Old maids have more appeal: Effects of age and pheromone source on mate attraction in an orb-web spider

Anna-Lena Cory, Jutta M Schneider

Background. In many insects and spider species, females attract males with volatile sex pheromones, but we know surprisingly little about the costs and benefits of female pheromone emission. Here we test the hypothesis that mate attraction by females is dynamic and strategic in the sense that investment in mate attraction is matched to the needs of the female. We use the orb-web spider Argiope bruennichi in which females risk the production of unfertilised egg-clutches if they do not receive a copulation within a certain time-frame. **Methods.** We designed field experiments to compare mate attraction by recently matured (young) females with females close to oviposition (old). In addition, we experimentally separated the potential sources of pheromone transmission, namely the female body and the web silk. **Results**. In accordance with the hypothesis of strategic pheromone production, the probability of mate attraction and the number of males attracted differed between age classes. While the bodies and webs of young females were hardly found by males, the majority of old females attracted up to two males within two hours. Old females not only increased pheromone emission from their bodies but also from their webs. Capture webs alone, spun by old females were significantly more efficient in attracting males than webs of younger females. **Discussion.** Our results suggest that females modulate their investment in signalling according to the risk of remaining unmated and that they thereby economize on the costs associated with pheromone production and emission.

1 **Old maids have more appeal: Effects of age and pheromone source on mate attraction in**

2 **an orb-web spider**

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12 **Abstract**

13 **Background.** In many insects and spider species, females attract males with volatile sex

14 pheromones, but we know surprisingly little about the costs and benefits of female pheromone

15 emission. Here we test the hypothesis that mate attraction by females is dynamic and strategic in

16 the sense that investment in mate attraction is matched to the needs of the female. We use the

17 orb-web spider *Argiope bruennichi* in which females risk the production of unfertilised egg-

18 clutches if they do not receive a copulation within a certain time-frame.

19 **Methods.** We designed field experiments to compare mate attraction by recently matured

20 (young) females with females close to oviposition (old). In addition, we experimentally separated

21 the potential sources of pheromone transmission, namely the female body and the web silk.

22 **Results.** In accordance with the hypothesis of strategic pheromone production, the probability of

23 mate attraction and the number of males attracted differed between age classes. While the bodies

24 and webs of young females were hardly found by males, the majority of old females attracted up

25 to two males within two hours. Old females not only increased pheromone emission from their

26 bodies but also from their webs. Capture webs alone, spun by old females were significantly

27 more efficient in attracting males than webs of younger females.

28 **Discussion.** Our results suggest that females modulate their investment in signalling according to

29 the risk of remaining unmated and that they thereby economize on the costs associated with

30 pheromone production and emission.

33   **Introduction**

34   Mating partners have to meet in order to mate and reproduce. This prerequisite may be a trivial

35   aspect in group-living animals, but it can constitute a significant factor within the overall costs of

36   sexual reproduction in other taxa (Kokko & Wong 2007; Magnhagen 1991). Encounters with

37   sexual partners can be facilitated by a wide range of mechanisms, e.g. meeting at certain

38   locations (Hoglund & Alatalo 1995; van Ginneken & Maes 2005) or producing long-range

39   signals that attract potential mates (Gerhardt 1994; Roelofs 1995). The latter can occur via

40   different communication channels (visual, acoustic or chemical), but which modality evolved

41   will depend on the distance the signal has to travel in addition to other biotic and strategic

42   selection pressures on signal design (Endler 1993). Here, we are concerned with chemical

43   communication that is considered to be an evolutionary ancient modality demanding the lowest

44   cost in comparison to acoustic and visual signals (Bradbury & Vehrencamp 1998). However, this

45   assumption is largely untested.

46   Both males and females can produce sex pheromones to attract mates (Tillman et al. 1999; Wyatt

47   2014), but particularly mate attraction by females is poorly understood. In many moths and

48   spiders, females produce volatile or substrate bound pheromones, and males follow these traces

49   (Gaskett 2007; Greenfield 1981; Wall & Perry 1987). Selection on male signal perception has

50   led to astounding sensory sensibility and accuracy, as exemplified by the receptor density and

51   functionality on the antennae of the male moth, *Bombyx mori* (Kaissling & Priesner 1970;

52   Steinbrecht 1970). The solid understanding of natural selection on males as signal recipients is

53   opposed by a rudimentary comprehension of the female perspective. It is largely unstudied but

54   nevertheless debated, whether pheromones emitted for mate attraction by females can be

55   classified as sexual signals that actively transmit information to potential mates or as cues

56   exploited by males (Andersson 1994; Harari & Steinitz 2013; Williams 1992; Zuk & Kolluru

57   1998). We use the orb-web spider *Argiope bruennichi* Scopoli, 1772 to test predictions from the

58   hypothesis that pheromones are costly sexual signals.

59   Umbers, Symonds and Kokko (2015) recognized that the emission of cues that reveal one's

60   presence and location will not only reach the intended receiver, but likely attracts parasitoids and

61   predators in addition to mates (e. g. Arakaki et al. 1996; Noldus et al. 1991; Zegelman et al.

62   1993). Besides increased mortality, the attraction of an adversely high number of mates also

63   involves costs (Arnqvist & Nilsson 2000). In spiders, the presence of males may be costly

64   because of kleptoparasitism (Erez, Schneider, & Lubin, 2005), web damage (Anava & Lubin,

65   1993; Harari, Ziv, & Lubin, 2009; Watson, 1986), mate guarding (Calbacho-Rosa, Cordoba-

66   Aguilar, & Peretti, 2010; Fromhage & Schneider, 2005; Herberstein et al., 2005), and sexual

67   harassment (Foellmer & Fairbairn 2003; Lubin 1986; Robinson & Robinson 1973). Hence, even

68   if the physiological costs of pheromone production would be low, extrinsic costs could be strong

69   enough to select for a strategic use of pheromones.

70   In many spider species, including *A. bruennichi*, females are under pressure to attract males soon

71   after maturation because egg maturation advances regardless of whether or not females have

72   copulated. Hence, females that have not been found by a male in time will produce unfertilised

73   egg clutches (Welke & Schneider 2012). Therefore, if virgin females use pheromones

74   strategically they should increase signalling efforts with increasing number of days passed since

75   maturation (here called age).

76   Following this line of reasoning, apart from their location, female spiders can be expected to

77   inform males about receptivity, mating status and perhaps even quality by regulating attraction

78   signals. There is ample evidence that male spiders distinguish between virgin and mated females

79 (Schulte et al. 2010; Stoltz et al. 2007; Tuni & Berger-Tal 2012; Watson 1986). Indeed, in many

80 species females seem to loose receptivity after mating (Herberstein et al. 2002; Rabaneda-Bueno

81 et al. 2008), but they may regain receptivity when sperm supplies require replenishing

82 (Perampaladas et al. 2008). Some studies have shown that virgin females produce sex

83 pheromones that are absent in mated females (Chinta et al. 2010; Jerhot et al. 2010; Schulz &

84 Toft 1993). However, it is unknown whether the emission of sex pheromones by mated females

85 discontinues because females are in control or because males avoid sperm competition by

86 transferring pheromonostatic compounds (Arnqvist & Rowe 2005; Thomas 2011).

87 Note that in spiders, pheromones for long-range attraction are volatile but seem not to be

88 released by a specific gland, rather they are found in the body and the silk (Gaskett 2007; Schulz

89 2013; Schulz et al. 2004). Many studies demonstrated that chemical compounds on female spider

90 silk contain important information for males (Johnson et al. 2011; Stoltz et al. 2007; Sweger et

91 al. 2010; Tuni & Berger-Tal 2012). Gaskett (2007) speculated that silk-based pheromones are

92 less costly while the emission of body pheromones might be more adjustable. Beyond

93 speculations, no studies on web-building spiders compared if female bodies and web silk differ

94 in the efficiency of mate attraction.

95 Here, we investigated age-dependency of male attraction by pheromones associated with the web

96 or the female body. We conducted a field experiment with the European wasp spider *A.*

97 *bruennichi*, in which the sex pheromone is known and found on the body and web of virgin

98 females (Chinta et al. 2010). Using the synthesized pheromone, Chinta et al. (2010) also found

99 that male attraction was dose-dependent. The presence of a response to pheromone concentration

100 might imply that variation occurs in nature and that females vary in their pheromone emission.

101 We predict that the longer females remain unmated, the more they should increase their

102   investment in sex pheromone emission to attract males from larger distances and to outcompete

103   other females in the proximity. If female signalling is costly for *A. bruennichi* females, we would

104   expect that old females regularly attract more males than necessary but accept the costs because

105   laying unfertilized egg clutches is even more costly. Note that only one mating event is needed to

106   fertilize all eggs (Schneider et al. 2005) and that due to efficient mate plugging by males, females

107   are limited to use sperm of two males at most (Nessler et al. 2007). Field experiments are ideal

108   for testing this prediction since they not only allow the qualitative assessment of wide-range

109   attraction but also provide information about the intensity of mate attraction by counting the

110   number of male arrivals. Females would benefit from regulating the emission within a short time

111   frame because the mating season only lasts for 3-4 weeks (Zimmer et al. 2012). Strategic

112   signalling implies that with increasing age and pressure to attract males, unmated females should

113   emit more pheromones.

114   Following this, we hypothesise that (1) mate attraction by virgin females increases with post-

115   maturation age. To further understand how females enhance mate attraction, we tested females

116   and their webs separately. Modulating pheromone content of webs requires rebuilding of webs

117   and is, hence, less rapid than direct emission from the body. Thus, we hypothesise that (2)

118   females adjust pheromone production on their web later than on the female body when the need

119   of signalling is strikingly increased.

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121   Material & Methods

122   *Collection and Maintenance*

123   We collected 59 juvenile and sub-adult *Argiope bruennichi* females on a natural meadow near

124   Nebenstedt, Lower Saxony (N 53°097255, E 11°13019) on June the 30th 2012. They were

125 housed under laboratory conditions at ambient temperature and natural photoperiod and were

126 sprayed with water five days a week. Depending on their body size, females were kept in 250 ml

127 or 500 ml cups that had a hole stuffed with cotton wool on the top. Sub-adult females were

128 transferred into Perspex frames (35 x 35 x 6 cm) where they build a normal orb-web. Small

129 juveniles were fed with approximately 20 *Drosophila hydei* two times a week. Large juvenile,

130 sub-adult and adult females received three *Calliphora* two times a week (for exception see

131 below).

132 *Female body measurements*

133 Sub-adult females were inspected for moults on at least five days a week. Moults to maturity

134 were recognized by inspection of the genitalia. The external genitalia of adult females have a

135 spoon-shaped structure, the scape. In the sub-adult stage, the scape starts to develop but is

136 covered with a thin-skinned layer. Mature females lose this layer, and the scape is exposed. At

137 maturity, females were allocated in a pre-set order to the three age treatments. On the first day

138 after maturation females were weighed on a calibrated scale (Mettler Toledo AB54-S) with an

139 accuracy of 0.1 mg. Each female was also weighed on the day of the test (test weight).

140 Spider size was determined after the experiments. Females were brought back to the laboratory

141 where they were kept and well supplied with food and water until natural death occurred. After

142 death, the first pair of legs was removed and photographed under a stereomicroscope. We

143 measured the length of the two segments tibia and patella with the Leica IM500 Imaging

144 software (version 4.0; Leica Microsystems Imaging Solution Ltd., Cambridge, UK). We used

145 this length as an approximation for the overall spider size. Legs and other sclerotized body parts

146 do only change through moulting but not between moults. As an approximation of female

147   condition, we used the residuals of the regression of body weight at maturation or at the test day

148   and size of the females (Schulte-Hostedde et al. 2005). To achieve a normal distribution, we log-

149   transformed the data for test weight.

150   *Pheromone source*

151   To disentangle the female body or the web as the source of pheromones, we used the webs of

152   sub-adult females as "neutral webs" because they do not contain pheromones (Chinta et al.

153   2010). In the "female body" trials, virgin females were placed into these neutral webs. Most

154   females showed little activity on the web and seemed to accept it. However, bad weather

155   conditions led to more activity such that some females either stabilized the web with silk threads

156   or destroyed the neutral web (see "Data analysis"). For the "web" trials, freshly constructed webs

157   by virgins were used without the female. Generally, the web and its producer female were used

158   on the same day (exceptions arise when webs are destroyed before they can be placed in the

159   meadow). We ensured that the web was freshly built on the day of the trial by destroying webs

160   on the evening before the trial. Females built their webs in the early morning, and we had

161   introduced a *Calliphora* fly into the frame to stimulate the production of a capture web.

162   *Female age classes*

163   Oviposition can already occur 10-16 days after maturation. We used this time window to define

164   three age classes of virgin females. Age is given as the number of days that had passed since

165   maturation. "Young females" were 1-2 days adult, "middle-aged females" 3-7 days adult (5.8 ±

166   1.4), and "old females" had matured 8 or more days ago (10.0 ± 2.1). The webs were produced

167   by females that were 1-2 days old in the groups of young females, by middle-aged females of 5.0

168   ± 1.3 days and by old females of the average age of 10.0 ± 2.8 days. We used a 2-day time

169 window for the category of young females because binary choice experiments in the laboratory

170 have shown that males do not distinguish between sub-adult females and females younger than

171 three days (Schneider et al. 2016). A plausible explanation for this result is that females do not

172 produce the pheromone until the 3rd day after maturation. Nevertheless, females readily copulate

173 with males on the day of sexual maturity (Welke et al. 2012). An age of at least eight days was

174 used for the category "old females" to include females with high pressure to mate because

175 oviposition is very close and the risk of producing unfertilised eggs is very high. Each female

176 was used once for only a single age class.

177 A comparison of mate attraction by females of different ages requires testing a matched sample

178 of females from all age classes every day. This is important since conditions on subsequent days

179 are rarely the same: males disappear from the mating pool as they get cannibalised and male

180 activity depends on weather conditions. However, females mature very synchronously which

181 makes it difficult to find young virgins towards the end of the mating season (Zimmer et al.

182 2012). To achieve a balanced availability of young and old females during the test period, we

183 included a short period of food restriction during the final instars for half of the females which

184 received three *Calliphora* flies once instead of twice a week for about two weeks. Spiders are

185 adapted to endure periods of prey shortage since they regularly occur in nature. A short hunger

186 period does not negatively affect spider survival or fecundity. However, it is sufficient to

187 postpone moulting into maturity for a few days. We payed attention to feed all females well

188 shortly before adulthood to standardize hunger level and distributed females from the two

189 feeding regimes across all three age classes to avoid a systematic bias.

190 To account for the possibility that differences in juvenile feeding history might have an impact

191 on the condition at maturation (Hector & Nakagawa 2012) and influenced our age treatments, we

192 tested whether the body condition on the first day of sexual maturity differed between females of

193 different age classes. We found no significant differences (Table 1) but in the web trial, there

194 was a trend that females allocated to the "middle-aged" group initially had a higher condition

195 than the others, while young females had a lower condition. However, this would be only

196 problematic if we found that middle-aged females attracted the most males.

197 Naturally, body condition increases with age (ANOVA on the weight of females across the age

198 classes: $F_{2,26}$=21.44, p<0.0001; ANOVA on the weight of females that produced the webs:

199 $F_{2,29}$=21.32, p<0.0001) such as body weight does. This increase is due to feeding and egg-

200 maturation. Hence, age and condition are confounding factors and cannot be separated. In an

201 attempt to dissociate the effects of female age and condition, we tested the effect of female

202 condition on male attraction separately within each age class.

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212 Table 1: Female weight and female size information within the age classes and statistical

213 comparisons (ANOVA) of female weight and female size between the age classes

	Adult weight (mg)[1]		Size (mm)[2]		Condition[3]	
	N	Mean ± SD	N	Mean ± SD	N	Mean ± SD
Female body trials						
Young	12	92.2 ± 25.5	10	6.2 ± 0.6	10	-5.6 ± 5.9
Middle-aged	12	94.0 ± 25.3	10	6.1 ± 0.6	10	4.5 ± 17.3
Old	12	83.4 ± 25.3	10	6.0 ± 0.6	10	1.5 ± 7.1
ANOVA	$F_{2,33}$=0.68, p=0.5148		$F_{2,26}$=0.35, p=0.7053		$F_{2,26}$=2.10, p=0.1425	
Web trials						
Young	14	96.0 ± 27.5	11	6.2 ± 0.6	11	-6.6 ± 6.3
Middle-aged	14	98.2 ± 27.9	11	6.2 ± 0.6	11	4.4 ± 16.4
Old	13	80.1 ± 23.2	10	5.9 ± 0.6	10	2.9 ± 11.4
ANOVA	$F_{2,38}$=1.88, p=0.1664		$F_{2,29}$=1.22, p=0.3105		$F_{2,29}$=2.88, p=0.0725	

214 [1]The adult weight was measured shortly after maturity. [2]For the size, we used the tibia-patella

215 length (segments of the leg) as a good approximation for the general size of spiders. [3]Residuals

216 of the regression of body weight shortly after maturation and size of the females.

217

Test preparation (field)

219 Field experiments were conducted between 12th and 26th July 2012 on a natural meadow in

220 Buxtehude, Lower Saxony (N 53°453995, E 9°674402). Our experimental period covered most

221   of the mating season, although some males were still active when the trials ended. We observed

222   male activity a few days before the experiments started and picked the experimental areas

223   according to similar male activity.

224   The design was balanced in that we tested females of all age classes on each test day. Females

225   were transported in containers (volume of 120 ml) to the field site and the webs for transported

226   within the frames in specially designed transport boxes to minimise damage. On the test

227   meadow, the Perspex frames with webs of the study females ("web" trial) or non-pheromone

228   webs ("female body" trial) were fixed with tent pegs to the ground in an upright position. To

229   ensure that the web was not destroyed by tall grasses, a small area around the frame was

230   flattened. The distribution of the frames on the meadow was random although we ensured that

231   the distance between the frames and local adult females was larger than 50 cm. For the "female

232   body" trial, we carefully placed the females into the prepared neutral webs after the frames were

233   securely fixed on the ground.

234   *Test run*

235   "Female body" trials and "web" trials were conducted apart from each other to avoid pheromone

236   contamination. Either the two web categories had a distance of at least eight metres, or they were

237   put out at different times of the day. The order and position was reversed every day so that each

238   category was tested at each time of the day and in different parts of the meadow. The distance of

239   eight metres was dictated by the size and structure of the test meadow.

240   We observed each web for two hours and counted the number of male visitors. Since not all

241   males found their way into the web, we also counted males that apparently attempted to reach the

242   females or their webs for at least 5 minutes. An attempt was only counted when the male

243 wandered around grass lying in close vicinity to the female. To avoid pseudo-replication, we

244 collected male visitors for the duration of the test. The males were released a few metres away

245 from the test area after the test run was ended. There was a half-hour break between subsequent

246 test runs so that the males could acclimatize in the field.

247 *Data analysis*

248 Although we were especially interested in the effects of female age and pheromone source, we

249 also analysed the effects of size, test weight, and condition. However, female weight and

250 condition are correlated and produced very similar results. We therefore excluded female weight

251 from the analysis.

252 Most likely due to windy and humid weather conditions, we had a high proportion of zero

253 counts. We divided the analysis into two levels and firstly used a binary response variable

254 namely whether or not males approached. Secondly, we explored the treatment effects on the

255 number of male visitors and reduced the sample to only include females that had male visitors.

256 The design was originally balanced with each 42 webs and females, but some webs and neutral

257 webs were destroyed before the tests could start. Overall, we could test 41 webs and 39 females

258 on neutral webs. However, particularly on rainy days some females escaped from the neutral

259 webs mostly and observation time was less than 120 minutes. Since, we found that most first

260 male visitors (92 %) appeared within the first hour of the test run, we discarded only 3 cases that

261 were shorter than 60 minutes and retained 36 tests that lasted for at least one hour for the first

262 analysis of male appearance as a binary variable. However, for the test of treatment effects on the

263 number of male visitors, we considered only females and their webs that were observed for the

264 full two hours and in which males visited, at least, the web or the female (n=14).

265 Statistical analyses were performed with the program R version 3.0.3. All variables were tested

266 for normal distribution with the Shapiro-Wilk test. We used parametric tests for normally

267 distributed data and nonparametric tests for analyses of non-normal and paired data. All tests

268 were two-tailed. Since only a part of the "female" and "web" data was paired, we performed a

269 generalised estimating equation (GEE) to explore whether females or webs were more likely to

270 attract males. We created a binary model with pheromone source as testing variable and defined

271 female ID as a random term (grouping variable). The correlation structure was specified as

272 "independent". We simplified the model by excluding the variable "pheromone source" and

273 compared whether the original model significantly differed from the simplified model by using

274 the Wald test. A significant difference between both models would be indicative for a significant

275 effect of the pheromone source on the probability of being visited by a male.

276

277 Results

278 Only 23 of 77 females or webs were visited by males during the 2h-observation period, most of

279 them (22 of 23) within the first 60 minutes. Presumably, male activity was low due to very

280 humid and windy weather conditions. We included all trials into the analyses unless stated

281 otherwise.

282 Females on neutral webs attracted visitors with a significantly higher probability (14 of 36

283 females) than webs alone (8 of 41 webs) (GEE: X^2=6.44, N=77, p=0.0111; Fig. 1). A paired

284 comparison of the number of male visitors attracted by a given female in a neutral web and by

285 her own web was not significant (Wilcoxon signed rank test: T=58.5, N=14, p=0.3724)

286 suggesting that pheromone emission from the body directly or via the silk of the web were not

287   necessarily linked. However, the sample size was low as we only considered 14 pairs in which

288   males visited, at least, the web or the female.

289   *Female body*

290   We used Fisher exact tests for pairwise comparisons of the proportion of females on neutral

291   webs visited per age class. Although the probability of male visits increased with increasing age

292   class (Fig. 1), we only found a significant difference between young and old females (N=24,

293   p=0.03607). Female fixed size (t-test: t_{27}=-0.67, p=0.5079) did not influence the probability of a

294   male visit while female condition had significant positive effects (t-test: t_{27}=-3.49, p=0.0017, Fig.

295   2). Note that any potential effect of female body condition was confounded by age (see

296   methods). In an attempt to separate age and condition, we tested the effects of female condition

297   within the three age classes and found no significant differences in condition of females that

298   were or were not visited (young females: no value; middle-aged females: t-test: t_7=-0.32,

299   p=0.7554, old females: logistic regression: X^2=0.99, N=10, p=0.3187).

300   Excluding females that were never visited, females received 1 to 4 male visits (median=2) within

301   two hours. Due to the unbalanced sample sizes within the age classes (young females were

302   hardly visited at all), we dropped the analysis with the category "age class". Instead, we used

303   "post-mature age" as a continuous variable, but we did not find a significant correlation between

304   age and the number of male visitors (Spearman rank correlations: r_s=0.49, N=13, p=0.0909, Fig.

305   3). Interestingly, while visitation rates did not correlate with female size (Spearman rank

306   correlations: r_s=0.82, N=13, p=0.789), the number of male visitors significantly increased with

307   female condition (Spearman rank correlations: r_s=0.68, N=13, p=0.0103).

308   *Web silk*

309   To compare the attractivity of webs without females, we used, as above, Fisher exact tests for

310   pairwise comparisons of the probability of being visited between female age classes. Since the

311   14 webs of middle-aged females and the 14 webs of young females hardly attracted males at all

312   (only a single web was visited in each of the two groups), we compared each of both categories

313   only with the category "old females". The tests revealed that webs of old females (6 of 13 webs)

314   attracted males significantly more often than webs of young females and webs of middle-aged

315   females (for both pairwise comparisons we used the same statistic: $N=27$, $p=0.0329$, Fig. 1).

316   While size (t-test: $t_{30}=0.81$, $p=0.4224$) of the females that had produced the webs did not

317   significantly differ between visited and non-visited webs, the effect of condition was significant

318   (t-test: $t_{30}=-2.81$, $p=0.0086$; Fig. 2) suggesting that females in better condition produced webs

319   that were more attractive to males. However, remember that females in better condition were

320   significantly older (Spearman rank correlations: $r_s=0.74$, N=32, p<0.0001) and 8 of 10 webs

321   came from old females.

322   Most female webs were visited by one or two males (80 %), although the maximum was five.

323   Visitation rates cannot be compared between the age classes because 80 % of the visited female

324   webs were from old females, as already mentioned above. As with the female body, we

325   conducted the analysis with the continuous variable "post-mature age". The post-mature age

326   correlated positively with the number of males that approached the web (Spearman rank

327   correlations: $r_s=0.69$, $N=10$, $p=0.0271$). From a post-mature age of 14 days onwards, some

328   females were visited by more than two males (Fig. 3). Neither female size (Spearman rank

329   correlations: $r_s=0.09$, $N=10$, $p=0.8149$) nor female condition (Spearman rank correlations:

330    $r_s=0.61$, $N=10$, $p=0.0613$) were significantly related to the number of male visitors.

331

332 Discussion

333 Our field assays provided support for the hypothesis of strategic pheromone emission. In

334 accordance with our prediction, females close to oviposition (old and heavy) were most

335 successful in mate attraction. Females used both, their body and their web to transmit

336 pheromones, although the latter became more relevant with increasing age of the female. As

337 oviposition came close, the attractivity of the web for mate attraction increased. Female fixed

338 size estimated from sclerotized body parts seemed to be irrelevant for male attraction while it

339 was indeed the body condition that caused increased numbers of males to visit a female and her

340 web. However, because of the correlative nature between female age and female condition, it is

341 difficult to separate both factors.

342 Our results are in accordance with model predictions by Umbers and colleagues (2015) in that

343 females increase their investment in signalling if they age without having encountered a mate

344 yet. Young virgin *A. bruennichi* females, particularly at the beginning of the mating season and

345 under high densities (Zimmer et al. 2012), are likely found haphazardly by the protandrous

346 roving males and females may save the costs of pheromone production. This is suggested by the

347 high frequency of mate guarding of sub-adult females in the field (Uhl et al. 2015). As females

348 age, egg maturation progresses and by mating too late, oviposition will take place, albeit laying

349 unfertilised eggs. Females should be under selection to avoid this fate. Thus, it is adaptive to

350 increase signalling effort if no males have appeared within a few days after maturation. Such

351 females may find themselves in a low-density patch or too late in the season when most of the

352 males are already mated and consumed (Zimmer et al. 2012).

353 Extrinsic costs of signalling can result from the attraction of parasitoids or an detrimentally high

354 number of mates. Male presence on the web is known to have costs for female spiders because

355   prey capture is reduced, and predation risk is increased (Herberstein et al. 2002). In nature, *A.*

356   *bruennichi* females mate with one or two males (Zimmer et al. 2012) and a single copulation is

357   sufficient to fertilise all eggs (Schneider et al. 2005). During copulation males plug the paired

358   genital openings of females to prevent sperm competition. The mating plugs are highly effective

359   so that the majority of females can store and use sperm of two males at most (Nessler et al.

360   2007). In our study, old females attracted many more males than needed for fertilisation and

361   more than of the maximal number of sires. Furthermore, webs were especially used for mate

362   attraction when oviposition was very close. In fact, those webs attracted up to five males. The

363   larger surface of the web may increase pheromone distribution. Hence, females may use

364   pheromones strategically and remain hidden for males if they do not benefit from mate

365   attraction. We did not test whether old females build larger webs compared to young females.

366   Therefore, further studies should consider that females might increase pheromone distribution by

367   extending the web surface or by adding more pheromones onto the web.

368   While extrinsic costs are most likely present, it is unclear whether pheromone production in

369   these spiders has physiological costs as well. If signal production involves physiological costs,

370   females in better condition may emit more and would honestly signal their quality. A purely

371   strategic use does not require honesty because it would only imply increased signalling with

372   increasing age and pressure to attract males. However, it is difficult to separate female condition

373   from female age because females become fat as egg maturation proceeds. Accordingly, both

374   variables reflect the time pressure on females to secure fertilisation by increasing investment into

375   signalling. A more important influence of age is confirmed by binary choice tests in *A.*

376   *bruennichi,* in which males showed no significant preference when presented with a choice

377   between two young females of the same age that differed in condition and size (Schulte et al.

378   2010). Investment in mate attraction may only become important at a later stage, while young

379   females rather use their energy for feeding and producing eggs. Due to the low sample sizes and

380   the low activity of males in that year, we did not find effects of condition within the class of old

381   females. More experiments are necessary to investigate whether females of different condition

382   but of the same age attract more males.

383   Our findings are inconsistent with the result from Chinta and Schulz (unpublished data; reviewed

384   in Schulz 2013) that the production of the sex pheromone (trimethyl (2R,3S)-methylcitrate) of *A.*

385   *bruennichi* increases until the fourth day after the final moult and then decreases. Since male

386   attraction by this sex pheromone follows a concentration-dependent matter (Chinta et al. 2010),

387   the middle-aged females of our study should have attracted more males than the old females. An

388   explanation for these inconsistencies could be that females close to oviposition attract males with

389   another semiochemical than trimethyl (2R, 3S)-methylcitrate. At least one other female specific

390   compound is known (Chinta et al. 2010). Whether or not increased mate attraction of old *A.*

391   *bruennichi* females is based on trimethyl (2R, 3S)-methylcitrate has to be resolved in further

392   studies.

393   The use of a different semiochemical shortly before oviposition could be based on a signal or cue

394   mechanism. Old females may actively enhance signalling with a further, perhaps more potent

395   pheromone, if the risk of laying unfertilized eggs outweighs the costs of attracting too many

396   males. Alternatively, females may passively emit oviposition cues or stress hormones, if the risk

397   of laying unfertilized eggs increased. Note that it is also adaptive for males to find virgin females

398   close to oviposition because the probablility might be lower, that females die before they

399   deposited their first egg sac (Rittschof 2011).

400 Research on the praying mantis *Pseudomantis albofimbriata* (Barry 2010; Barry et al. 2010)

401 suggests that pheromone production could be directly linked to egg production and hence be

402 considered a cue rather than a signal. In a simultaneous choice test, male mantises displayed a

403 preference for females that carried more eggs, which correlates with body condition. Given a

404 choice between two females in equally poor condition, the males still picked the one with more

405 eggs in their ovaries (Barry 2010; Barry et al. 2010). This link between pheromone release and

406 fecundity could be mediated by juvenile hormone titers (Barry 2010). This explanation is

407 unlikely to apply to spiders with strong first male sperm precedence such as in *A. bruennichi*

408 (Austad 1982; Jones & Parker 2008; Nessler et al. 2007; Uhl et al. 2014). Most females will mate

409 shortly after maturation and are no longer visited by males, most likely because mated females

410 stop producing pheromones (Chinta et al. 2010). Copulation with mated females promises low

411 fitness returns for males since the genital openings are blocked with genital parts of the

412 predecessor (Nessler et al. 2007) and unlike in mantises where a male constitutes a significant

413 nutritional benefit (Barry et al. 2008; Birkhead et al. 1988; Maxwell 2000), males of *Argiope* are

414 very small and provide only a small addition to the diet (Blamires 2011; Fromhage et al. 2003).

415 However, egg maturation is a continuous process and independent of mating. Hence, females

416 that mate shortly after maturation and are no longer interesting for males, still develop their eggs

417 at much the same speed as unmated females. To understand the mechanisms of dynamic

418 pheromone emission, we need studies that examine the underlying physiological processes.

419 Pheromone emission in spiders is poorly understood. The glands responsible for sex pheromone

420 secretion and the mechanism to attach them to the web silk are still unknown. Just a few studies

421 investigated if female spiders use both their body and their web for mate attraction and no study

422 compared the efficiency. For wide range-attraction, volatile pheromones rather than contact

423 pheromones are used (Kasumovic & Andrade 2004; Olive 1982; Riechert & Singer 1995; Searcy

424 et al. 1999). Prouvost and colleagues (1999) found that in the house spider *Tegenaria atrica*, the

425 body cuticle and the web differ in some chemical compounds. Possibly, the importance of female

426 or web pheromones for mate attraction depends on the mobility of the spider species. In orb-web

427 spiders, females are sessile and mainly use volatile pheromones for mate attraction while web

428 silk might give males more specific information about the female upon contact. In wandering

429 spiders, silk, especially from draglines, may have a higher relevance for mate attraction and mate

430 search than the silk of orb-webs (discussed in Baruffaldi et al. 2010).

431 As far as we know, this is the first study that compared the efficiency of pheromones transmitted

432 by web silk and body in the field. Beyond that, our study complements the picture of

433 mechanisms of mate attraction in spiders. In most studies, age-dependent mate attraction was

434 explained by female receptivity (Klein et al. 2012; Papke et al. 2001; Roberts & Uetz 2005). We

435 can exclude this explanation because *A. bruennichi* females are always receptive (Schneider et

436 al. 2006). We can show that beside moths (Umbers et al. 2015), female spiders adjust mate

437 attraction to the pressure of achieving a copulation. Future research should combine female age

438 and mating status to test when females stop signalling. *A. bruennichi* females are polyandrous

439 and benefit from attracting males after the first copulation.

440

441 Conclusion

442 We conclude that female signalling is a dynamic process and depends on the time spent

443 unfertilised. Female signalling seems to be strategic implying the presence of costs. We found

444 that the signal strength variation was adaptive and likely adjusted to the females' needs.

445

446 Acknowledgements

447 We thank Tomma Dirks, Angelika Taebel-Hellwig, Gabriele Uhl, and Stefanie Zimmer for their

448 crucial support during the experimental planning, the collection of spiders, and the experimental

449 conduction in the field. Furthermore, we like to thank Jasmin Ruch and two anonymous

450 reviewers for helpful comments on the manuscript.

452 REFERENCES

453 Andersson M. 1994. Sexual Selection. *Princeton Univ Press*.

454 Arakaki N, Wakamura S, and Yasuda T. 1996. Phoretic egg parasitoid, *Telenomus euproctidis*
455 (Hymenoptera: Scelionidae), uses sex pheromone of tussock moth *Euproctis taiwana*
456 (Lepidoptera: Lymantriidae) as a kairomone. *Journal of Chemical Ecology* 22:1079-
457 1085. 10.1007/BF02027946

458 Arnqvist G, and Nilsson T. 2000. The evolution of polyandry: multiple mating and female fitness
459 in insects. *Animal Behaviour* 60:145-164. 10.1006/anbe.2000.1446

460 Arnqvist G, and Rowe L. 2005. Sexual conflict. *Princton Univ Press*.

461 Austad SN. 1982. First Male Sperm Priority in the Bowl and Doily Spider, *Frontinella*
462 *pyramitela* (Walckenaer). *Evolution* 36:777. 10.2307/2407891

463 Barry KL. 2010. Influence of female nutritional status on mating dynamics in a sexually
464 cannibalistic praying mantid. *Animal Behaviour* 80:405-411.
465 10.1016/j.anbehav.2010.05.024

466 Barry KL, Holwell GI, and Herberstein ME. 2008. Female praying mantids use sexual
467 cannibalism as a foraging strategy to increase fecundity. *Behavioral Ecology* 19:710-715.
468 10.1093/beheco/arm156

469 Barry KL, Holwell GI, and Herberstein ME. 2010. Multimodal mate assessment by male praying
470 mantids in a sexually cannibalistic mating system. *Animal Behaviour* 79:1165-1172.
471 10.1016/j.anbehav.2010.02.025

472 Baruffaldi L, Costa FG, Rodriguez A, and Gonzalez A. 2010. Chemical Communication in
473 *Schizocosa malitiosa*: Evidence of a Female Contact Sex Pheromone and Persistence in
474 the Field. *Journal of Chemical Ecology* 36:759-767. 10.1007/s10886-010-9819-x

475   Birkhead TR, Lee KE, and Young P. 1988. Sexual Cannibalism in the Praying Mantis *Hierodula*

476   *membranacea*. *Behaviour* 106:112-118. Doi 10.1163/156853988x00115

477   Blamires SJ. 2011. Nutritional implications for sexual cannibalism in a sexually dimorphic orb

478   web spider. *Austral Ecology* 36:389-394. 10.1111/j.1442-9993.2010.02161.x

479   Bradbury JW, and Vehrencamp SL. 1998. *Principles of animal communication*.

480   Chinta SP, Goller S, Lux J, Funke S, Uhl G, and Schulz S. 2010. The sex pheromone of the wasp

481   spider *Argiope bruennichi*. *Angewandte Chemie-International Edition* 49:2033-2036.

482   10.1002/anie.200906311

483   Endler JA. 1993. Some general comments on the evolution and design of animal communication

484   systems. *Philos Trans R Soc Lond B Biol Sci* 340:215-225. 10.1098/rstb.1993.0060

485   Foellmer MW, and Fairbairn DJ. 2003. Spontaneous male death during copulation in an orb-

486   weaving spider. *Proceedings of the Royal Society B Biological sciences* 270 Suppl

487   2:S183-185. 10.1098/rsbl.2003.0042

488   Fromhage L, Uhl G, and Schneider JM. 2003. Fitness consequences of sexual cannibalism in

489   female *Argiope bruennichi*. *Behavioral Ecology and Sociobiology* 55:60-64.

490   10.1007/s00265-003-0656-6

491   Gaskett AC. 2007. Spider sex pheromones: emission, reception, structures, and functions.

492   *Biological Reviews* 82:26-48. 10.1111/j.1469-185X.2006.00002.x

493   Gerhardt HC. 1994. The Evolution of Vocalization in Frogs and Toads. *Annual Review of*

494   *Ecology and Systematics* 25:293-324. DOI 10.1146/annurev.es.25.110194.001453

495   Greenfield MD. 1981. Moth Sex-Pheromones - an Evolutionary Perspective. *Florida*

496   *Entomologist* 64:4-17. Doi 10.2307/3494597

497   Harari AR, and Steinitz H. 2013. The evolution of female sex pheromones. *Current Zoology*

498   59:569-578.

499   Hector KL, and Nakagawa S. 2012. Quantitative analysis of compensatory and catch-up growth

500   in diverse taxa. *Journal of Animal Ecology* 81:583-593. 10.1111/j.1365-

501   2656.2011.01942.x

502   Herberstein ME, Schneider JM, and Elgar MA. 2002. Costs of courtship and mating in a sexually

503   cannibalistic orb-web spider: female mating strategies and their consequences for males.

504   *Behavioral Ecology and Sociobiology* 51:440-446. 10.1007/s00265-002-0460-8

505   Hoglund J, and Alatalo RV. 1995. *Monographs in Behavior and Ecology: Leks*.

506   Jerhot E, Stoltz JA, Andrade MC, and Schulz S. 2010. Acylated serine derivatives: a unique class

507   of arthropod pheromones of the Australian redback spider, *Latrodectus hasselti*.

508   *Angewandte Chemie-International Edition* 49:2037-2040. 10.1002/anie.200906312

509   Johnson JC, Trubl P, Blackmore V, and Miles L. 2011. Male black widows court well-fed

510   females more than starved females: silken cues indicate sexual cannibalism risk. *Animal*

511   *Behaviour* 82:383-390. 10.1016/j.anbehav.2011.05.018

512   Jones TC, and Parker PG. 2008. First male sperm precedence in multiply-mated females of the

513   cooperative spider *Anelosimus studiosus* (Araneae, Theridiidae). *Journal of Arachnology*

514   36:527-532. Doi 10.1636/St06-63.1

515   Kaissling KE, and Priesner E. 1970. Olfactory Threshold of Silk Moths. *Naturwissenschaften*

516   57:23-&.

517   Kasumovic MM, and Andrade MCB. 2004. Discrimination of airborne pheromones by mate-

518   searching male western black widow spiders (*Latrodectus hesperus*): species- and

519   population-specific responses. *Canadian Journal of Zoology-Revue Canadienne De*

520   *Zoologie* 82:1027-1034. 10.1139/Z04-081

521 Klein AL, Trillo MC, and Albo MJ. 2012. Sexual receptivity varies according to female age in a

522   Neotropical nuptial gift-giving spider. *Journal of Arachnology* 40:138-140.

523 Kokko H, and Wong BBM. 2007. What determines sex roles in mate searching? *Evolution*

524   61:1162-1175. 10.1111/j.1558-5646.2007.00090.x

525 Lubin YD. 1986. Courtship and Alternative Mating Tactics in a Social Spider. *Journal of*

526   *Arachnology* 14:239-257.

527 Magnhagen C. 1991. Predation Risk as a Cost of Reproduction. *Trends in Ecology & Evolution*

528   6:183-185. Doi 10.1016/0169-5347(91)90210-O

529 Maxwell MR. 2000. Does a single meal affect female reproductive output in the sexually

530   cannibalistic praying mantid *Iris oratoria*? *Ecological Entomology* 25:54-62.

531   10.1046/j.1365-2311.2000.00227.x

532 Nessler SH, Uhl G, and Schneider JM. 2007. Genital damage in the orb-web spider *Argiope*

533   *bruennichi* (Araneae : Araneidae) increases paternity success. *Behavioral Ecology*

534   18:174-181. 10.1093/beheco/arl074

535 Noldus L, Potting RPJ, and Barendregt HE. 1991. Moth sex pheromone adsorption to leaf

536   surface: bridge in time for chemical spies. *Physiological Entomology* 16:329-344.

537   10.1111/j.1365-3032.1991.tb00571.x

538 Olive CW. 1982. Sex Pheromones in Two Orbweaving Spiders, (Aranaeae, Araneidae) - An

539   Experimental Field Study. *Journal of Arachnology* 10:241-245.

540 Papke MD, Riechert SE, and Schulz S. 2001. An airborne female pheromone associated with

541 male attraction and courtship in a desert spider. *Animal Behaviour* 61:877-886.

542 10.1006/anbe.2000.1675

543 Perampaladas K, Stoltz JA, and Andrade MCB. 2008. Mated redback spider females re-advertise

544 receptivity months after mating. *Ethology* 114:589-598. 10.1111/j.1439-

545 0310.2008.01513.x

546 Prouvost O, Trabalon M, Papke M, and Schulz S. 1999. Contact sex signals on Web and cuticle

547 of *Tegenaria atrica* (Araneae, Agelenidae). *Archives of Insect Biochemistry and*

548 *Physiology* 40:194-202. 10.1002/(sici)1520-6327(1999)40:4<194::aid-arch4>3.0.co;2-p

549 Rabaneda-Bueno R, Rodriguez-Girones MA, Aguado-de-la-Paz S, Fernandez-Montraveta C, De

550 Mas E, Wise DH, and Moya-Larano J. 2008. Sexual Cannibalism: High Incidence in a

551 Natural Population with Benefits to Females. *PLoS ONE* 3.

552 10.1371/journal.pone.0003484

553 Riechert SE, and Singer FD. 1995. Investigation of potential male mate choice in a monogamous

554 spider. *Animal Behaviour* 49:715-723. 10.1016/0003-3472(95)90046-2

555 Rittschof CC. 2011. Mortality risk affects mating decisions in the spider *Nephila clavipes*.

556 *Behavioral Ecology* 22:350-357. 10.1093/beheco/arq222

557 Roberts JA, and Uetz GW. 2005. Information content of female chemical signals in the wolf

558 spider, *Schizocosa ocreata*: male discrimination of reproductive state and receptivity.

559 *Animal Behaviour* 70:217-223. 10.1016/j.anbehav.2004.09.026

560 Robinson MH, and Robinson B. 1973. Ecology and behaviour of the giant wood spider *Nephila*

561 *maculata* (Fabricius) in New Guinea. *Smithsonian Contributions to Zoology* No. 149:1-

562 75.

563   Roelofs WL. 1995. Chemistry of sex attraction. *Proceedings of the National Academy of*

564   *Sciences* 92:44-49. 10.1073/pnas.92.1.44

565   Schneider JM, Fromhage L, and Uhl G. 2005. Extremely Short Copulations Do Not Affect

566   Hatching Success in *Argiope bruennichi* (Araneae, Araneidae). *Journal of Arachnology*

567   33:663-669. 10.1636/s03-32.1

568   Schneider JM, Gilberg S, Fromhage L, and Uhl G. 2006. Sexual conflict over copulation

569   duration   in   a   cannibalistic   spider. *Animal   Behaviour*   71:781-788.

570   10.1016/j.anbehav.2005.05.012

571   Schneider JM, Zimmer SM, Gatz AL, and Sauerland K. 2016. Context- and State-Dependent

572   Male Mate Choice in a Sexually Cannibalistic Spider. *Ethology* 122:257-266. DOI:

573   10.1111/eth.12466

574   Schulte-Hostedde AI, Zinner B, Millar JS, and Hickling GJ. 2005. Restitution of mass-size

575   residuals: Validating body condition indices. *Ecology* 86:155-163. Doi 10.1890/04-0232

576   Schulte KF, Uhl G, and Schneider JM. 2010. Mate choice in males with one-shot genitalia:

577   limited   importance   of   female   fecundity. *Animal   Behaviour*   80:699-706.

578   10.1016/j.anbehav.2010.07.005

579   Schulz S. 2013. Spider Pheromones - a Structural Perspective. *Journal of Chemical Ecology*

580   39:1-14. 10.1007/s10886-012-0231-6

581   Schulz S, Cardé RT, and Millar JG. 2004. Semiochemistry of spiders. *Advances in Insect*

582   *Chemical Ecology*:110-150. 10.1017/cbo9780511542664.005

583   Schulz S, and Toft S. 1993. Identification of a Sex Pheromone from a Spider. *Science* 260:1635-

584   1637. 10.1126/science.260.5114.1635

585 Searcy LE, Rypstra AL, and Persons MH. 1999. Airborne chemical communication in the wolf

586   spider *Pardosa milvina*. *Journal of Chemical Ecology* 25:2527-2533. Doi

587   10.1023/A:1020878225553

588 Steinbrecht RA. 1970. Morphometric Studies on Antenna of Silk Moth, *Bombyx mori* L. -

589   Number and Distribution of Olfactory Sensilla. *Zeitschrift Fur Morphologie Der Tiere*

590   68:93-126.

591 Stoltz JA, McNeil JN, and Andrade MCB. 2007. Males assess chemical signals to discriminate

592   just-mated females from virgins in redback spiders. *Animal Behaviour* 74:1669-1674.

593   10.1016/j.anbehav.2007.03.011

594 Sweger AL, Marr M, Satteson A, Rypstra AL, and Persons MH. 2010. The effects of predation

595   risk on female silk deposition and male response to predator-cued conspecifics in the

596   wolf spider, *Pardosa milvina* (Araneae: Lycosidae). *Journal of Arachnology* 38:393-397.

597   Doi 10.1636/Hi09-89.1

598 Thomas ML. 2011. Detection of female mating status using chemical signals and cues.

599   *Biological Reviews* 86:1-14. 10.1111/j.1469-185X.2010.00130.x

600 Tillman JA, Seybold SJ, Jurenka RA, and Blomquist GJ. 1999. Insect pheromones - an overview

601   of biosynthesis and endocrine regulation. *Insect Biochemistry and Molecular Biology*

602   29:481-514. 10.1016/s0965-1748(99)00016-8

603 Tuni C, and Berger-Tal R. 2012. Male preference and female cues: males assess female sexual

604   maturity and mating status in a web-building spider. *Behavioral Ecology* 23:582-587.

605   10.1093/beheco/ars001

606 Uhl G, Kunz K, Vocking O, and Lipke E. 2014. A spider mating plug: origin and constraints of

607   production. *Biological Journal of the Linnean Society* 113:345-354. 10.1111/bij.12359

608 Uhl G, Zimmer SM, Renner D, and Schneider JM. 2015. Exploiting a moment of weakness:
609   male spiders escape sexual cannibalism by copulating with moulting females. *Scientific*
610   *Reports* 5. 10.1038/srep16928

611 Umbers KD, Symonds MR, and Kokko H. 2015. The mothematics of female pheromone
612   signaling: strategies for aging virgins. *Am Nat* 185:417-432. 10.1086/679614

613 van Ginneken VJT, and Maes GE. 2005. The European eel (*Anguilla anguilla*, Linnaeus), its
614   Lifecycle, Evolution and Reproduction: A Literature Review. *Reviews in Fish Biology*
615   *and Fisheries* 15:367-398. 10.1007/s11160-006-0005-8

616 Wall C, and Perry JN. 1987. Range of Action of Moth Sex-Attractant Sources. *Entomologia*
617   *Experimentalis Et Applicata* 44:5-14.

618 Watson PJ. 1986. Transmission of a Female Sex-Pheromone Thwarted by Males in the Spider
619   *Linyphia litigiosa* (Linyphiidae). *Science* 233:219-221. 10.1126/science.3726530

620 Welke KW, and Schneider JM. 2012. Sexual cannibalism benefits offspring survival. *Animal*
621   *Behaviour* 83:201-207. 10.1016/j.anbehav.2011.10.027

622 Welke KW, Zimmer SM, and Schneider JM. 2012. Conditional monogyny: female quality
623   predicts male faithfulness. *Frontiers in Zoology* 9. 10.1186/1742-9994-9-7

624 Williams GC. 1992. *Natural selection: domains, levels and challenges*.

625 Wyatt TD. 2014. *Pheromones and Animal Behavior: Chemical Signals and Signatures, 2nd*
626   *Edition*.

627 Zegelman L, Hassner A, Mendel Z, and Dunkelblum E. 1993. Synthesis and Field Bioassay of
628   the Israeli Pine Bast Scale, *Matsucoccus joseph*i, Female Sex-Pheromone. *Tetrahedron*
629   *Letters* 34:5641-5644. Doi 10.1016/S0040-4039(00)73904-9

630   Zimmer SM, Welke KW, and Schneider JM. 2012. Determinants of Natural Mating Success in

631   the Cannibalistic Orb-Web Spider *Argiope bruennichi*. *PLoS ONE* 7.

632   10.1371/journal.pone.0031389

633   Zuk M, and Kolluru GR. 1998. Exploitation of sexual signals by predators and parasitoids.

634   *Quarterly Review of Biology* 73:415-438. 10.1086/420412

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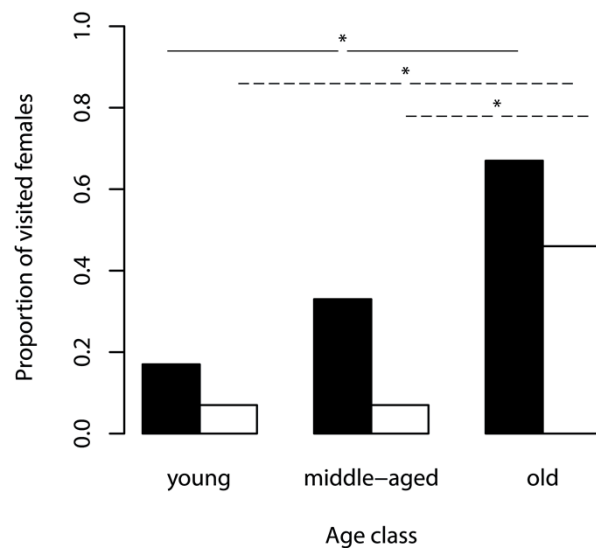
637

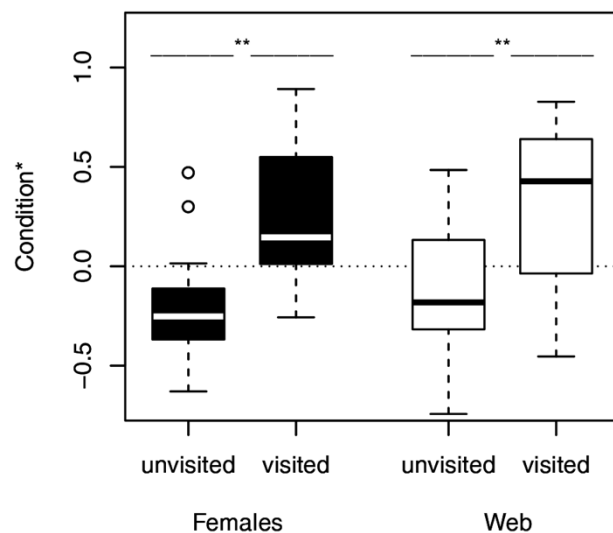
638 **FIGURES**

639

640 Figure 1: Proportion of visited females (black bars) and visited female webs (white bars)

641 depending on the female age. The solid lines (female body) and dashed lines (web silk) show

642 significant results between age classes. * = significant differences.

644

645

646   Figure 2: Condition of females separated into the occurrence of male visitation. Females and

647   their webs attracted more males, when they were in good condition. *The condition is shown as

648   the log-transformed residuals from the regression of weight and the size of the females. The

649   dotted line marks the zero line.

651

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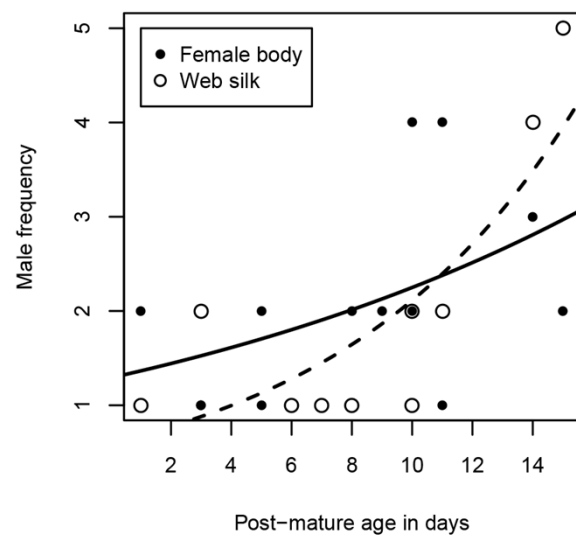
653   Figure 3: Number of male visitors depending on female post-mature age. * The lines show the

654   functional graphs for the "female body" data (solid line), the "web silk" data (dashed line).

655