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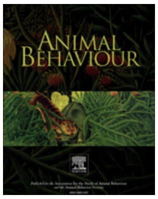

Sexual harassment by males reduces female fecundity in the alfalfa leafcutting bee, *Megachile rotundata*

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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.

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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.

Figure 1. The (a) outside and (b) inside of the outdoor cages, including the artificial nest block.

1994). The bee density was varied to control for the possible effect of overcrowding in the cages.

We monitored the bees' activities at the nest box for 2–3 days until at least 75% of the females had initiated nests. Females do not initiate nests until after they have mated. Frequent chases and occasional mountings of females by males were observed, but it is unknown if these resulted in successful copulations. We then monitored the nesting females for 7 days. In total, we monitored 34 females in eight cages. To measure male harassment, we counted the number of male-initiated chases of any females within 50 cm of the nest in 10 min observations twice a day in each cage. Observations were made from 1000 to 1500 hours (MDT), the bees' active period (Klostermeyer & Gerber 1969). The exact time of observation was varied from day to day to represent every part of the active period in the data sets and ensure that each cage was observed during the same times of day. 'Harassment rate' was defined as the mean number of male-initiated chases per day divided by the number of females (known to be present that day).

We videotaped (using Sony Digital-8 camcorders) each nest box for 1 h each day during one of three time periods: 1000–1130, 1130–1230, and 1230–1500 hours. We used the number of times that each female entered and exited her nest to calculate the number of trips taken per hour, the average duration of foraging trips, and the total time spent foraging during the hour.

We also measured other aspects of female nesting behaviour. The type of foraging trip (e.g. for leaves or pollen/nectar) was recorded by noting whether the female performed a specific 'turn-around' manoeuvre after arriving. When a female has collected pollen, it is held in the hairs of her scopa, or pollen-carry apparatus, on the underside of the abdomen. The female first enters her nest headfirst, to regurgitate nectar into the cell. Then the female backs out of the tunnel, turns around and moves into the tunnel abdomen-first, so that she can scrape pollen from the scopa and pack it into a mass provision. Thus, if this turn-around manoeuvre is observed, the female must have just been on a nectar/pollen-collecting trip. Pollen and nectar are usually collected on the same trips (Klostermeyer & Gerber 1969; Klostermeyer et al. 1973). Also, we counted the number of 'mistakes' females made as they returned to their tunnel. Because females usually work on only one nest at a time (Klostermeyer & Gerber 1969), if a female entered a tunnel that was not her nest prior to finding her own nest tunnel, this was counted as a mistake.

Table 1
The types and numbers of each cage treatment

Sex ratio (males:females)	Bee density (total number bees)	Number of trials with these treatments
0.5:1	12	2
3:1	8	2
3:1	12	2
3:1	16	1
4:1	10	1

Ambient temperature and food availability (floral resources) were monitored because they are well known to affect bee activity levels (reviewed in Willmer & Stone 2004) and reproduction (Kim 1996, 1999; Richards 1996; Peterson & Roitberg 2006) positively. Ambient temperature was monitored using a Hobo data logger (Onset Computer Corp., Pocasset, MA, U.S.A.) that was placed inside each domicile in each cage. For analyses, we used the mean temperature for each day during the bees' active period from 1000 to 1500 hours.

We estimated the floral resources by counting open, unvisited flowers every other day in four 0.25 m^2 quadrats placed in four evenly spaced locations within each cage. When an alfalfa flower is visited by a bee, pressure on the keel petal causes the flower to 'trip', meaning the sexual column is released. The bee can then collect both pollen and nectar from the flower (Larkin & Graumann 1954). Thus, we used untripped flowers, identified by the exposed sexual columns, as representative samples for the floral resources available to bees. We conducted the first flower survey before bees were introduced to ensure floral resources were adequate for nesting success to occur and to determine the maximum floral resources available to bees.

Males and females were counted every night in nest tunnels. Although the numbers of males and females remained relatively stable, the sex ratios and bee densities did vary from the initial starting values. The mean sex ratio and bee density were calculated for each cage and each female (across the days she lived), and those values were used in our analyses. Also at night, we removed each nest tunnel's paper straw and measured the distance from the back end of the nest to the end of any nest construction to determine the progress made each day by each bee. From these data we calculated the mean nest progression (mm) for each female.

To determine total offspring production, we removed and X-radiographed each nest at the conclusion of trials. X-radiography has no significant, negative effects on developing offspring (Stephen & Undurraga 1976; Maki et al. 1990). In the X-ray images, developing offspring are clearly visible within the individual cells (Fig. 2). The nest-building distances from each day were compared to the X-ray images to measure offspring production, which was defined as the number of offspring each female produced each day. An offspring (of either sex) was counted if it developed to at least the prepupal stage. It was not possible, from the X-radiographs, to identify offspring that died before developing to this stage because

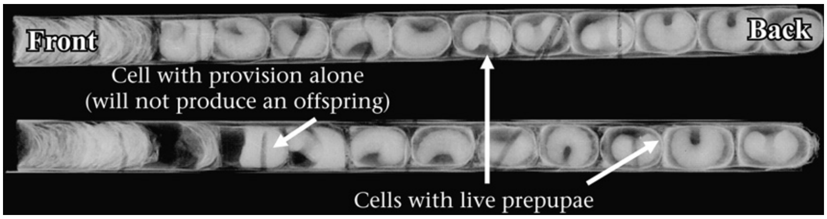

Figure 2. X-radiographs of nest straws were used to count the number of offspring produced based on the length of nest that was built each day. Cells with live prepupae can be distinguished from cells that contain only provision and will not produce a live offspring (either because the female never laid an egg in that cell or the offspring died).

females will also produce cells without an egg (Pitts-Singer 2004). All adult bees were removed and frozen, so we could take further morphological measurements that were not used in these analyses.

Data Analysis

To determine which factors affected variables measured at the cage (treatment; e.g. harassment rate and food availability), we conducted linear and nonlinear (when appropriate) regression analyses level. $N = 8$ unless otherwise indicated.

To determine the factors affecting variables measured at the individual female level (e.g. nest progression, offspring production, and female foraging variables), we used a linear regression model, the generalized linear model (GLM; Laird 2004), with robust standard errors adjusting for possible cage effects (SPSS 15; SPSS, Inc., Chicago, IL, U.S.A.). $N = 34$ unless otherwise indicated.

The values for sex ratio and bee density differed from their initial starting values (Table 1) because of disappearances of males or females and were calculated as means over the 7 days. Five females in three of the cages were not present for the entire 7 days, because they either died or escaped, so separate harassment rates, temperatures, and floral resources were calculated for each of them using measures only from the days they were present.

RESULTS

Factors Affecting Harassment Rate

The male:female sex ratio did not affect mean harassment rate (linear regression: $F_{1,6} = 3.913$, $R^2 = 0.294$, $P = 0.0953$; power 0.38; Fig. 3), although the trend was in the expected direction. The distribution suggested that a quadratic fit was more appropriate. Using this model, sex ratio did affect harassment rate, with the highest harassment rates at intermediate sex ratios (quadratic regression: $F_{1,5} = 7.336$, $R^2 = 0.746$, $P = 0.0326$).

Mean harassment rate increased with mean temperature (linear regression: $F_{1,6} = 18.209$, $N = 8$, $R^2 = 0.835$, $P = 0.0053$), but not with food availability (linear regression: $F_{1,5} = 1.201$, $N = 7$, $P = 0.3231$). Total bee density (includes both males and females) had no significant effect on harassment (linear regression: $F_{1,6} = 1.572$, $R^2 = 0.076$, $P = 0.2566$; power 0.19) or offspring production rate (GLM: $B + \mathrm{SE} = -0.037 + 0.050$, $R^2 = 0.035$, $P = 0.4687$).

Effect of Harassment and Sex Ratio on Fecundity

As mean harassment rate increased, offspring production decreased (GLM: $B + \mathrm{SE} = -0.351 + 0.042$, $R^2 = 0.442$, $P < 0.0001$; Fig. 4) and nest progression decreased (GLM: $B + \mathrm{SE} = -3.192 + 0.341$, $R^2 = 0.483$, $P < 0.0001$). Offspring production increased with nest progression (GLM: $B + \mathrm{SE} = -3.192 + 0.341$, $R^2 = 0.725$, $P < 0.0001$).

As sex ratio increased, offspring production decreased (GLM: $B + \mathrm{SE} = -0.116 + 0.060$, $R^2 = 0.165$, $P = 0.0523$; Fig. 5). However, the relationship may not be best described as linear. Therefore, we added a second-order term of sex ratio as a main effect, thus enabling the testing of a quadratic model using a linear GLM. This significantly improved the fit of the model, with the lowest offspring production at intermediate sex ratios (GLM: $R^2 = 0.481$, $N = 34$; Fig. 5, Table 2).

Effect of Harassment on Female Foraging Behaviour

Mean harassment rate had a negative effect on the mean number of nest visits (GLM: $B + \mathrm{SE} = -1.343 + 0.378$, $R^2 = 0.214$, $P = 0.0004$) and foraging trips (GLM: $B + \mathrm{SE} = -1.879 + 0.580$, $R^2 = 0.218$, $P = 0.0012$) per female per day. Offspring production was positively affected by the number of nest visits (GLM: $B + \mathrm{SE} = 0.134 + 0.026$, $R^2 = 0.299$, $P < 0.0001$) and foraging trips (GLM: $B + \mathrm{SE} = 0.060 + 0.019$, $R^2 = 0.271$, $P = 0.0014$).

Mean harassment rate had a positive effect on the mean durations of nest visits (GLM: $B + \mathrm{SE} = 48.650 + 17.910$, $R^2 = 0.236$, $P = 0.0066$) and foraging trips (GLM: $B + \mathrm{SE} = 117.663 + 46.550$, $R^2 = 0.269$, $P = 0.0115$). Offspring production was lower in nest visits and foraging trips of longer durations (nest visits: GLM: $B + \mathrm{SE} = -0.002 + 0.001$, $R^2 = 0.206$, $P = 0.0341$; foraging trips: GLM: $B + \mathrm{SE} = -0.002 + 0.0002$, $R^2 = 0.459$, $P < 0.0001$).

There was no significant effect of mean harassment rate on total time spent in the nest (GLM: $B + \mathrm{SE} = -89.032 + 68.994$, $P = 0.1969$) or on foraging trips (GLM: $B + \mathrm{SE} = 85.102 + 66.425$, $P = 0.2001$) per day. Offspring production was not significantly affected by total time spent in the nest (GLM: $B + \mathrm{SE} < 0.001 + 0.0002$, $P = 0.1083$) or on foraging trips (GLM: $B + \mathrm{SE} < 0.001 + 0.0002$, $P = 0.1083$).

Mean harassment rate positively affected the proportion of pollen trips (GLM: $B + \mathrm{SE} = 0.046 + 0.017$, $R^2 = 0.035$, $P = 0.0077$),

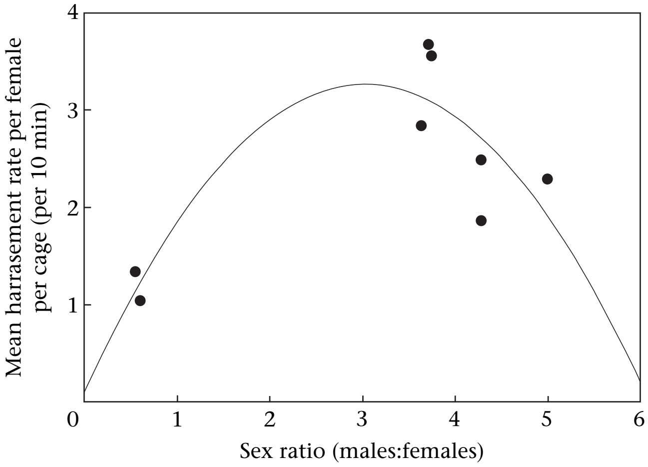

Figure 3. Effect of sex ratio (males:females) on the mean harassment rate (quadratic regression: $F_{1,5} = 7.336$, $R^2 = 0.746$, $P = 0.0326$). Harassment rate was highest at intermediate sex ratios.

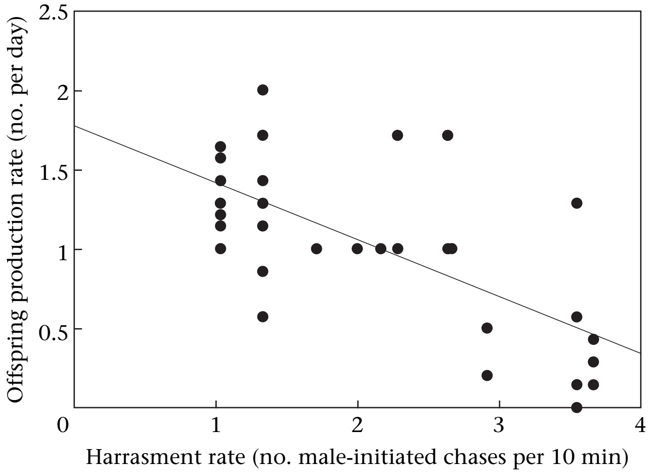

Figure 4. Harassment rate reduced the offspring production rate per female (GLM: $B + \mathrm{SE} = -0.351 + 0.042$, $R^2 = 0.442$, $P < 0.0001$).

but did not affect offspring production (GLM: $B + \mathrm{SE} = -0.033 + 0.212$, $R^2 = 0.026$, $P = 0.8758$). Harassment did not affect the number of mistakes a female made when returning to the nest (GLM: $B + \mathrm{SE} < 0.001 + 0.027$, $P = 0.9964$).

Effects of Temperature, Floral Resources, and Female Body Size

As mean temperature increased, offspring production decreased (GLM: $B + \mathrm{SE} = -0.053 + 0.014$, $R^2 = 0.297$, $P = 0.0002$). Nest progression also decreased as mean temperature increased (GLM: $B + \mathrm{SE} = -0.525 + 0.079$, $R^2 = 0.389$, $P < 0.0001$).

Data analyses from the seven cages in which floral resources were recorded revealed that offspring production increased with the mean number of untripped flowers (GLM: $B + \mathrm{SE} = 0.019 + 0.010$, $N = 30$, $R^2 = 0.160$, $P = 0.0404$). Bee density did not affect the number of untripped flowers (linear regression: $F_{1,5} = 3.512$, $N = 7$, $R^2 = 0.30$, $P > 0.1198$), nor was there any significant effect of sex ratio on the mean number of untripped flowers per female (linear regression: $F_{1,5} = 0.019$, $N = 7$, $R^2 < 0.01$, $P > 0.8944$). All cages used in our analyses contained at least 1650 untripped flowers per female each day, the minimum number of flowers needed by a female to produce a single provision (J. H. Cane, unpublished data), and contained from 4113 to 39 287 untripped flowers each day. Thus, females did not seem to have been limited by floral resources.

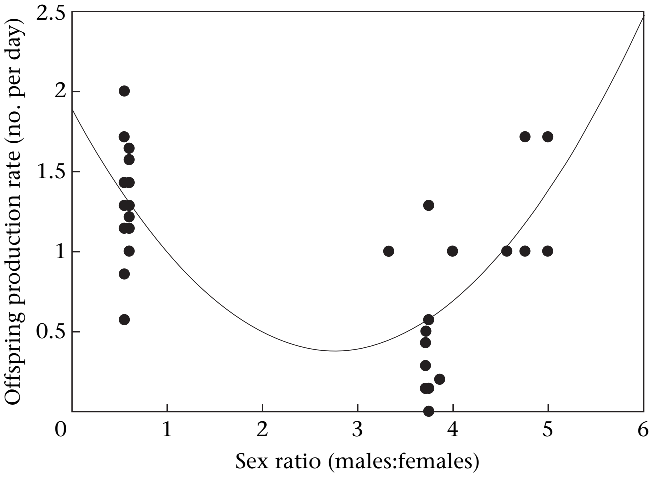

Figure 5. Effect of sex ratio (males:females) on the offspring production rate per female. Offspring production rates were lowest at intermediate sex ratios (GLM of sex ratio and its second-order term: $R^2 = 0.481$, $N = 34$; Table 2).

Table 2

Quadratic model for the effect of sex ratio on offspring production (GLM: $R^2 = 0.481$, $N = 34$)

Parameter	*B*	SE	95% Wald confidence interval		Hypothesis test		
			Lower	Upper	Wald chi-square	*df*	*P*
Intercept	1.963	0.062	1.841	2.086	988.885	1	<0.0001
Sex ratio	−1.183	0.121	−1.419	−0.946	95.951	1	<0.0001
Sex ratio^2	0.215	0.023	0.170	0.260	88.253	1	<0.0001
Scale	0.153						

For this GLM analysis, our model uses Wald test statistics to test the significance of the coefficient and control for possible cage effects. Dependent variable: offspring production rate.

A principal component analysis of the female body size measurements was conducted (SPSS 15) and produced three independent measures of body size (see the Appendix). There was no significant effect of any of the direct adult female body size measures or any of the principal component measures of body size on offspring production (GLM: $P > 0.05$).

DISCUSSION

Our results show that male harassment imposes a fecundity cost on female alfalfa leafcutting bees. Importantly, this cost is directly related to sex ratio, in that intermediate sex ratio treatments result in the highest harassment rates. Sex ratio is known to affect costs of harassment (Rowe et al. 1994), and harassment is known to affect negatively female longevity (Meader & Gilburn 2008) and fecundity (McLain & Pratt 1999; Sakurai & Kasuya 2008). However, this is the first demonstration of a direct link between sex ratio and fecundity under field conditions within realistic sex ratio bounds. By resisting mating attempts and fleeing from males, females made fewer foraging trips and took longer to make the number of foraging trips necessary to build each cell. Thus, the females harassed more frequently built their nests and laid eggs at a slower rate, resulting in lower reproductive success.

The foraging costs that resulted from females fleeing males are similar to those observed in seed-eating true bugs (McLain & Pratt 1999) and the solitary bee *Anthophora plumipes* (Stone 1995). Because females are not known to mate while nest-building, this resistance probably serves to reduce the cost of male mating attempts. If females did not flee and allowed approaching males to mount them, they would have to endure even longer time costs as they worked to dislodge males. Fleeing reduces these potential time costs by preventing the mounting of males. The cost of male mating attempts probably cannot be completely eliminated by females because males patrol in areas essential to females such as their nests and foraging sites.

Male harassment may impact female foraging through time costs of fleeing males and the gathered resources that are lost. We observed that females returning to nests with a leaf piece would often drop it when pounced upon or chased by a male. Once she escaped, she then had to retrieve a new leaf piece, so a part of that foraging trip had to be repeated. There was no correlation between harassment and mistakes, or females entering the wrong nest cavity upon return from a foraging trip, so a female's ability to identify her own nest cavity correctly did not seem affected by pressure to flee harassing males.

Harassment rates peaked in the 3:1 sex ratio cages. The slightly reduced harassment rate in the 4:1 cages might have been due to male–male competition. At high densities, male bees are known to compete directly with other males more to maintain access to areas containing females such as nests or foraging sites (Thornhill & Alcock 1983; Larsson 1991; Stone et al. 1995; Willmer & Stone 2004). Although we measured only male chases of females, males did chase other males. We did not mark males, so it is unclear if these chases were attempts to defend territories, attempts to exclude other males from the male's current vicinity, or mistaken mating attempts. In any case, when the male density is very high, male–male chases may increase, which would reduce the frequency of male chases of females. A similar pattern was found in mosquitofish (Smith 2007), in which male–male agonistic displays were more frequent and copulation attempts less frequent at higher male densities.

Environmental factors also influenced reproductive success. Food availability correlated with offspring production, which was expected based on previous work on alfalfa leafcutting bees (Peterson & Roitberg 2006) and their sister species *M. apicalis* (Kim 1996, 1999). Also, food limitation did not influence our results because all cages had at least the minimum required flowers for each female each day to build cells and produce offspring. In all the experiments we observed females foraging throughout the cages. Indeed, males patrolled and chased females everywhere in the cages. There were no areas that were free of males, and thus females could not shift foraging areas to avoid males.

Mean temperature reduced nest and offspring production, although temperature was previously shown to increase nest and offspring production in alfalfa leafcutting bees (Richards 1996). Additional studies of solitary bees that found positive effects of temperature on bee activity (Stone et al. 1995; Abrol 1998) were conducted at lower temperatures. The bees in our experiments may have suffered from overheating (Willmer & Stone 2004), with some cages reaching maximum temperatures as high as 44 °C.

Adult body size did not correlate with offspring production in our study. This is similar to findings in another cavity-nesting, solitary megachilid, *Osmia lignaria* Say (Tepedino & Torchio 1982). However, in alfalfa leafcutting bees (Klostermeyer et al. 1973) and *M. apicalis* (Kim 1997), body size did have an effect. Also, when bees were selected for each cage, attempts were made to keep the average fresh weight of each cage's females close to that of the other cages. We often needed to use whatever bees had emerged within the past few days to ensure that bees in each cage were of identical ages. To detect the effects of body size on offspring production in the context of high and low harassment rates, we would need to test many females in more cages with intentionally selected larger- or smaller-sized females.

We did not examine the longevity of females, another component of fitness. Although it is possible that the females that were harassed at a higher rate may have ended up living longer and making up the difference in fecundity, we would predict that if the energetic costs of escaping harassing males had any effect on longevity, it would be to reduce it (because of physical injuries and increased predation risk), not increase it. The relationship between factors promoting female longevity and lifetime fecundity are fertile grounds for future investigation.

Indirect Benefits in a Haplodiploid System

If there are indirect benefits of female resistance, they would come from those first few days posteclosion when females do mate with a single male despite appearing resistant to all mating attempts. Now that there is confirmation that costs exist, this experiment can be repeated and multiple generations monitored to measure possible indirect benefits of female resistance.

Hymenoptera are haplodiploid, meaning that sons develop from unfertilized eggs and daughters from fertilized eggs. As a result, a female's sons do not inherit any genes from her mate. Any 'sons effect' must be expressed through grandsons (i.e. a 'grandsons effect'). Nevertheless, the male effect is still genetically similar to that of diploid organisms because the relatedness of a haplodiploid father to his grandson is the same as a diploid father to his son ($r = 0.5$). Alternatively, indirect benefits could appear as a 'daughters effect' in which daughters inherit increased strength or vigour that aggressive, coercive males might possess. Thus, females could accrue benefits from mating with effectively harassing males through either daughters or grandoffspring. This study has shown that females pay an immediate cost from male harassment. This opens the opportunity for future work to explore whether they can recoup those costs through the reproductive success of their daughters and especially through the reproductive success of grandsons (B. H. Rossi, unpublished data). If the costs are outweighed by such indirect benefits, then the function of female resistance would be for mate choice in addition to reducing male-induced costs of sexual conflict.

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APPENDIX

Eigenvalues from the principal components analysis of female body size measures

Component	Initial eigenvalues		Cumulative (%)	Extraction sums of squared loadings		
	Total	% of variance		Total	% of variance	Cumulative (%)
1	2.690	67.25	67.25	2.69	67.25	67.25
2	0.623	15.58	82.83			
3	0.540	13.50	96.34			
4	0.147	3.67	100.00			

Only the first three principal components were used in analyses. Extraction method, principal component analysis.