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

51 KEYWORDS

52

53 sexual conflict, sexual coercion, sexual harassment, female resistance, sex ratio, fecundity, cost,

54 alfalfa leafcutting bee, *Megachile rotundata*, Hymenoptera

55 Sexual conflict can drive the evolution of males and females in ways completely different
56 from traditional mate choice. Under traditional mate choice, males evolve traits to lure and entice
57 females, and female preferences evolve because choosy females receive direct and/or indirect
58 benefits from males (Andersson 1994). In contrast, under sexual conflict, males evolve
59 adaptations that increase their own fitness while imposing costs on females. Females then evolve
60 counter-adaptations to resist mating attempts, which, in turn, reduces the fitness of these
61 manipulative males. Though these male-induced costs have been measured in several species,
62 there is currently a debate over whether examples of sexual conflict represent true conflict. This
63 is due to the possibility that female resistance may be a mechanism for mate choice to only allow
64 the highest quality males to mate (Eberhard 2002; Chapman et al. 2003; Kokko et al. 2003;
65 Eberhard 2005; Parker 2006; Peretti & Cordoba-Aguilar 2007). If females receive indirect
66 benefits through offspring, the observed female resistance behaviours may actually function to
67 screen out lower quality males. Females that are highly resistant to coercive males would end up
68 mating with only the most coercive males. If coercion ability in males is heritable, those highly
69 resistant females would produce highly coercive sons. Females could thus “gain by losing”
70 through this “sons effect” (a.k.a., “sexy son”) benefit (Weatherhead & Robertson 1979; Wedell
71 & Tregenza 1999; Huk & Winkel 2008). Females could also receive good genes benefits by
72 mating with the most vigorous, aggressive males. Such indirect benefits to females are thought to
73 be weak compared to the direct costs because they are expressed only through sons (Parker
74 2006). Females may also receive direct benefits due to their resistance by avoiding low quality
75 males that do not provide high direct benefits such as nuptial gifts (Thornhill 1980). It is
76 essential that costs *and* benefits to females are measured in the same species to determine
77 whether direct costs are outweighed by indirect benefits (Eberhard 2005; Hosken & Tregenza

78 2005). If the indirect benefits do outweigh the costs, this would suggest that female choice is
79 operating. If not, then sexual conflict is operating (Parker 2006). Both female choice and sexual
80 conflict could be operating simultaneously, but the net cost or benefit would indicate which is
81 primarily responsible for the evolution of male and female traits.

82

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

95

96 Our study species was the alfalfa leafcutting bee (*Megachile rotundata* Fabricius;
97 Hymenoptera: Megachilidae), a solitary bee. Males pursue females at their nests and foraging
98 sites. Females put up active resistance to all mating attempts and usually mate only once (Gerber
99 & Klostermeyer 1972; Blanchetot 1992), though they are capable of mating multiply. Thus, if
100 males impose a fitness cost on females, this can be easily observed and quantified because

101 females build linear nests making daily measurements of reproduction possible. The frequency
102 of harassment from male bees can be manipulated under natural conditions in outdoor cages by
103 varying the sex ratio within the species' normal range. If male harassment impairs a female's
104 foraging ability, then females housed with relatively more males should be harassed more
105 frequently and need to take more or longer foraging trips to build and provision each cell. As a
106 result, the more frequently harassed females should produce offspring at a slower rate. Reduced
107 fecundity would represent a quantifiable measure of the direct fitness cost of sexual conflict,
108 measured in interacting groups of bees under natural conditions.

109

110 METHODS

111

112 *Study species*

113

114 Alfalfa leafcutting bees are sexually dimorphic being easily distinguished by color and
115 size (Gerber & Akre 1969; Akre et al. 1982; Richards 1984), with females an average of 1.2
116 times larger than males (Klostermeyer & Gerber 1969; Klostermeyer et al. 1973). After
117 emergence as adults, most females live approximately 30 days, and males live 15-23 days,
118 though many individuals of either sex live longer (Richards 1984). Adult sex ratios range from
119 1:1 to 5:1 (males: females) depending on environmental and nesting conditions of the parents
120 (Gerber & Klostermeyer 1972; Richards 1993; Pitts-Singer & James 2005) and drops towards
121 the end of the season when males die off before females (Richards 1984). Females nest
122 gregariously (under wild and captive conditions) and build linear nests in pre-existing tunnels.
123 Females forage for leaves, nectar and pollen near their nests. The tunnels are lined with leaf-

124 cuttings used to form individual brood cells, which are provisioned with nectar and pollen. A
125 single egg is laid in each cell, which is then sealed off with leaf discs before the initiation of the
126 next cell (Gerber & Klostermeyer 1972; Richards 1984).

127

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

135

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

145

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

152

153 *General procedure*

154

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

164

165 Bees were obtained from a commercial bee supplier (JWM Leafcutters, Inc., Nampa, ID,
166 U.S.A.) as prepupae in leaf-covered cocoons. Alfalfa leafcutting bees overwinter as prepupae
167 and are stored in this state over the winter season (Gerber & Klostermeyer 1972). Prepupae were
168 incubated (in staggered batches of approximately 60 bees) individually in clear gelatine capsules

169 (size 00, Capsuline Inc., Pompano Beach, FL, U.S.A.) at 30 degrees C for 2-3 weeks until they
170 emerged as adults (Pankiw et al. 1979; Richards 1984). Only bees (males and females) of equal
171 age were used in each cage for each trial.

172

173 We uniquely colour-marked each female upon emergence and took four body size
174 measures – fresh weight at emergence, head width, intertegular width (Cane 1987) and wing
175 length. Virgin males and females were released into cages and allowed to freely interact, mate,
176 examine nest tunnels, forage and build nests. Variations in sex ratio and bee density represented
177 different treatment conditions (Table 1) and included possible sex ratios of 0.5:1, 3:1 and 4:1
178 (male: female) and bee densities of 8, 10, 12 and 16 total bees (males and females). This is
179 similar to what has been done in studies of sexual harassment in water striders (Rowe et al.
180 1994). The bee density was varied to control for the possible effect of overcrowding in the cages.

181

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

192 mean number of male-initiated chases per day divided by the number of females (known to be
193 present that day).

194

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

200

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

214

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

221

222 We estimated the floral resources by counting open, unvisited flowers every other day in
223 four 0.25 m² quadrats placed in four evenly-spaced locations within each cage. When an alfalfa
224 flower is visited by a bee, pressure on the keel petal causes the flower to “trip,” meaning the
225 sexual column is released. The bee can then collect both pollen and nectar from the flower
226 (Larkin & Graumann 1954). Thus, we used untripped flowers, identified by the exposed sexual
227 columns, as representative samples for the floral resources available to bees. We conducted the
228 first flower survey before bees were introduced to ensure floral resources were adequate for
229 nesting success to occur and determine the maximum floral resources available to bees.

230

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

238

239 To determine total offspring production, we removed and x-radiographed each nest at the
240 conclusion of trials. X-radiography has no significant, negative effects on developing offspring
241 (Stephen & Undurraga 1976; Maki et al. 1990). In the x-ray images, developing offspring are
242 clearly visible within the individual cells (Fig. 2). The nest-building distances from each day
243 were compared to the x-ray images to measure offspring production, which was defined as the
244 number of offspring each female produced each day. An offspring (of either sex) was counted if
245 it developed to at least the prepupal stage. It was not possible, from the x-radiographs, to identify
246 offspring that died before developing to this stage because females will also produce cells
247 without an egg (Pitts-Singer 2004). All adult bees were removed and frozen, so we could take
248 further morphological measurements that were not used in these analyses.

249

250 *Data analysis*

251

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

255

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

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The values for sex ratio and bee density differed from their initial starting values (Table 1) due to disappearances of males or females and were calculated as means over the seven days. Five females in three of the cages were not present for the entire seven days, because they either died or escaped, so separate harassment rates, temperatures and floral resources were calculated for each of them only using measures from 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), though 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: $B+SE = -0.037+0.050$, $R^2 = 0.035$, $p = 0.4687$).

285 *Effect of harassment and sex ratio on fecundity*

286

287 As mean harassment rate increased, offspring production decreased (GLM: $B+SE = -$
288 $0.351+0.042$, $R^2 = 0.442$, $p < 0.0001$; Fig. 4) and nest progression decreased (GLM: $B+SE = -$
289 $3.192+0.341$, $R^2 = 0.483$, $p < 0.0001$). Offspring production increased with nest progression
290 (GLM: $B+SE = -3.192+0.341$, $R^2 = 0.725$, $p < 0.0001$).

291

292 As sex ratio increased, offspring production decreased (GLM: $B+SE = -0.116+0.060$, R^2
293 $= 0.165$, $p = 0.0523$; Fig. 5). However, the relationship may not be best described as linear.
294 Therefore, we added a second order term of sex ratio as a main affect, thus enabling the testing
295 of a quadratic model using a linear GLM. This significantly improved the fit of the model with
296 the lowest offspring production at intermediate sex ratios (GLM: $R^2 = 0.481$, $N = 34$; Fig. 5,
297 Table 2).

298

299 *Effect of harassment on female foraging behaviour*

300

301 Mean harassment rate had a negative effect on the mean number of nest visits (GLM:
302 $B+SE = -1.343+0.378$, $R^2 = 0.214$, $p = 0.0004$) and foraging trips (GLM: $B+SE = -1.879+0.580$,
303 $R^2 = 0.218$, $p = 0.0012$) per female per day. Offspring production was positively affected by the
304 number of nest visits (GLM: $B+SE = 0.134+0.026$, $R^2 = 0.299$, $p < 0.0001$) and foraging trips
305 (GLM: $B+SE = 0.060+0.019$, $R^2 = 0.271$, $p = 0.0014$).

306

307 Mean harassment rate had a positive effect on the mean durations of nest visits (GLM:
308 $B+SE = 48.650+17.910$, $R^2 = 0.236$, $p = 0.0066$) and foraging trips (GLM: $B+SE =$
309 $117.663+46.550$, $R^2 = 0.269$, $p = 0.0115$). Offspring production was lower in nest visits and
310 foraging trips of longer durations (nest visits: GLM: $B+SE = -0.002+0.001$, $R^2 = 0.206$, $p =$
311 0.0341 ; foraging trips: GLM: $B+SE = -0.002+0.0002$, $R^2 = 0.459$, $p < 0.0001$).

312

313 There was no significant effect of mean harassment rate on total times spent in the nest
314 (GLM: $B+SE = -89.032+68.994$, $p = 0.1969$) or on foraging trips (GLM: $B+SE =$
315 $85.102+66.425$, $p = 0.2001$) per day. Offspring production was not significantly affected by total
316 times spent in the nest (GLM: $B+SE < 0.001+0.0002$, $p = 0.1083$) or on foraging trips (GLM:
317 $B+SE < 0.001+0.0002$, $p = 0.1083$).

318

319 Mean harassment rate positively affected the proportion of pollen trips (GLM: $B+SE =$
320 $0.046+0.017$, $R^2 = 0.035$, $p = 0.0077$), but did not affect offspring production (GLM: $B+SE = -$
321 $0.033+0.212$, $R^2 = 0.026$, $p = 0.8758$). Harassment did not affect the number of mistakes a
322 female made when returning to the nest (GLM: $B+SE < 0.001+0.027$, $p = 0.9964$).

323

324 *Effect of temperature, floral resources and female body size*

325

326 As mean temperature increased, offspring production decreased (GLM: $B+SE = -$
327 $0.053+0.014$, $R^2 = 0.297$, $p = 0.0002$). Nest progression also decreased as mean temperature
328 increased (GLM: $B+SE = -0.525+0.079$, $R^2 = 0.389$, $p < 0.0001$).

329

330 Data analyses from the seven cages in which floral resources were recorded revealed that
331 offspring production increased with the mean number of untripped flowers (GLM: $B+SE =$
332 $0.019+0.010$, $N = 30$, $R^2 = 0.160$, $p = 0.0404$). Bee density did not affect the number of untripped
333 flowers (Linear regression: $F_{1,5} = 3.512$, $N = 7$, $R^2 = 0.30$, $p > 0.1198$), nor was there any
334 significant effect of sex ratio on the mean number of untripped flowers per female (Linear
335 regression: $F_{1,5} = 0.019$, $N = 7$, $R^2 < 0.01$, $p > 0.8944$). All cages used in our analyses contained
336 at least 1650 untripped flowers per female each day, the minimum number of flowers needed by
337 a female to produce a single provision (Cane 2005), and contained from 4113 to 39287 untripped
338 flowers each day. Thus, females did not appear to have been limited by floral resources.

339

340 A principal component analysis of the female body size measurements was conducted
341 (SPSS 15) and produced three independent measures of body size (Table S1). There was no
342 significant effect of any of the direct adult female body size measures or any of the principal
343 component measures of body size on offspring production (GLM: $p > 0.05$).

344

345 DISCUSSION

346

347 Our results demonstrate that male harassment imposes a fecundity cost on female alfalfa
348 leafcutting bees. Importantly, this cost is directly related to sex ratio, where intermediate sex
349 ratio treatments result in the highest harassment rates. Sex ratio is known to affect costs of
350 harassment (Rowe et al. 1994), and harassment is known to negatively affect female longevity
351 (Meader & Gilburn 2008) and fecundity (McLain & Pratt 1999; Sakurai & Kasuya 2008).
352 However, this is the first demonstration of a direct link between sex ratio and fecundity under

353 field conditions within realistic sex ratio bounds. By resisting mating attempts and fleeing from
354 males, females made fewer foraging trips and took longer to make the number of foraging trips
355 necessary to build each cell. Thus, the females harassed more frequently built their nests and laid
356 eggs at a slower rate resulting in lower reproductive success.

357

358 The foraging costs that resulted from females fleeing males are similar to those observed
359 in seed-eating true bugs (McLain & Pratt 1999) and the solitary bee *Anthophora plumipes* (Stone
360 1995). Because females are not known to mate while nest-building, this resistance likely serves
361 to reduce the cost of male mating attempts. If females did not flee and allowed approaching
362 males to mount them, they would have to endure even longer time costs as they worked to
363 dislodge males. Fleeing reduces these potential time costs by preventing the mounting of males.
364 The cost of male mating attempts probably cannot be completely eliminated by females because
365 males patrol in areas essential to females such as their nests and foraging sites.

366

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

374

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

386

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

395

396 Mean temperature reduced nest and offspring production, though temperature was
397 previously shown to increase nest and offspring production in alfalfa leafcutting bees (Richards

398 1996). Additional studies of solitary bees that found positive effects of temperature on bee
399 activity (Stone et al. 1995; Abrol 1998) were conducted at lower temperatures. The bees in our
400 experiments may have suffered from overheating (Willmer & Stone 2004) with some cages
401 reaching maximum temperatures as high as 44° C.

402

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

412

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

419

420 *Indirect benefits in a haplodiploid system*

421

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

426

427 Hymenoptera are haplodiploid, meaning sons develop from unfertilized eggs and
428 daughters from fertilized eggs. As a result, a female's sons do not inherit any genes from her
429 mate. Any "sons effect" must be expressed through grandsons (i.e. a "grandsons effect").
430 Nevertheless, the male effect is still genetically similar to diploid organisms because the
431 relatedness of a haplodiploid father to his grandson is the same as a diploid father to his son ($r =$
432 0.5). Alternatively, indirect benefits could appear as a "daughters effect" in which daughters
433 inherit increased strength or vigour that aggressive, coercive males might possess. Thus, females
434 could accrue benefits from mating with effectively harassing males either through daughters or
435 grandoffspring. This study has shown that females pay an immediate cost from male harassment.
436 This opens the opportunity for future work to explore whether they can recoup those costs
437 through the reproductive success of their daughters and especially through the reproductive
438 success of grandsons (Rossi, B. H. unpublished data). If the costs are outweighed by such
439 indirect benefits, then the function of female resistance would be for mate choice in addition to
440 reducing male-induced costs of sexual conflict.

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575

576

577 TABLES

578 **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

579

580

581 **Table 2.** Quadratic model for the effect of sex ratio on offspring production (GLM: $R^2 = 0.481$,
582 $N = 34$). For this GLM analysis, our model uses wald test statistics to test the significance of the
583 coefficient and control for possible cage effects.

Parameter	B	Std. Error	95% Wald Confidence Interval		Hypothesis Test		
			Lower	Upper	Wald Chi- Square.	df	Sig.
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						

584 Dependent Variable: Offspring production rate

585 FIGURE LEGENDS

586

587 **Figure 1.** The outside (left) and inside (right) of the outdoor cages, including the artificial nest
588 block.

589

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

594

595 **Figure 3.** Effect of sex ratio (males: females) on the mean harassment rate (Quadratic regression:
596 $F_{1,5} = 7.336$, $R^2 = 0.746$, $p = 0.0326$). Harassment rate was highest at intermediate sex ratios.

597

598 **Figure 4.** Harassment rate reduced the offspring production rate per female (GLM: $B+SE = -$
599 $0.351+0.042$, $R^2 = 0.442$, $p < 0.0001$).

600

601 **Figure 5.** Effect of sex ratio (males: females) on the offspring production rate per female.

602 Offspring production rates were lowest at intermediate sex ratios (GLM of sex ratio and its
603 second order term: $R^2 = 0.481$, $N = 34$; Table 2).

604