

PIETRO POLLO

**Not what you expect: assortative male choice without
assortative pairing pattern in a *Nephila* spider**



São Paulo

2018

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Não é o que você espera: escolha assortativa do macho sem padrão de pareamento assortativo em uma aranha *Nephila*



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assortative pairing pattern in a *Nephila* spider**

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Resumo

A escolha de parceiras por machos ocorre em espécies em que machos sofrem altos custos associados ao acasalamento, o que gera poucas oportunidades para copular. Adicionalmente, se a competição entre machos é intensa, a escolha de parceiras por machos deveria ser afetada pela habilidade de luta dos machos. Bons competidores deveriam escolher fêmeas de alta qualidade porque eles seriam capazes de afastar rivais, enquanto maus competidores deveriam escolher fêmeas de má qualidade. Em aranhas da espécie *Nephila clavipes*, machos possuem um estoque limitado de esperma e lutam pelo acesso a fêmeas. Usando experimentos de campo e dados observacionais, testamos se a qualidade das fêmeas e o tamanho dos machos (variável operacional de habilidade de luta) afetam a probabilidade de pareamento dos machos. Nos nossos experimentos, vimos que machos não expressam escolha de parceiras inicialmente, mas, depois de algumas horas, escolhem parceiras baseado no tamanho e no estado recente de pareamento delas. A direção e intensidade da escolha de parceiras por machos variou com o tamanho dos machos, pois machos grandes guardaram fêmeas maiores que não estavam pareadas recentemente, enquanto machos pequenos guardaram fêmeas menores que estavam pareadas recentemente. Nossos dados observacionais forneceram pouca evidência para pareamento assortativo referente a tamanho corporal. Com nossos experimentos de campo, mostramos que informação crucial pode ser coletada avaliando a escolha de parceiras em diferentes momentos. Em conjunto, nossos resultados revelam a importância de considerar características dos machos e contexto social no entendimento da variação na escolha de parceiras por machos, que é comumente negligenciada em estudos empíricos.

Palavras-chave: preferência dos machos por parceiras, escolha de parceiras por machos, fecundidade, competição intrasexual, competição espermática.

Abstract

Male mate choice occurs in species in which males face high mating costs, leading to few opportunities to copulate. Additionally, if male-male competition is strong, male mate choice should be influenced by male fighting ability. Good competitors should choose high quality females because they may be able to fight off contenders, while poor competitors should choose lower quality females. In *Nephila clavipes* spiders, males have limited sperm supply and fight for access to females. Here, using field experiments and observational data, we tested whether female quality and male size (a proxy of fighting ability) affect the pairing likelihood of males. In our experiments, we found that males did not express mate choice initially, but, after a few hours, chose their mates based on female size and female recent pairing status. Importantly, male mate choice direction and intensity varied with male size, as large males guarded larger females that were not recently paired and small males guarded smaller females that were recently paired. The observational data provided little evidence of assortative pairing regarding body size. With our field experiments, we show that crucial information can be collected by assessing mate choice in multiple moments. Taken together, our findings reveal the importance of considering male traits and the social context in understanding variation in male mate choice, which is often neglected in empirical studies.

Keywords: male mate preference, male mate choice, fecundity, intrasexual competition, sperm competition.

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Introduction

The Darwin-Bateman paradigm that underlies the traditional sexual selection theory posits that male reproductive potential is virtually unbounded because males can, in principle, fertilize countless females (reviewed in Parker and Pizzari 2015). According to this paradigm, male reproductive potential is limited by female availability. Thus, males are predicted to seek copulations with as many females as possible, irrespective of female quality. However, the main premise of this argument that female availability is the main limitation to male fitness, is not always true (Dewsbury 2005; Tang-Martinez and Ryder 2005; Tang-Martinez 2016). Several factors may restrict male reproductive potential, such as sperm limitation (Dewsbury 1982; Wedell et al. 2002; Perry et al. 2013), provision of paternal care (Berglund et al. 1989), provision of nuptial gifts to females (Cratsley et al. 2003), and sexual cannibalism by females (Andrade 1996). These factors can reduce the number of females a male can copulate with or diminish the benefits of copulating with a high number of females. In such scenarios, if there is variation in female quality (*i.e.*, some females provide more fitness benefits than others to males), one would expect the evolution of male mate choice (Bonduriansky 2001; Edward and Chapman 2011).

Male mate choice, when it occurs, is often based on the number or quality of offspring that a female can produce (Bonduriansky 2001; Edward and Chapman 2011). In systems in which male mate choice occurs, males often use female body size or age as a mate choice criterion, because larger, younger females typically possess more ova which could be inseminated by the male (Arnaud and Haubruge 1999; Bonduriansky 2001; Wedell et al. 2002). Moreover, the presence of another male (a competitor) with a female can affect male mating decisions due to two main reasons (Mautz and Jennions 2011). First, in some mating systems a male accompanying a female may aggressively defend this

female from other males, so that approaching an accompanied female would result in fighting with the resident male, which may be energetically costly. Second, even if a male manages to access a recently accompanied female, he will probably face sperm competition, which can decrease the benefit of copulating with that female, especially if there is first-male sperm precedence (Bonduriansky 2001). Therefore, males generally prefer females that are not being guarded or that have not mated recently (*e.g.*, Schwagmeyer and Parker 1990; Schneider et al. 2011).

Male mate choice should occur more frequently in species with female-biased operational sex ratio (OSR, the ratio of ready-to-mate males to ready-to-mate females; (Emlen and Oring 1977) as greater mate availability reduces the costs of mate choosiness (Dougherty and Shuker 2015). However, male mate choice can occur even when the OSR is male-biased and male intra-sexual competition is strong (*e.g.*, Bel-Venner et al. 2008; Venner et al. 2010). The occurrence of both male mate choice and male competition among males is predicted to generate an assortative mating pattern, in which males that are good competitors copulate with high quality females while males that are bad competitors copulate with low quality females (Bel-Venner et al. 2008; Venner et al. 2010; Edward and Chapman 2011). This pattern of assortative mating, however, can be generated by two slightly distinct mechanisms, both involving male mate choice. First, all males may choose high quality females, but poor competitors are displaced by good competitors and thus are restricted to low quality females. Alternatively, male mate choice may vary, so that good competitors choose high quality females, while poor competitors choose low quality females or simply do not express choice (*i.e.*, fighting ability dependent male mate choice), as a strategy to reduce competition and enhance mating success (Fawcett and Johnstone 2003; Venner et al. 2010). These two mechanisms are poorly studied, yet they are important, as they show whether variation in male mate choice exists in animal systems.

In golden silk orb-weaver spiders of the genus *Nephila*, adult males live on female webs and fight against male intruders to monopolize access to the female (Christenson and Goist 1979). This female-defense behavior ultimately reduces sperm competition, especially early in the mating season when the OSR is male-biased (Higgins 2000; Kasumovic et al. 2008). In *N. clavipes*, males also face great mating costs and severe mate limitation for several reasons. For instance, males may suffer high mortality rates when traveling between webs in search of mates (Vollrath and Parker 1992). Furthermore, males are strongly sperm limited because spermatogenesis ceases after maturation, meaning that once a male uses all sperm in his pedipalps, he becomes permanently sperm depleted (Christenson 1989; Michalik and Rittschof 2011). Moreover, adult females vary widely in body size, which is correlated with fecundity, and are polyandrous, so that there is risk of sperm competition (Vollrath 1980), a factor that should influence the males' mating decisions. Thus, we expect *N. clavipes* males to perform male mate choice, because their reproductive potential is severely restricted by sperm limitation and females vary in their quality.

As other nephilid species, *N. clavipes* males show an enormous variance in size, the weight of the heaviest male in a sample can be more than 20 times greater than that of the lightest male (this study; Elgar et al. 2003). Larger males possess greater fighting ability and are more effective in guarding a female (Constant et al. 2011). However, larger males do not necessarily achieve higher fitness. For example, in *N. senegalensis*, males of different sizes achieve similar paternity by employing distinct mating tactics (Neumann and Schneider 2015). Although there are no empirical investigations on variation of mating strategies of *N. clavipes* males, a model proposed by Rittschof et al. (2012) predicted that larger males should be choosy while smaller ones should be indiscriminate regarding mates. While there is one empirical study that examines male mate choice in *N. clavipes*, it

focuses exclusively on female proximity to oviposition, and does not investigate the effect of male characteristics in mate choice intensity (see Rittschof 2011). In this study, we conducted field experiments and observations to investigate male mate choice in *N. clavipes*. We hypothesize that female quality and male fighting ability (*i.e.*, size) affect pairing likelihood. We expect that males with great fighting ability will choose high quality females, whilst males with low fighting ability may be (1) equally choosy, (2) less choosy than males with great fighting ability, (3) indifferent toward females' quality (*i.e.*, non-choosy) or, even (4) prefer low quality females as a strategy to avoid male-male competition. We investigated male mate choice performing field experiments, in which we offered two females to each male, and noted the male behaviour in two moments: at the beginning of the experiment and 16-22 hours after that. In addition, we performed field observations to inspect patterns of pairing in the study population that could be generated by male mate choice, such as assortative pairing (*i.e.*, larger females paired with larger males).

Material and methods

Study species

In our study site, *N. clavipes* is univoltine and its reproductive season begins in December and ends in June (P.P. pers. obs.). Females live for three to four months as adults (Christenson and Cohn 1988), and they take 30 to 40 days to lay their first clutch after their last molt. In laboratory conditions, females are capable of laying up to five clutches (Christenson et al. 1985; Higgins 2000). Adult males live considerably less than females (ca. three weeks after the last moult) and search for sexual partners through all this period (Brown 1985). In our study population, males can visit up to six female webs during their

lives (L. A. Del Matto and E. S. A. Santos, unpublished data). However, as males have a limited sperm supply (Michalik and Rittschof 2011), it is probable that they can only fertilize a few females. In some cases, males can become monogynous if they use all their sperm with one female (Christenson and Cohn 1988).

Study site and maintenance of study animals

We conducted our experimental trials (see details in the *Male mate choice experiment* section below) on the webs of female *N. clavipes* occurring naturally on the gardens surrounding the Zoology Department building of the Institute of Biosciences in the main campus of the University of São Paulo, in São Paulo, Brazil (23.564° S, 46.729° W). We conducted the experiments in two reproductive seasons (March to May of 2017; February and March of 2018). Early in both seasons (December and January), we collected immature males on the same gardens where we conducted our trials. We kept these males in the laboratory until sexual maturation to use them as subjects in our experimental trials. Males were individually kept in 250 ml plastic cups in controlled conditions (14h:10h light/dark photoperiod, 25° C) on a diet of *Drosophila* flies. We provided three flies and sprayed the male spiders with water every two days. All cups had an opening on the top that was covered with fine mesh. The inside of the cups was roughened to facilitate climbing of the male spiders and attachment of silk. Each cup was labeled, and thus we could observe when males became adult by assessing the date of the last molt of each individual. When *N. clavipes* males become adult, their body and palp coloration darken.

Female quality proxies and morphometric measurements

We evaluated female quality using two proxies: female body size and recent pairing status (see below). Female body size is positively related to the amount of eggs a

female can produce (*i.e.*, fecundity; Honěk 1993; Head 1995). Consequently, female body size is positively related to female quality. From the point of view of an approaching male, a recently paired female represents two disadvantages: a possible fight with the resident male and also a greater risk of sperm competition (due to probable recent copulation; there is evidence of first-male sperm precedence in *N. clavipes*; Christenson and Cohn 1988). As males deposit their own silk on the female web, an approaching male may perceive the resident's chemical cues even if the resident is not there anymore (Schneider et al. 2011). Hence, the quality of a potential female mate that has been recently paired should be lower than that of a female that has not been previously guarded (*i.e.*, recently unpaired female).

To assess female body size, we photographed each female with a scale bar on the day of the beginning of each trial. We used these photographs to measure female cephalothorax width (mm) using ImageJ (US National Institutes of Health, Bethesda, MD, <http://imagej.nih.gov/ij>). We assessed female recent pairing status by recording whether there was at least one male on each female's web immediately before we started each trial. We note that the variable recent pairing status is different from reproductive status. We did not collect systematic data about each female's sexual history, thus we cannot infer whether unpaired females were virgin. In fact, it is highly likely that all of the females used in our experiments had already copulated at least once before the trials (*i.e.*, non-virgin). Hence, what we classified as “recently unpaired females” did not have any male on their web on the day of the beginning of trials, but possibly copulated before.

To test the effect of male body size (positively related to male fighting ability; Constant et al. 2011) in male mate choice, we measured body mass of focal males that were used in our experiments, using a digital scale (to the nearest 0.1 mg) on the day of the beginning of each trial. When we encountered males on the webs of females that would be

used in the trials, we measured their body mass to use in our observational analyses (see details in the section *Observational data*, below).

Male mate choice experiment

To test for the occurrence of male mate choice in *N. clavipes*, virgin adult males had the opportunity to choose between two females that differed in body size. Focal females could also differ regarding their recent pairing status. Females often build their webs intertwined to other female webs (*i.e.*, aggregated webs; Robinson and Mirick 1971), and we used these natural aggregations to conduct our trials. For each trial, we selected two females that had their webs close to one another and that shared at least one silk-thread. We individually marked these females on the dorsal side of their abdomen with water-based paint (Ziggs' Posterman markers) in order to identify them during the trial. We also removed any males that were on experimental webs prior to the beginning of the trials, so the males being tested (*i.e.*, focal males) would be in a scenario free of competition.

We initiated each trial by gently placing a virgin male at the intersection of silk-threads connecting the webs of the two females. Male choice was assessed at two moments. First, we assessed the *initial male choice* by noting to which female the male moved towards within 15 minutes after the beginning of the experiment. We only considered that a male made a choice when he moved at least 30 cm towards one of the females. On the following day (16 to 22 hours after the beginning of the trial), we observed the *late male choice* by noting which female the male was guarding. We only considered late male choice trials as valid when both experimental females were present on the original trial site on their individual web and the webs were still connected to one another. Moreover, we also excluded from our analyses trials in which non-experimental males

(*i.e.*, other than the focal male) were present with any of the experimental females, as they could influence the focal male mating decisions.

Statistical analysis of male mate choice experiment

We investigated the hypothesis that male mate choice in *N. clavipes* depends on female cephalothorax width, female recent pairing status and male body mass by testing a set of alternative predictions (Figure 1). We invariably predict that high body mass males will choose recently unpaired females with great cephalothorax width. The reasoning being that these high body mass males should have high resource holding power that allows them to monopolize their mates by fighting off intruders, leading to exclusive paternity with a high quality female. Additionally, we have four distinct alternative predictions about mate choice in low body mass males. First, low body mass males may choose recently unpaired females with great cephalothorax width with the same intensity as heavy males do (*i.e.*, no inter-individual variation scenario; prediction 1, Figure 1B). Second, low body mass males may choose recently unpaired females with great cephalothorax, but are less choosy than heavy males (prediction 2; Figure 1C). Third, low body mass males may not be selective regarding female cephalothorax width and recent pairing status (*i.e.*, may not exert mate choice; prediction 3; Figure 1D). Fourth, low body mass males may choose recently paired females with small cephalothorax width, the opposite choice of heavy males (prediction 4; Figure 1E).

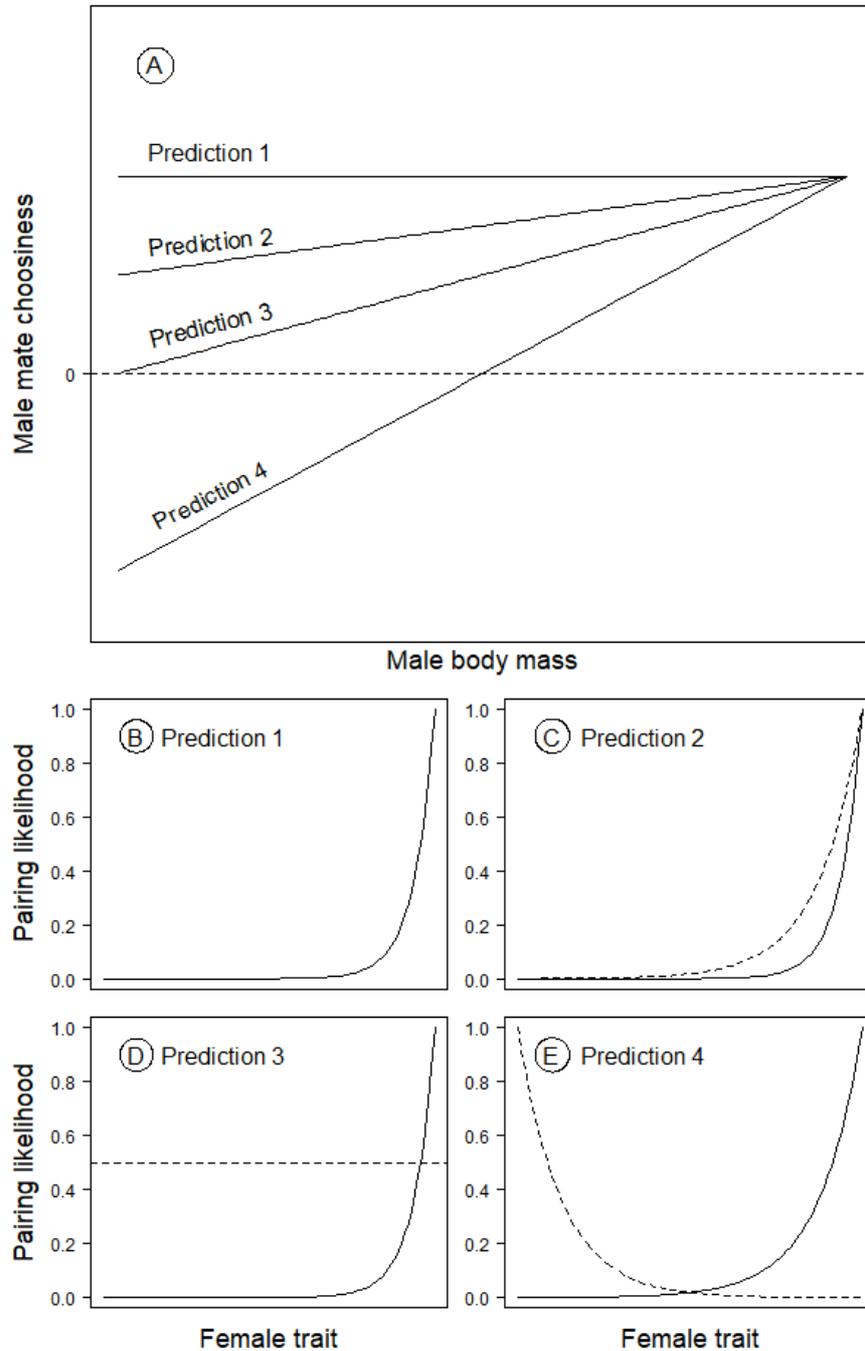


Figure 1. Predictions regarding the relationship between male body mass and male mate choosiness. **(A)** Relationship between male body mass and male choosiness for female quality according to our four alternative predictions. Choosiness value of zero represents no mate choice, positive values represent preference for high-quality females and negative values represent preference for low-quality females. **(B-E)** The x-axis represent female quality, whereas the y-axis is pairing likelihood, which is proportional to the probability

that the male will choose a given female. In these plots, the dashed lines represent males with the lowest body mass in plot A, whereas continuous lines represent the males with highest body mass.

To test these predictions, we used a multinomial model of choice as described by Muniz et al. (2017). We used this model because it assumes that the choosing agent (in our case, the males) exerts choice by comparing among the available options, as happens in our experiment. We ran separate but identical models for our initial and late male choice trials (see details in the section *Male mate choice experiment* above). Our predictor variables for these models were (1) male body mass, (2) female cephalothorax width, (3) female recent pairing status, and (4) the breeding season in which the trials were conducted. We standardized all continuous predictor variables to zero mean and unit variance (Schielezeth 2010). Although we had no reasons to expect differences in male choosiness between years, we added the season in which each trial was conducted as a predictor in the models to control for this potential source of variation. In each trial, we assumed that the male i could choose among the two available females (j and k) and we calculated a probability of mate choice for each female based on the predictor variables. Specifically, in the models, we assumed that male selectivity for each female trait (cephalothorax width and pairing status) has a linear relationship with male body mass, so that we calculated the selectivity values S_1 and S_2 for each male i as:

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

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

Where $m_{(i)}$ is male body mass of male i and $y_{(i)}$ is the season in which the trial was conducted. Then, we incorporated these preference components into the multinomial model equation for the probability of choice for a given female j over a female k :

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

Where $f_{(j)}$ and $f_{(k)}$ represent cephalothorax width (continuous variable) of each female in the trial, and $g_{(j)}$ and $g_{(k)}$ represent female recent paired status (categorical variable; 0: recently paired females, 1: recently unpaired females). Because males in our experiments could only choose between two females (j and k), the probability to choose female k is complementary to the probability to choose female j as:

$$P_{(i,k)} = 1 - P_{(i,j)} \text{ (Eq. 4)}$$

On equation 1, the intercept A represents general male choice for female cephalothorax width, in which positive values represent higher general male preference for females with larger cephalothorax. Whereas, the slope B represents how male choice for larger female cephalothorax width increases with male body mass, in which positive values represent greater preference intensity for larger female cephalothorax width as male body mass increases. Similarly, in equation 2, the intercept D represents general male choice for recently unpaired females, in which positive values represent higher general male choice for recently unpaired females. The slope E represents how male choice for recently unpaired females increases with male body mass, in which positive values represent greater preference intensity for recently unpaired females as male body mass increases.

The relationship between our alternative predictions (Fig. 1) and the values of these coefficients is summarized in Table 1.

Table 1. Summary table containing the predictions investigated in this study. Confer Fig. 1 for a visual representation of the predictions regarding the choosiness of small body mass males. The third and fourth columns represent the direction and magnitude of expected model coefficients for each prediction. Column three (coefficients *A* and *D*) represent effects of general male choice for female cephalothorax width and for recently unpaired females, respectively. Column four (coefficients *B* and *E*) represent how male choice for female cephalothorax width and for recently unpaired females, respectively, increase with male body mass.

Prediction	Choosiness of small body mass males	Coefficients <i>A</i> and <i>D</i>	Coefficients <i>B</i> and <i>E</i>
1	Positive, equal to choosiness of large body mass males	Positive	Zero
2	Positive, smaller than choosiness of large body mass males	Positive	Positive, small
3	Zero	Positive	Positive, large
4	Negative	Zero	Positive

We implemented the model using the *stan* modelling language (Stan Development Team 2017a) and fit the models by Markov-Chain Monte-Carlo (MCMC) using a Bayesian framework in the software R 3.4.1 (R Core Team 2017) using the package *rstan* (Stan Development Team 2017b). For each model, we ran three MCMC chains with 10,000 iterations each, plus 10,000 burn-in iterations. We adopted *Stan*'s standard

uninformative improper priors. For more details on model fitting, please see the tutorial in Muniz *et al.* (2017). We evaluated convergence of the chains by inspecting R values (all < 1.01) and visually inspecting the chains for each parameter in our model (Supplementary figures 1 and 2). Because we centered and scaled all predictor variables, coefficients can be interpreted as estimates of effect size. We considered coefficients to be statistically significant when 95% credible intervals (95% CI) did not overlap zero.

Observational data recording and analysis

We recorded observational data by collecting males that were on focal females' webs immediately before our experimental trials. We then used the (i) presence of males on each web and (ii) the mass of these males to investigate whether naturally occurring pairings could be a product of male mate choice for female size.

Depending on how male mate choice occurs, we can observe two distinct patterns regarding pairing of individuals. Firstly, if all males generally choose females with larger cephalothorax width, females with great cephalothorax width will have more males on their webs than females with small cephalothorax width (hereby observational prediction 1). Furthermore, if heavy males are more selective towards females with great cephalothorax width than light males, we would expect assortative pairing (*i.e.*, a positive relationship between male body mass and female cephalothorax width in pairings; observational prediction 2).

To test whether our observational data matches any of these two predictions, we built one generalized linear mixed-effect model (GLMM) for each prediction using the *glmer* function of the *lme4* package (Bates et al. 2015). The model corresponding to our observational prediction 1 used a Poisson distribution and its response variable was the number of males on focal female webs. The model corresponding to our observational

prediction 2 used a Gaussian distribution and its response variable was the body mass of males that were closer to each focal female. We used the same predictor variables in both models: standardized female cephalothorax width, the season in which we collected individuals and their interaction. Moreover, in both models we used the identity of the trial as a random predictor variable, because females from the same trial were close to one another and therefore were not totally independent.

Results

Male mate choice experiment

In the initial male mate choice trials (total initial choice trials: $N = 73$, trials in which focal females had the same recent pairing status: $N = 51$, trials in which focal females differed in recent pairing status: $N = 22$), male decision was not influenced by female body size, the previous presence of male partners or the experimental males' own body size (Table 2). This means that initial male decisions, upon encountering a female web, did not support any of our predictions in our experiments.

However, in our late male choice trials (total late choice trials: $N = 30$, trials in which focal females had the same recent pairing status: $N = 18$, trials in which focal females differed in recent pairing status: $N = 12$), males decision was influenced by female cephalothorax width, recent pairing status and the male's own body mass (Table 3). We found that heavier focal males had a significantly greater probability to pair with females of larger cephalothorax width and that were unpaired before the trial. Lighter focal males, however, had a higher probability of pairing with females of lower cephalothorax width and that were paired before the trial. These results support our fourth prediction (Fig. 1E).

We did not find any differences on male mate choice between trials conducted on different seasons.

Table 2. Results of the multinomial model investigating initial male mate choice (*i.e.*, which female thread the experimental male climbed) in *Nephila clavipes*. More details about the parameters in Material and methods section.

Predictor variable	Parameter symbol	Mean estimate	95% credible interval
General male choice for female cephalothorax width (S_1 intercept)	<i>A</i>	-0.454	-0.997 to 0.045
Male choice for female cephalothorax width related to male body mass (S_1 slope)	<i>B</i>	-0.342	-0.875 to 0.129
Male choice for female cephalothorax width related to which season the experiment was conducted (S_1 slope)	<i>C</i>	-0.540	-1.415 to 0.280
General male choice for recently unpaired females (S_2 intercept)	<i>D</i>	-0.399	-1.806 to 0.940
Male choice for recently unpaired females related to male body mass (S_2 slope)	<i>E</i>	0.551	-0.709 to 1.831
Male choice for recently unpaired female related to which season the experiment was conducted (S_2 slope)	<i>F</i>	-0.724	-3.167 to 1.695

Table 3. Results of the multinomial model to investigate late male mate choice (*i.e.*, which female the focal male was guarding after 16 to 22 hours of the beginning of the experiment) in *Nephila clavipes*. More details on parameters in Material and methods section.

Predictor variable	Parameter symbol	Mean estimate	95% credible interval
General male choice for female cephalothorax width (S_1 intercept)	<i>A</i>	0.455	-0.399 to 1.449
Male choice for female cephalothorax width related to male body mass (S_1 slope)	<i>B</i>	1.287	0.184 to 2.787
Male choice for female cephalothorax width related to which season the experiment was conducted (S_1 slope)	<i>C</i>	1.736	-0.547 to 4.627
General male choice for recently unpaired females (S_2 intercept)	<i>D</i>	1.705	-0.722 to 4.814
Male choice for recently unpaired females related to male body mass (S_2 slope)	<i>E</i>	2.823	0.059 to 6.386
Male choice for recently unpaired female related to which season the experiment was conducted (S_2 slope)	<i>F</i>	-0.795	-8.608 to 5.894

Observational data

Only the season in which we collected males influenced the number of males in focal females' webs (*observational prediction 1* model), as in the 2018 season there were, on average, 1.67 males on focal females' webs compared to, on average, 0.41 males on focal females' webs in the 2017 season (Table 4). The season in which we collected males also influenced the body mass of the male that was closer to females on their webs (*observational prediction 2* model; Table 5), as the body mass of the closest males to females were, on average, 18.3 mg in 2017 and 25.8 mg in 2018. However, the focal

females' cephalothorax width had little influence on the body mass of the male that was closer to them on their webs.

Table 4. Results of the generalized linear model with mixed-effects to investigate female attractiveness (*i.e.*, the number of males per female web; N = 146).

Predictor variable	Mean estimate	Standard error	Z-value	p-value
Intercept	-0.868	0.203	-4.274	< 0.001
Female cephalothorax width (standardized)	0.278	0.160	1.734	0.082
2018 season in relation to 2017 season	1.386	0.220	6.289	< 0.001
Interaction between female cephalothorax width (standardized) and season	-0.166	0.188	-0.883	0.377

Table 5. Results of the generalized linear model with mixed-effects to investigate assortative pairing (*i.e.*, relationship between female cephalothorax width and the body mass of the male that was closest to female; N = 92).

Predictor variable	Mean estimate	Standard error	t-value	p-value
Intercept	-0.510	0.207	-2.465	0.016
Female cephalothorax width (standardized)	0.258	0.171	1.510	0.140
2018 season in relation to 2017 season	0.663	0.239	2.767	0.007
Interaction between female cephalothorax width (standardized) and season	-0.245	0.217	-1.127	0.268

Discussion

In this study, we tested the hypothesis that female quality and male fighting ability (*i.e.*, body mass) would influence male pairing decisions in *N. clavipes*. Using field experiments, we observed that, on a first moment (*i.e.*, when males are first exposed to silk threads of different female webs), males do not exert mate choice. However, after a period of a few hours, we observed that heavier males were more likely to guard the larger female among those available. Also, when females differed in their recent pairing status, heavier males preferred to guard the female that had not been recently paired to other male. Lighter males, on the other hand, were more likely to guard the smaller female, and also, when given an option, preferred a previously paired female to the one that was not recently guarded by other male. Therefore, there is variation in male mate choice, and lighter males showed a preference pattern opposite to that of heavier males. Using the observational data, we observed that both the number of males on female webs and the weight of the closest male to females were greater in the 2018 season than in the 2017 season. These differences probably occurred because we collected data later in the reproductive season in 2017 than in 2018. In both years, however, we found that larger females did not have more males on their webs nor were paired with heavier males (*i.e.*, no evidence for assortative pairing).

Our experimental design allowed us to investigate whether male mate choice for female traits occurred shortly after males encountered a pair of females or whether males need more time to access females once they encounter them. In our field experiments, we found differences in male mate choice along the duration of trials, in which males were initially indifferent to the female traits we measured, but later expressed mate choice depending on their own size. Males of many spider species can access female information through silk strands produced by females (reviewed in Gaskett 2007). For instance,

Rittschof (2011) found, with laboratory experiments, that *N. clavipes* males prefer females that are closer to oviposition using only cues present in female silk. However, because we conducted our experiments in the field, males might have been subjected to naturally occurring confounding factors that are absent in laboratory experiments. For example, as we did not have information on when females built their webs, it is possible that males just chose more recently weaved threads due to their fresher chemical cues. Furthermore, because females occasionally take over webs from other females (P.P. pers. obs.), silk components may not be reliable cues to inform the quality of a resident female. As male spiders may gather crucial information on female quality from vibrational cues or from cuticular pheromones (Robinson 1982), *N. clavipes* males may need time to evaluate female quality in a natural situation. This may explain why we found male mate choice only after males approached females and had time to collect more information on their quality. Generally, animals gather information on mate quality using multisensory cues (Rowe 1999), but several mate choice investigations allow only one sensory input information to focal individuals (*e.g.*, insects: Goubault and Burlaud 2017; fish: Gasparini et al. 2013). Thus, our findings highlight the importance of using experimental designs that assess choice in more than one moment and that allow males to receive multisensory female cues in a proper time frame.

We found that males differ in the direction and intensity of their mate choice depending on their own body size. Only great body mass males behaved according to what is generally predicted by the mate choice literature (reviewed in Bonduriansky 2001); that is, these males chose larger females that had been recently unpaired. If high quality females are expected to offer more benefits, why do some males choose lower quality females instead? Under an equal sex ratio scenario, if several males chose to copulate with a high quality female, they would share the paternity of her eggs. Consequently, the

advantage of copulating with high quality females over low quality ones would be reduced. Thus, mate choice for higher quality females is only advantageous if the choosing male can effectively guard his mate and increase his likelihood of siring a large proportion of the female's clutch. In *N. clavipes*, large males have higher fighting ability and can guard their mate more effectively than small males (Constant et al. 2011). Thus, only large males would receive the benefits of mating with a high quality female. In *N. clavipes* males can guard only one female at a time, which means that low quality females would become available as larger males are busy guarding high quality females. Thus, the most profitable tactic for small males may be to choose lower quality females, as a way to avoid competition with other males.

We also found that small males choose females that were recently being guarded by another male, which is a more unexpected result. This could be a second layer of avoidance of large males. The logic may be that a recently paired female was abandoned by her prior male, and will not be approached by competitive males, increasing the likelihood that a small male approaching her will be the last male to copulate with her. Alternatively, the choice for recently paired females by smaller males could mean that these males are more prone to employ a sneaking tactic, in which they stay on the web periphery and attempt to copulate with a guarded female unnoticed by the guarding male (Christenson and Goist 1979). Although we do not know how successful this strategy can be, smaller males appear to get less attention from guarding males when compared to larger males (P.P. pers. obs.). In any case, smaller males are apparently employing a different mating tactic than larger males. Here, we only observed their pairing decisions, but males of different fighting abilities may also differ in tactics regarding female search, sperm allocation, and mate guarding tactics. Smaller males may compensate the lower quality of their partners by being more promiscuous than larger males. If that is the case,

we would expect them to (i) move more frequently between female webs than larger males, as a strategy to copulate with more females, and to (ii) invest less in each female, in terms of sperm allocation and mate-guarding time.

Using observational data that was collected prior to the onset of the experimental trials, we found little evidence of assortative pairing based on body size; that is, there was no relationship between male body size and the body size of their mate. The incongruence between our experimental findings and the observational results could arise from several factors. It is likely that *N. clavipes* males vary in the number of females they sample before making mating decisions because females vary greatly in how clustered they are in space (*i.e.*, females can be solitary or aggregated to other females, Rypstra 1985). When choosing individuals have access to a very limited sample of options, mate choice patterns become very hard to detect via simple correlations (Benton and Evans 1998; Muniz et al. 2017). Therefore, assortative pairing patterns are more likely to emerge in scenarios in which males have the opportunity to sample a wide range of females that vary considerably in their phenotype. If *N. clavipes* males usually have access to a low number of females, which could occur due to high mate searching costs, this would lead to a lower likelihood of observing an assortative mating pattern (*i.e.*, correlation between male and female phenotypes). Moreover, if males choose females based on an additional criterion, such as social context (*i.e.*, the presence of other males), assortative pairing based on a phenotypic trait could be disrupted. In our experiments, we found that males chose females more intensively based on females' recent pairing status than based on female size (Table 3). Finally, male mate choice can change with male previous experiences. For example, *Argiope bruennichi* non-virgin males express mate choice regarding female age, but virgin males do not (Schneider et al. 2016). In our experiments, all tested males were virgins, while the males found previously in the females' webs may not necessarily have been. If

experienced males change their mate choice patterns, this could also suppress assortative pairing patterns.

In conclusion, our experimental setup allowed us to observe that *N. clavipes* males are choosy toward females and that they base their mate choice on both female size (related to female fecundity) and female recent pairing status (which may influence risk and intensity of sperm competition). Moreover, we observed that males vary in mate choice depending on their own fighting ability: good competitors choose higher quality females, which is probably a strategy to maximize the number of eggs fertilized per copula, while bad competitors choose lower quality females, likely as a strategy to avoid competition with better competitors. However, using observational data, we observed that male choosiness does not generate a pattern of assortative pairing by size in the population, probably because of limited mate sampling by males or because males use more than one criterion during mate choice. We suggest that males may employ different mate searching and choosing tactics according to their fighting ability, and highlight the importance of field experiments in the detection of mate choosiness and of acknowledging that males may vary in their mate preferences.

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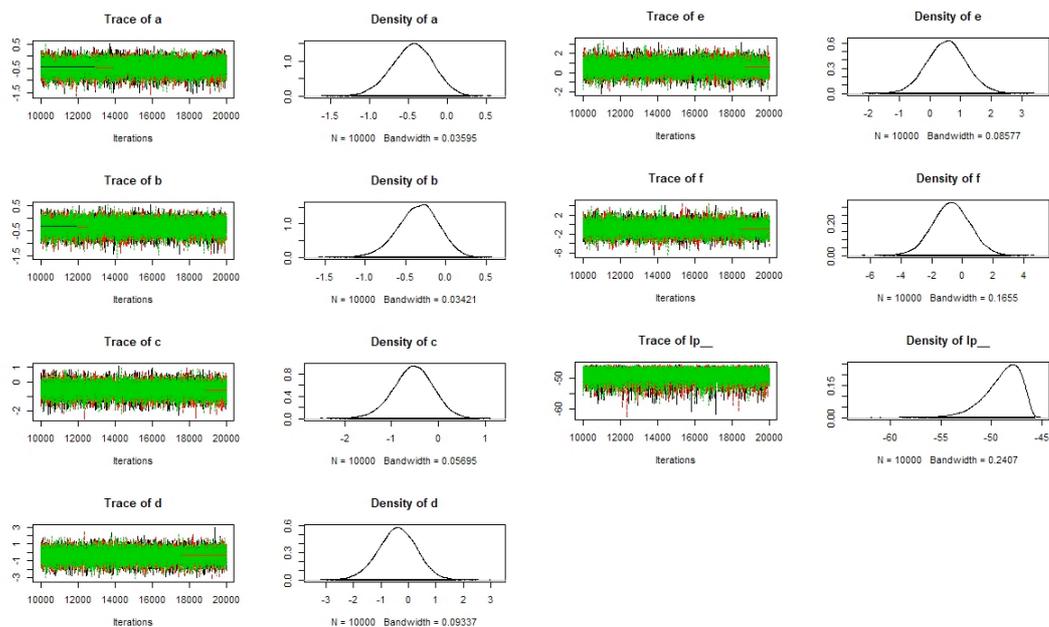
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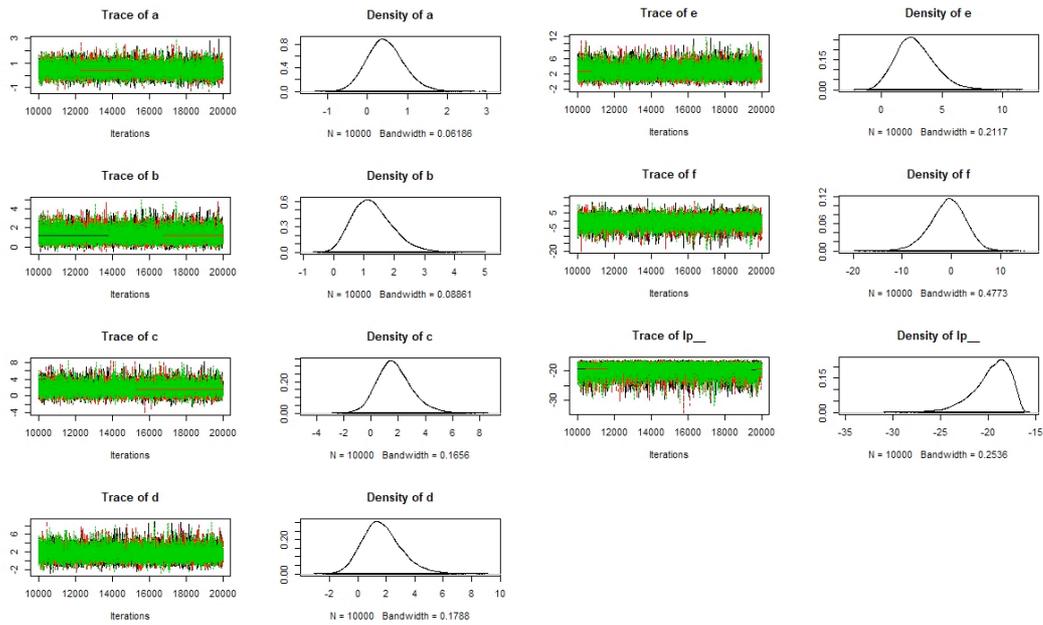
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Supplementary material



Supplementary figure 1. Trace plots and density plots of posterior samples illustrating all parameters of the multinomial model used to test initial male mate choice in *Nephila clavipes*.



Supplementary figure 2. Trace plots and density plots of posterior samples illustrating all parameters of the multinomial model used to test late male mate choice in *Nephila clavipes*.