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**

3   Anna-Lena Cory[1], Jutta M. Schneider[1]

4   [1]Zoologisches Institut, Universität Hamburg, Hamburg, Hamburg, Germany

6   Corresponding author:

7   Anna-Lena Cory[1]

8   Martin-Luther-King-Platz 3, Hamburg, 20146, Germany

9   Email address: anna_lena.cory@yahoo.de

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.

Introduction

Mating partners have to meet in order to mate and reproduce. This prerequisite may be a trivial aspect in group-living animals, but it can constitute a significant factor within the overall costs of sexual reproduction in other taxa (Kokko & Wong 2007; Magnhagen 1991). Encounters with sexual partners can be facilitated by a wide range of mechanisms, e.g. meeting at certain locations (Hoglund & Alatalo 1995; van Ginneken & Maes 2005) or producing long-range signals that attract potential mates (Gerhardt 1994; Roelofs 1995). The latter can occur via different communication channels (visual, acoustic or chemical), but which modality evolved will depend on the distance the signal has to travel in addition to other biotic and strategic selection pressures on signal design (Endler 1993). Here, we are concerned with chemical communication that is considered to be an evolutionary ancient modality demanding the lowest cost in comparison to acoustic, and visual signals (Bradbury & Vehrencamp 1998). However, this assumption is largely untested.

Both males and females can produce sex pheromones to attract mates (Tillman et al. 1999; Wyatt 2014), but particularly mate attraction by females is poorly understood. In many moths and spiders, females produce volatile or substrate bound pheromones, and males follow these traces (Gaskett 2007; Greenfield 1981; Wall & Perry 1987). Selection on male signal perception has led to astounding sensory sensibility and accuracy, as exemplified by the receptor density and functionality on the antennae of the male moth, *Bombyx mori* (Kaissling & Priesner 1970; Steinbrecht 1970). The solid understanding of natural selection on males as signal recipients is opposed by a rudimentary comprehension of the female perspective. It is largely unstudied but nevertheless debated, whether pheromones emitted for mate attraction by females can be 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 with the time a female remains unmated, females should increase the investment

102   in sex pheromone emission to enhance mate attraction over other females or to attract males

103   perhaps from larger distances. If female signalling is costly for *A. bruennichi* females, we would

104   expect that females attract more males than necessary. Note that only one mating event is needed

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

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

107   are ideal for testing this prediction since they not only allow the qualitative assessment of wide-

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

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

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

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

112   emit more pheromones.

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

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

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

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

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

118   of signalling is strikingly increased.

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120   Material & Methods

121   *Collection and Maintenance*

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

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

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

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

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

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

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

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

130   below).

131   *Female body measurements*

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

148 transformed the data for test weight.

Pheromone source

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

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

152 2010). In the "female body" trials, virgin females were placed into these neutral webs. Most

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

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

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

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

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

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

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

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

Female age classes

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

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

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

165 1.4), and "old females" had matured 8 or more days ago (10.0 ± 2.1). The webs were produced

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

167 ± 1.3 days and by old females of the average age of 10.0 ± 2.8 days. We used a 2-day time

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

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

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

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

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

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

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

175   was used once for only a single age class.

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

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

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

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

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

181   2012). To achieve a balanced availability of young and old females during the test period, we

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

201   condition on male attraction separately within each age class.

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

212   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	$F_{2,38}=1.88, p=0.1664$		$F_{2,29}=1.22, p=0.3105$		$F_{2,29}=2.88, p=0.0725$	

213   [1]The adult weight was measured shortly after maturity. [2]For the size, we used the tibia-patella

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

215   of the regression of body weight shortly maturation and size of the females.

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217   *Test preparation (field)*

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

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

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

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

222   according to similar male activity.

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

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

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

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

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

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

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

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

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

232   securely fixed on the ground.

233   *Test run*

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

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

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

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

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

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

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

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

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

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

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

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

246   *Data analysis*

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

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

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

250   from the analysis.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

275

276   Results

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

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

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

280   otherwise.

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

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

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

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

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

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

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

288 *Female body*

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

307 *Web silk*

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

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

310   14 webs of middle-aged females and the 14 webs of young females hardly attracted males at all

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

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

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

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

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

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

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

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

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

320   came from old females.

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

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

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

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

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

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

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

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

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

330

331   Discussion

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

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

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

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

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

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

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

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

340   difficult to separate both factors.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

359   2007). In our study, old females attracted many more males than needed for fertilisation and

360   more than of the maximal number of sires. Furthermore, webs were rather used for mate

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

377   2010). Investment in mate attraction may only become important at a later stage, while young

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

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

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

381   but of the same age attract more males.

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

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

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

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

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

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

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

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

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

391   studies.

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

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

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

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

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

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

398   deposited their first egg sac (Rittschof 2011).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

438   and benefit from attracting males after the first copulation.

439

440   Conclusion

441   We conclude that female signalling is a dynamic process and depend on the time spent

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

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

444

445   Acknowledgements

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

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

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

449   reviewers for helpful comments on the manuscript.

451   REFERENCES

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

453   Arakaki N, Wakamura S, and Yasuda T. 1996. Phoretic egg parasitoid, *Telenomus euproctidis*

454   (Hymenoptera: Scelionidae), uses sex pheromone of tussock moth *Euproctis taiwana*

455   (Lepidoptera: Lymantriidae) as a kairomone. *Journal of Chemical Ecology* 22:1079-

456   1085. 10.1007/BF02027946

457   Arnqvist G, and Nilsson T. 2000. The evolution of polyandry: multiple mating and female fitness

458   in insects. *Animal Behaviour* 60:145-164. 10.1006/anbe.2000.1446

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

460   Austad SN. 1982. First Male Sperm Priority in the Bowl and Doily Spider, *Frontinella*

461   *pyramitela* (Walckenaer). *Evolution* 36:777. 10.2307/2407891

462   Barry KL. 2010. Influence of female nutritional status on mating dynamics in a sexually

463   cannibalistic praying mantid. *Animal Behaviour* 80:405-411.

464   10.1016/j.anbehav.2010.05.024

465   Barry KL, Holwell GI, and Herberstein ME. 2008. Female praying mantids use sexual

466   cannibalism as a foraging strategy to increase fecundity. *Behavioral Ecology* 19:710-715.

467   10.1093/beheco/arm156

468   Barry KL, Holwell GI, and Herberstein ME. 2010. Multimodal mate assessment by male praying

469   mantids in a sexually cannibalistic mating system. *Animal Behaviour* 79:1165-1172.

470   10.1016/j.anbehav.2010.02.025

471   Baruffaldi L, Costa FG, Rodriguez A, and Gonzalez A. 2010. Chemical Communication in

472   *Schizocosa malitiosa*: Evidence of a Female Contact Sex Pheromone and Persistence in

473   the Field. *Journal of Chemical Ecology* 36:759-767. 10.1007/s10886-010-9819-x

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

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

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

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

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

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

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

481   10.1002/anie.200906311

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

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

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

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

486   2:S183-185. 10.1098/rsbl.2003.0042

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

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

489   10.1007/s00265-003-0656-6

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

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

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

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

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

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

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

497   59:569-578.

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

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

500   2656.2011.01942.x

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

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

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

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

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

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

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

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

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

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

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

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

513   36:527-532. Doi 10.1636/St06-63.1

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

515   57:23-&.

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

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

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

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

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

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

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

523   61:1162-1175. 10.1111/j.1558-5646.2007.00090.x

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

525   *Arachnology* 14:239-257.

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

527   6:183-185. Doi 10.1016/0169-5347(91)90210-O

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

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

530   10.1046/j.1365-2311.2000.00227.x

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

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

533   18:174-181. 10.1093/beheco/arl074

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

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

536   10.1111/j.1365-3032.1991.tb00571.x

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

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

539 Papke MD, Riechert SE, and Schulz S. 2001. An airborne female pheromone associated with
540   male attraction and courtship in a desert spider. *Animal Behaviour* 61:877-886.
541   10.1006/anbe.2000.1675

542 Perampaladas K, Stoltz JA, and Andrade MCB. 2008. Mated redback spider females re-advertise
543   receptivity months after mating. *Ethology* 114:589-598. 10.1111/j.1439-
544   0310.2008.01513.x

545 Prouvost O, Trabalon M, Papke M, and Schulz S. 1999. Contact sex signals on Web and cuticle
546   of *Tegenaria atrica* (Araneae, Agelenidae). *Archives of Insect Biochemistry and*
547   *Physiology* 40:194-202. 10.1002/(sici)1520-6327(1999)40:4<194::aid-arch4>3.0.co;2-p

548 Rabaneda-Bueno R, Rodriguez-Girones MA, Aguado-de-la-Paz S, Fernandez-Montraveta C, De
549   Mas E, Wise DH, and Moya-Larano J. 2008. Sexual Cannibalism: High Incidence in a
550   Natural Population with Benefits to Females. *PLoS ONE* 3.
551   10.1371/journal.pone.0003484

552 Riechert SE, and Singer FD. 1995. Investigation of potential male mate choice in a monogamous
553   spider. *Animal Behaviour* 49:715-723. 10.1016/0003-3472(95)90046-2

554 Rittschof CC. 2011. Mortality risk affects mating decisions in the spider *Nephila clavipes*.
555   *Behavioral Ecology* 22:350-357. 10.1093/beheco/arq222

556 Roberts JA, and Uetz GW. 2005. Information content of female chemical signals in the wolf
557   spider, *Schizocosa ocreata*: male discrimination of reproductive state and receptivity.
558   *Animal Behaviour* 70:217-223. 10.1016/j.anbehav.2004.09.026

559 Robinson MH, and Robinson B. 1973. Ecology and behaviour of the giant wood spider *Nephila*
560   *maculata* (Fabricius) in New Guinea. *Smithsonian Contributions to Zoology* No. 149:1-
561   75.

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

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

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

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

566   33:663-669. 10.1636/s03-32.1

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

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

569   10.1016/j.anbehav.2005.05.012

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

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

572   10.1111/eth.12466

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

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

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

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

577   10.1016/j.anbehav.2010.07.005

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

579   39:1-14. 10.1007/s10886-012-0231-6

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

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

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

583   1637. 10.1126/science.260.5114.1635

584 Searcy LE, Rypstra AL, and Persons MH. 1999. Airborne chemical communication in the wolf
585   spider *Pardosa milvina*. *Journal of Chemical Ecology* 25:2527-2533. Doi
586   10.1023/A:1020878225553

587 Steinbrecht RA. 1970. Morphometric Studies on Antenna of Silk Moth, *Bombyx mori* L. -
588   Number and Distribution of Olfactory Sensilla. *Zeitschrift Fur Morphologie Der Tiere*
589   68:93-&.

590 Stoltz JA, McNeil JN, and Andrade MCB. 2007. Males assess chemical signals to discriminate
591   just-mated females from virgins in redback spiders. *Animal Behaviour* 74:1669-1674.
592   10.1016/j.anbehav.2007.03.011

593 Sweger AL, Marr M, Satteson A, Rypstra AL, and Persons MH. 2010. The effects of predation
594   risk on female silk deposition and male response to predator-cued conspecifics in the
595   wolf spider, *Pardosa milvina* (Araneae: Lycosidae). *Journal of Arachnology* 38:393-397.
596   Doi 10.1636/Hi09-89.1

597 Thomas ML. 2011. Detection of female mating status using chemical signals and cues.
598   *Biological Reviews* 86:1-14. 10.1111/j.1469-185X.2010.00130.x

599 Tillman JA, Seybold SJ, Jurenka RA, and Blomquist GJ. 1999. Insect pheromones - an overview
600   of biosynthesis and endocrine regulation. *Insect Biochemistry and Molecular Biology*
601   29:481-514. 10.1016/s0965-1748(99)00016-8

602 Tuni C, and Berger-Tal R. 2012. Male preference and female cues: males assess female sexual
603   maturity and mating status in a web-building spider. *Behavioral Ecology* 23:582-587.
604   10.1093/beheco/ars001

605 Uhl G, Kunz K, Vocking O, and Lipke E. 2014. A spider mating plug: origin and constraints of
606   production. *Biological Journal of the Linnean Society* 113:345-354. 10.1111/bij.12359

607 Uhl G, Zimmer SM, Renner D, and Schneider JM. 2015. Exploiting a moment of weakness:

608   male spiders escape sexual cannibalism by copulating with moulting females. *Scientific*

609   *Reports* 5. 10.1038/srep16928

610 Umbers KD, Symonds MR, and Kokko H. 2015. The mothematics of female pheromone

611   signaling: strategies for aging virgins. *Am Nat* 185:417-432. 10.1086/679614

612 van Ginneken VJT, and Maes GE. 2005. The European eel (*Anguilla anguilla*, Linnaeus), its

613   Lifecycle, Evolution and Reproduction: A Literature Review. *Reviews in Fish Biology*

614   *and Fisheries* 15:367-398. 10.1007/s11160-006-0005-8

615 Wall C, and Perry JN. 1987. Range of Action of Moth Sex-Attractant Sources. *Entomologia*

616   *Experimentalis Et Applicata* 44:5-14.

617 Watson PJ. 1986. Transmission of a Female Sex-Pheromone Thwarted by Males in the Spider

618   *Linyphia litigiosa* (Linyphiidae). *Science* 233:219-221. 10.1126/science.3726530

619 Welke KW, and Schneider JM. 2012. Sexual cannibalism benefits offspring survival. *Animal*

620   *Behaviour* 83:201-207. 10.1016/j.anbehav.2011.10.027

621 Welke KW, Zimmer SM, and Schneider JM. 2012. Conditional monogyny: female quality

622   predicts male faithfulness. *Frontiers in Zoology* 9. 10.1186/1742-9994-9-7

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

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

625   *Edition*.

626 Zegelman L, Hassner A, Mendel Z, and Dunkelblum E. 1993. Synthesis and Field Bioassay of

627   the Israeli Pine Bast Scale, *Matsucoccus joseph*i, Female Sex-Pheromone. *Tetrahedron*

628   *Letters* 34:5641-5644. Doi 10.1016/S0040-4039(00)73904-9

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

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

631   10.1371/journal.pone.0031389

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

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

634

636

FIGURES

638

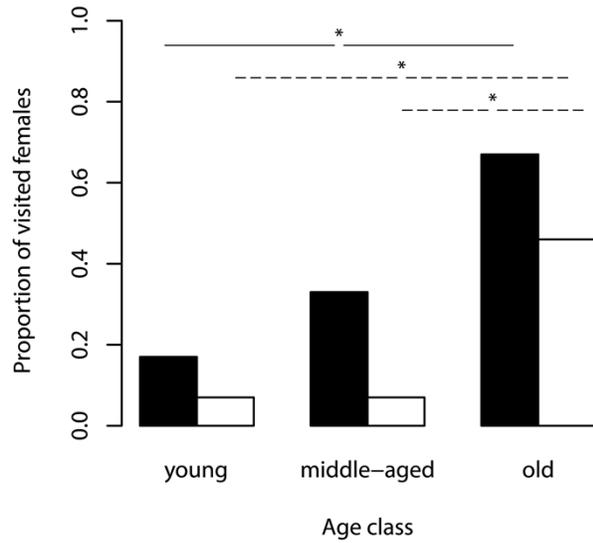
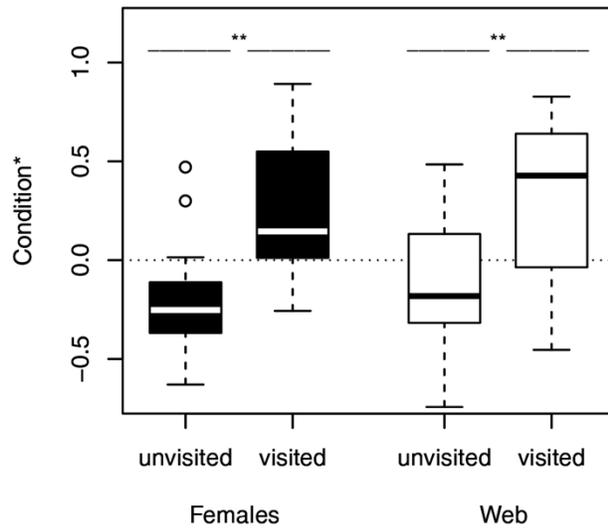



Figure 1: Proportion of visited females (black bars) and visited female webs (white bars)

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

significant results between age classes. * = significant differences.

643

644

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

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

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

648   dotted line marks the zero line.

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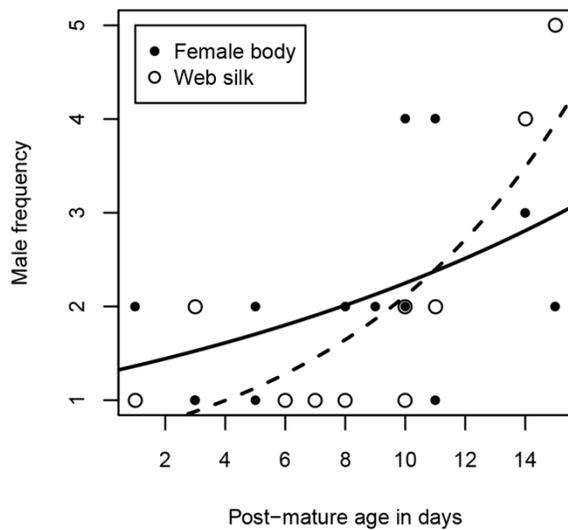
651

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

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

654