Mate-guarding duration is mainly influenced by the risk of sperm competition and not by female quality in a golden orb-weaver spider

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Males are expected to mate with as many females as possible, but can maximize their reproductive success through strategic mating decisions. For instance, males can increase their own fitness by mating with high quality females that produce more offspring. Additionally, males can adjust mating effort based on the relative distribution of females and male competitors. To test factors that influence male mate choice, we assessed male mating decisions in the golden silk orb-weaver spider, *Trichonephila clavipes* (Nephilidae), a species in which females are polyandrous, males guard females before and after copulation occurs and large males are the most successful at guarding mates. We tested the hypothesis that males spend more time guarding high quality females that are spatially isolated, and when the risk of sperm competition is higher. We also hypothesized that this effect increases with male body size. We assessed solitary and aggregated female webs in the field and quantified female quality (i.e. female body condition), male size (i.e. male body size), the risk of sperm competition (i.e. number of males in each female web), and mate-guarding duration (i.e. number of days each male spent in each web). We found that mate-guarding behaviour is largely influenced by the presence of male competitors. In addition, male body size seems to moderately influence male guarding decisions, with larger males guarding for a longer time. Finally, female body condition and type of web (i.e. solitary or aggregated) seem to play small roles in mate-guarding behaviour. As mate-guarding duration increased by 0.718 day per each additional male competitor in the web, and guarding behaviour prevents males from seeking additional mates, it seems that guarding females can be considerably costly. We conclude that failing to guard a sexual partner promotes high costs derived from sperm competition, and a male cannot recover his relative loss in fertilization success by seeking and fertilizing more females. In addition,

the search for more sexual partners can be constrained by possible high costs imposed by weight loss and fights against other males, which may explain why the type of web only moderately influenced male mate choice. Following the same rationale, if high-quality females are not easy to find and/or mating with a high-quality female demands much effort, males may search females and guard them regardless of female quality. In conclusion, the factor that most influences male mate-guarding behaviour among *T. clavipes* in the field is the risk of sperm competition.

1 **Mate-guarding duration is mainly influenced by the risk of sperm competition**

2 **and not by female quality in a golden orb-weaver spider**

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18 **Abstract**

19 Males are expected to mate with as many females as possible, but can maximize their reproductive

20 success through strategic mating decisions. For instance, males can increase their own fitness by

21 mating with high quality females that produce more offspring. Additionally, males can adjust

22 mating effort based on the relative distribution of females and male competitors. To test factors

23 that influence male mate choice, we assessed male mating decisions in the golden silk orb-weaver

24 spider, *Trichonephila clavipes* (Nephilidae), a species in which females are polyandrous, males

25 guard females before and after copulation occurs and large males are the most successful at

26 guarding mates. We tested the hypothesis that males spend more time guarding high quality

27 females that are spatially isolated, and when the risk of sperm competition is higher. We also

28 hypothesized that this effect increases with male body size. We assessed solitary and aggregated

29 female webs in the field and quantified female quality (i.e. female body condition), male size (i.e.

30 male body size), the risk of sperm competition (i.e. number of males in each female web), and

31 mate-guarding duration (i.e. number of days each male spent in each web). We found that mate-

32 guarding behaviour is largely influenced by the presence of male competitors. In addition, male

33 body size seems to moderately influence male guarding decisions, with larger males guarding for

34 a longer time. Finally, female body condition and type of web (i.e. solitary or aggregated) seem to

35 play small roles in mate-guarding behaviour. As mate-guarding duration increased by 0.718 day

36 per each additional male competitor in the web, and guarding behaviour prevents males from

37 seeking additional mates, it seems that guarding females can be considerably costly. We conclude

38 that failing to guard a sexual partner promotes high costs derived from sperm competition, and a

39 male cannot recover his relative loss in fertilization success by seeking and fertilizing more

40 females. In addition, the search for more sexual partners can be constrained by possible high costs

41   imposed by weight loss and fights against other males, which may explain why the type of web

42   only moderately influenced male mate choice. Following the same rationale, if high-quality

43   females are not easy to find and/or mating with a high-quality female demands much effort, males

44   may search females and guard them regardless of female quality. In conclusion, the factor that

45   most influences male mate-guarding behaviour among *T. clavipes* in the field is the risk of sperm

46   competition.

47

48

Introduction

49

50   The classical theory of sexual selection predicts that male reproductive success is positively

51   influenced by the number of females a male copulates with (Darwin, 1871; Bateman, 1948).

52   Therefore, following the Darwin-Bateman paradigm, males were initially predicted to mate with

53   the maximum number of females they can have access to (Dewsbury, 2005). However, factors

54   such as the quality of a potential mate, the difficulty in finding additional matings, or the intensity

55   of sperm competition should all influence the expected reproductive success of males (Scharf,

56   Peter & Martin, 2013). Consequently, developments in sexual selection theory lead to predictions

57   that males should adjust their mating investment based on expected reproductive success (Parker,

58   1998; Bonduriansky, 2001). This strategic adjustment of male mating investment should occur

59   especially in species in which males face high costs of mating or limited mating opportunities

60   (Schneider, 2014). Thus, male reproductive success is not always maximized by copulating with

61   as many females as possible.

62   Male strategic mating decisions are likely influenced by female quality (Bonduriansky,

63   2001; Edward & Chapman, 2011). For males, high quality females may be the females with the

64   best body condition, given that they are more likely to survive until oviposition and provide

65   resources for the eggs (Rittschof, 2011). Female body size can indicate female condition for males,

66   and it is also a predictor of female fecundity in arthropods (Honěk, 1993; Foellmer & Moya-

67   Laraño, 2007). Thus, copulating with large females could increase a male's reproductive success

68   as a result of a greater likelihood of female survival until oviposition and also as a result of an

69   increased number of viable eggs a male can fertilize. In scenarios in which female condition is

70   variable, males can optimize their reproductive success by choosing to mate with females that are

71   in the best condition (Reinhold, Kurtz & Engqvist, 2002). Thus, mating with a few high-quality

72   females may yield a greater reproductive success than mating with many low- or average-quality

73   females (Bonduriansky, 2001).

74   Complementarily, male strategic mating decisions may be influenced by the spatial

75   distribution of females and male competitors. For instance, whether females are scattered or

76   aggregated around a habitat should influence how males encounter females. This spatial

77   distribution dictates whether males encounter females in a more simultaneous or sequential

78   manner, and male mate choice is expected to evolve under the former condition (Barry & Kokko,

79   2010). For males that have to search for females, finding more than one viable partner can be easier

80   when females are "clumped", as there can be "hot-spots" of reproductive opportunity (Emlen &

81   Oring, 1977). However, when the costs of finding females are low, the probability of facing male

82   competitors and the risk of sperm competition tend to increase (Gage, 1995; Kokko & Rankin,

83   2006). Thus, when investigating aspects of male mate choice, one should take into account the

84   distribution of potential mates as well as the distribution of competitors. Whenever different

85   females can be easily found, males could optimize their reproductive success by investing in a

86   mating strategy of searching for several mates (Kasumovic & Jordan, 2013). On the other hand,

87   whenever several males compete for the same female, or females are hard to find, males could

88   optimize their reproductive success by defending the access to the female(s) (Seibt & Wickler,

89   1979).

90   In the golden silk orb-weaver spider, *Trichonephila clavipes*, females can mate with

91   multiple males (Rittschof *et al*., 2012) and males guard females both before mating, when females

92   are immature (Christenson & Goist, 1979), and after mating (Cohn, Balding & Christenson, 1988).

93   Females construct webs that can be physically connected to the webs built by other females or can

94   be completely isolated (Rypstra, 1985). Males are able to move to different webs and sample

95   females, which may allow them to choose the best options among potential sexual partners (Pollo,

96   Muniz & Santos, 2019). Additionally, males become sexually mature sooner than females in the

97   breeding season, thus they can mate-guard juvenile females and wait to copulate when they

98   become sexually mature (Christenson & Goist, 1979). Importantly, it is known that large males

99   have an advantage in intrasexual contests (Rittschof, 2010; Constant, Valbuena & Rittschof, 2011),

100   assuming hub male status and guarding females more frequently than small (satellite) males

101   (Christenson & Goist, 1979). Moreover, male competitive ability has been shown to influence

102   male mate choice. Large males have a preference for large females and small males prefer small

103   females (Pollo, Muniz & Santos, 2019). This assortative pattern is particularly relevant because

104   large females are more fecund (Rittschof, 2010). Therefore, good competitors (i.e., large males)

105   tend to achieve higher reproductive success than small (satellite) males.

106   Given that males in *T. clavipes* can adopt different strategies (i.e., guarding behaviour *vs*.

107   searching behaviour) and the outcome of these strategies depend on how much females vary in

108   quality and on how easy it is to find females, we asked: why do some *T. clavipes* males spend

109   more time guarding a given female instead of searching for more females, while other males do

110   not? Our hypothesis is that males spend more time guarding high quality females that are spatially

111   isolated, when the risk of sperm competition is higher and when the guarding male is larger.

112   Therefore, we predict that males will guard females for longer periods of time when (1) females

113   are in solitary webs that are not physically connected to other female webs, (2) there are more

114   satellite males in the female web, (3) females are in a better body condition, and (4) guardian males

115   are larger. We also test predictions based on two-way interactions between these variables, as these

116   are of biological interest. Males (especially large males) should invest more time guarding females

117   in good body condition when in solitary webs, and when there are more satellite males.

118

Methods

Data collection

121   We conducted our study in the gardens that surround the Zoology Department building at

122   Instituto de Biociências (23.564°S, 46.729°W) on the main campus of the University of São Paulo,

123   in São Paulo, Brazil. The garden is delimited by the Zoology Department building and by the

124   access roads around it. *T. clavipes* individuals present in the area build their webs using trees,

125   shrubs, lamp poles and walls as anchoring substrate. We made observations twice a day, at

126   approximately 9 A.M. and at 2 P.M. between the 11th of February and the 23rd of May of 2015.

127   We did not collect data (see below, for details) when there was heavy rain, because under these

128   conditions females can eat up to half of their webs (LADM, personal observation), influencing the

129   position of males, and, thus, our data collection.

130   We limited our data collection to those webs that were built between 0 and 2 m above the

131   ground (measured from the hub of the web to the ground) in order to allow us to individually mark

132   individuals and conduct accurate observations. During each visit to a web, we classified it as

133   "solitary" or "aggregated". If the web did not share threads with other webs, we considered it as a

134   solitary web. If the web shared threads with other webs, we considered it as an aggregated web.

135   The type of web could change from day to day, with new females sharing threads with a previously

136   solitary web or females becoming isolated because other females on the aggregation died. We

137   identified females by combinations of colours painted in the dorsal side of their abdomens using

138   acrylic paint (Testors). We conducted this procedure without removing the females from the web

139   to avoid disturbances that may cause females to abandon their webs. In some aggregations,

140   painting females' abdomen was not possible without removing threads or destroying adjacent

141   webs. Therefore, some females were identified daily by their web position in the aggregation, as

142   positions hardly changed from day to day (Vollrath, 1985; LADM, personal observation).

143   Additionally, because aggregations prevented us from accessing some females without damaging

144   webs, we could not accurately identify female age by assessing their epigynum. Nevertheless,

145   female age (penultimate instar females *vs*. moulting females *vs*. mature females) has little influence

146   on female attractiveness in another Brazilian population of *T. clavipes* (Almeida & Peixoto, 2021).

147   We also identified males with fine dots of acrylic paint on the dorsal side of their abdomen.

148   Each male received a unique combination of two or three colour dots that allowed individual

149   identification. In order to mark males when monitoring female webs, we removed the male from

150   the web with a fine paintbrush and placed him inside a Petri dish with a small scale bar if the male

151   was not uniquely identified before. We placed this Petri dish on top of ice and kept it inside an ice-

152   cooler for approximately two minutes. We used this cooling procedure to make males less active

153   in order to facilitate individual colour-marking and photographing males for posterior measuring.

154   Each male received a unique combination of two or three colour dots that allowed individual

155   identification. We recorded males as "guardians" if they were occupying a central position on the

156   web and were the closest male to the female. We identified males as "satellites" if they were on

157   sustaining threads or on the periphery of the female web and were not the closest male to the

158   female. We returned males by placing them individually inside a plastic cup and holding it as close

159   as possible to the male's original position on the web. Later, with the photos we took of males, we

160   used the software Image J (Schneider, Rasband & Eliceiri, 2012) to calculate male cephalothorax

161   width (mm), our proxy for male body size.

162   We photographed females at intervals of approximately 10 days. All photos contained a

163   scale bar for posterior measuring. We measured cephalothorax width (mm), abdomen width (mm),

164   and abdomen length (mm) of females using Image J (Schneider, Rasband & Eliceiri, 2012) to infer

165   body condition, our proxy for female quality, as larger females produce more offspring (Rittschof,

166   2010) and females with higher body condition are more attractive (Rittschof, 2011). If more than

167   one photo of a female was taken, the photos used for measurement were the ones that were taken

168   closer to the period a male stayed with the female. Female abdomen volume was estimated based

169   on abdomen length and width, and assuming its shape to be equivalent to the volume of a cylinder

170    $V = \pi \times r^2 \times h$. We used residuals from a regression between the logarithm of abdomen volume

171   (response variable) and cephalothorax width (predictor) to calculate a body condition index. This

172   body condition index provides an estimate that is uncorrelated with body size, and this approach

173   is widely used in studies with spiders (Taylor, Price & Wedell, 2014; Macedo-Rego *et al.*, 2016).

174   Negative values represent females with smaller predicted abdomen volume given their

175   cephalothorax width, whereas positive values represent females with larger predicted abdomen

176   volume given their cephalothorax width.

177

178   **Data analysis**

179   To test our predictions about the amount of time guarding males spent with females, we

180   ran generalized linear mixed models (GLMMs) with model selection and model averaging based

181   on AIC (Burnham & Anderson, 2002). The response variable of the models was time spent (in

182   days) by a guardian male with the same female. Thus, we fitted GLMMs with a Poisson error

183   structure and a log link function. In all models, we used male identity as a random factor to account

184   for the repeated observations made on individual males. Moreover, to account for overdispersion,

185   we included an observation level random effect in the model. We included the type of web

186   (aggregated or solitary) as a categorical binary predictor. The risk of sperm competition was

187   included as a predictor variable and was coded as the maximum number of males present on the

188   web during the time a guarding male spent with the same female. We also included female body

189   condition index and male body size as continuous predictors. We included two-way interactions

190   between female condition, male body size, number of males, and type of web in the global model.

191   We standardized all input variables with the function rescale from the package *arm* (Gelman &

192   Su, 2016) to be able to directly compare effect sizes of the predictors and to allow comparison of

193   the effects when interactions are present.

194   We used model averaging (Burnham & Anderson, 2002; Grueber *et al*., 2011), with the

195   functions available in the package *MuMIn* (Bartón, 2018), to determine what were the most

196   important predictors in our GLMM. We built a global model with all predictors and interactions

197   based on our predictions, and then derived a set of models with all combinations of explanatory

198   variables. We defined our top model set as those models that fell within two AICC of the best

199   model in the set. We used the natural-average method to conduct model averaging (Burnham &

200   Anderson, 2002; Grueber *et al*., 2011; Nakagawa & Freckleton, 2011). Model averaging yields

201   two outputs, the standardized coefficients (and their unconditional standard errors, which

202   incorporate model-selection uncertainty) and the relative importance of each coefficient for

203   explaining the variance in the response variable (Grueber *et al*., 2011). We present estimated

204   parameters along with their 95% compatibility intervals (CIs) and discuss our findings interpreting

205   the parameter point estimates, while at the same time acknowledging their uncertainty

206   (Wasserstein, Schirm & Lazar, 2019). All GLMMs were built using the *lme4* package (Bates *et*

207   *al*., 2015) in the R programming environment (R Core Team, 2017).

208

209 Results

210   We sampled a total of 83 males and we classified 39 of them as guardian males. We

211 sampled a total of 31 females (22 aggregated and 14 solitary; type of web sums to 36 webs because

212 some females changed between types). The median number of males — including guarding and

213 satellite males — in a web was 1 (range: 1 to 5; aggregated webs: 1 [1 to 3] male; solitary webs: 1

214 [1 to 5] male). We re-sighted 25 guardian males in at least another web of a different female than

215 the one he was originally observed with. Female body condition, estimated by the residual of

216 abdomen volume and cephalothorax width, ranged from -492.14 to 516.46 (mean female body

217 condition = -6.94, S.D. = 243.0). Male body size ranged from 1.08 to 2.68 mm (mean male body

218 size = 1.97 mm, S.D. = 0.37 mm). The average distance between focal webs and the closest

219 neighbour web was 9.21 ± 6.55 m S.D. for solitary webs and 10.5 ± 8.5 m S.D. for aggregated

220 webs.

221   We generated a model set (including the null model) from the global model that resulted

222 in 49 models (see Supplemental Table S1). Including the best model, we had three models within

223 two AICC top model set (Table 1). All the top models contained the predictors "number of males",

224 "female condition" and the interaction between "number of males" and "female condition". Two

225 models contained the predictor "male body size", and one model contained the interaction between

226 "male body size" and "female condition" (Table 2).

227   The parameter estimate for the number of satellite males on the web was positive,

228 indicating that the amount of time a guarding male spent with a female increased as the number of

229 males on the web increased (Table 2, Fig. 1). The 95% CI for the number of males on the web

230 ranged from 0.357 to 1.079, suggesting that our data is compatible with small to large positive

231 effects of the presence of satellite males on the time spent guarding (Table 2). The male body size

232 parameter was also positive, but the confidence interval overlapped zero (Table 2). The interaction

233 between the number of satellite males on a web and female condition was negative, meaning that

234 mate-guarding duration decreases as both the number of competitor males and female quality

235 increase. The respective 95% CI ranged from -1.998 to -0.596 which is compatible with a small to

236 large negative effect of this interaction term on the time spent guarding (Table 2). The female

237 condition parameter and the interaction between male body size and female condition were

238 negative but presented unconditional standard error estimates (which incorporates model-selection

239 uncertainty) that were large relative to their effect sizes. Thus, the 95% compatibility intervals for

240 these estimates overlapped zero, suggesting that these results are most likely compatible with no

241 important effects (Table 2).

242

243 **Discussion**

244   Here, in a population of *T. clavipes*, we showed that the number of males cohabiting a

245 female web is the factor that, alone, best explained the variation in male mate-guarding behaviour.

246 In other words, males seem to guard females for longer periods when the risk of sperm competition

247 is higher, and sperm competition seems to be the factor that influences male mating decisions the

248 most. Importantly, we also showed that the intrinsic quality of females interacts negatively with

249 the number of males on a web, which indicates that guardian males reduce guarding time when

250 higher quality females have more satellite males on their webs. Additionally, we showed that male

251 body size may moderately influence male mating decisions, with larger males investing more time

252 in guarding position, but note that the compatibility interval of this estimate overlapped zero.

253 Regarding the interaction between male body size and female quality, we found a small reduction

254 in mate-guarding duration among larger males as higher the female quality was, but the

255 compatibility interval overlapped zero. Similarly, we showed that female quality alone seems to
256 have a small negative influence on mate-guarding duration, but the compatibility interval
257 overlapped zero, again. Finally, we found that web connectivity had little to no influence in mate-
258 guarding duration, once none of the selected models included the type of web. Below, we discuss
259 why the risk of sperm competition is more effective in shaping male mate-guarding behaviour than
260 web connectivity, female quality and male size.

261 As the number of satellite males on a web increases, it is more likely that some of these
262 males will manage to copulate with the female, leading to sperm competition for the fertilization
263 of her eggs. This is a probable scenario given that it is now well-known that females mate multiply
264 (Gowaty, 2006; Taylor, Price & Wedell, 2014), with several examples among spiders (*e.g.*, Prokop
265 & Maxwell, 2009; González, Costa & Peretti, 2019), including *T. clavipes* (Rittschof *et al.*, 2012).
266 A male may avoid the risk of sperm competition by guarding a female and, therefore, preventing
267 competitors from mating with her, as widely documented for spiders (*e.g.*, Herberstein *et al.*, 2005;
268 Elias *et al.*, 2014). Moreover, the higher the number of competitors, the higher should be the effort
269 employed by guarding males. Accordingly, in our study, mate-guarding time increased 0.718 day
270 per each additional satellite male on the web, which corresponds to 3.42% to 5.13% of a male's
271 life as an adult (Brown, 1985). Therefore, if a male is guarding a female that attracted several
272 satellite males, this guardian male tends to spend a great part of his adult life guarding one single
273 female, instead of increasing his reproductive success by mating with a second female. In addition,
274 despite the occurrence of sperm limitation in *T. clavipes* (Michalik & Rittschof, 2011), males might
275 not transfer all their sperm to a single female (Christenson & Cohen, 1988; Cohn, 1990; Rittschof,
276 2011; Schneider *et al.*, 2011), which allows them to mate again. Consequently, losing the
277 opportunity to mate with a second female can impose a high fitness cost. Given this, we can ask:

278   if guarding females can be so costly, why do *T. clavipes* males guard females? Probably because

279   the fitness costs of seeking additional mates and facing sperm competition are higher than the costs

280   of mate-guarding behaviour, and males have a higher fitness return when reducing their lifetime

281   mating success and guarding their current sexual partners.

282   Contrary to the number of satellite males present, whether a web is clustered or isolated

283   seems to be a less important factor in determining the amount of time that a male spends guarding

284   a female in *T. clavipes*. At first glance, one could consider that both the number of males and the

285   type of web inform the risk of sperm competition and should influence mate-guarding duration.

286   However, we know that the number of satellite males on a web is not related (see Supplemental

287   Material) to the type of web, and that males in *T. clavipes* arrive at aggregated and solitary webs

288   in equal frequencies (Meraz, Hénaut & Elgar, 2012). Therefore, the type of web is not a good

289   proxy for the risk of sperm competition and any influence of web type on mate-guarding is

290   probably explained by other variables. However, guardian males on aggregated webs still have a

291   clear path to at least one other female in case they decide to leave their current partner, while

292   guardian males on solitary webs are not able to access other female webs easily, and would be less

293   keen on leaving their web. Surprisingly, the effect of web connectivity alone on mate-guarding

294   duration is small to non-existent, which may indicate that, despite the connectivity provided by

295   aggregated webs, leaving a given web and mating multiply brings high costs for males, regardless

296   of web type. These costs of moving among webs and searching for additional mates might include

297   weight loss (Meraz, Hénaut & Elgar, 2012) and fighting with competitors (Rittschof, 2010). These

298   may explain why the type of web has little to no influence on mate-guarding behaviour in the

299   studied spider. Another possibility is that connectivity between webs is not as important as the

300   distances between them. As the distances are not affected by web connectivity in the studied

301   population, this can explain why mate-guarding behaviour was little or not influenced by web type.

302   The possible costs derived from looking for high-quality mates and fighting with many

303   competitors may also explain why males did not spend more time guarding high-quality females.

304   If the search for females incurs severe survival costs for males (see Kasumovic *et al.*, 2007; Berger-

305   Tal & Lubin, 2011), the distribution of males among female webs may be little influenced by

306   female quality, especially if high-quality females are not easy to find. Given this and that looking

307   for a high-quality female probably means facing extreme intrasexual competition, males might

308   avoid leaving a current sexual partner, regardless of its quality. However, one should not conclude

309   from our study that males in *T. clavipes* never express mate choice. Recent papers show that male

310   mate choice is more common in nature than previously expected (Edward & Chapman, 2011), and

311   field experiments have shown that large *T. clavipes* males prefer large females when given the

312   option to guard one of two females of different body size (Pollo, Muniz & Santos, 2019). In

313   addition, we highlight that our study is restricted to mate-guarding behaviour. Therefore, it is

314   possible that male mate choice occurs in contexts other than mate-guarding, such as pre-mating

315   courtship (*e.g.*, which female should the male court?) or post-mating competition (*e.g.*, how much

316   sperm should the male transfer to a female?). In fact, Rittschof (2011) showed that *T. clavipes*

317   males transfer more sperm to females that are close to oviposition. Interestingly, females close to

318   oviposition are the ones that will have less opportunity to mate with additional mates, which means

319   that their sexual partners tend to face lower sperm competition. Therefore, because we demonstrate

320   that mate-guarding behaviour is mainly determined by the density of male competitors, our results

321   and the results provided by Rittschof (2011) reinforce the idea that male reproductive effort in *T.*

322   *clavipes* is mostly influenced by the risk of sperm competition.

323   As mate-guarding duration is mainly influenced by the risk of sperm competition and

324   female quality has little to no influence, it would be reasonable to expect increasing mate-guarding

325   duration in response to concomitant increases in female quality and sperm competition risk.

326   However, we found the opposite. Why would a male reduce his guarding effort when mate-

327   guarding is more important (i.e. there are more competitors on the web) and the potential fitness

328   benefits are higher (i.e. high-quality females are more fecund, Rittschof, 2010)? Two non-

329   exclusive hypotheses can explain this result. First, regarding the number of males on a web, there

330   may be a guarding threshold, above which guardian males cease mate-guarding and leave the web

331   (analogously, across animals, aggressive behaviour increases as the OSR becomes more male-

332   biased and reduces after reaching a threshold; Weir, Grant & Hutchings, 2011). Such hypothetical

333   threshold may be easily reached on webs of high-quality females, as they are more attractive to

334   large males (Pollo, Muniz & Santos, 2019), and large males are the best competitors when it comes

335   to fighting for the guardian position (Constant, Valbuena, & Rittschof, 2011). Second, it is possible

336   that satellite males behave differently according to female quality, being more aggressive towards

337   the guardian male when competing for a high-quality female (increased male aggressive behaviour

338   due to high female quality has been demonstrated for other taxa; Weiss & Dubin, 2018; Kapranas

339   *et al.*, 2020), which would make guarding effort more costly and would reduce guarding duration.

340   In addition, as high-quality females attract more good competitors (i.e., large males; Pollo, Muniz

341   & Santos, 2019), it is possible that satellite males become even more aggressive and/or bold due

342   to increased pre-mating competition, leading to an even lower mate-guarding duration.

343   Similarly to the interaction between the number of competitor males and female quality,

344   the interaction between male body size and female quality is negatively correlated with mate-

345   guarding duration. However, the influence of this interaction is much lower and the compatibility

346   interval overlaps zero. Therefore, from a guardian's perspective, it seems that self-evaluation plays

347   a less important role than evaluating external factors, such as quantity and quality of conspecifics.

348   While male body size alone has a moderate positive influence on mate-guarding duration,

349   indicating that self-evaluation has some relevance and corroborating the evidence that larger males

350   are better fighters (Constant, Valbuena & Rittschof, 2011), the influence of sperm competition risk

351   alone is more than three times higher, reinforcing the idea that external factors are the ones that

352   best modulate mate-guarding duration. More specifically, because the number of male competitors

353   showed to be the most important factor influencing mate-guarding, our results provide additional

354   evidence that post-mating competition plays an important role in determining male reproductive

355   investment in animals (Parker & Pizzari, 2010). In this sense, as guarding behaviour in *T. clavipes*

356   can occur before (Christenson & Goist, 1979) and after (Cohn, Balding & Christenson, 1988)

357   mating takes place and both forms of guarding behaviour influence the sperm competition faced

358   by males, future experimental studies should assess whether the risk of sperm competition

359   influences pre- and post-mating guarding effort in a similar manner. Additionally, as pre- and post-

360   ejaculatory investments may be traded-off (Parker, 2020), future studies should also evaluate how

361   investment in mate-guarding and investment in ejaculate are related in *T. clavipes* and other

362   species. Complementarily, given the relevance of post-mating events in male decisions and that

363   spiders are good systems to study cryptic female choice (Eberhard, 2004), future studies should

364   address how mate-guarding duration influence the way females use the sperm of each sexual

365   partner in *T. clavipes* and other spider species.

366

367 **References**

368 Almeida JGL, Peixoto PEC. 2021. In search of the perfect web? Males of the golden silk orb-web

369  spider *Trichonephila clavipes* do not aggregate in webs of high-quality females. *Ethology*

370  127:342–349. DOI: 10.1111/eth.13132

371 Barry KL, Kokko H. 2010. Male mate choice: why sequential choice can make its evolution

372  difficult. *Animal Behaviour* 80:163–169. DOI: 10.1016/j.anbehav.2010.04.020

373 Bartón K. 2018. *MuMIn: Multi-model inference*. R package version 1.40.4.

374 Bateman AJ. 1948. Intra-sexual selection in *Drosophila*. *Heredity* 2:349–368. DOI:

375  10.1038/hdy.1948.21

376 Bates D, Mächler M, Bolker BM, Walker SC. 2015. Fitting linear mixed-effects models using

377  lme4. *Journal of Statistical Software* 67:1–48. DOI: 10.18637/jss.v067.i01

378 Berger-Tal R, Lubin Y. 2011. High male mate search costs and a female-biased sex ratio shape

379  the male mating strategy in a desert spider. *Animal Behaviour* 82:853–859. DOI:

380  10.1016/j.anbehav.2011.07.021

381 Bonduriansky R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and

382  evidence. *Biological Reviews* 76:305–339. DOI: 10.1017/S1464793101005693

383 Brown SG. 1985. Mating behavior of the golden-orb-weaving spider, *Nephila clavipes*: II. Sperm

384  capacitation, sperm competition, and fecundity. *Journal of Comparative Psychology* 99:167–

385  175. DOI: 10.1037/0735-7036.99.2.167

386 Burnham KP, Anderson DR. 2002. *Model selection and multimodel inference: A practical*

387  *information-theoretic approach*. New York, United States: Springer US. DOI:

388  10.2307/3803117

389   Christenson TE, Goist KC. 1979. Costs and benefits of male-male competition in the orb weaving

390   spider, *Nephila clavipes*. *Behavioral Ecology and Sociobiology* 5:87–92. DOI:

391   10.1007/BF00302697

392   Christenson TE, Cohen J. 1988. Male advantage for egg fertilization in the golden orb-weaving

393   spider (*Nephila clavipes*). *Journal of Comparative Psychology* 102:312–318. DOI:

394   10.1037/0735-7036.102.4.312

395   Cohn J. 1990. Is it the size that counts? Palp morphology, sperm storage, and egg hatching

396   frequency in *Nephila clavipes* (Araneae, Araneidae). *The Journal of Arachnology* 18:59–71.

397   Cohn J, Balding FV, Christenson TE. 1988. In defense of *Nephila clavipes*: Postmate guarding by

398   the male golden orb-weaving spider. *Journal of Comparative Psychology* 102:319–325. DOI:

399   10.1037/0735-7036.102.4.319

400   Constant N, Valbuena D, Rittschof CC. 2011. Male contest investment changes with male body

401   size but not female quality in the spider *Nephila clavipes*. *Behavioural Processes* 87:218–

402   223. DOI: 10.1016/j.beproc.2011.04.003

403   Darwin C. 1871. *The descent of man and selection in relation to sex*. London, United Kingdom: J.

404   Murray.

405   Dewsbury DA. 2005. The Darwin-Bateman paradigm in historical context. *Integrative and*

406   *Comparative Biology* 45:831–837. DOI: 10.1093/icb/45.5.831

407   Eberhard WG. 2004. Why study spider sex: special traits of spiders facilitate studies of sperm

408   competition and cryptic female choice. *Journal of Arachnology* 32:545–556. DOI:

409   10.1636/0161-8202(2004)032[0545:WSSSST]2.0.CO;2

410   Edward DA, Chapman T. 2011. The evolution and significance of male mate choice. *Trends in*

411   *Ecology and Evolution* 26:647–654. DOI: 10.1016/j.tree.2011.07.012

412   Elias DO, Sivalinghem S, Mason AC, Andrade MCB, Kasumovic MM. 2014. Mate-guarding

413   courtship behaviour: Tactics in a changing world. *Animal Behaviour* 97:25–33. DOI:

414   10.1016/j.anbehav.2014.08.007

415   Emlen ST, Oring LW. 1977. Ecology, sexual selection, and the evolution of mating systems.

416   *Science* 197:215–223.

417   Foellmer MW, Moya-Laraño J. 2007. Sexual size dimorphism in spiders: patterns and processes.

418   In: Fairbairn DJ, Blanckenhorn WU, Székely T eds. *Sex, size and gender roles: Evolutionary*

419   *studies of sexual size dimorphism*. Oxford, United Kingdom: Oxford University Press, 71–

420   81.

421   Gage MJG. 1995. Continuous variation in reproductive strategy as an adaptive response to

422   population density in the moth *Plodia interpunctella*. *Proceedings of the Royal Society B:*

423   *Biological Sciences* 261:25–30. DOI: 10.1098/rspb.1995.0112

424   Gelman A, Su Y-S. 2016. *arm: Data analysis using regression and multilevel/hierarchical models.*

425   R package version 1.9-3.

426   González M, Costa FG, Peretti A V. 2019. Different levels of polyandry in two populations of the

427   funnel-web wolf spider *Aglaoctenus lagotis* from South America. *Journal of Ethology*

428   37:325–333. DOI: 10.1007/s10164-019-00606-5

429   Gowaty PA. 2006. Beyond extra-pair paternity: Constraints, fitness components, and social mating

430   systems. In: Lucas JR, Simmons LW eds. *Essays in Animal Behaviour - Celebrating 50 Years*

431   *of Animal Behaviour*. Burlington: Elsevier Academic Press, 221–254.

432   Grueber CE, Nakagawa S, Laws RJ, Jamieson IG. 2011. Multimodel inference in ecology and

433   evolution: challenges and solutions. *Journal of Evolutionary Biology* 24:699–711. DOI:

434   10.1111/j.1420-9101.2010.02210.x

435   Herberstein ME, Barry KL, Turoczy MA, Wills E, Youssef C, Elgar MA. 2005. Post-copulation

436   mate guarding in the sexually cannibalistic St. Andrew's Cross spider (Araneae Araneidae).

437   *Ethology Ecology and Evolution* 17:17–26. DOI: 10.1080/08927014.2005.9522612

438   Honĕk A. 1993. Intraspecific variation in body size and fecundity in insects: a general relationship.

439   *Oikos* 66:483–492. DOI: 10.2307/3544943

440   Kapranas A, Zenner ANRL, Mangan R, Griffin CT. Objective and subjective components of

441   resource value in lethal fights between male entomopathogenic nematodes. *Animal Behaviour*

442   164:149–154. DOI: 10.1016/j.anbehav.2020.04.015

443   Kasumovic MM, Bruce MJ, Herberstein ME, Andrade MCB. 2007. Risky mate search and mate

444   preference in the golden orb-web spider (*Nephila plumipes*). *Behavioral Ecology* 18:189–195.

445   DOI: 10.1093/beheco/arl072

446   Kasumovic MM, Jordan LA. 2013. Social factors driving settlement and relocation decisions in a

447   solitary and aggregative spider. *American Naturalist* 182:532–541. DOI: 10.1086/671930

448   Kokko H, Rankin DJ. 2006. Lonely hearts or sex in the city? Density-dependent effects in mating

449   systems. *Philosophical Transactions of the Royal Society B: Biological Sciences* 361:319–

450   334. DOI: 10.1098/rstb.2005.1784

451   Macedo-Rego RC, Costa-Schmidt LE, Santos ESA, Machado G. 2016. Negative effects of

452   prolonged dietary restriction on male mating effort: nuptial gifts as honest indicators of long-

453   term male condition. *Scientific Reports* 6:1–8. DOI: 10.1038/srep21846

454   Meraz LC, Hénaut Y, Elgar MA. 2012. Effects of male size and female dispersion on male mate-

455   locating success in *Nephila clavipes*. *Journal of Ethology* 30:93–100. DOI: 10.1007/s10164-

456   011-0300-2

457   Michalik P, Rittschof CC. 2011. A comparative analysis of the morphology and evolution of

458   permanent sperm depletion in spiders. *PLoS ONE* 6:e16014. DOI:

459   10.1371/journal.pone.0016014

460   Nakagawa S, Freckleton RP. 2011. Model averaging, missing data and multiple imputation: a case

461   study for behavioural ecology. *Behavioral Ecology and Sociobiology* 65:103–116. DOI:

462   10.1007/s00265-010-1044-7

463   Parker GA. 1998. Sperm competition and the evolution of ejaculates: towards a theory base. In:

464   Birkhead TR, Møller AP eds. *Sperm competition and sexual selection*. London, United

465   Kingdom: Academic Press, 3–54.

466   Parker GA. 2020. Conceptual developments in sperm competition: a very brief synopsis.

467   *Philosophical Transactions of the Royal Society B: Biological Sciences* 375:1–10. DOI:

468   10.1098/rstb.2020.0061

469   Parker GA, Pizzari T. 2010. Sperm competition and ejaculate economics. *Biological Reviews*

470   85:897–934. DOI: 10.1111/j.1469-185X.2010.00140.x.

471   Pollo P, Muniz DG, Santos ESA. 2019. Be prudent if it fits you well: male mate choice depends

472   on male size in a golden orb-weaver spider. *Animal Behaviour* 156:11–20. DOI:

473   10.1016/j.anbehav.2019.06.030

474   Prokop P, Maxwell MR. 2009. Female feeding regime and polyandry in the nuptially feeding

475   nursery web spider, *Pisaura mirabilis*. *Naturwissenschaften* 96:259–265. DOI:

476   10.1007/s00114-008-0477-6

477   R Core Team. 2017. R: A language and environment for statistical computing.

478   Reinhold K, Kurtz J, Engqvist L. 2002. Cryptic male choice: sperm allocation strategies when

479   female quality varies. *Journal of Evolutionary Biology* 15:201–209. DOI: 10.1046/j.1420-

480   9101.2002.00390.x

481   Rittschof CC. 2010. Male density affects large-male advantage in the golden silk spider, *Nephila*

482   *clavipes*. *Behavioral Ecology* 21:979–985. DOI: 10.1093/beheco/arq099

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

484   *Behavioral Ecology* 22:350–357. DOI: 10.1093/beheco/arq222

485   Rittschof CC, Hilber SA, Tudor MS, St Mary CM. 2012. Modeling male reproductive strategies

486   and optimal mate number in an orb-web spider. *Behavioral Ecology* 23:1–10. DOI:

487   10.1093/beheco/arr142

488   Rypstra AL. 1985. Aggregations of *Nephila clavipes* (L.) (Araneae, Araneidae) in relation to prey

489   availability. *Journal of Arachnology* 13:71–78.

490   Scharf I, Peter F, Martin OY. 2013. Reproductive trade-offs and direct costs for males in

491   arthropods. *Evolutionary Biology* 40:169–184. DOI: 10.1007/s11692-012-9213-4

492   Schneider JM. 2014. Sexual cannibalism as a manifestation of sexual conflict. *Cold Spring Harbor*

493   *Perspectives in Biology* 6:1–16. DOI: 10.1101/cshperspect.a017731

494   Schneider JM, Lucass C, Brandler W, Fromhage, L. 2011. Spider males adjust mate choice but not

495   sperm allocation to cues of a rival. *Ethology* 117:970–978. DOI: 10.1111/j.1439-

496   0310.2011.01960.x

497   Schneider CA, Rasband WS, Eliceiri KW. 2012. NIH Image to ImageJ: 25 years of image analysis.

498   *Nature Methods* 9:671–675. DOI: 10.1038/nmeth.2089

499   Seibt U, Wickler W. 1979. The biological significance of the pair-bond in the shrimp *Hymenocera*

500   *picta*. *Zeitschrift für Tierpsychologie* 50:166–179. DOI: 10.1111/j.1439-0310.1979.tb01024.x

501   Taylor ML, Price TAR, Wedell N. 2014. Polyandry in nature: a global analysis. *Trends in Ecology*

502   *and Evolution* 29:376–383. DOI: 10.1016/j.tree.2014.04.005

503   Vollrath, F. 1985. Web spider's dilemma: a risky move or site dependent growth. *Oecologia* 68:69–

504   72. DOI: 10.1007/BF00379476

505   Wasserstein RL, Schirm AL, Lazar NA. 2019. Moving to a world beyond "p < 0.05". *The*

506   *American Statistician* 73:1–19. DOI: 0.1080/00031305.2019.1583913

507   Weir LK, Grant JWA, Hutchings JA. 2011. The influence of operational sex ratio on the intensity

508   of competition for mates. *American Naturalist* 177:167-176. DOI: 10.1086/657918

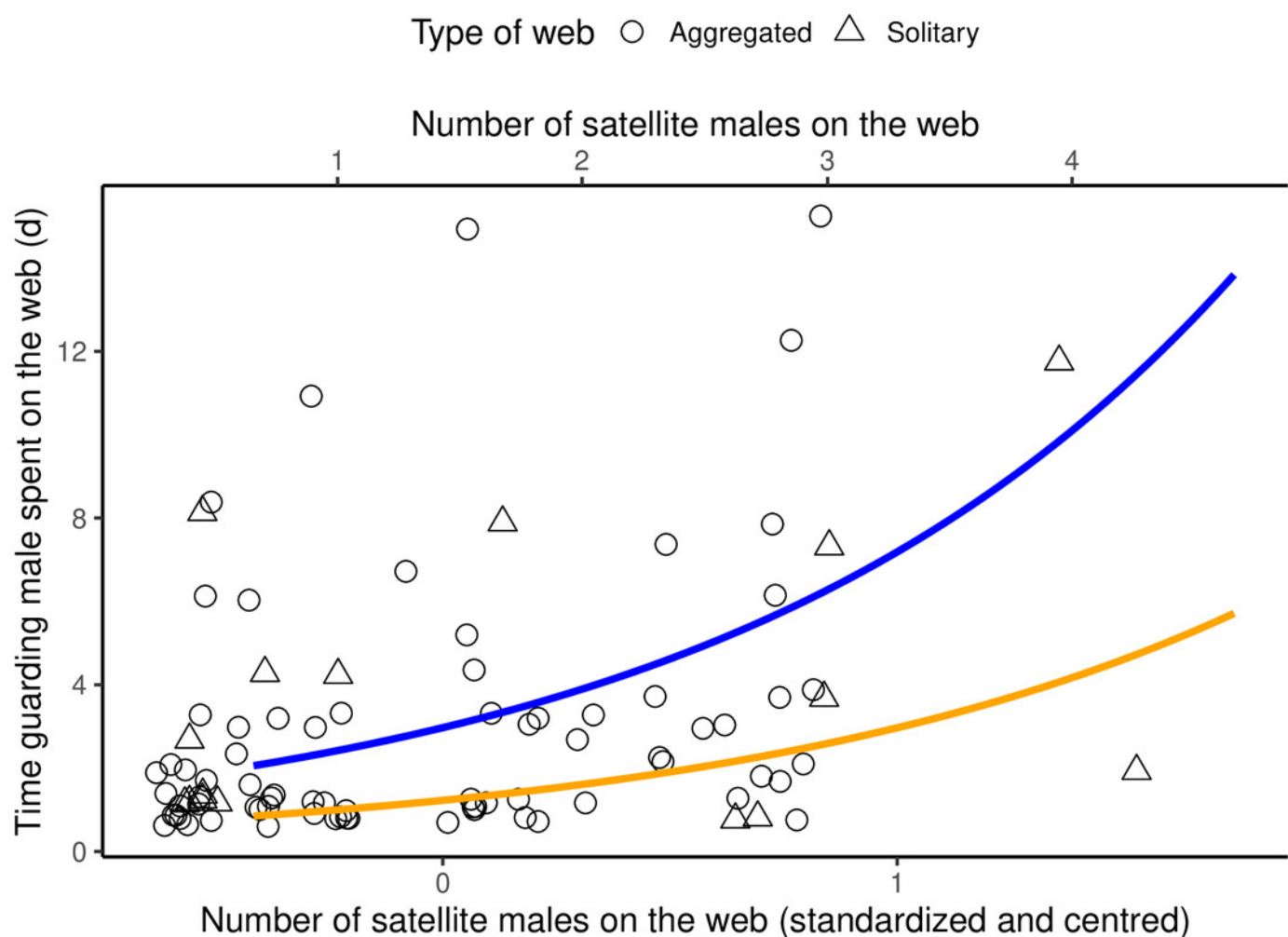
509   Weiss SL, Dubin M. 2018. Male mate choice as differential investment in contest competition is

510   affected   by   female   ornament   expression.   *Current   Zoology*   64:335-344.   DOI:

511   10.1093/cz/zoy023

Figure 1

Relationship between the number of males (primary x-axis: centred and scaled; secondary x-axis: original data) on the female web and the time the guarding male spent (in hours) with a female on her web.

Aggregated webs are represented by circles; solitary webs by triangles. Regression line (in blue) shown from coefficients of average model. Original points have been jittered horizontally to minimize overlap. One observation in which a male spent 32 days in a female web was removed from the plot for better visualization.

Top model set (ΔAIC$_c$ < 2.0) for the number of days a male *Trichonephila clavipes* spent guarding a female (n = 93 observations).

All models include the random effects of the male identity and also observation level. Models are ranked by increasing order of their Akaike information criterion corrected for small sample size (AIC$_c$).

Fixed effect predictors	AIC$_C$	K	ΔAIC$_C$	Weight
Male body size + number of males + female condition + number of males × female condition	392.47	7	0.00	0.42
Number of males + female condition + number of males × female condition	392.61	6	0.14	0.39
Male body size + number of males + female condition + number of males × female condition + male body size × female condition	394.13	8	1.67	0.18

1   K = number of parameters, ΔAIC$_C$ = difference between the AIC$_C$ value of each model and the
2   AIC$_C$ value of the most parsimonious model, and weight = AIC$_C$ weight of each model. The
3   symbols + and × represent additive and interaction between variables, respectively.

Standardized predictors, from the averaged model, of the number of days a male *Trichonephila clavipes* spent guarding a female.

Results shown are model predictors derived after averaging submodels within 2 AIC_c of the best model.

Parameter	β	SE	95% CI
intercept	0.895	0.118	0.660 to 1.130
Male body size	0.191	0.124	-0.055 to 0.438
Number of males	0.718	0.182	0.357 to 1.079
Female condition	-0.233	0.199	-0.629 to 0.163
Number of males × female condition	-1.297	0.353	-1.998 to -0.596
Male body size × female condition	-0.151	0.176	-0.500 to 0.198

2 β, standardized coefficient for model predictors; SE, unconditional standard error; 95% CI, 95%
3 compatibility interval.