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Why do male *Callosobruchus maculatus* harm their mates?

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Males of the bruchid beetle *Callosobruchus maculatus* have spines on their intromittent organs that puncture the female reproductive tract during mating. Females kick their mates during copulation. If females are prevented from kicking the males, copulations last longer and the injuries females sustain are more severe. We tested whether or not these injuries represent real fitness costs that can be mitigated by kicking and also what males gain by inflicting them. Our results show that females do indeed suffer lowered lifetime fecundity if they are prevented from kicking. However, we could find no evidence that males gain benefits through harming their mates. It has been suggested that the way females respond to the harm may benefit the male causing it. Injured females may be less willing to remate to avoid sustaining further injuries, or they may respond by increasing their rate of oviposition if they perceive the injuries as a threat to their survival. In our study, however, females that were prevented from kicking did not respond by delaying remating or increasing their rate of oviposition. Furthermore, preventing females from kicking during their second copulation did not make their second mates more successful in sperm competition. This suggests that the spines have evolved for other reasons than harming the females, such as serving as an anchor during copulation, and that the harm they cause is a side effect of a male adaptation and is not itself adaptive for either sex. *Key words: Callosobruchus maculatus*, female resistance, harmful male traits, mating costs, sexual conflict, sperm competition. *[Behav Ecol 16:788–793 (2005)]*

Among the multitude of male copulatory behaviors and reproductive traits are some that appear to be harmful to females. For example, Chapman et al. (1995) showed that substances in the seminal fluid of male *Drosophila melanogaster* are toxic to females, causing a reduction of their longevity. Other examples come from the bruchid beetle *Callosobruchus maculatus* and the dung fly *Sepsis cynipsea.* Males of both these species have spines on their intromittent organs that harm the female reproductive tract during mating and leave scars (Blanckenhorn et al., 2002; Crudgington and Siva-Jothy, 2000).

Male traits that are harmful to females can evolve for two different reasons. The harm can be a pleiotropic side effect of a trait that is beneficial to males in some other way. Male traits that have the side effect of being harmful to females can be favored by selection, provided the benefits to males from having these traits are greater than the costs males incur by reducing the offspring production of their mates (Morrow et al., 2003; Parker, 1979). Alternatively, males may actually benefit from harming females by altering their mate's optimal reproductive strategy in favor of greater investment in offspring from their current mate (Constantz, 1984). Recent treatments (Johnstone and Keller, 2000; Lessells, 1999, 2005) show that this is theoretically possible and may happen in two ways. First, if there are escalating costs to females of repeated matings, then mate harm can be maintained because it reduces female remating propensity (Johnstone and Keller, 2000). Second, females may perceive the injuries as a threat to their survival and respond by increasing their current reproductive effort in order to maximize offspring production before they die (Lessells, 1999; Michiels, 1998). In both cases, males would benefit through the increased number of eggs fertilized by their sperm before the females remate. This is especially important when last-male sperm precedence is high and previous mates gain little paternity after female remating.

There is currently no direct experimental evidence in support of the idea that the infliction of harm per se is a male adaptation. Indeed, there is very little evidence for male traits that have overall negative effects on female lifetime reproductive success at natural mating frequencies. There are many studies that indicate that matings have negative effects, such as reducing female longevity, but in most cases matings also increase female egg-laying rate (e.g., Chapman et al., 1998). The coevolution of male and female reproductive traits is a central part of the theory of sexual selection. Our limited knowledge of the evolution of harmful male reproductive traits and behaviors represents a major gap in our understanding of male-female interactions and mating behavior. In an attempt to evaluate the potential effects of injuries on female reproduction, Morrow et al. (2003) wounded or snipped body parts off females of three insect species (*D. melanogaster, Tribolium castaneum,* and *C. maculatus*) immediately after they had mated. This did not cause females to delay remating or increase their reproductive rate (Morrow et al., 2003) and hence failed to support the idea that males might gain by harming their mates in a similar fashion. That experimentally inflicted physical harm to females does not seem to benefit their latest mates begs the question whether or not this is also true for harm inflicted by copulating males.

C. maculatus (Coleoptera, Bruchidae) is a polyandrous beetle with high last-male sperm precedence (Eady and Tubman, 1996). Copulating females usually start kicking their mates vigorously approximately two thirds into the copulation and continue kicking until the copulation is terminated (Eady, 1991a; Qi and Burkholder, 1982; Tufton, 1993). If females are prevented from kicking their mates, copulations are prolonged and the wounds caused by the spines on the male genitalia are more extensive (Crudgington, 2001). Although

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negative effects on female fitness of such damage have not been reported, some costs to females seem likely. It has hence been suggested that mate kicking has evolved to mitigate the costs of mating in *C. maculatus* (Crudgington, 2001). Previous studies have not demonstrated any benefits to male *C. maculatus* from harming their mates. For females, mating appears both to carry costs and convey benefits. This is manifest in the complex way in which mating rate affects longevity and lifetime offspring production. Crudgington showed that females mated twice (Crudgington and Siva-Jothy, 2000), but not females mated three times (Crudgington, 2001), suffered reduced longevity compared to singly mated females. Arnqvist et al. (2005) found no effects of mating rate on longevity but did find the analogous effect that females with an intermediate mating rate (average number of copulations: 1.82) had a significantly reduced lifetime offspring production compared to females with low (1 copulation) and high mating rates (average number of copulations: 2.6 to 3.9). There is evidence suggesting that nutrients in the male ejaculate are responsible for the benefits of mating (Savalli and Fox, 1999), but whether or not the wounds caused by the spines on the male genitalia are responsible for the costs is not clear. All together, this makes *C. maculatus* an intriguing model organism in which to study copulatory interactions and the evolution of harmful male traits.

In this study, we report the results of two experiments assessing the effects of preventing female mate kicking in *C. maculatus*. We tested whether or not the relatively extensive injuries males inflict when mating with females unable to kick them benefit males through increased female refractoriness, rate of offspring production, or sperm precedence. We also evaluated the importance of mate kicking to female lifetime fecundity and hence mating costs.

METHODS

C. maculatus is a widely distributed pest on stored legumes. Its eggs are attached to beans, and the larvae develop inside. We used the *Brazil* strain in this study, and cultures were maintained on black-eyed beans *Vigna unguiculata* at 28°C (with a 19:5 h light:dark photoperiod). Experimental matings were also carried out at 28°C in a constant-temperature room. We ablated female hind legs as described below to prevent mate kicking. This method has previously been found to result in prolonged copulations and more severe injuries to the central region of the female reproductive tract (Crudgington, 2001). Crudgington (2001) showed that these effects were not simply artifacts of females being traumatized before mating because females that had had two other legs ablated prior to mating showed normal copulation durations and levels of injury.

Experiment 1—mating and remating

We assigned virgin females randomly to two groups. Females assigned to the treatment group had their hind legs ablated immediately before their first mating, and females assigned to the control group had their hind legs ablated immediately after their first mating. Females having their legs ablated were immobilized on ice, and their hind legs were cut off at the midpoint of the tibia with a pair of microscissors. When females belonging to the treatment group had their legs ablated, control females were also immobilized on ice and vice versa. Matings were staged in petri dishes with 30 mm diam. We measured copulation duration and recorded when females started to kick their mates ("time until kicking") and the length of the time period between the onset of kicking and the termination of the copulation ("duration of kicking"). After the first mating, females were transferred to petri dishes containing 60 black-eyed beans and allowed to oviposit. Every 6 h, females were placed in a 30-mm petri dish together with a new male. If a mating occurred within 10 min, we recorded copulation duration and kicking duration as above. Females that did not mate were transferred to a new petri dish containing 60 fresh, black-eyed beans. This process was repeated until all females had remated. We recorded oviposition for every 6-h period by counting the number of eggs attached to beans.

Experiment 2—sperm precedence and ejaculate size

The color morphs tan and black were used in this experiment to enable paternity determination. Hybrids from matings between tan and black beetles are readily distinguishable from homozygous individuals by their color pattern (see Eady, 1991b). The beetles were maintained, and the matings were conducted under the same conditions as above. Virgin black females were mated to virgin black males. Females were then provided with 60 fresh black-eyed beans and were allowed to oviposit for 24 h. The number of eggs laid was later counted by visual inspection of the beans. Females were mated again 24 h after the first mating, this time to tan males. Half of the females had their hind legs ablated, in the same way as described above, approximately 2 h before the second mating. Females that did not have their legs ablated at this time were also immobilized on ice. Their legs were ablated immediately after the second mating at which time the females of the other group were also immobilized on ice. We recorded copulation duration and kicking duration at both matings as described above. This time, we also estimated the size of the transferred ejaculate by weighing males and females immediately prior to and immediately after matings using a Cahn 28 microbalance with an accuracy of <10 μg. After their second mating, females were transferred to a petri dish containing 60 fresh black-eyed beans and were allowed to oviposit until they died. Adult offspring were counted and scored for body color when they emerged. This enabled us to calculate lifetime offspring production and the proportion of offspring sired by the second male to mate (*P2*).

GLIM was used to estimate generalized linear models, and other statistical analyses were carried out using SYSTAT 10.

RESULTS

Experiment 1—mating and remating

Females with ablated hind legs copulated for significantly longer periods of time than did normal females (mean = 341 ± 18.6 s and mean = 261 ± 11.9 s; $t_{2,48} = -3.61$, $p = .001$). Increased copulation duration was caused by a prolongation of the time between the onset of female kicking and the termination of copulation (mean = 181 ± 19.4 s and mean = 80 ± 10.6 s; $t_{2,48} = -4.53$, $p < .001$). Leg ablation did not have a significant effect on time until remating (see Table 1) or on the rate of oviposition over the 6 h after the first mating (Mann-Whitney $U_{25,25} = 294$, $p = .71$).

Experiment 2—size of transferred ejaculate

There was a strong correlation between male weight lost during copulation and female weight gained (first mating: $r = .907$, $p < .0001$), indicating that we were able to make reliable measures of the size of the transferred ejaculate. We analyzed variation in ejaculate size in the first mating, measured as female weight gain, in a general linear model (Table 2). Duration of kicking, but not time until kicking, had a

Table 1

A contingency table of time to remating for females of the two treatments with actual and expected number of females remating at 6-h intervals after an initial mating

	Time (h)									
Treatment	6	12	18	24	30	36	42	48	54	Total
Normal	7 (5)	5 (6.5)	3 (3.5)	9 (9)	1 (0.5)	0 (0)	0 (0)	0 (0)	0 (0.5)	25
Ablated	3 (5)	8 (6.5)	4 (3.5)	9 (9)	0 (0.5)	0 (0)	0 (0)	0 (0)	1 (0.5)	25
Table	10	13	7	18	1	0	0	0	1	50

χ^2_a has been calculated from this table. χ^2_b has been calculated from a table where the columns have been merged into two columns (6–12 h and 18–54 h) and all the expected frequencies hence are larger than 10. $\chi^2_a = 4.435$; $v = 8$; $p > .75$. $\chi^2_b = 0.0805$; $v = 1$; $p > .75$.

significant positive association with the size of the transferred ejaculate in the first mating (Table 2). To produce a model only including variables with a significant effect on ejaculate size, we performed stepwise backwards elimination of the nonsignificant variables in Table 2, starting with the least significant variable. Duration of kicking was still the only significant variable after the stepwise elimination of the nonsignificant variables and the exclusion of one extreme outlier ($p = .003$, $r^2 = .27$, $N = 30$). The outlier had a studentized residual of 3.3 and did not change the significance of the models. In the second mating, males copulating with females with intact hind legs transferred ejaculates that were smaller on average (mean = 0.160 ± 0.010 mg) than did males copulating with females with ablated hind legs (mean = 0.18 ± 0.012 mg), but the difference was not significant ($t_{2,22} = -1.40$, $p = .18$).

Experiment 2—offspring production

Variance in initial rate of oviposition and lifetime offspring production was analyzed in general linear models (Tables 3 and 4). The initial rate of oviposition, measured over the first 24 h after the first mating, was positively associated with female weight and negatively with copulation duration (especially time until kicking) (Table 3). The effect of female weight remained significant, and time until kicking was marginally nonsignificant after stepwise elimination of the nonsignificant variables ($p_{\text{female weight}} = .009$ and $p_{\text{time until kicking}} = .052$, $r^2 = .37$, $N = 31$). The negative association between time until kicking and initial rate of oviposition was also significant in a Spearman rank correlation ($r_s = -.58$, $N = 31$, $p < .001$).

Female weight had a significant positive association with lifetime offspring production; heavier females produced more offspring. Leg ablation also had a significant effect on lifetime offspring production (Table 4). Stepwise elimination of the nonsignificant variables resulted in a model with leg ablation as a fixed factor and female weight as the only covariate ($p_{\text{female weight}} = .004$ and $p_{\text{leg ablation}} = .018$, $r^2 = .51$, $N = 22$). Females that were unable to kick their mates during their second copulation produced fewer offspring than females that were able to kick their mates (mean = 86.7 ± 4.6 s and mean = 103 ± 4.0 s, $t_{2,21} = 2.61$, $p = .016$). Offspring production was not associated with the size of the two ejaculates (Table 4).

Experiment 2—second male sperm precedence, *P2*

Males copulating with females having ablated hind legs did not achieve higher *P2* scores than did males copulating with normal females (mean actual *P2* values = 0.92 ± 0.020 and 0.93 ± 0.026, respectively; $t_{2,21} = 0.127$, $p = .90$ (*P2* values are arcsine square root transformed). We analyzed variance in *P2* (i.e., the number of offspring fathered by the second male to mate) among females in a generalized linear model, using binomial errors and a logit link function, with the total number of offspring produced after the second mating as the binomial denominator (Table 5). To compensate for overdispersion (McCullagh and Nelder, 1989), we employed the method of Williams (1982). There were no significant interactions between any of the variables in the analysis. Again, *P2* was not affected by leg ablation. In the full model, *P2* had a highly significant positive association with duration of kicking in the first mating and a marginally significant negative association with duration of kicking in the second mating. However, duration of kicking in the first mating was the only variable that could not be removed from the full model without reducing its fit significantly (duration of kicking in the first mating: $\chi^2_1 = 17.75$, $p < .001$; duration of kicking in the second mating: $\chi^2_1 = 3.22$, $p = .073$). Stepwise elimination of the nonsignificant variables resulted in a model only including duration of kicking in the first mating and time until kicking in the first mating. Removal of either of these variables had a highly significant effect on the fit of this reduced model (duration of kicking in the first mating: $\chi^2_1 = 50.504$, $p < .001$; time until kicking in the first mating: $\chi^2_1 = 9.48$, $p = .002$).

Table 2

Results of a general linear model of the factors affecting the size of the ejaculate received at the first mating

Effect	Coefficient	Standard error	t	p
Constant	0.175	0.106	1.635	.110
Duration of kicking	0.000	0.000	2.068	.049
Time until kicking	−0.000	0.000	−0.092	.928
Female weight	−0.017	0.012	−1.496	.147
Male weight	0.029	0.018	1.599	.122

$r^2 = .263$; $N = 31$.

Table 3

Results of a general linear model of the factors affecting number of eggs laid over the first 24 h after the first copulation

Effect	Coefficient	Standard error	t	p
Constant	16.838	15.493	1.087	.288
Ejaculate size	49.833	30.863	1.615	.119
Duration of kicking	−0.044	0.023	−1.912	.067
Time until kicking	−0.055	0.025	−2.160	.041
Female weight	6.869	2.001	3.433	.002

$r^2 = .468$; $N = 30$.

Table 4

The results of a general linear model with female lifetime offspring production as the response variable, leg ablation as a fixed categorical factor, and the other variables as covariates

Effect	Sum of squares	df	*F* ratio	*p*
Ejaculate size (1)	211.305	1	1.604	.228
Ejaculate size (2)	267.226	1	2.028	.178
Time until kicking (1)	21.950	1	0.167	.690
Duration of kicking (1)	446.572	1	3.389	.089
Time until kicking (2)	276.766	1	2.100	.171
Duration of kicking (2)	62.795	1	0.477	.502
Female weight	959.411	1	7.281	.018
Leg ablation	1027.623	1	7.798	.015
Error	1713.038	13		

The numbers in parentheses refer to the first (1) and second (2) matings.

$r^2 = .708$; $N = 22$.

DISCUSSION

We found that females that had their hind legs ablated prior to their second mating had a lower lifetime fecundity than females that had their hind legs ablated immediately after their second mating. This difference could be due to some general costs associated with carrying an injury during mating. However, this seems unlikely given the short duration of mating and the finding of Crudgington (2001) that whereas removal of the hind legs leads to prolonged copulations and more severe injuries to the female reproductive tract, removal of any other two legs prior to mating does not have this effect. A more likely explanation is that our treatment effects are due to the inability of females with ablated legs to kick their mates. This is the first evidence that reduced female resistance to a presumably harmful male trait (Crudgington, 2001) represents an actual fitness cost to females. Mate kicking shortens copulations and may thereby reduce the extent of injuries. Furthermore, it is possible that kicking has additional effects on depth of penetration of male genitalia etc., which could in turn also affect the severity of injuries inflicted by the males. As of yet, it is only possible to speculate over the relative importance of potential mechanisms. However, we can infer that because copulation duration does not appear to influence lifetime fecundity, the influence of mate kicking on fecundity is not likely to be just through its effect on copulation duration.

Table 5

The results of a generalized linear model, using binomial errors and a logit link function, of the proportion of offspring fathered by the second male to mate (*P2*) with leg ablation as a fixed categorical factor

Effect	Estimate	Standard error	*t*	*p*
Time until kicking (1)	0.00936	0.00759	1.232	.121
Duration of kicking (1)	0.0199	0.00577	3.455	.002
Time until kicking (2)	0.00208	0.00337	0.618	.274
Duration of kicking (2)	−0.00199	0.00109	1.822	.047
Ejaculate size (1)	3.501	6.326	0.553	.295
Ejaculate size (2)	−8.418	7.526	1.119	.143
Female weight	−0.6154	0.5813	1.059	.155
Leg ablation	0.3250	0.5473	0.594	.282

The numbers in parentheses refer to the first (1) and second (2) matings.

Log-likelihood ratio test of full model: $\chi^2_8 = 36.13$, $p < .001$, $r^2 = .705$.

We did not find support for any benefits to males from harming their mates in our study. It has been suggested that females may reduce their remating propensity in response to male-induced harm to avoid sustaining further injuries (Johnstone and Keller, 2000). This would benefit males through a decreased risk of having to compete with sperm from other males. Results from our first experiment show that leg ablation does not result in a delayed remating. Apparently, male *C. maculatus* cannot extend the refractory period of their mates by prolonging matings and inflicting more severe injuries.

Males have been suggested to benefit from harming their mates as a consequence of females perceiving the injuries as a threat to their survival and responding by increasing their current reproductive effort in order to maximize offspring production before they die (Lessells, 1999; Michiels, 1998). In neither of our two experiments did ablation of female hind legs prior to mating result in an increased rate of offspring production. This outcome suggests that male *C. maculatus* cannot manipulate the reproductive effort of their mates to their advantage by prolonging copulations and inflicting more severe injuries.

Males would benefit from harming their mates if females for some reason responded to the harm in a way that helped males achieve precedence over sperm of other males. In our second experiment, males that mated second to females with ablated hind legs did not achieve higher *P2* scores than did second males mated to normal females. This suggests that prolonging copulations beyond a certain point and inflicting more severe injuries will not make males more successful at competing with sperm from previous matings. We found that *P2* was affected by the duration of the first copulation. Both time until kicking and duration of kicking seem to be important. Counterintuitively, the duration of the first copulation was positively associated with *P2*; long first copulations gave a high *P2* score. This result has now been confirmed by another study on *C. maculatus* where copulation duration was manipulated by the researchers (Edvardsson M and Canal D, unpublished data). First males to mate that were allowed to copulate for relatively long periods of time were less successful, that is, *P2* scores were higher, than males that were allowed to copulate for relatively short periods of time. As in the present study, duration of the second copulation did not have a strong effect on *P2*.

For there to be a positive relation between length of the first copulation and *P2*, long first copulations must have a negative impact on the first males' relative reproductive success. This is a difficult finding to explain, but it could happen for a number of reasons. Smaller amounts of sperm may be transferred because male problems with ejaculate transfer lead to long copulations. However, small ejaculates were not associated with long copulations in the present study. Female usage of sperm between the first and second mating may be altered by stimuli and injuries sustained during copulation in such a way that fewer sperm remain in storage at the time of the second mating. This is also an unlikely explanation, however, because a relatively high rate of oviposition was associated with short copulations rather than with long ones. Finally, copulation duration may somehow affect the amounts of sperm being stored at either the first or the second mating through some unknown mechanism.

One possibility is that copulation duration of the first mating, perhaps through the severity of the sustained injuries, somehow influences sperm uptake and storage at the second mating. If copulating for a long time increases the number of stored sperm at both the present copulation and also at subsequent copulations, this would create a trade-off between replacing sperm from previous matings (sperm offense) and avoiding replacement by sperm from future matings (sperm defense). Further research is needed to determine whether or not that is the case in *C. maculatus*.

Perhaps somewhat surprisingly, we failed to find any significant effects on *P2* of the relative sizes of the ejaculates from first and second matings. Part of the explanation for this could be that an ejaculate normally contains more sperm than is needed to fill the spermatheca, which will therefore be full of sperm after the first mating regardless of the size of the ejaculate (Eady, 1995). This will diminish the importance of variance in size of the first ejaculate for *P2*.

We did not test for any effects of ejaculate size on the refractory period of females in this experiment. There is evidence suggesting that males benefit from transferring a large ejaculate through prolonged refractory periods of their mates in *C. maculatus* (see Savalli and Fox, 1999). We found that duration of kicking was positively associated with size of the transferred ejaculate in the first mating, whereas time until kicking was not. There was a positive relationship between duration of kicking and the size of the ejaculate. Apparently, variance in the size of the transferred ejaculate is determined by events during this part of copulation and not by what takes place during the initial period when the females are not kicking. This suggests that perhaps females start kicking when they have received a certain volume of ejaculate rather than after a certain amount of time. In the second mating, males mating with females with ablated hind legs did not transfer significantly larger ejaculates than males mating with normal females in spite of their longer copulations. It appears that even though ejaculate transfer continues after kicking has started, extending copulations beyond a certain point does not increase the size of the transferred ejaculate.

In conclusion, our results suggest that female mate kicking in *C. maculatus* indeed mitigates the costs of the injuries males inflict on females during mating. It therefore seems likely that the kicking behavior evolved in response to the harmful male spines. Avoidance of male-imposed costs of mating has been suggested to be an important driving force behind the evolution of female traits in a number of species (see Chapman et al., 2003). However, it is also possible that female kicking originally evolved for other reasons. For instance, females may have used kicking to exercise mate choice (Eberhard, 1996). The males best able to carry out copulation and sperm transfer in spite of female kicking would presumably be the ones of good condition and high genetic quality. This could in turn lead to selection on males to evolve spiny genitalia to be used as anchors during copulation as discussed below. The female kicking behavior may then have been modified to mitigate the injuries caused by the spines.

Our results suggest that males do not benefit from harming their mates. Because we did not find any disadvantages of mating with kicking females compared to nonkicking females, it is possible that kicking is currently beneficial both to males and females because of its positive effects on female fecundity. However, there are some potential benefits to the males from harming their mates that are still unexplored. For instance, females can increase their reproductive output not only by laying more eggs but also by investing more in each egg (Cunningham and Russell, 2000). Males could benefit through increased survival of their offspring if the eggs they fertilized were heavier. We only examined the effects of female leg ablation on the offense component of sperm competition. It is possible that prolonged matings and more severe injuries make males more successful when competing with sperm from subsequent copulations. This seems unlikely, however, because we found a significant negative association between copulation duration of the first mating and the proportion of offspring sired by the first male to mate. It has been suggested that the puncture wounds caused by male genitalia may speed up and facilitate uptake of seminal substances into the hemolymph of female *C. maculatus* (Crudgington, 2001; Lewis and Pollock, 1975). If these substances reduce female receptivity to other males, then this could reduce the time it takes females to become unreceptive.

If male *C. maculatus* do not benefit from harming their mates, then the harm must be regarded as a side effect of another function of the spines. Male genital structures are sometimes involved in the removal of sperm from previous matings (Simmons, 2001), although Eady (1994) showed that this is not the function of the genital spines of male *C. maculatus*. A perhaps more likely scenario is that the injuries sustained by females are side effects of male efforts to remain in copula for long enough to finish ejaculate transfer (Crudgington, 2001; Simmons, 2001; Siva-Jothy et al., 1996). The spines on the male genitalia may, when the genitalia are inflated inside the female, serve as an anchor. Copulating males are attached very firmly to females (Crudgington, 2001; Edvardsson M and Canal D, personal observation). This would have provided males with a selective advantage if females had evolved kicking as a mate-choice mechanism as discussed above. However, even in the absence of female kicking, being firmly attached to the female during mating is important because male *C. maculatus* frequently and aggressively try to mate with females that are already copulating. This may well be the reason why the spines evolved in the first place.

Our results fit the conclusions of Morrow et al. (2003), who found that harming female insects immediately after mating does not cause any responses that would be favorable to males. They are also in accord with Hosken et al. (2003), who found that the number of previous copulations, and hence the total amount of damage caused by male genitalia, did not have a positive association either with female reproductive investment or with female reluctance to remate in the dung fly *S. cynipsea*. This suggests that the harmful spines on the male genitalia of *S. cynipsea* as well as of *C. maculatus* have evolved for other reasons than harming the females. Taken together, the current study and the work of Morrow et al. (2003) and Hosken et al. (2003) cast doubt over the suggestion that harming females per se has been an important driving force behind the evolution of harmful male traits in insects.

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