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Sexual harassment by males reduces female fecundity in the alfalfa leafcutting bee, *Megachile rotundata*

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Under sexual conflict, males evolve traits to increase their mating and reproductive success that impose costs on females. Females evolve counteradaptations to resist males and reduce those costs. Sexual harassment is a form of sexual conflict in which males make repeated, costly attempts to mate. Costs to female foraging or predation risk have been measured in several species, but quantitative measurements of direct fitness costs are rare. In the alfalfa leafcutting bee, *Megachile rotundata* (Fabricius; Hymenoptera: Megachilidae), males harass females, and females resist all mating attempts. We placed bees in large, outdoor cages with various male-biased sex ratios. Harassment rate, nest progression, offspring production, temperature, and food availability were measured daily for 7 days. Harassment rates were highest at intermediate sex ratios. Harassment reduced the number of foraging trips and increased the duration of foraging trips made by females. Females produced offspring at a slower rate when subjected to higher rates of harassment. This shows a direct link from sex ratio to harassment to female fitness under natural conditions. We also discuss an alternative explanation that female resistance is a mechanism for mate choice for high-quality males, which would require that indirect benefits accrue through either daughters or grandsons, because all sons in haplodiploid species arise from unfertilized eggs.

Sexual conflict can drive the evolution of males and females in ways completely different from traditional mate choice. Under traditional mate choice, males evolve traits to lure and entice females, and female preferences evolve because choosy females receive direct and/or indirect benefits from males (Andersson 1994). In contrast, under sexual conflict, males evolve adaptations that increase their own fitness while imposing costs on females. Females then evolve counteradaptations to resist mating attempts, which, in turn, reduces the fitness of these manipulative males. Although these male-induced costs have been measured in several species, there is currently a debate over whether examples of sexual conflict represent true conflict. This may be because female resistance may be a mechanism for mate choice to allow only the highest-quality males to mate (Eberhard 2002, 2005; Chapman et al. 2003; Kokko et al. 2003; Parker 2006; Peretti & Cordoba-Aguilar 2007). If females receive indirect benefits through offspring, the observed female resistance behaviours may actually function to screen out lower-quality males. Females that are highly resistant to coercive males would end up mating with only the most coercive males. If coercion ability in males is heritable, those highly resistant females would produce highly coercive sons. Females could thus 'gain by losing' through this 'sons effect' (a.k.a., 'sexy son') benefit (Weatherhead & Robertson 1979; Wedell & Tregenza 1999; Huk & Winkel 2008). Females could also receive good genes benefits by mating with the most vigorous, aggressive males. Such indirect benefits to females are thought to be weak compared to the direct costs because they are expressed only through sons (Parker 2006). Females may also receive direct benefits due to their resistance by avoiding low-quality males that do not provide high direct benefits such as nuptial gifts (Thornhill 1980). It is essential that costs *and* benefits to females are measured in the same species to determine whether direct costs are outweighed by indirect benefits (Eberhard 2005; Hosken & Tregenza 2005). If the indirect benefits do outweigh the costs, this would suggest that female choice is operating. If not, then sexual conflict is operating (Parker 2006). Both female choice and sexual conflict

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could be operating simultaneously, but the net cost or benefit would indicate which is primarily responsible for the evolution of male and female traits.

One form of sexual conflict is sexual coercion, by which males attempt to copulate through physical force and harassment (Clutton-Brock & Parker 1995). Through harassment, males make repeated, costly mating attempts, which induce females to mate rather than continue resisting. The cost of male harassment to females has been measured in several species in terms of physical injuries to the female (Rowe et al. 1994; Blanckenhorn et al. 2002; Mühlhäuser & Blanckenhorn 2002), increased predation (Rowe et al. 1994; Mühlhäuser & Blanckenhorn 2002), and foraging costs (Rowe et al. 1994; Stone 1995; Schlupp et al. 2001). Rowe et al. (1994) found that changes in the population sex ratio in water striders resulted in higher rates of harassment and higher potential costs to females. The few studies that directly measured fitness in terms of longevity and fecundity were performed in the laboratory under artificial conditions and measured costs by pairing the subjects (e.g. a male and a female versus two females; McLain & Pratt 1999; Meader & Gilburn 2008; Sakurai & Kasuya 2008; Gay et al. 2009).

Our study species was the alfalfa leafcutting bee, *Megachile rotundata* (Fabricius; Hymenoptera: Megachilidae), a solitary bee. Males pursue females at their nests and foraging sites. Females put up active resistance to all mating attempts and usually mate only once (Gerber & Klostermeyer 1972; Blanchetot 1992), although they are capable of mating multiple times. Thus, if males impose a fitness cost on females, this can be easily observed and quantified because females build linear nests, making daily measurements of reproduction possible. The frequency of harassment from male bees can be manipulated under natural conditions in outdoor cages by varying the sex ratio within the species' normal range. If male harassment impairs a female's foraging ability, then females housed with relatively more males should be harassed more frequently and need to take more or longer foraging trips to build and provision each cell. As a result, the more frequently harassed females should produce offspring at a slower rate. Reduced fecundity would represent a quantifiable measure of the direct fitness cost of sexual conflict, measured in interacting groups of bees under natural conditions.

METHODS

Study Species

Alfalfa leafcutting bees are sexually dimorphic, being easily distinguished by colour and size (Gerber & Akre 1969; Akre et al. 1982; Richards 1984), with females an average of 1.2 times larger than males (Klostermeyer & Gerber 1969; Klostermeyer et al. 1973). After emergence as adults, most females live approximately 30 days, and males live 15–23 days, although many individuals of either sex live longer (Richards 1984). Adult sex ratios range from 1:1 to 5:1 (males:females), depending on environmental and nesting conditions of the parents (Gerber & Klostermeyer 1972; Richards 1993; Pitts-Singer & James 2005), and drops towards the end of the season when males die off before females (Richards 1984). Females nest gregariously (under wild and captive conditions) and build linear nests in preexisting tunnels. Females forage for leaves, nectar, and pollen near their nests. The tunnels are lined with leaf cuttings used to form individual brood cells, which are provisioned with nectar and pollen. A single egg is laid in each cell, which is then sealed off with leaf discs before the initiation of the next cell (Gerber & Klostermeyer 1972; Richards 1984).

The mating system appears to be a form of scramble competition, and the male mating strategy resembles sexual coercion through harassment with apparent attempts at forced copulations (Gerber & Klostermeyer 1972). Males patrol and chase females near nesting and foraging sites, and they pounce on females found resting, foraging at flowers, entering nest tunnels, or flying nearby. This harassment seems to interfere with females' nesting activities (Gerber & Klostermeyer 1972). Once a male captures a female, he moves to mount the female dorsally and copulate (Wittmann & Blochtein 1995).

The females' behaviour suggests intense resistance to all mating attempts by males. When a female is seized, a struggle ensues as the female tries to dislodge the male using rapid abdominal thrusts (Wittmann & Blochtein 1995) and leg kicks (B. H. Rossi, personal observation). These struggles can last from a few seconds to several minutes and end after copulation or with the male dislodged. Most females will mate with only one male within the first few days posteclosion before nest-building begins (Gerber & Klostermeyer 1972; Richards 1984), providing them with a lifetime supply of sperm (Richards 1994), although some females may mate multiple times (Blanchetot 1992). Observations suggest that females may become more resistant to mating attempts after they mate (Gerber & Klostermeyer 1972).

Many features of struggles during sexual encounters remain unexplained and may include a combination of male coercive and luring behaviours. When mounting a female, alfalfa leafcutting bee males press their front legs over the female's eyes and antennae. Odour glands on the front legs may be used to send signals to the female through her antennae, perhaps to stimulate her rather than physically overcome her resistance (Wittmann & Blochtein 1995). Males will also beat their wings intermittently throughout the event (B. H. Rossi, personal observation).

General Procedure

In the summers of 2006 and 2007, eight $2 \times 6 \times 6$ m ($h \times w \times l$) outdoor screened cages were placed in a field of alfalfa (*Medicago sativa*) in Logan, Utah (U.S.A.) and each was equipped with a small domicile that housed a polystyrene nest board with prefabricated tunnels (Fig. 1). Nest tunnels were 10 cm deep and 6 mm in diameter. We cut nest boards to size so that four nest tunnels were provided for each female and two nest tunnels for each male to prevent overcrowding and provide space for both sexes to rest in tunnels at night (Stephen 1981). Paper straws were inserted in the available nest tunnels to allow the progress of each nest to be monitored, as described below. Alfalfa leafcutting bees forage close to their nest (Richards 1984), so this cage setup resembled their natural conditions.

Bees were obtained from a commercial bee supplier (JWM Leafcutters, Inc., Nampa, ID, U.S.A.) as prepupae in leaf-covered cocoons. Alfalfa leafcutting bees overwinter as prepupae and are stored in this state over the winter season (Gerber & Klostermeyer 1972). Prepupae were incubated (in staggered batches of approximately 60 bees) individually in clear gelatin capsules (size 00; Capsuline, Inc., Pompano Beach, FL, U.S.A.) at 30 °C for 2–3 weeks until they emerged as adults (Pankiw et al. 1979; Richards 1984). Only bees (males and females) of equal age were used in each cage for each trial.

We uniquely colour-marked each female upon emergence and took four body size measures, fresh weight at emergence, head width, intertegular width (Cane 1987), and wing length. Virgin males and females were released into cages and allowed to freely interact, mate, examine nest tunnels, forage, and build nests. Variations in sex ratio and bee density represented different treatment conditions (Table 1) and included possible sex ratios of 0.5:1, 3:1, and 4:1 (male:female) and bee densities of 8, 10, 12 and 16 total bees (males and females). This is similar to what has been done in studies of sexual harassment in water striders (Rowe et al.