Savalli & Fox 1999), suggesting that females receive material benefits from the absorbed ejaculate. These benefits of polyandry appear to consist of water (rather than nutrients) (Edvardsson 2007; Ursprung et al. 2009). Experimental variation in degree of male presence may explain the differences in effects of polyandry on female fitness. The amount of variation that females experience in natural populations is unknown.

Some evidence suggests that the material benefits that C. maculatus females receive from this nuptial gift are unable to offset mating and harassment costs imposed by the presence of multiple males (Gay et al. 2009; this study). Other studies have found that male ejaculate size does not affect female fecundity or several other components of female fitness (Eady 1995; Fox et al. 2006; Rönn et al. 2008). Fox et al. (2006) suggested that large ejaculates primarily function to reduce female propensity to remate, a hypothesis bolstered by Miyatake & Matsumura’s (2004) finding that females reduce their refractory period and remate much sooner when matings are interrupted. Females may, however, be remating sooner to gain a nuptial seminal gift, and this has been supported by several studies, where thristy female C. maculatus remate sooner (Edvardsson 2007; Ursprung et al. 2009).

Sakurai & Kasuya (2008) studied the related species C. chinensis, and were able to separate mating costs from harassment costs by pairing females with males that could not physically mate but could harass females for copulations. In contrast to the present study, they found that male harassment alone significantly reduced female fitness, and there was no evidence of direct costs (or benefits) of copulation in this species. The differences between our studies probably arise from the differences in the reproductive system between the two congeners. Ejaculate materials transferred by male C. chinensis are much smaller (1.21% of their body weight; Takakura 1999) than those transferred by C. maculatus males (5% of their body weight; Fox et al. 1995).

Rönn et al. (2006) examined the effects of male cohabitation (in addition to other costs of mating and reproduction) on female fitness in six species of Callosobruchus, including two geographically disparate stains of C. maculatus. In that study, males were unmanipulated and could freely harass and copulate with the focal female. They found that in comparison to females that were mated once and allowed to oviposit in isolation, females experiencing both harassment and polyandry had reduced life span and fecundity. However, there was a significant effect of strain on fecundity, where only one strain of C. maculatus experienced a significant drop in fecundity when cohabitating with males. These results are similar to ours, where females in the 4-intact treatment suffered reduced fitness when cohabitating with multiple intact males. Although Rönn et al. (2006) were unable to partition the costs of multiple mating and male sexual harassment separately in their experimental design, they found that the net costs and benefits to mating can vary greatly between strains of the same species, and they cautioned against using a single species (such as C. maculatus) to generalize concepts. It is worth exploring the possibility that many of the inconsistencies in previous studies may be attributed to strain differences within C. maculatus.

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