

1 **Be prudent if it fits you well: male mate choice depends on male size in a *Nephila* spider**

2 **Pietro Pollo<sup>1</sup>, Danilo G. Muniz<sup>2</sup> & Eduardo S. A. Santos<sup>1</sup>**

3 <sup>1</sup> BECO Lab, Department of Zoology, Institute of Biosciences, University of São Paulo, São  
4 Paulo, SP, 05508-090, Brazil

5 <sup>2</sup> LAGE Lab, Department of Ecology, Institute of Biosciences, University of São Paulo, São  
6 Paulo, SP, 05508-090, Brazil

7 **Abstract**

8 Male preference for high-quality females is expected to evolve when male reproductive potential  
9 is restricted. However, when there is competition among males, some models predict the  
10 evolution of assortative male mate choice, in which good competitors choose high quality  
11 females while poor competitors choose lower quality females to avoid competition. In *Nephila*  
12 *clavipes* spiders, males have limited sperm supply and fight for access to females. Here, using  
13 field experiments in which males could choose among two available females, we tested whether  
14 female quality and male size (a proxy of fighting ability) influence male decisions in *N. clavipes*.  
15 We found that males choose their mates based on female size and female recent pairing status  
16 (whether the female was accompanied by a male before the experiment). Importantly, male mate  
17 choice varied with male size, as large males preferred larger females that were recently unpaired,  
18 medium-sized males showed no preference, and small males preferred smaller, recently paired  
19 females. Because all females appear to attract males, we predict that variation on male mate

20 choice attenuates sexual selection on females. Our findings confirm the prediction of variable  
21 male mate choice when there is male-male competition and male reproductive potential is  
22 restricted, a pattern that may be common, but hard to detect.

23 **Key-words:** male mate preference, mating tactics, intrasexual competition, fecundity, sperm  
24 competition.

25

## 26 **Introduction**

27 The Darwin-Bateman paradigm that underlies the traditional sexual selection theory posits that  
28 male reproductive potential is virtually unbounded, and male reproductive success is limited only  
29 by female availability (reviewed in [1]). Thus, males are predicted to seek copulations with as  
30 many females as possible, irrespective of female quality. This prediction assumes that copulation  
31 is virtually without costs for males, which is not necessarily true [2–4]. Several factors may  
32 restrict male reproductive potential, such as sperm limitation [5–7], costly mate search [8,9],  
33 provision of paternal care [10], provision of nuptial gifts to females [11], and sexual cannibalism  
34 by females [12]. These factors can limit the number of females a male can copulate with or  
35 diminish the benefits of copulating with a high number of females. When male reproductive  
36 potential is constrained, if there is variation in female quality (*i.e.*, some females provide more  
37 fitness benefits than others to males), one would expect the evolution of male mate choice  
38 [13,14].

39 Male mate choice is often based on the number or quality of offspring that a female can  
40 produce [13,14]. In populations in which male mate choice occurs, males often choose females  
41 based on their body size, as larger females typically possess more ova [6,13,15]. Moreover,

42 males may also use female social context as a mate choice criterion. For instance, the presence of  
43 another male with a female could influence male mating decisions due to two main reasons [16].  
44 First, in some mating systems a male accompanying a female may aggressively defend her from  
45 other males, so that approaching an accompanied female would result in fighting with the  
46 resident male, which may be energetically costly [17,18]. Second, even if a male manages to  
47 access a recently accompanied female, he will probably face sperm competition, which can  
48 decrease the benefit of copulating with that female, especially if there is first-male sperm  
49 precedence [13]. Therefore, males generally prefer females that are not being guarded or that  
50 have not mated recently (*e.g.*, [19,20]).

51 Male mate choice is often associated with restricted male reproductive potential [13],  
52 absence of male-male competition (*e.g.*, [21]) and high female availability [22]. However, male  
53 mate choice can occur even when male intra-sexual competition is strong (*e.g.*, [23]). Theoretical  
54 models predict that the occurrence of male-male competition is one of the mechanisms that can  
55 generate variation in male mate choice [24–26]. By incorporating male-male competition,  
56 theoretical models predict that good competitors would prefer high quality females, whereas bad  
57 competitors would prefer low quality females [24–26]. In this scenario, bad competitors would  
58 be performing prudent mate choice (*sensu* [25]), in which low-quality males actively choose to  
59 mate with low-quality females as a mechanism to avoid competition with other males (*e.g.*, [27]).

60 Here, we investigate mate choice by males of the golden silk orb-weaver spider *Nephila*  
61 *clavipes*. In *N. clavipes*, adult males live on female webs and fight against male intruders to  
62 monopolize access to the female [28]. Males of this species face high mating costs and severe  
63 mate limitation for two main reasons. First, males suffer high mortality risk when traveling  
64 between female webs [29,30], and second, males are strongly sperm limited because

65 spermatogenesis ceases after maturation [31,32]. Also, *Nephila clavipes* males show great  
66 variation in body size: within a population, one male can be 20 times heavier than other males  
67 (this study). Larger males possess higher fighting ability and are more effective in guarding a  
68 female [33]. Moreover, adult females in this species vary widely in body size, which is correlated  
69 with fecundity [34,35]. Females are also polyandrous, so that there is risk of sperm competition  
70 [29], a factor that should influence males' mating decisions. We expect *N. clavipes* males to  
71 perform male mate choice, because their reproductive potential is severely restricted and females  
72 vary in quality. Additionally, it is also possible that *N. clavipes* males vary in their choosiness  
73 according to body size, because there is male-male competition for access to females and male  
74 body size is correlated with their competitive ability.

75         In this study, we performed field experiments to investigate male mate choice in *N.*  
76 *clavipes*. In these experiments, we tested whether female quality and male fighting ability (*i.e.*,  
77 size) influence the likelihood that a male would choose a given female. We have two alternative  
78 hypotheses. Because all males suffer high mating costs, our first hypothesis is that all males are  
79 equally choosy and select high quality females. However, considering the strong competition  
80 among males, our second hypothesis is that male mate choice varies according to male  
81 competitive ability, so that the most competitive males prefer high-quality females, whereas the  
82 least competitive males would prefer low-quality females, while males with average competitive  
83 ability would likely be non-choosy.

84

## 85 **Material and methods**

86 *Study species*

87 In our study site, *N. clavipes* is univoltine (*i.e.*, one generation per year) and its reproductive  
88 season begins in December and ends in June (P.P. pers. obs.). Females live for three to four  
89 months as adults [36], and then take 30 to 40 days to lay their first clutch after their last moult.  
90 Adult males live considerably less than females (ca. three weeks after the last moult) and stop  
91 constructing their own webs to search for sexual partners [37]. In our study population, males  
92 can visit up to six female webs during their lives (Del Matto and Santos, pers. obs.). However, as  
93 males have a limited sperm supply [32], it is probable that they can only fertilize a few females.  
94 In some cases, males can become monogynous if they use all their sperm with one female [36].

95

#### 96 *Study site and maintenance of study animals*

97 We conducted our experimental trials (see details below) on the webs of female *N. clavipes*  
98 occurring naturally on the gardens surrounding the Zoology Department building in the campus  
99 of the University of São Paulo, in São Paulo, Brazil (23.564° S, 46.729° W). We conducted the  
100 experiments in two consecutive reproductive seasons (March to May 2017; February and March  
101 2018). Early in both seasons (December and January), we collected immature males on the  
102 gardens and kept these males in the laboratory until sexual maturation to use them in the  
103 experimental trials. Males were individually kept in 250 ml plastic cups in controlled conditions  
104 (14h:10h light/dark photoperiod, 25° C) on a diet of *Drosophila* flies. We provided three flies  
105 and sprayed the male spiders with water every two days. All cups had their tops covered with  
106 fine mesh and the inside of the cups was roughened to facilitate climbing of the spiders and silk  
107 attachment. We recorded the date of the last moult of each male.

#### 108 *Female quality proxies and morphometric measurements*

109 We use the term “female quality” to refer to the fitness output that a male gains when mating  
110 with a female. Thus, female quality can be influenced by both female phenotypic traits and the  
111 female’s social context. Here, we evaluated female quality using two independent proxies:  
112 female body size and recent pairing status (*i.e.*, if she had a male in her web prior to the mate  
113 choice experiment). Female body size is positively related to fecundity [34,35]. Consequently,  
114 female body size is positively related to female quality. Moreover, because body size does not  
115 change after maturation in this species, female body size represents an intrinsic and fixed  
116 component of female quality. On the other hand, female pairing status (whether a female is  
117 accompanied by a male or not) can vary throughout female adult life, as males come and go from  
118 females’ webs. We assume that recently paired females were of lower quality, because from the  
119 point of view of an approaching male, a recently paired female represents two disadvantages: a  
120 possible fight with the resident male and a greater risk of sperm competition due to probable  
121 recent copulation. As males deposit their own silk on the female web, an approaching male may  
122 perceive chemical cues of the presence of a competitor male, even if the competitor is not there  
123 anymore [20].

124         To assess female body size, we photographed each female on the day of the beginning of  
125 each trial (a ruler was placed by the female for scale). We used these photographs to measure  
126 female cephalothorax width (mm) using the software ImageJ (US National Institutes of Health,  
127 Bethesda, MD, <http://imagej.nih.gov/ij>). We assessed female recent pairing status by recording  
128 whether there was at least one male on each female’s web immediately before we started each  
129 trial. We note that the variable recent pairing status is different from reproductive status. We did  
130 not collect systematic data about each female's mating history, thus we cannot infer whether  
131 unpaired females were virgin. In fact, it is highly likely that all of the females used in our

132 experiments had already copulated at least once before the trials. Hence, what we classified as  
133 “recently unpaired females” did not have any male on their web on the day of the beginning of  
134 trials, but possibly copulated before. To estimate male fighting ability (positively related to male  
135 body size; [33]), we measured body mass of focal males that were used in our experiments using  
136 a digital scale (to the nearest 0.1 mg) on the day of the beginning of each experimental trial.

### 137 *Male mate choice experiment*

138 To investigate male mate choice in *N. clavipes*, we conducted a field experiment in which we  
139 allowed virgin adult males to choose between two females that differed in body size. Focal  
140 females could also differ regarding their recent pairing status. Females often build their webs  
141 intertwined to other female webs (*i.e.*, aggregated webs; [38]), and we used these natural  
142 aggregations to conduct our trials. For each trial, we selected two females that had their webs  
143 close to one another and that shared at least one silk-thread. We individually marked these  
144 females on the dorsal side of their abdomen with water-based paint (Ziggs’ Posterman markers)  
145 in order to identify them during the trial. We also removed any males that were on the webs prior  
146 to the beginning of the trials, so the males being tested (hereafter, focal males) would be in a  
147 scenario free of competition. However, because males deposit their own silk on the female web  
148 [20], we assume that focal males can detect the cues of previous males in the web.

149         Before we started each trial, we placed the focal male in a plastic container to carry him  
150 from the lab to the trial site. We initiated each trial by placing a wooden stick in the container so  
151 that the focal male could climb it, leading the male to an intersection of silk-threads connecting  
152 the webs of the two females. We evaluated male choice in two moments. We assessed the *initial*  
153 *male choice* by recording to which female the male moved towards within 15 minutes after the  
154 focal male reached the intersection of silk-threads (*i.e.*, beginning of the experiment). Our total

155 sample size for the initial choice trials was 73; both focal females had the same recent pairing  
156 status in 51 trials, whereas focal females differed in recent pairing status in the remaining 22  
157 trials. We only considered that a male made a choice when he moved at least 30 cm towards one  
158 of the females. We chose this distance arbitrarily because males would often stop moving and  
159 consequently would take a long time to reach a female web, especially in windy days. Our  
160 methodology is very similar to most investigations on male mate choice in spiders that are  
161 conducted in laboratory conditions and mainly evaluate male decisions at a single moment using  
162 female silk (*e.g.*, [39–41]). However, because we conducted the experiment in the field, in which  
163 female web construction date was not manipulated, and to account for possible manipulation  
164 stress on focal males, we also decided to assess male choice at a posterior moment. Thus, on the  
165 following day (16 to 22 hours after the beginning of the trial), we observed the *late male choice*  
166 by noting which female the male was guarding. Our total sample size for the late choice trials  
167 was 30; both focal females had the same recent pairing status in 18 trials, whereas focal females  
168 differed in recent pairing status in the remaining 12 trials. The decrease in sample size (72 to 30  
169 trials) is because we only considered late male choice trials as valid when both experimental  
170 females were present on the original trial site on their individual web and the webs were still  
171 connected to one another. Moreover, we excluded from our analyses trials in which non-  
172 experimental males (*i.e.*, other than the focal male) were present with any of the experimental  
173 females, as they could influence the focal male mating decisions.

#### 174 *Statistical analysis of male mate choice experiment*

175 We investigated whether male mate choice in *N. clavipes* depends on female  
176 cephalothorax width, female recent pairing status and male body mass by testing two alternative  
177 predictions. We used male body mass as a continuous variable in all of our analyses. However,



178 we use categorical terms of this continuum (*i.e.*, high, medium, and low body mass males)  
179 simply as a way to better explain our predictions. Our first prediction is that all males, regardless  
180 of body mass, due to their high mating costs will pair preferentially with recently unpaired  
181 females with wider cephalothorax. Alternatively, our second prediction is that male body mass  
182 influences male mate choice in a way that high body mass males pair preferentially with recently  
183 unpaired females with wider cephalothorax, medium body mass males are unselective regarding  
184 female cephalothorax width and recently pairing status, and low body mass males pair  
185 preferentially with recently paired females with narrower cephalothorax (the opposite choice of  
186 heavy males). The reasoning being that as male body mass increases, so does male capacity to  
187 monopolize their mates by fighting off intruders, leading to exclusive paternity with a female.

188         We tested these predictions using a modified version of the model proposed by [42],  
189 which is a statistical model of comparative mate choice (*i.e.*, it assumes that the choosing  
190 individuals perform their decisions by comparing at least two available options). The response  
191 variable of the model was the identity of the chosen female, and the predictors included  
192 characteristics of focal males and of the females available in each trial. Therefore, we built our  
193 statistical model based on three main assumptions: (1) that males performed choice by  
194 comparing the two available females; (2) that males can assess female size and recent pairing  
195 status; and (3) that pairing decisions can be influenced by the traits of the available females and  
196 male selectivity, whereas male selectivity can be influenced by male traits. We included two  
197 female traits in the model: cephalothorax width (continuous) and pairing status (categorical:  
198 recently paired or recently unpaired, see details in the “Female quality proxies and morphometric  
199 measurements” section above). Hence, the preferences of a male are represented by two  
200 selectivity values:  $S_1$ , selectivity for female cephalothorax width; and  $S_2$ , selectivity for female

201 recent pairing status. Positive  $S_1$  values represent preference for females with wider  
 202 cephalothorax, while negative values represent preference towards females with narrower  
 203 cephalothorax. Similarly, positive  $S_2$  values represent preference towards previously unpaired  
 204 females, whereas negative  $S_2$  values represent preference for previously paired females. In both  
 205 cases, a value of zero represents no male choice.

206         Given that we hypothesized that male body size could influence their pairing decisions,  
 207 we assumed that both  $S_1$  and  $S_2$  were functions of male body mass. Additionally, although we  
 208 had no reasons to expect differences in male choosiness between years, we added the season in  
 209 which each trial was conducted as a predictor in the models to control for this potential source of  
 210 variation (parameters  $C$  and  $F$  below). Given all that, we calculated  $S_1$  and  $S_2$  values for each  
 211 male with the following equations:

$$212 \qquad S_{1(i)} = A + B * m_{(i)} + C * y_{(i)} \text{ (Eq. 1)}$$

$$213 \qquad S_{2(i)} = D + E * m_{(i)} + F * y_{(i)} \text{ (Eq. 2)}$$

214

215

216 Where  $m_{(i)}$  is the mass of male  $i$  and  $y_{(i)}$  is the season in which the trial was conducted, and  $A$ ,  $B$ ,  
 217  $C$ ,  $D$ ,  $E$ , and  $F$  are the model parameters (that we needed to fit). Given these selectivity values,  
 218 the probability  $P_{(i,j)}$  that a male  $i$  will choose female  $j$ , in a trial with females  $j$  and  $k$ , is a function  
 219 of  $S_{1(i)}$ ,  $S_{2(i)}$  and the values of female cephalothorax width  $f$ , and female recent pairing status  $g$  of  
 220 both females. The probability  $P_{(i,j)}$  was calculated as follows:

221

$$222 \qquad P_{(i,j)} = \frac{\exp(S_{1(i)} * f_{(j)} + S_{2(i)} * g_{(j)})}{\exp(S_{1(i)} * f_{(j)} + S_{2(i)} * g_{(j)}) + \exp(S_{1(i)} * f_{(k)} + S_{2(i)} * g_{(k)})} \text{ (Eq. 3)}$$

223

224 Whereas the probability that the male will choose female  $k$  is calculated as  $P_{(i,k)} = 1 - P_{(i,j)}$ .

225           The model parameters can be interpreted as follows. On equation 1, the intercept  $A$   
226 represents general male choice regarding female cephalothorax width, in which positive values  
227 represent higher general male choice for females with wider cephalothorax. Whereas, the slope  $B$   
228 represents how male choice for wider female cephalothorax increases with male body mass, in  
229 which positive values represent greater preference intensity for wider female cephalothorax as  
230 male body mass increases. Similarly, in equation 2, the intercept  $D$  represents general male  
231 choice regarding female recent pairing status, in which positive values represent higher  
232 preference for recently unpaired females. The slope  $E$  represents how male choice for recently  
233 unpaired females increases with male body mass, in which positive values represent greater  
234 preference intensity for recently unpaired females as male body mass increases. The relationship  
235 between our alternative predictions and the values of these parameters is summarized in Table 1.  
236 Our analysis allowed coefficient values that would represent additional scenarios, not included in  
237 the predictions. For example, it would be possible to find that all males do prefer females with  
238 wider cephalothorax and that greater male body mass increases selectivity. However, we did not  
239 have *a priori* theoretical reasons to expect these other possibilities.

240  
241 **Table 1.** Summary of the predictions investigated in this study and their relationship with model  
242 parameter values. Columns two, three, and four represent male selectivity regarding female  
243 cephalothorax width and female recent pairing status according to male body mass. Positive  
244 selectivity represents preference for recently unpaired females with wider cephalothorax, zero  
245 selectivity represents no preference and negative selectivity represents preference for recently  
246 paired females with narrower cephalothorax. The fifth and sixth columns represent the expected  
247 values of model parameters according to each alternative prediction (see text for details).

248

Prediction	Selectivity of high body mass males	Selectivity of medium body mass males	Selectivity of small body mass males	Coefficients <i>A</i> and <i>D</i>	Coefficients <i>B</i> and <i>E</i>
1 - all males equally choosy	Positive	Positive	Positive	Positive	Zero
2 - variable male mate choice	Positive	Zero	Negative	Zero	Positive

249

250

We implemented the model using the *stan* modelling language [43] and fit the models by

251 Markov-Chain Monte-Carlo (MCMC) using a Bayesian framework in the software R 3.4.1 [44]

252 using the package *rstan* [45]. Prior to model fitting we standardized all continuous predictor

253 variables (male body mass and female cephalothorax width) to zero mean and then divided by

254 two times its standard deviation (following [46]). Binary variables (recent pairing status and

255 season) were set as 0 (recently paired females and 2017) and 1 (recently not paired females and

256 2018), respectively. For each model, we ran three MCMC chains with 10,000 iterations each,

257 plus 10,000 burn-in iterations. We adopted *Stan*'s standard uninformative improper priors. For

258 more details on model fitting, please see the tutorial in [42]. We evaluated convergence of the

259 chains by inspecting R values (all < 1.01) and visually inspecting the chains for each parameter

260 in our model (Supplementary Figures 1 and 2). Because we centred and scaled all predictor

261 variables, coefficients can be interpreted as estimates of effect size. We considered coefficients

262 to be different than zero when 95% credible intervals (95% CI) did not overlap zero.

## 263 Results

264 In all trials, focal males successfully chose one of the two females available within 15 minutes.

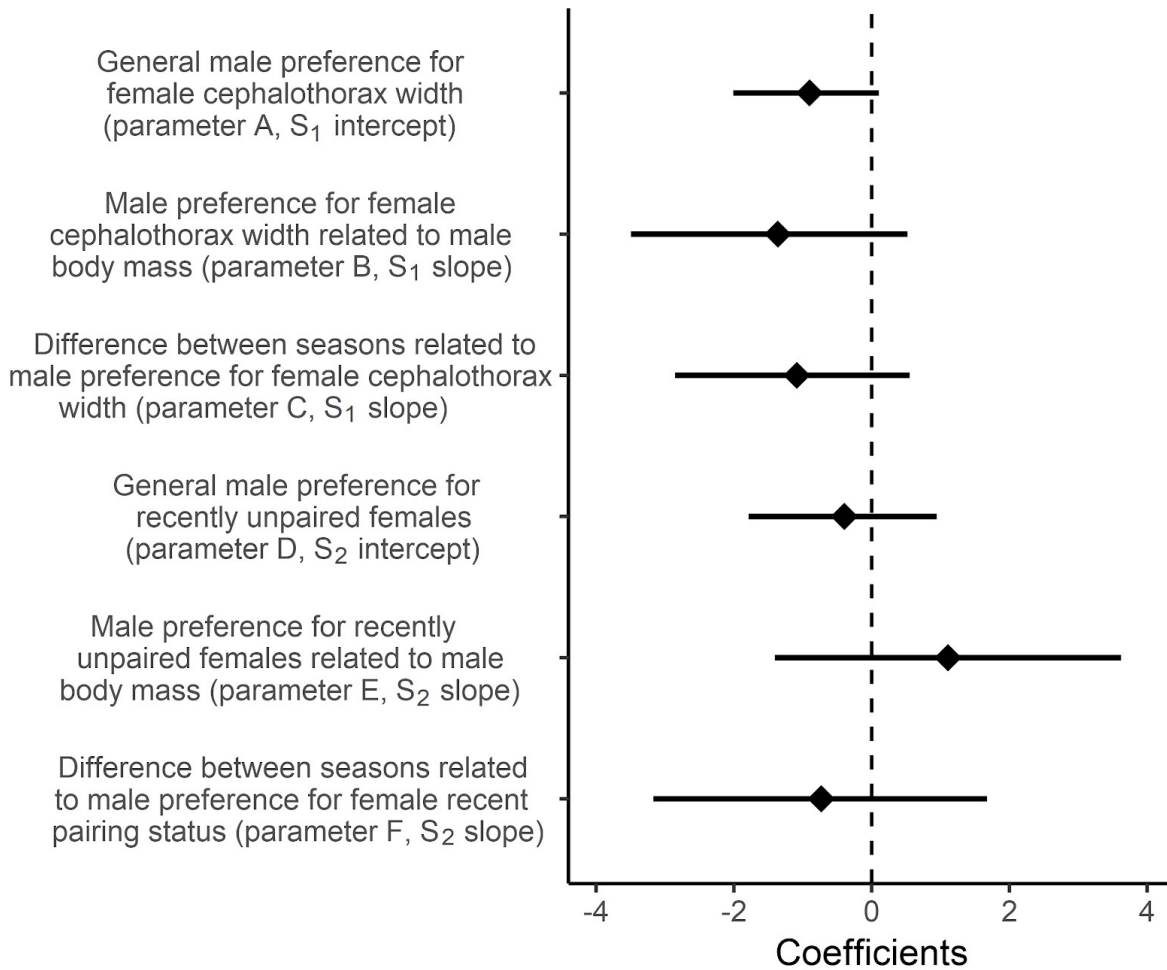
265 On average, males took  $2.36 \pm 3.16$  minutes (mean  $\pm$  SD) to make this initial decision,

266 depositing their own silk where they went. In the initial male mate choice trials, we found little

267 evidence that male decision was influenced by female cephalothorax width, the previous  
268 presence of male partners or the experimental males' own body mass (Figure 1). This means that  
269 initial male decisions, upon encountering a female web, did not support any of our predictions.

270         In the late male choice trials, we found evidence that male pairing decision was  
271 influenced by female cephalothorax width, recent pairing status and male body mass (Figures 2  
272 and 3). We found that greater than average male body mass increased the pairing probability  
273 with females of wider cephalothorax and that were unpaired before the trial (Figure 2).  
274 Additionally, smaller than average male body mass increased the pairing probability with  
275 females of narrower cephalothorax and that were paired before the trial (Figure 2). These results  
276 support our second prediction that male mate choice is assortative. We found little evidence of  
277 differences in male mate choice when trials were conducted on different seasons (Figures 1 and  
278 2).

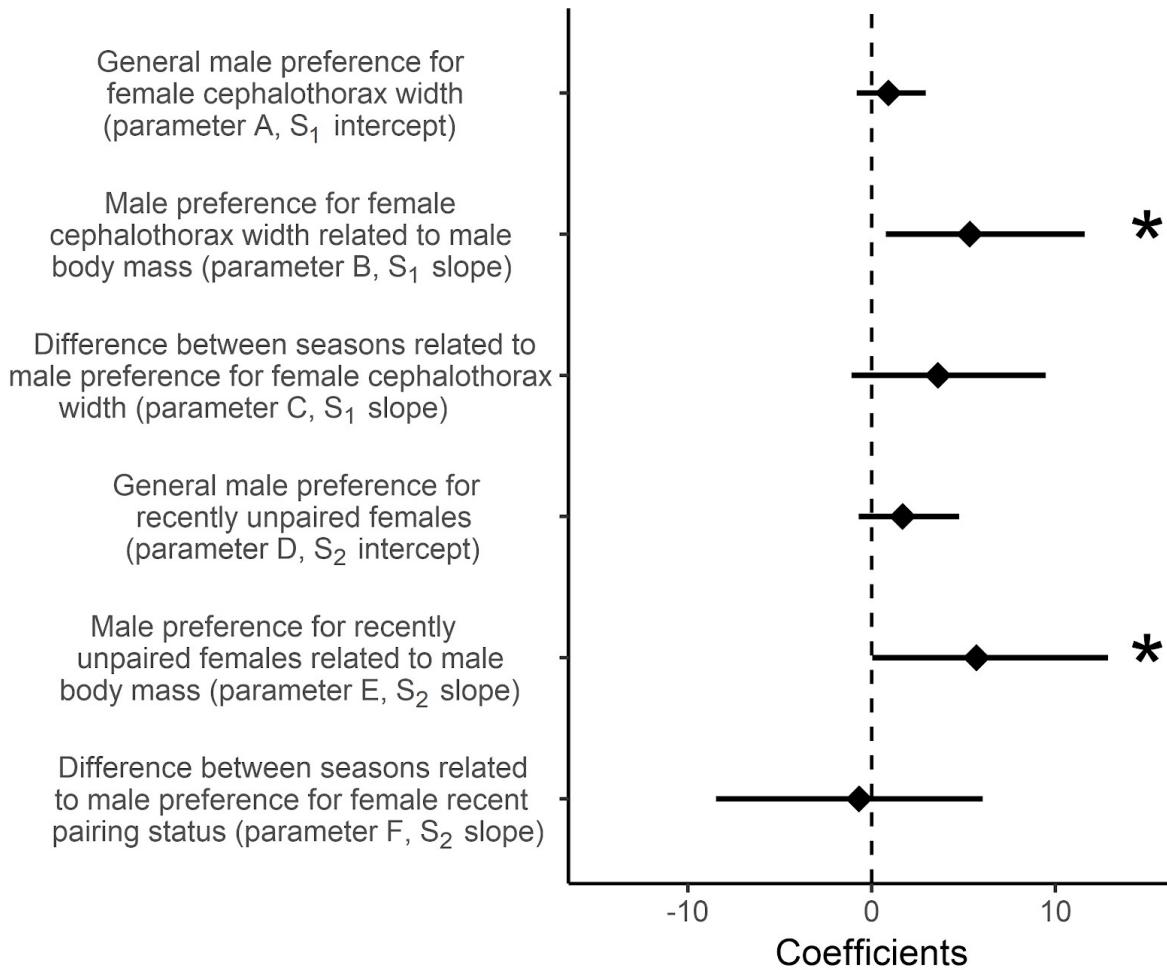
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280

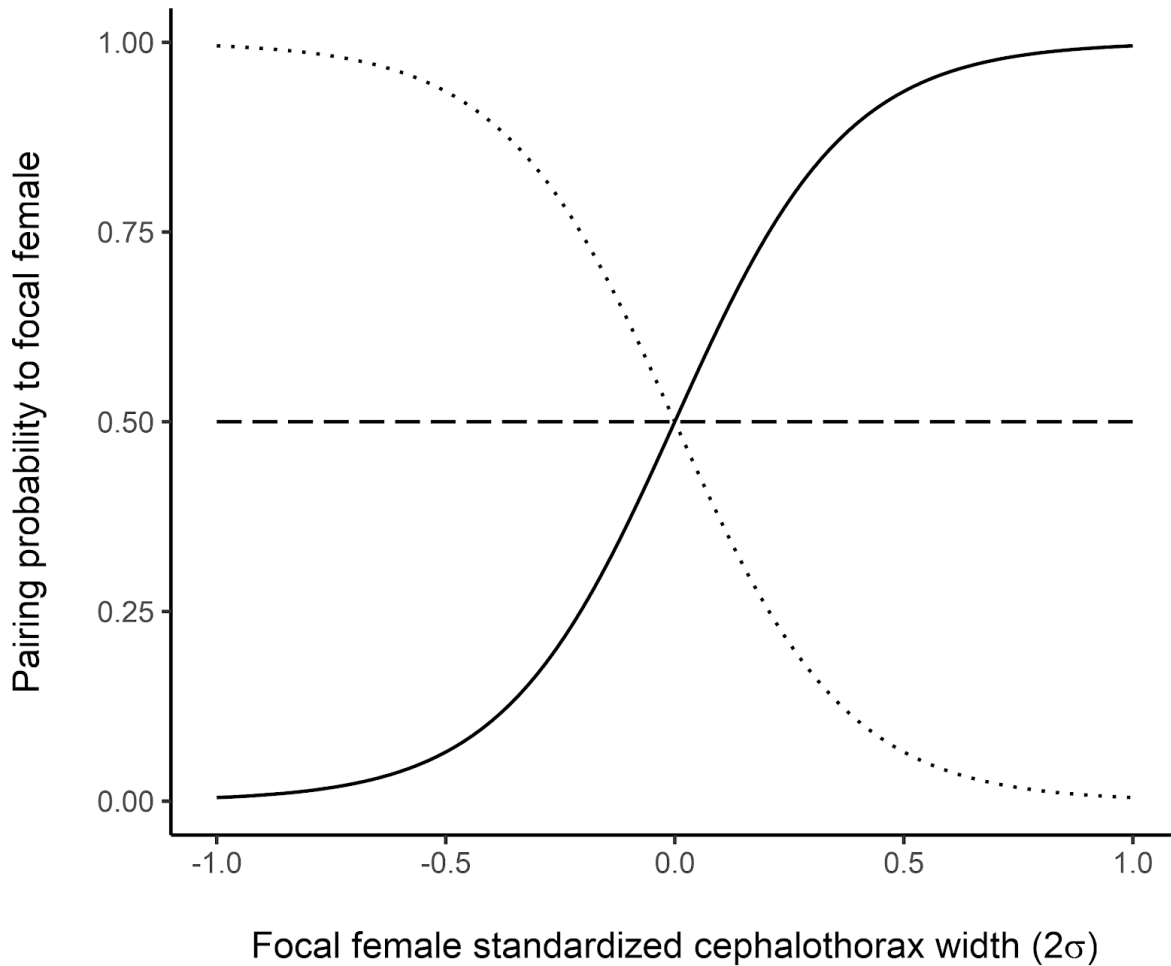
281 **Figure 1.** Results of the multinomial model investigating initial male mate choice (*i.e.*, which  
 282 female thread the experimental male climbed) in *Nephila clavipes*. Points and segments represent  
 283 mean estimated parameter value and 95% credible interval. See details about what each  
 284 parameter represents in the *Statistical analysis of male mate choice experiment* section.

285



286

287 **Figure 2.** Results of the multinomial model to investigate late male mate choice (*i.e.*, which  
 288 female the focal male was guarding after 16 to 22 hours of the beginning of the experiment) in  
 289 *Nephila clavipes*. Points and segments represent mean estimated parameter value and 95%  
 290 credible interval. Stars highlight the model parameters for which the 95% credible interval did  
 291 not overlap zero. See details about what each parameter represents in the *Statistical analysis of*  
 292 *male mate choice experiment* section.



293

294 **Figure 3.** Pairing probability of a male with a focal female depending on male body mass and  
 295 focal female cephalothorax width. In this scenario, a male may choose between the focal female  
 296 and another female of standardized cephalothorax width of zero value (*i.e.*, an average-sized  
 297 female). Moreover, females do not differ in previous pairing status in this scenario. The  
 298 probability that a high body mass male (solid line) will pair with the focal female increases as  
 299 focal female cephalothorax width increases. In contrast, the probability that a low body mass  
 300 male (dotted line) will pair with the focal females decreases as focal female cephalothorax width  
 301 increases. The probability that medium body mass males (dashed line) will pair with the focal  
 302 female does not depend on focal female cephalothorax width and is always 50% (random). We



303 used coefficient values in which 95% credible interval did not overlap zero in the late male mate  
304 choice model to estimate the predicted values displayed in this figure.

305

## 306 **Discussion**

307 In this study, we tested whether female quality and male fighting ability (*i.e.*, body size) would  
308 influence male pairing decisions in the golden silk orb-weaver spider *Nephila clavipes*. Using  
309 field experiments, we found little evidence that, on a first moment (*i.e.*, when males are first  
310 exposed to silk threads of different female webs), males exert mate choice. However, after a  
311 period of a few hours, we found evidence that males express mate choice based on their own  
312 fighting ability in an assortative manner. For instance, large males were more likely to guard the  
313 larger female among those available. Also, when females differed in their recent pairing status,  
314 large males preferred to guard the female that had not been paired to other male recently. Small  
315 males, on the other hand, were more likely to guard the smaller female, and also, when given an  
316 option, preferred a previously paired female to the one that was not recently guarded by other  
317 male. Medium-sized males, however, guarded females randomly regarding female size and  
318 female previous pairing status. Therefore, our results indicate that there is variation in male mate  
319 choice with small males showing preference patterns opposite to the large males, while medium-  
320 sized males showed no mating preferences.

321       Our experimental design allowed us to investigate whether male mate choice for female  
322 traits occurred shortly after males encountered a pair of females or whether males need more  
323 time to access females once they encounter them. We found differences in male mate choice  
324 along the duration of trials, in which males were initially indifferent to the female traits we  
325 evaluated, but later expressed mate choice depending on their own size. Males of many spider

326 species can access female information through silk strands produced by females (reviewed in  
327 [39]). For instance, [40] found, with laboratory experiments, that *N. clavipes* males can  
328 distinguish females that are closer to oviposition using only cues present in female silk.  
329 However, because we conducted our experiments in the field, males might have been subjected  
330 to naturally occurring confounding factors that are absent in laboratory experiments. For  
331 example, as we did not have information on when females built their webs, it is possible that  
332 males chose more recently weaved threads due to their fresher chemical cues. Furthermore,  
333 because females occasionally take over webs from other females (P.P. pers. obs.), silk  
334 components may not be reliable cues to inform the quality of a resident female. Therefore, *N.*  
335 *clavipes* males may need time to evaluate female quality in a natural situation by gathering  
336 information on female quality from vibrational cues or from cuticular pheromones [47]. This  
337 may explain why we found male mate choice only after males approached females and had time  
338 to collect more information on their quality. Generally, animals gather information on mate  
339 quality using multisensory cues [48], but several mate choice investigations allow only one  
340 sensory input information to focal individuals (*e.g.*, insects: [49]; fish: [50]). Thus, our findings  
341 highlight the importance of using experimental designs that assess choice in more than one  
342 moment and that allow males to receive multisensory female cues in a proper time frame.

343         Traditional mate choice literature predicts that when individuals express mate preference,  
344 they should invariably choose high quality mates [51]. Yet, empirical studies that found intra-  
345 population variation on mate choice have been accumulating [52]. Among several factors that  
346 may promote mate choice variation, theoretical models (*e.g.*, [24–26]) identified that intrasexual  
347 competition may be a critical influence on male mate choice variation. Here, we found that *N.*  
348 *clavipes* males differ in the direction and intensity of their mate choice according to their own

349 size. Only large males behaved according to what is generally predicted by the traditional mate  
350 choice literature: these males chose larger females that had not been recently paired. Whereas  
351 small males preferred smaller females that had been recently paired to other males. Why should  
352 these less competitive males choose lower quality females? If males invariably choose to  
353 copulate with high quality females, these females would probably mate multiply, and males  
354 would probably share the paternity of the eggs. Consequently, the advantage of copulating with  
355 high quality females over low quality ones would be reduced. Thus, mate choice for higher  
356 quality females is only advantageous if the choosing male can effectively guard the female and  
357 increase his likelihood of siring most of her offspring. In *N. clavipes*, guarding efficiency is  
358 positively related to male size [33]. Therefore, the larger the male, the greater is the likelihood  
359 that he receives the benefits of mating with a high quality female.

360         *Nephila clavipes* males can guard only one female at a time, which means that lower  
361 quality females would become available as large males are busy guarding high quality females.  
362 Thus, the most profitable tactic for small males may be to choose lower quality females, as a way  
363 to avoid competition with other males (*i.e.*, prudent mate choice; [25]). This rationale is  
364 supported by theoretical models that explore the influence of male competitive ability on male  
365 mate choice, which usually focus on the extremes of male phenotypes using two male categories  
366 (*e.g.*, high/low quality in [24]; large/small in [25]; high/low competitive ability in [26]). Our  
367 results on the choice expressed by males of extremes sizes (large/small) match the assortative  
368 choice predicted by these theoretical studies under certain parameters. However, these models do  
369 not clarify what is expected of average competitors. Here, we found that *N. clavipes* medium-  
370 sized males are unselective regarding female quality. Following the rationale that competitive  
371 ability determines whether males prefer or avoid high-quality females, medium-sized males

372 stand in the middle. That is, because medium-sized males are displaced by half of their  
373 competitors, they may gain the advantages of pairing with high-quality females sometimes, but  
374 not frequently enough to promote preference nor rarely enough to promote avoidance to these  
375 females. On the other hand, it would be interesting to explore whether males express consistent  
376 preference for a particular female phenotype. This is because our findings that medium-sized  
377 males are not selective towards female body size could also be explained by individual variation  
378 among medium-sized males. Overall, our results match precisely the results of a theoretical study  
379 on mutual mate choice in a scenario in which females are weakly sperm limited, female mating  
380 costs are low and male mating costs are high (see Figure 2 in [53]). Surprisingly, [53] did not  
381 consider pre-copulatory aggression explicitly in their model, revealing that our findings may also  
382 be justified with other features, such as sperm competition.

383         We found that the smaller the male, the greater is the probability that he chooses females  
384 that were recently being guarded by another male. It is possible that choosing recently paired  
385 females is a mechanism that decreases the risk of being displaced by larger males (in addition to  
386 preferring small females). Males using this strategy would copulate mostly with non-virgin  
387 females, and therefore would share the paternity of the offspring. This choice for recently paired  
388 females could indicate that smaller males tend to employ a sneaking tactic, in which the male  
389 stays on the periphery of a guarded female's web and attempts to copulate unnoticed by the  
390 guarding male [28]. In this scenario, smaller males would be interpreting the cues of male  
391 presence as a sign that the female is currently being guarded, and would be choosing to be  
392 sneakers rather than guardians. Although we do not know how successful this strategy can be,  
393 small males appear to get less attention from guarding males when compared to large males (P.P.  
394 pers. obs.). In any case, males are apparently employing different mating tactics depending on

395 their own body size. Here, we only observed their pairing decisions, but males of different  
396 fighting abilities may also differ in tactics regarding female search, sperm allocation, and mate  
397 guarding tactics. Small males may compensate the lower quality of their partners by being more  
398 promiscuous. If that is the case, we would expect that male size is also (i) negatively related to  
399 movement between female webs, as a strategy to copulate with more females, and (ii) negatively  
400 related to investment in each female, in terms of sperm allocation and mate-guarding time.

401         Our results show that males similarly consider female size and female social context  
402 when choosing their mates. The combination of these two variables makes it difficult to estimate  
403 female quality in certain scenarios (*e.g.*, a recently paired large female may be of similar quality  
404 to a not recently paired small female). Hence, a simple assortative pairing pattern by body size  
405 alone may not necessarily occur in natural conditions. Furthermore, our results indicate that all  
406 females, regardless of size, are able to attract males to mate, although it seems that they attract  
407 more males of a particular fighting ability than randomly expected. Thus, even though male mate  
408 choice can generate sexual selection on females and even favor the evolution of female sexual  
409 ornaments (*e.g.*, [54]), variation in male mate choice may attenuate sexual selection on females,  
410 especially if some males perform prudent mate choice. Therefore, scenarios of variable male  
411 mate choice may be common, but hard to detect. Detection of variation in male mate choice is  
412 made difficult by two main reasons: (1) it generates weak or no sexual selection on females and  
413 (2) it requires specific experimental and statistical protocols (as the ones we employed here). We  
414 argue that variation in male mate choice may be relatively common, as theory predicts that it will  
415 evolve in populations in which mating is costly for males and there is strong intrasexual  
416 competition among males [24–26]. These conditions occur in *N. clavipes*, and may also be met  
417 by other species in which males cannot monopolize groups of females, so that these males are

418 continuously searching for new mates, or in mating systems in which males can guard a single  
419 female at a time. Such non-territorial mating systems are less studied than territorial ones, but are  
420 the most common among animals [55]. Therefore, future studies employing an experimental  
421 protocol similar to ours may discover that variation in male mate choice is very common in  
422 natural populations.

423

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429

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