

Adaptation versus pleiotropy: why do males harm their mates?

Edward H. Morrow,^a Göran Arnqvist,^a and Scott Pitnick^b

^aDepartment of Animal Ecology, Evolutionary Biology Centre, University of Uppsala, Norbyvägen 18D, SE-752 36 Uppsala, Sweden, and ^bDepartment of Biology, Syracuse University, 108 College Place, Syracuse, NY 13244, USA

Recent studies have documented male traits that cause physical harm to their mates during copulation. Such harm has been suggested to either (1) arise as a negative pleiotropic side effect of adaptations that give males a reproductive advantage in another context or (2) represent a male adaptation per se. In other words, male traits that cause harm to their mates may become established despite the fact that they cause harm or because they do so. A critical assumption of the latter hypotheses is that females respond to infliction of harm in a manner that is beneficial to their mates: by reducing their propensity to remate and/or by elevating their current reproductive rate. In the present study, we test this assumption by experimentally inflicting various forms of harm to females immediately after copulation in three different insect species. We reveal that females do not delay remating or increase their reproductive rate after being harmed but, on the contrary, remate sooner and lay fewer eggs in some cases. We conclude that selection for infliction of harm to females per se is unlikely. Instead, available empirical evidence supports the hypothesis that harmful male traits arise as negative pleiotropic side effects of adaptations that yield other selective advantages to males during reproductive competition. *Key words:* accessory gland substances, *Callosobruchus maculatus*, *Drosophila melanogaster*, genitalia, sexual conflict, sperm competition, terminal investment, *Tribolium castaneum*. [*Behav Ecol* 14:802–806 (2003)]

Sexual reproduction is not always a harmonious affair between the sexes. Rather, evolutionary conflicts of interest between the sexes are ubiquitous (Chapman and Partridge, 1996; Parker, 1979; Rice, 1996, 2000). Sexual conflict is predicted to result in sexually antagonistic coevolution (Chapman and Partridge, 1996; Rice, 1996) whenever adaptations to one sex compromise the fitness interests of the other. When that is the case, we expect the evolution of counter-adaptations in the latter sex, aimed at reducing the cost imposed by such harmful adaptations. Novel harmful adaptations will then be selected, leading to potentially perpetual coevolutionary arms races between the sexes (Rice, 1998, 2000).

During the past decade, several studies of insects have documented male adaptations that inflict harm on females during copulation. Such male traits include seminal “toxins” in *Drosophila* fruitflies (Chapman et al., 1995), nematodes (Gems and Riddle, 1996), and beetles (Das et al., 1980) and injurious genital spines in beetles (Crudginton and Siva-Jothy, 2000) and dung flies (Blanckenhorn et al., 2002; Mühlhäuser and Blanckenhorn, 2002). The origin and maintenance of such harmful male adaptations can be understood in two distinct ways. First, Parker (1979) suggested that such traits may be favored in males if harm to females is a negative pleiotropic side effect of a trait that is otherwise beneficial to males (the “pleiotropic harm hypothesis”). He pointed out that a male mutation that gives bearers a reproductive advantage (e.g., in terms of sperm competition), but which simultaneously harms their mates, will be favored by selection provided that the reproductive benefit to males of this mutation outweighs the reproductive cost bearers suffer owing to a reduced reproductive output of their mates.

Second, two recent models suggest that inflicting post-copulatory harm to their mates may be beneficial per se to males (the “adaptive harm hypotheses”). The first of these models (Johnstone and Keller, 2000) suggests a link between increased harm to females and reduced probability of remating for females. Male infliction of harm could invade and become established in a population if females responded to harm by lowering their remating rate. Inflicting harm would thus directly benefit males in terms of reduced sperm competition from other males. Another model (Lessells, 1999; Michiels, 1998) suggests that females respond to harm by reallocating resources from maintenance to current reproduction, because future reproductive value is decreased when injured (i.e., a “terminal investment” effect). Inflicting harm would thus directly benefit males in terms of an increased current reproductive output of their mates. Both mechanisms of the adaptive harm hypothesis thus assume that females respond to postmating injury in certain ways, owing to general life-history trade-offs. This response essentially constitutes “hidden preferences” (cf. Ryan, 1991) favoring males that cause them harm. Males then evolve to exploit these preferences by harming their mates. Unfortunately, empirical studies of female reproductive response to postmating harm are absent, and it is thus not possible to evaluate this critical assumption.

The aim of the present study was to examine whether female insects generally do respond to postmating harm in a manner that is favorable to their mates, as assumed by the adaptive harm hypothesis. We experimentally inflicted sublethal post-mating harm to females, and then assessed both female remating behavior and their short-term reproductive output. We replicated this experiment with three different species, two in which postmating harm to females have been documented (*Drosophila melanogaster* and *Callosobruchus maculatus*) and one in which it has not (*Tribolium castaneum*). Although the injuries inflicted experimentally may not realistically simulate the kinds or levels of injuries produced by males of these species, they nevertheless address the key hypothesis of a generalized physiological and life-history response to harm.

Address correspondence to E.H. Morrow, who is now at the Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106, USA. E-mail: morrow@lifesci.ucsb.edu.

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Table 1
ANOVA of the effects of harming treatment on the reproductive rate of females, performed separately for each species

Species	Source	df	F ratio	p
<i>Tribolium castaneum</i>	Treatment	5	2.56	.037
	Error	55		
<i>Callosobruchus maculatus</i>	Treatment	5	0.10	.99
	Error	42		
<i>Drosophila melanogaster</i>	Treatment	5	2.66	.025
	Error	144		

METHODS

General

In each species, virgin females were mated once with a virgin male and then randomly assigned to one of five possible harmful treatments, in which harm was inflicted immediately after copulation, or to a control treatment, in which females were similarly handled but not harmed. The females were then allowed to oviposit for a species-specific period of time and then given the opportunity to remate with another male. In this way, we obtained measures of both reproductive rate and female remating interval. The postmating harm treatments used were (1) ablation of a mesothoracic leg, (2) ablation of an antenna, (3) ablation of a wing, (4) puncture of the abdomen, and (5) puncture of the thorax. Ablations of legs, antennae, and elytra were carried out by using microscissors, and punctures were made by using the tip of fine stainless steel entomological pins (maximum diameter, 0.2 mm). We made every effort to inflict postmating harm to females in a manner that was consistent across species.

Tribolium castaneum

The Georgia wild-type strain, provided by the *Tribolium* stock center at the US Grain Marketing Research Laboratory in Manhattan, Kansas, USA (<http://bru.usgmlr.ksu.edu/proj/tribolium/index.asp>), was used in the current experiments. Pupae were sexed and isolated from stocks to ensure male and female virginity. Sixty-one virgin females were mated 4–5 days after eclosion. Only females copulating for 35 s or more were used in this experiment (see Edvardsson and Arnqvist, 2000). When inflicting harm, the tibia of a midleg was cut medially, the antennae basally, and the elytra medially. Puncture wounds were made on the ventral side, slightly lateral to the midline. Females were then isolated individually and allowed to oviposit for 4 days in Petri dishes (9-cm diameter) with 12 g of standard medium. Females were then exposed to two virgin males in a mating chamber (4-cm diameter), and the time until remating was recorded. The number of larvae produced by each female was subsequently counted over a period of 4 days. All animals were reared in standard medium (95% wheat flour and 5% brewer's yeast, w/w) and maintained at 30°C and 70% relative humidity, except during matings, which were performed at 20°C and under natural light conditions.

Callosobruchus maculatus

Individuals of *C. maculatus* were obtained from a wild-type stock population maintained at the Department of Animal Ecology, University of Uppsala. Adults from the stocks were allowed to oviposit for 2 days on black-eyed beans (*Vigna unguiculata*). After oviposition, beans carrying one egg only were removed and isolated, ensuring virginity of the emerging adults. Adults emerging from these beans were used in the experiment within 24–48 h of eclosion. Forty-eight virgin

males and females were placed in a 3-cm-diameter Petri dish for 45 min or until they mated. Any females that did not mate were discarded. When inflicting harm, the midlegs were cut medially through the femur, the antennae basally, and the elytra medially. Punctures of the abdomen were made medial-ventrally, and punctures of the thorax were made laterally at the base of the left foreleg. Females were then transferred to a Petri dish containing approximately 3 g of mung beans (*Vigna radiata*) as an oviposition substrate. After 24 h of oviposition, females were exposed to a novel male for 45 min once every 6 h until they remated. The number of eggs laid by each female during the first 24-h period were then counted. All animals were maintained at 27°C and 65% relative humidity, 12-h/12-h light/dark photoperiod, but matings and experimental manipulations were performed at 20°C and under natural light conditions.

Drosophila melanogaster

A large outbred population (LH_M) of *D. melanogaster* was used, which had adapted to laboratory conditions for over 200 generations. This strain was generously provided by A. Chippindale and W. R. Rice (University of California, Santa Barbara) and has been maintained in the laboratory (of S.P.) since its arrival in 2001 in a population cage supporting more than 1000 individuals with overlapping generations on standard cornmeal-molasses-agar medium with a supplement of live yeast. All experimental flies were collected after a single generation of rearing in 200-ml bottles with a low-to-moderate density of larvae. Virgin males and females were collected on the day of eclosion after anesthetization with CO₂. The experiments began with 4-day-old flies. One hundred fifty virgin males and females were taken from the stock population and singly mated. All females were anesthetized with CO₂ for harming or control handling. The mesothoracic leg was cut in the middle of the tibia; the puncture in the thorax was mid-dorsum and shallow so as not to damage the underlying flight musculature, and the puncture in the abdomen was in the plueron, approximately mid-way from anterior to posterior. Importantly, care was taken to insert the pin at a shallow oblique angle so as not to make contact with any part of the underlying reproductive tract. Reproductive output was examined for 48 h after the initial mating, with a fresh vial on the second day. Females were then tested for remating by placing one virgin male per vial, for 2 h each morning until they remated. All animals were maintained at 25°C and 50% relative humidity, 12-h/12-h light/dark photoperiod, but matings and experimental manipulations were performed at room temperature and natural humidity and light conditions.

RESULTS

Female reproductive rate

Experimental harm had no significant overall effect on the reproductive rate of females ($F_{5,241} = 1.25$; $p = .29$). However, there was significant heterogeneity among the variances from each species ($F_{\max 0.05 [3,48]} = 27.1$; $p < .01$). Thus, it was more appropriate to analyze the data from each species separately. These analyses showed that the reproductive rate of *T. castaneum* and *D. melanogaster* did appear to be affected by the experimental treatments (Table 1), with females that experienced abdominal (*T. castaneum*), or abdominal and thoracic wounds (*D. melanogaster*), tending to lay the smallest number of eggs (Figure 1). We also performed more focussed tests of the general effects of injury by conducting planned post-hoc contrasts, comparing control females to all injured females simultaneously. Injured and control females only dif-

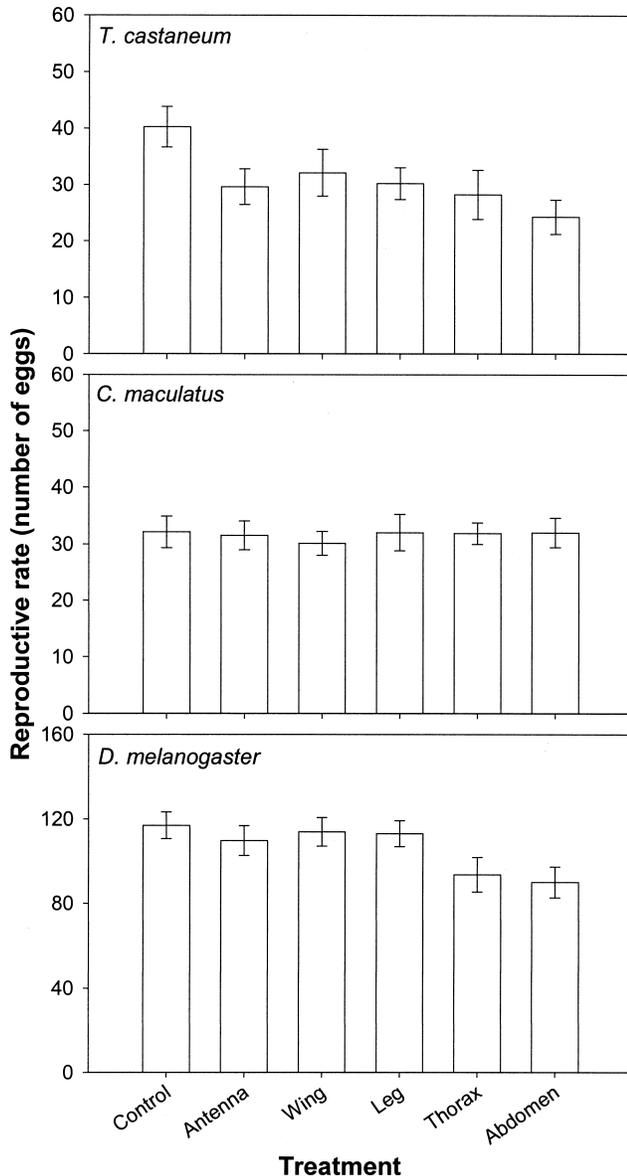


Figure 1
The reproductive rate for the three species, measured as offspring production during a certain period after mating, for control females and for females in which various forms of postmating harm was inflicted. Error bars represent SE.

ferred significantly in *T. castaneum* (*D. melanogaster*: $F_{1,144} = 2.82$, $p = .10$; *T. castaneum*: $F_{1,55} = 9.78$, $p = .003$; *C. maculatus*: $F_{1,42} = 0.05$, $p = .82$), in which control females laid more eggs than did injured. To summarize, there was clearly no tendency for females to elevate their reproductive rate, as assumed by the adaptive harm hypothesis. On the contrary, injury tended to affect short-term reproductive output negatively (Figure 1).

Female remating propensity

Data on remating intervals were log-transformed before analyses. When analyzed jointly, there was no significant overall effect of experimental harm on female remating behavior ($F_{5,239} = 0.44$; $p = .82$). However, the variances were also found to be heterogeneous ($F_{\max} 0.05[3,48] = 4.25$; $p < .01$); a separate analysis for each species was thus more

Table 2
ANOVA of the effects of harming treatment on the propensity to remate among females, performed separately for each species

Species	Source	df	F ratio	p
<i>Tribolium castaneum</i>	Treatment	5	0.14	.98
	Error	55		
<i>Callosobruchus maculatus</i>	Treatment	5	1.12	.36
	Error	42		
<i>Drosophila melanogaster</i>	Treatment	5	2.38	.04
	Error	142		

appropriate. These separate analyses indicated that remating interval was affected by treatment in *D. melanogaster* (Table 2), such that females with ablated legs remated sooner than did other females (Figure 2). Nonetheless, planned post-hoc contrasts comparing control females to all injured females simultaneously showed that in no case did control females differ from injured females (*D. melanogaster*: $F_{1,142} = 0.29$, $p = .59$; *T. castaneum*: $F_{1,55} = 0.48$, $p = .49$; *C. maculatus*: $F_{1,42} = 0.77$, $p = .39$). Because remating interval is known to be associated with reproductive output in insects (Arnqvist and Nilsson, 2000), we also performed ANCOVA of remating interval, using treatment as a factor and egg productivity as a covariate. When thus controlling for egg production, there were no significant effects of our experimental harm treatment on remating interval in any of the three species (*D. melanogaster*: $F_{5,136} = 1.20$, $p = .32$; *T. castaneum*: $F_{5,49} = 1.12$, $p = .37$; *C. maculatus*: $F_{5,36} = 0.45$, $p = .81$). In summary, postmating harm had no significant general effects on females' remating behavior. It is nevertheless worth noting that injury tended to decrease females' time to remate (Figure 2), contrary to the assumptions of the adaptive harm hypothesis.

DISCUSSION

Our results show that females did not increase their rate of egg production or their remating interval when harmed after mating. On the contrary, our data provide evidence for the opposite effect in a few cases. These results contradict the scenario envisioned in the adaptive harm hypothesis (Johnstone and Keller, 2000) and suggest that females do not generally respond to infliction of harm in a manner beneficial to their mates. Without such preexisting female "preferences" for harmful males, the adaptive evolution of traits with a primary function to harm females during copulation seems unlikely.

In a stochastic dynamic model of adaptive male harm, in which females were able to choose both oviposition rate and remating interval, the evolutionarily stable state included an increase in oviposition rate but, perhaps counter-intuitively, a decrease in female remating interval with increasing levels of harm (Lessells CM, personal communication). Only the pleiotropic harm hypothesis predicts a decrease in both the remating interval and the rate of oviposition as observed in the current study.

Our results are consistent with previous studies using *D. melanogaster*, which have found that, despite larger males inflicting greater harm on their mates, females mated to relatively large males do not increase their oviposition rate or their remating interval (Friberg U and Arnqvist G, unpublished data; Pitnick, 1991; Pitnick and García-González, 2002). Moreover, a recent experimental investigation of female responses to male harm in another species, the dung

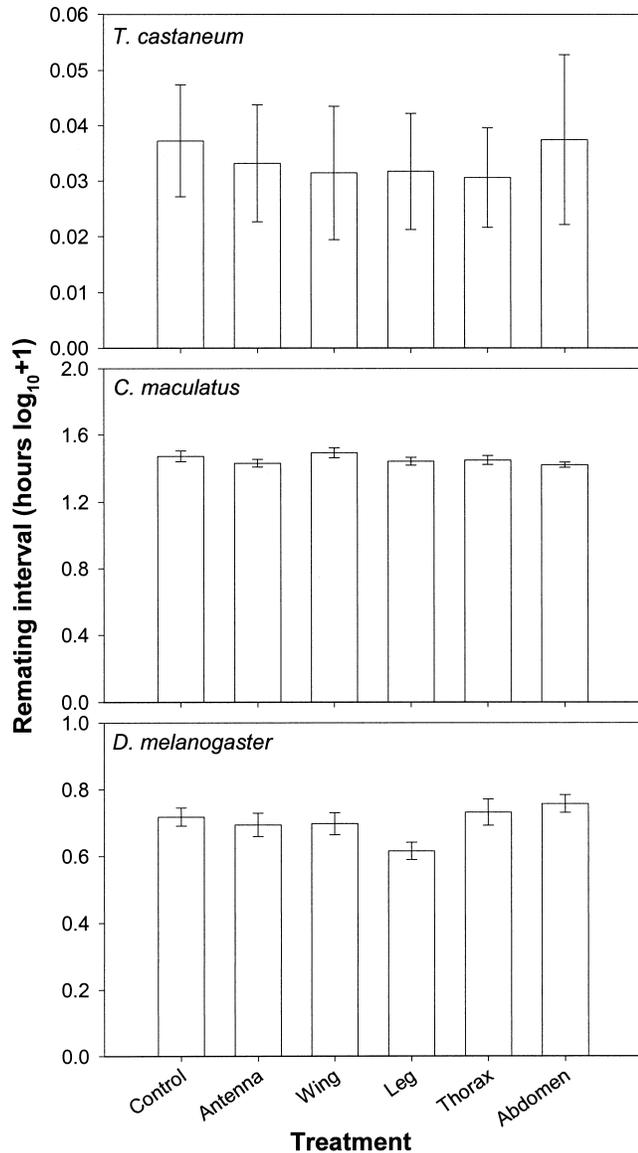


Figure 2

The propensity to remate for the three species, measured as the time until remating occurred, for control females and for females in which various forms of postmating harm was inflicted. Error bars represent SE.

fly *Sepsis cynipsea*, provides additional support for the pleiotropic harm hypothesis (Hosken et al., 2003). Mating in this species is characterized by precopulatory struggles, and males of this species are known to damage the female reproductive tract during copula in a manner that greatly increases the probability of female death (Blanckenhorn et al., 2002). Hosken et al. (2003) observed that female *S. cynipsea* became less reluctant to remate as the number of previous copulations increased. Furthermore, female reproductive output was found to be unaffected by the number of copulations a female experienced.

Why do females not respond to harm inflicted in the manner assumed by the adaptive harm hypothesis? With regard to remating, resisting courting males incurs a range of different costs to females (Arnqvist and Nilsson, 2000). We suggest that it is frequently less profitable for injured females, or they may be less able, to resist male courtship. For example, female *Drosophila* suffering lowered viability as a result of

inbreeding depression are known to accept matings after fewer courtships and hence remate more frequently (Bryant, 1979; Harmsen and Clark, 1987; Van Den Berg et al., 1984). With regard to reproductive effort, life-history theory assumes that females allocate resources to current reproduction, maintenance, and growth (Roff, 1992), and the trade-offs between these different goals can be very complex. It is easy to envision scenarios in which the optimal female response to an injury is to temporarily invest in wound repair and/or healing, which would then decrease investment in current reproduction. Our data show that this is true, suggesting that females generally respond to harm in a manner that compromises the interests of their mates.

Other evidence casts doubt on the validity of the adaptive harm hypothesis. First, despite ample opportunity for the infliction of “intentional” postmating harm to females in animals, we are unaware of any unambiguous examples. In the few cases in which there is some understanding of male postmating harm to females, the primary function of male traits is not to cause harm. For example, genital spines in insects most likely function either to provide an internal grasp for males, an “anchor” that might even prolong copulations beyond the female optimum duration (Lloyd, 1979; Simmons, 2001), or to increase the diffusion rate of seminal substances into the females’ hemolymph by causing punctures/abrasions in the vaginal wall (Crudginton and Siva-Jothy, 2000; Eberhard, 1998). Similarly, the seminal “toxins” of *Drosophila* (see Chapman et al., 1995) and other insects (Das et al., 1980) seem to serve other primary functions (Chapman, 2001; Civetta and Clark, 2000; Lung et al., 2002). In *Drosophila*, such toxins seem to be protease inhibitors that function to protect sperm and/or seminal fluid substances from enzymatic attack in the female genital tract (Lung et al., 2002). The toxic effect is thought to arise as a result of diffuse interference with essential enzymatic processes in the female body, because these seminal substances are known to enter the female hemolymph by leakage through the vaginal wall (Lung and Wolfner, 1999).

Second, in theory, the adaptive harm scenarios will only predict the maintenance of harmful male traits if one does not allow evolution of the relationship between harm inflicted and reproductive benefit achieved (cf. Johnstone and Keller, 2000). For example, a male mutation that achieves the same sperm competition goal (i.e., reduced remating and/or elevated reproductive rate) while harming females less, would be strongly favored by selection: a male that transfers a “signal” would outcompete one that achieves the same goal by harming its mates. We would then generally expect the evolution of males that achieve the same reproductive benefit to a lower cost to females (i.e., more benign males), and mate-harming would not be maintained.

Potential criticisms of our experiments are that the treatments used do not realistically simulate the kinds of harmful traits that males exhibit in nature and that the species selected are not representative. We do not consider either criticism valid for three reasons. First, although the ablations and punctures used in our experiments were subjectively different from the harm males actually inflict on their mates, they should generate exactly the same kind of immune response as those induced by genital wounds from spiny or barbed genitalia (Blanckenhorn et al., 2002; Crudginton and Siva-Jothy, 2000): the prophenoloxidase activating system and subsequent melanization follow both kinds of wounding (Gillespie et al., 1997; Lai-Fook, 1966; Sugumaran, 2002). Second, males of two of the species included here exhibit precisely the kinds of adaptations that the models aim to explain the evolution of: the spiny genitalia of *Callosobruchus* (Crudginton and Siva-Jothy, 2000) and the toxic ejaculates of

Drosophila (Chapman et al., 1995). Third, and perhaps most importantly, the adaptive harm hypothesis clearly rests on the assumption that females have generalized life-history responses to harm which favor their mates. This hypothesis cannot be rescued by arguing that a male trait (e.g., a seminal toxin) that causes some highly specific form of harm may have reproductive effects in females that are qualitatively opposite to those effects of harm in general.

In summary, models that claim that males can gain by harming their mates are based on the premise that females respond to being harmed in a way that gives harming males a selective advantage over more benign males (Johnstone and Keller, 2000; Lessells, 1999). We have attempted to test whether females respond in the way assumed in three insect species and found that they appear not to. In combination with some other difficulties with the adaptive harm hypothesis, our results suggest that infliction of postmating harm to females is more likely to represent negative pleiotropic effects of male adaptations with other selected functions (Parker, 1979).

Another distinction between the adaptive harm and pleiotropic harm hypotheses involves the nature of sexually antagonistic coevolution underlying trait evolution. If harm were adaptive, female resistance to male harm would always compromise male interests. In contrast, female resistance to male adaptations that cause harm as a pleiotropic side effect may or may not compromise male interests. If female resistance to given male adaptation reduces its efficacy in terms of its selected primary function, this will compromise male interests. However, if resistance is instead targeted only at alleviating the costly side effects of a male adaptation, this will be in the interest of both sexes. We thus expect selection in both sexes to diminish negative pleiotropic side effects. For this reason, we predict that (1) male traits that cause physical harm to females should only occur when harm represents a more or less unavoidable side effect of their primary function, and (2) the magnitude of harm inflicted to females should be limited.

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