Be prudent if it fits you well: male mate choice depends on male size in a golden orb-weaver spider

Pietro Pollo[1,2*], Danilo G. Muniz[3] & Eduardo S. A. Santos[2]

[1] Evolution & Ecology Research Centre, School of Biological, Earth & Environmental Sciences, University of New South Wales, Sydney 2052 NSW, Australia

[2] BECO Lab, Department of Zoology, Institute of Biosciences, University of São Paulo, São Paulo, SP, 05508-090, Brazil

[3] LAGE Lab, Department of Ecology, Institute of Biosciences, University of São Paulo, São Paulo, SP, 05508-090, Brazil

[*] Corresponding author: pietro_pollo@hotmail.com

Abstract

Male preference for high-quality females is expected to evolve when male reproductive potential is restricted. However, when there is competition among males, some models predict the evolution of assortative male mate choice, in which good competitors choose high quality females while poor competitors choose lower quality females to avoid competition. In *Trichonephila clavipes* spiders, males have limited sperm supply and fight for access to females. We tested whether female quality and male size (a proxy of fighting ability) influence male decisions in *T. clavipes*. We used field experiments in which males could choose among two available females in a scenario free of competition. We found that males choose their mates based on both female size and female recent pairing status (whether the female was accompanied by a male before the experiment). Importantly, male mate choice exhibited plasticity, and varied with male size, as large males preferred larger females that were recently unpaired, medium-sized males showed no preference, and small males preferred smaller, recently paired females. Because all females appear to attract males, we predict that variation on male mate choice attenuates sexual selection on females. Our findings confirm the prediction of variable male mate choice when there is male-male competition and male reproductive potential is restricted, a pattern that may be common, but hard to detect.

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

Introduction

The Darwin-Bateman paradigm that underlies the traditional sexual selection theory posits that male reproductive potential is virtually unbounded, and male reproductive success is limited only by female availability (reviewed in Parker & Pizzari, 2015). Thus, males are predicted to seek copulations with as many females as possible, irrespective of female quality. This prediction assumes that copulation is virtually without costs for males, which is not necessarily true (Dewsbury, 2005; Tang-Martinez, 2016; Tang-Martinez & Ryder, 2005). Several factors may restrict male reproductive potential, such as sperm limitation (Dewsbury, 1982; Perry, Sirot, & Wigby, 2013; Wedell, Gage, & Parker, 2002), costly mate search (Kasumovic, Bruce, Herberstein, & Andrade, 2007; Lane, Boutin, Speakman, & Humphries, 2010), provision of paternal care (Berglund, Rosenqvist, & Svensson, 1989), provision of nuptial gifts to females (Cratsley, Rooney, & Lewis, 2003), and sexual cannibalism by females (Andrade, 1996). These factors can limit the number of females a male can copulate with or diminish the benefits of copulating with a high number of females. When male reproductive potential is constrained, 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 & Chapman, 2011).

Male mate choice is often based on the number or quality of offspring that a female can produce (Bonduriansky, 2001; Edward & Chapman, 2011). In populations in which male mate choice occurs, males often choose females based on their body size, as larger females typically possess more ova (Arnaud & Haubruge, 1999; Bonduriansky, 2001; Wedell et al., 2002). Moreover, the presence of other males may influence male mating decisions (Mautz & Jennions, 2011). First, in some mating systems a male accompanying a female may aggressively defend her from other males, so that approaching an accompanied female would result in fighting with the resident male, which may be energetically costly (Austad, 1983; Kelly, 2006). Second, if a male copulates with a recently mated, 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., Schneider, Lucass, Brandler, & Fromhage, 2011; Schwagmeyer & Parker, 1990).

Male mate choice is often associated with restricted male reproductive potential (Bonduriansky, 2001), absence of male-male competition (e.g., Gwynne, 1981) and high female availability (Dougherty & Shuker, 2015). However, male mate choice can occur even when male intra-sexual competition is strong (e.g., Bel-Venner, Dray, Allainé, Menu, & Venner, 2008). Theoretical models predict that the occurrence of male-male competition is one of the mechanisms that can generate variation in male mate preference (Fawcett & Johnstone, 2003; Härdling & Kokko, 2005; Venner, Bernstein, Dray, & Bel-Venner, 2010). By incorporating male-male competition, theoretical models predict that good competitors would prefer high quality females, whereas bad competitors would prefer low quality females (Fawcett & Johnstone, 2003; Härdling & Kokko, 2005; Venner et al., 2010). In this scenario, bad competitors would be performing prudent mate choice (*sensu* Härdling & Kokko, 2005), in which low-quality males actively choose to mate with low-quality females as a mechanism to avoid competition with other males (e.g., Wada, Arashiro, Takeshita, & Shibata, 2010).

Here, we investigate mate choice by males of the golden silk orb-weaver spider *Trichonephila clavipes* (formerly known as *Nephila clavipes*, see Kuntner et al., 2018). In *T. clavipes*, adult males live on female webs and fight against male intruders to monopolize access to the female (Christenson & Goist, 1979). Males of this species face high mating costs and severe mate limitation for two main reasons. First, males suffer high mortality risk when traveling between female webs (Vollrath, 1980; Vollrath & Parker, 1992), and second, males are strongly sperm limited because spermatogenesis ceases after maturation (Christenson, 1989; Michalik & Rittschof, 2011). Also, *T. clavipes* males show great variation in body size: within a population, one male can be 20 times heavier than other males (this study). Larger males possess higher fighting ability and are more effective in guarding a female (Constant, Valbuena, & Rittschof, 2011). Moreover, adult females in this species vary widely in body size, which is correlated with fecundity (Head, 1995; Honěk, 1993). Females are also polyandrous, so that there is risk of sperm competition (Vollrath, 1980), a factor that should influence males' mating decisions. We expect *T. clavipes* males to perform male mate choice, because their reproductive potential is severely restricted and females vary in quality. Additionally, it is also possible that *T. clavipes* males vary in their choosiness according to body size, because there is male-male competition for access to females and male body size is correlated with their competitive ability.

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

Material and methods

Study species

In our study site, *T. clavipes* is univoltine (i.e., one generation per year) 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 & Cohn, 1988), and then take 30 to 40 days to lay their first clutch after their last moult. Adult males live considerably less than females (ca. three weeks after the last moult) and stop constructing their own webs following maturation to search for sexual partners (Brown, 1985). In our study population, males can visit up to six female webs during their lives (Del Matto and Santos, pers. obs.). However, as males have a limited sperm supply (Michalik & 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 & Cohn, 1988).

Study site and maintenance of study animals

We conducted our experimental trials (see details below) on the webs of female *T. clavipes* occurring naturally on the gardens surrounding the Zoology Department building in the campus of the University of São Paulo, in São Paulo, Brazil (23.564° S, 46.729° W). We conducted the experiments in two consecutive reproductive seasons (March to May 2017; February and March 2018). Early in both seasons (December and January), we collected immature males on the gardens and kept these males in the laboratory until sexual maturation to use them in the 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 their tops covered with fine mesh and the inside of the cups was roughened to facilitate climbing of the spiders and silk attachment. We recorded the date of the last moult of each male.

Female quality proxies and morphometric measurements

We use the term "female quality" to refer to the fitness output that a male gains when mating with a female. Thus, female quality can be influenced by both female phenotypic traits and the female's social context (i.e., her previous and present association with conspecifics, mainly males). Here, we evaluated female quality using two independent proxies: female body size and recent pairing status (i.e., if she had a male in her web prior to the mate choice experiment). Female body size is positively related to fecundity (Head, 1995; Honěk, 1993). Consequently, female body size is positively related to female quality. Moreover, because body size does not change after maturation in this species, female body size represents an intrinsic and fixed component of female quality. On the other hand, female pairing status (whether a female is accompanied by a male or not) can vary throughout female adult life, as males come and go from females' webs. We assume that recently paired females were of lower quality, because from the point of view of an approaching male, a recently paired female represents two disadvantages: a possible fight with the resident male and a greater risk of sperm competition due to probable recent copulation. As males deposit their own silk on the female web, an approaching male may perceive chemical cues of the presence of a competitor male, even if the competitor is not there anymore (Schneider et al., 2011).

To assess female body size, we photographed each female on the day of the beginning of each trial (a ruler was placed by the female for scale). We used these photographs to measure female cephalothorax width (mm) using the software 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 mating 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. 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 estimate male fighting ability (positively related to male body size; Constant et al., 2011), 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 experimental trial.

Male mate choice experiment

To investigate male mate choice in *T. clavipes*, we conducted a field experiment in which we allowed virgin adult males 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 & 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 the webs prior to the beginning of the trials, so the males being tested (hereafter, focal males) would be in a scenario free of competition. However, because males deposit their own silk on the female web (Schneider et al., 2011), we assume that focal males can detect the cues of previous males in the web.

Before we started each trial, we placed the focal male in a plastic container to carry him from the lab to the trial site. We initiated each trial by placing a wooden stick in the container so that the focal male could climb it, leading the male to an intersection of silk-threads connecting the webs of the two females. We evaluated male choice in two moments. We assessed the *initial male choice* by recording to which female the male moved towards within 15 minutes after the focal male reached the intersection of silk-threads (i.e., beginning of the experiment). Our total sample size for the initial choice trials was 73; both focal females had the same recent pairing status in 51 trials, whereas focal females differed in recent pairing status in the remaining 22 trials. We only considered that a male made a choice when he moved at least 30 cm towards one of the females. We chose this distance arbitrarily because males would often stop moving and consequently would take a long time to reach a female web, especially in windy days. This distance of 30 cm implies that not all focal males reached the initially chosen female's web immediately. Yet, males were at a close distance (less than 25 cm) from the initially chosen female within the first 3 hours of the trial in approximately 79% of the trials for which we have additional observations (N = 38). Our methodology is very similar to most investigations on male mate choice in spiders that are conducted in laboratory conditions that mainly evaluate male decisions at a single moment using female silk (e.g., Gaskett, 2007; Rittschof, 2011; Schneider, Zimmer, Gatz, & Sauerland, 2016). However, because we conducted the experiment in the field, in which female web construction date was not manipulated, and to account for possible manipulation stress on focal males, we also decided to assess male choice at a later time. We established that each focal male would have at least twelve hours to access the experimental females and to make their decision. But, we were unable to standardize the starting time of all trials as we conducted trials with several males on the same day, and we continuously searched for new female webs during the same period. Because all trials started between 12PM and 6PM and we were unable to make observations at night, 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. Our total sample size for the late choice trials was 32; both focal females had the same recent pairing status in 20 trials, whereas focal females differed in recent pairing status in the remaining 12 trials. The decrease in sample size (73 to 32 trials) is because we established a rigorous protocol to ensure that males had the chance to evaluate both focal females and choose between them during the entire trial. Thus, we excluded late male choice trials when at least one of the focal females was absent from the original trial site and/or the focal webs were disconnected from one another (30 exclusions of a total of 41). Moreover, we excluded from our analyses late male mate choice trials in which we could not find the focal male on the web of any of the focal females (six cases) or in which we found non-experimental males (i.e., other than the focal male) with any of the focal females, as they could influence the focal male mating decisions (five cases). There was no pattern in the relationship between male size or female size and the likelihood of an observation being excluded.

Statistical analysis of male mate choice experiment

We investigated whether male mate choice in *T. clavipes* depends on female cephalothorax width, female recent pairing status and male body mass by testing two alternative predictions. We used male body mass as a continuous predictor variable in all of our analyses. However, we use categorical terms of this continuum (i.e., high, medium, and low body mass males) simply as a way to better explain our predictions. Our first prediction is that all males, regardless of body mass, due to their high mating costs will pair preferentially with recently unpaired females with wider cephalothorax. Alternatively, our second prediction is that male body mass influences male mate choice in a way that high body mass males pair preferentially with recently unpaired females with wider cephalothorax, medium body mass males are unselective regarding female cephalothorax width and recently pairing status, and low body mass males pair preferentially with recently paired females with narrower cephalothorax (the opposite choice of heavy males). The reasoning being that as male body mass increases, so does male capacity to monopolize their mates by fighting off intruders, leading to exclusive paternity with a female.

We tested these predictions using a modified version of the model proposed by (Muniz, Santos, Guimarães, Nakagawa, & Machado, 2017), which is a statistical model of comparative mate choice (i.e., it assumes that the choosing individuals perform their decisions by comparing at least two available options). The response variable of the model was the identity of the chosen female, and the predictors included characteristics of focal males and of the females available in each trial. Therefore, we built our statistical model based on three main assumptions: (1) that males performed choice by comparing the two available females; (2) that males can assess female size and recent pairing status; and (3) that pairing decisions can be influenced by the traits of the available females and male selectivity, whereas male selectivity can be influenced by male traits. We included two female traits in the model: cephalothorax width (continuous) and pairing status (categorical: recently paired or recently unpaired, see details in the "Female quality proxies and morphometric measurements" section above). Hence, the preferences of a male are represented by two selectivity values: S_1, selectivity for female cephalothorax width; and S_2, selectivity for female recent pairing status. Positive S_1 values represent preference for females with wider cephalothorax, while negative values represent preference towards females with narrower cephalothorax. Similarly, positive S_2 values represent preference towards previously unpaired females, whereas negative S_2 values represent preference for previously paired females. In both cases, a value of zero represents no male choice.

Given that we hypothesized that male body size could influence their pairing decisions, we assumed that both S_1 and S_2 were functions of male body mass. Additionally, 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 (parameters C and F below). Given all that, we calculated S_1 and S_2 values for each male with the following equations:

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

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

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

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

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

The model parameters can be interpreted as follows. On equation 1, the intercept A represents general male choice regarding female cephalothorax width, in which positive values represent higher general male choice for females with wider cephalothorax. Whereas, the slope B represents how male choice for wider female cephalothorax increases with male body mass, in which positive values represent greater preference intensity for wider female cephalothorax as male body mass increases. Similarly, in equation 2, the intercept *D* represents general male choice regarding female recent pairing status, in which positive values represent higher preference 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 parameter values are not on any interpretable scale, they are dimensionless. Higher absolute values represent stronger preference and here we mainly interpret their sign (positive or negative). The relationship between our alternative predictions and the values of these parameters is summarized in Table 1. Our analysis allowed coefficient values that would represent additional scenarios, not included in the predictions. For example, it would be possible to find that all males do prefer females with wider cephalothorax and that greater male body mass increases selectivity. However, we did not have *a priori* theoretical reasons to expect these other possibilities.

Table 1. Summary of the predictions investigated in this study and their relationship with model parameter values.

Prediction	**Selectivity of high body mass males**	**Selectivity of medium body mass males**	**Selectivity of small body mass males**	**Coefficients *A* and *D***	**Coefficients *B* and *E***
1 - all males equally choosy	Positive	Positive	Positive	Positive	Zero
2 - variable	Positive	Zero	Negative	Zero	Positive

male mate choice

Columns two, three, and four represent male selectivity regarding female cephalothorax width and female recent pairing status according to male body mass. Positive selectivity represents preference for recently unpaired females with wider cephalothorax, zero selectivity represents no preference and negative selectivity represents preference for recently paired females with narrower cephalothorax. The fifth and sixth columns represent the expected values of model parameters according to each alternative prediction (see text for details).

We implemented the model using the *stan* modelling language (Carpenter et al., 2017) 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, 2018). Prior to model fitting we standardized all continuous predictor variables (male body mass and female cephalothorax width) to zero mean and then divided by two times its standard deviation (following Gelman, 2008). Binary variables (recent pairing status and season) were set as 0 (recently paired females and 2017) and 1 (recently not paired females and 2018), respectively. 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 (Figures A1 and A2). Because we centred and scaled all predictor variables, coefficients can be interpreted as estimates of effect size. We considered coefficients to be different than zero when 95% credible intervals (95% CI) did not overlap zero.

Ethical note

This research was approved by Chico Mendes Biodiversity Conservation Institute (permit 61620). Animals kept in captivity were released in the same area where they were collected (see details in the *Study site and maintenance of study animals* section).

Results

In all trials, focal males successfully chose one of the two females available within 15 minutes. On average, males took 2.36 ± 3.16 minutes (mean ± SD) to make this initial decision, depositing their own silk where they went. In the initial male mate choice trials, we found little evidence that male decision was influenced by female cephalothorax width, the previous presence of male partners or the experimental males' own body mass (Figures 1 and A3). This means that initial male decisions, upon encountering a female web, did not support any of our predictions.

In the late male choice trials, we found evidence that male pairing decision was influenced by female cephalothorax width, recent pairing status and male body mass (Figures 2, 3, and A4). We found that greater than average male body mass increased the pairing probability with females of wider cephalothorax and that were unpaired before the trial (Figure 2). Additionally, smaller than average male body mass increased the pairing probability with females of narrower cephalothorax and that were paired before the trial (Figure 2). These results support our second prediction that male mate choice is assortative. We found little evidence of differences in male mate choice when trials were conducted on different seasons (Figures 1 and 2).

Our late male mate choice results are not merely a product of data exclusion, as the focal male was guarding the female which he did not initially choose in 12 of the 32 late male mate trials. This change of females from the start to the end of our trials was not influenced by male size. We have more precise information on the moment the male changed the female in only half of these 12 cases: one occurred within one hour of the initial choice, two occurred between four and six hours after the initial choice, and the remaining three occurred after six hours of the initial choice (at night or early in the morning).

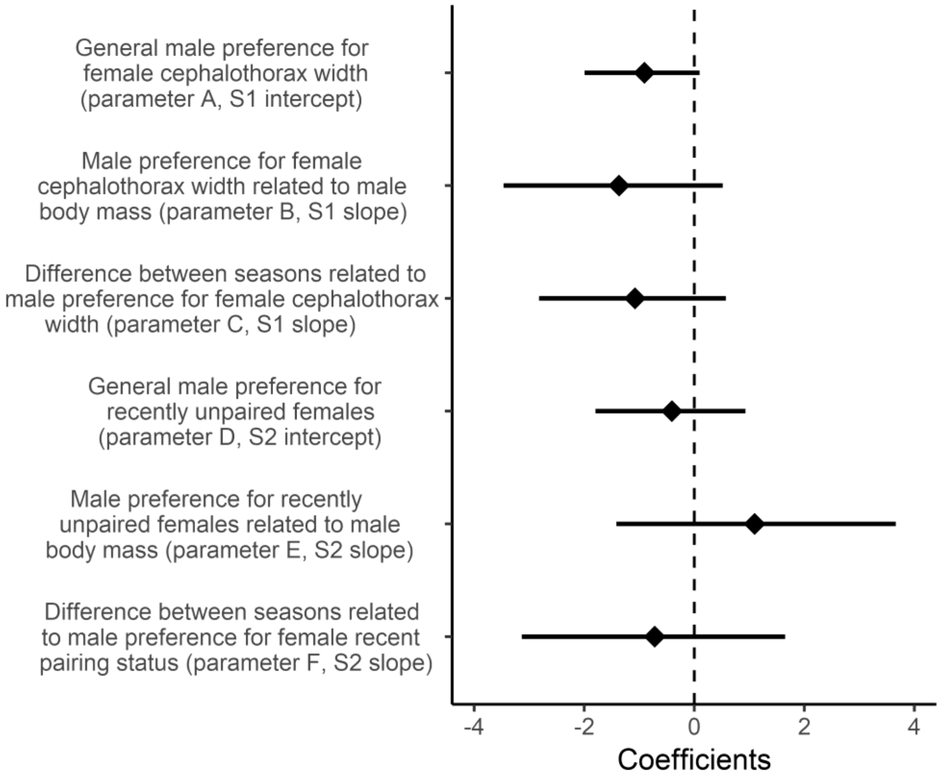

Figure 1. Results of the multinomial model investigating initial male mate choice (i.e., which female thread the experimental male climbed) in *Trichonephila clavipes*. Points and segments represent mean estimated parameter value and 95% credible interval. See details about what each parameter represents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

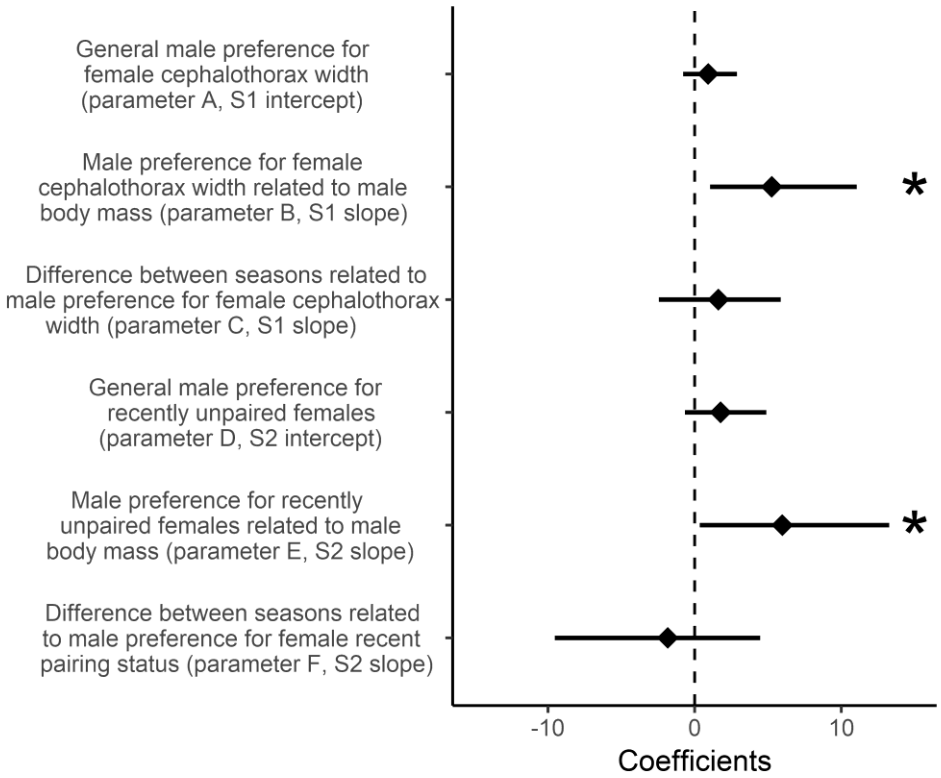

Figure 2. Results of the multinomial model to investigate late male mate choice (i.e., which female the focal male was guarding after 16 to 22 hours of the beginning of the experiment) in *Trichonephila clavipes*. Points and segments represent mean estimated parameter value and 95% credible interval.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

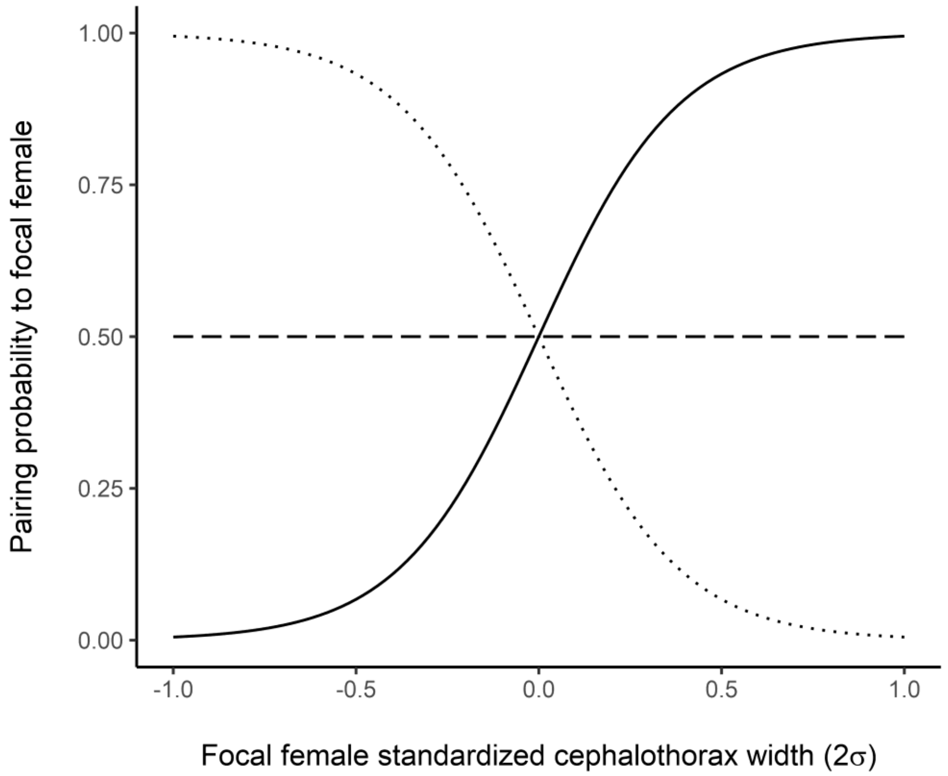

Figure 3. Pairing probability of a male with a focal female depending on male body mass and focal female cephalothorax width. In this scenario, a male may choose between the focal female and another female of standardized cephalothorax width of zero value (i.e., an average-sized female). Moreover, females do not differ in previous pairing status in this scenario. The probability that a high body mass male (solid line) will pair with the focal female increases as focal female cephalothorax width increases. In contrast, the probability that a low body mass male (dotted line) will pair with the focal females decreases as focal female cephalothorax width increases. The probability that medium body mass males (dashed line) will pair with the focal female does not depend on focal female cephalothorax width and is always 50% (random). We used coefficient values in which 95% credible interval did not overlap zero in the late male mate choice model to estimate the predicted values displayed in this figure.

Discussion

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

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. We found differences in male mate choice along the duration of trials, in which males were initially indifferent to the female traits we evaluated, 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 *T. clavipes* males can distinguish 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 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. Therefore, *T. clavipes* males may need time to evaluate female quality in a natural situation by gathering information on female quality from vibrational cues or from cuticular pheromones (Robinson, 1982). 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 & Burlaud, 2017; fish: Gasparini, Serena, & Pilastro, 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.

Traditional mate choice literature predicts that when individuals express mate preference, they should invariably choose high quality mates (Andersson, 1994). Yet, empirical studies that found intra-population variation on mate choice have been accumulating (Jennions & Petrie, 1997). Among several factors that may promote mate choice variation, theoretical models (e.g., Fawcett & Johnstone, 2003; Härdling & Kokko, 2005; Venner et al., 2010) identified that intrasexual competition may be a critical influence on male mate choice variation. Here, we found that *T. clavipes* males differ in the direction and intensity of their mate choice according to their own size. Only large males behaved according to what is generally predicted by the traditional mate choice literature: these males chose larger females that had not been recently paired. Whereas small males preferred smaller females that had been recently paired to other males. Why should these less competitive males choose lower quality females? If males invariably choose to copulate with high quality females, these females would probably mate multiply, and males would probably share the paternity of the 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 the female and increase his likelihood of siring most of her offspring. In *T. clavipes*, guarding efficiency is positively related to male size (Constant et al., 2011). Therefore, the larger the male, the greater is the likelihood that he receives the benefits of mating with a high quality female.

Trichonephila clavipes males can guard only one female at a time, which means that lower quality females would become available as large 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 (i.e., prudent mate choice; Härdling & Kokko, 2005). This rationale is supported by theoretical models that explore the influence of male competitive ability on male mate choice, which usually focus on the extremes of male phenotypes using two male categories (e.g., high/low quality in Fawcett & Johnstone, 2003; large/small in Härdling & Kokko, 2005; high/low competitive ability in Venner et al., 2010). Our results on the choice expressed by males of extremes sizes (large/small) match the assortative choice predicted by these theoretical studies under certain parameters. However, these models do not clarify what is expected of average competitors. Here, we found that *T. clavipes* medium-sized males are unselective regarding female quality. Following the rationale that competitive ability determines whether males prefer or avoid high-quality females, medium-sized males stand in the middle. That is, because medium-sized males are displaced by half of their competitors, they may gain the advantages of pairing with high-quality females sometimes, but not frequently enough to promote preference nor rarely enough to promote avoidance to these females. On the other hand, it would be interesting to explore whether males express consistent preference for a particular female phenotype. This is because our findings that medium-sized males are not selective towards female body size could also be explained by individual variation among medium-sized males. Overall, our results match precisely the results of a theoretical study on mutual mate choice in a scenario in which females are weakly sperm limited, female mating costs are low and male mating costs are high (see Figure 2 in Puurtinen & Fromhage, 2017). Surprisingly, Puurtinen & Fromhage (2017) did not consider pre-copulatory aggression explicitly in their model, revealing that our findings may also be justified with other features, such as sperm competition.

We found that the smaller the male, the greater is the probability that he chooses females that were recently being guarded by another male. It is possible that choosing recently paired females is a mechanism that decreases the risk of being displaced by larger males (in addition to preferring small females). Males using this strategy would copulate mostly with non-virgin females, and therefore would share the paternity of the offspring. This choice for recently paired females could indicate that smaller males tend to employ a sneaking tactic, in which the male stays on the periphery of a guarded female’s web and attempts to copulate unnoticed by the guarding male (Christenson & Goist, 1979). In this scenario, smaller males would be interpreting the cues of male presence as a sign that the female is currently being guarded, and would be choosing to be sneakers rather than guardians. Although we do not know how successful this strategy can be, small males appear to get less attention from guarding males when compared to large males (P.P. pers. obs.). In any case, males are apparently employing different mating tactics depending on their own body size. 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. Small males may compensate the lower quality of their partners by being more promiscuous. If that is the case, we would expect that male size is also (i) negatively related to movement between female webs, as a strategy to copulate with more females, and (ii) negatively related to investment in each female, in terms of sperm allocation and mate-guarding time.

Our results show that males similarly consider female size and female social context when choosing their mates. The combination of these two variables makes it difficult to estimate female quality in certain scenarios (e.g., a recently paired large female may be of similar quality to a not recently paired small female). Hence, a simple assortative pairing pattern by body size alone may not necessarily occur in natural conditions. Furthermore, our results indicate that all females, regardless of size, are able to attract males to mate, although it seems that they attract more males of a particular fighting ability than randomly expected. Thus, even though male mate choice can generate sexual selection on females and even favour the evolution of female sexual ornaments (e.g., Amundsen & Forsgren, 2003), variation in male mate choice may attenuate sexual selection on females, especially if some males perform prudent mate choice. Therefore, scenarios of variable male mate choice may be common, but hard to detect. Detection of variation in male mate choice is made difficult by two main reasons: (1) it generates weak or no sexual selection on females and (2) it requires specific experimental and statistical protocols (as the ones we employed here). We argue that variation in male mate choice may be relatively common, as theory predicts that it will evolve in populations in which mating is costly for males and there is strong intrasexual competition among males (Fawcett & Johnstone, 2003; Härdling & Kokko, 2005; Venner et al., 2010). These conditions occur in *T. clavipes*, and may also be met by other species in which males cannot monopolize groups of females, so that these males are continuously searching for new mates, or in mating systems in which males can guard a single female at a time. Such non-territorial mating systems are less studied than territorial ones, but are the most common among animals (Herberstein, Painting, & Holwell, 2017). Therefore, future studies employing an experimental protocol similar to ours may discover that variation in male mate choice is very common in natural populations.

Declaration of interest

The authors have no competing financial interests related to this research.

Acknowledgements

This study was supported by National Geographic Society (grant number WW-083ER-17), Brazil's Coordination of Superior Level Staff Improvement (CAPES; Finance Code 001) and Brazil's National Council for Scientific and Technological Development (CNPq). We thank Paulo E. C. Peixoto and Vinícius M. Caldart for feedback on the manuscript. We are thankful for comments and discussion on the development of this project by Glauco Machado, Cristiane H. Millán and Gustavo Requena. We also thank Clare C. Rittschof and Shakira Quiñones for tips on *Trichonephila* handling and maintenance in the lab.

Authors' contributions

P.P and E.S.A.S. conceived the idea of the study, P.P. collected the data, P.P. and D.G.M. analysed the data, P.P., D.G.M and E.S.A.S. wrote the manuscript.

Data accessibility

Data and analysis scripts are available at http://osf.io/6n7kt/.

References

Amundsen, T., & Forsgren, E. (2003). Male preference for colourful females affected by male size in a marine fish. *Behavioral Ecology and Sociobiology*, *54*(1), 55–64. https://doi.org/10.1007/s00265-003-0593-4

Andersson, M. (1994). *Sexual selection*. Princeton, New Jersey: Princeton University Press.

Andrade, M. C. B. (1996). Sexual selection for male sacrifice in the Australian redback spider. *Science*, *271*(5245), 70–72. https://doi.org/10.1126/science.271.5245.70

Arnaud, L., & Haubruge, E. (1999). Mating behaviour and male mate choice in *Tribolium castaneum* (Coleoptera, Tenebrionidae). *Behaviour*, *136*(1), 67–77. https://doi.org/10.1163/156853999500677

Austad, B. Y. S. N. (1983). A game theoretical interpretation of male combat in the bowl and doily spider (*Frontinella pyramitela*). *Animal Behaviour*, *31*, 59–73. https://doi.org/10.1016/S0003-3472(83)80173-0

Bel-Venner, M. C., Dray, S., Allainé, D., Menu, F., & Venner, S. (2008). Unexpected male choosiness for mates in a spider. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1630), 77–82. https://doi.org/10.1098/rspb.2007.1278

Berglund, A., Rosenqvist, G., & Svensson, I. (1989). Reproductive success of females limited by males in two pipefish species. *The American Naturalist*, *133*(4), 506–516. https://doi.org/10.1086/284932

Bonduriansky, R. (2001). The evolution of male mate choice in insects: a synthesis of ideas and evidence. *Biological Reviews*, *76*(3), 305–339. https://doi.org/10.1017/s1464793101005693

Brown, S. G. (1985). Mating behavior of the golden-orb-weaving spider, *Nephila clavipes*: II. Sperm capacitation, sperm competition, and fecundity. *Journal of Comparative Psychology*, *99*(2), 167–175. https://doi.org/10.1037/0735-7036.99.2.167

Carpenter, B., Gelman, A., Hoffman, M. D., Lee, D., Goodrich, B., Betancourt, M., … Riddell, A. (2017). Stan: a probabilistic programming language. *Journal of Statistical*

Software, *76*(1), 1–32. https://doi.org/10.18637/jss.v076.i01

Christenson, T. E. (1989). Sperm depletion in the orb-weaving spider *Nephila clavipes* (Araneae, Araneidae). *Journal of Arachnology*, *17*(1), 115–118.

Christenson, T. E., & Cohn, J. (1988). Male advantage for egg fertilization in the golden orb-weaving spider (*Nephila clavipes*). *Journal of Comparative Psychology*, *102*(4), 312–318. https://doi.org/10.1037/0735-7036.102.4.312

Christenson, T. E., & Goist, K. C. (1979). Costs and benefits of male-male competition in the orb weaving spider, *Nephila clavipes*. *Behavioral Ecology and Sociobiology*, *5*(1), 87–92. https://doi.org/10.1007/bf00302697

Constant, N., Valbuena, D., & Rittschof, C. C. (2011). Male contest investment changes with male body size but not female quality in the spider *Nephila clavipes*. *Behavioural Processes*, *87*(2), 218–223. https://doi.org/10.1016/j.beproc.2011.04.003

Cratsley, C. K., Rooney, J. A., & Lewis, S. M. (2003). Limits to nuptial gift production by male fireflies, *Photinus ignitus*. *Journal of Insect Behavior*, *16*(3), 361–370. https://doi.org/10.1023/A:1024876009281

Dewsbury, D. A. (1982). Ejaculate cost and male choice. *The American Naturalist*, *119*(5), 601–610. https://doi.org/10.2307/2461181

Dewsbury, D. A. (2005). The Darwin-Bateman paradigm in historical context. *Integrative and Comparative Biology*, *45*(5), 831–837. https://doi.org/10.1093/icb/45.5.831

Dougherty, L. R., & Shuker, D. M. (2015). The effect of experimental design on the measurement of mate choice: a meta-analysis. *Behavioral Ecology*, *26*(2), 311–319. https://doi.org/10.1093/beheco/aru125

Edward, D. A., & Chapman, T. (2011). The evolution and significance of male mate choice. *Trends in Ecology & Evolution*, *26*(12), 647–654. https://doi.org/10.1016/j.tree.2011.07.012

Fawcett, T. W., & Johnstone, R. A. (2003). Mate choice in the face of costly competition. *Behavioral Ecology*, *14*(6), 771–779. https://doi.org/10.1093/beheco/arg075

Gaskett, A. C. (2007). Spider sex pheromones: emission, reception, structures, and functions. *Biological Reviews*, *82*(1), 27–48. https://doi.org/10.1111/j.1469-185X.2006.00002.x

Gasparini, C., Serena, G., & Pilastro, A. (2013). Do unattractive friends make you look better? Context-dependent male mating preferences in the guppy. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1756), 20123072. https://doi.org/10.1098/rspb.2012.3072

Gelman, A. (2008). Scaling regression inputs by dividing by two standard deviations. *Statistics in Medicine*, *27*, 2865–2873. https://doi.org/10.1002/sim.3107

Goubault, M., & Burlaud, R. (2017). Do males choose their mates in the lekking moth *Achroia grisella*? Influence of female body mass and male reproductive status on male mate choice. *Insect Science*, *25*(5), 861–868. https://doi.org/10.1111/1744-7917.12471

Gwynne, D. T. (1981). Sexual difference theory: mormon crickets show role reversal in mate choice. *Science*, *213*(4509), 779–780. https://doi.org/10.1126/science.213.4509.779

Härdling, R., & Kokko, H. (2005). The evolution of prudent choice. *Evolutionary Ecology Research*, *7*(5), 697–715. https://doi.org/10.1046/j.1461-0248.1999.00075.x

Head, G. (1995). Selection on fecundity and variation in the degree of sexual size dimorphism among spider species (class Araneae). *Evolution*, *49*(4), 776–781. https://doi.org/10.2307/2410330

Herberstein, M. E., Painting, C. J., & Holwell, G. I. (2017). Scramble competition polygyny in terrestrial arthropods. In M. Naguib, J. Podos, L. W. Simmons, L. Barrett, S. D. Healy, & M. Zuk (Eds.), *Advances in the study of behavior* (Vol. 49, pp. 237–295). Cambridge, Massachusetts: Elsevier. https://doi.org/10.1016/bs.asb.2017.01.001

Honěk, A. (1993). Intraspecific variation in body size and fecundity in insects: a general relationship. *Oikos*, *66*(3), 483–492. https://doi.org/10.2307/3544943

Jennions, M. D., & Petrie, M. (1997). Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews*, *72,* 283–327. https://doi.org/10.1111/j.1469-185X.1997.tb00015.x

Kasumovic, M. M., Bruce, M. J., Herberstein, M. E., & Andrade, M. C. B. (2007). Risky mate search and mate preference in the golden orb-web spider (*Nephila plumipes*). *Behavioral Ecology*, *18*(1), 189–195. https://doi.org/10.1093/beheco/arl072

Kelly, C. D. (2006). Fighting for harems: assessment strategies during male e male contests in the sexually dimorphic Wellington tree weta. *Animal Behaviour*, *72,* 727–736. https://doi.org/10.1016/j.anbehav.2006.02.007

Kuntner, M., Hamilton, C. A., Cheng, R.-C., Gregorič, M., Lupše, N., Lokovšek, T., … Bond, J. E. (2018). Golden orbweavers ignore biological rules: phylogenomic and comparative analyses unravel a complex evolution of sexual size dimorphism. *Systematic Biology*, *0*(0), syy082. https://doi.org/10.1093/sysbio/syy082

Lane, J. E., Boutin, S., Speakman, J. R., & Humphries, M. M. (2010). Energetic costs of male reproduction in a scramble competition mating system. *Journal of Animal Ecology*, *79*(1), 27–34. https://doi.org/10.1111/j.1365-2656.2009.01592.x

Mautz, B. S., & Jennions, M. D. (2011). The effect of competitor presence and relative competitive ability on male mate choice. *Behavioral Ecology*, *22*(4), 769–775. https://doi.org/10.1093/beheco/arr048

Michalik, P., & Rittschof, C. C. (2011). A comparative analysis of the morphology and evolution of permanent sperm depletion in spiders. *Plos One*, *6*(1), e16014. https://doi.org/10.1371/journal.pone.0016014

Muniz, D. G., Santos, E. S. A., Guimarães, P. R., Nakagawa, S., & Machado, G. (2017). A multinomial network method for the analysis of mate choice and assortative mating in spatially structured populations. *Methods in Ecology and Evolution*, *8*(10), 1321–1331. https://doi.org/10.1111/2041-210X.12798

Parker, G. A., & Pizzari, T. (2015). Sexual selection: the logical imperative. In T. Hoquet (Ed.), *Current perspectives on sexual selection* (pp. 119–164). Amsterdam: Springer Netherlands. https://doi.org/10.1007/978-94-017-9585-2_7

Perry, J. C., Sirot, L., & Wigby, S. (2013). The seminal symphony: how to compose an ejaculate. *Trends in Ecology & Evolution*, *28*(7), 414–422. https://doi.org/10.1016/j.tree.2013.03.005

Puurtinen, M., & Fromhage, L. (2017). Evolution of male and female choice in polyandrous systems. *Proceedings of the Royal Society B: Biological Sciences*, *284*, 20162174. https://doi.org/10.1098/rspb.2016.2174

R Core Team. (2017). R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.r-project.org/

Rittschof, C. C. (2011). Mortality risk affects mating decisions in the spider *Nephila clavipes*. *Behavioral Ecology*, *22*(2), 350–357. https://doi.org/10.1093/beheco/arq222

Robinson, M. H. (1982). Courtship and mating behavior in spiders. *Annual Review of Entomology*, *27*, 1–20. https://doi.org/10.1146/annurev.en.27.010182.000245

Robinson, M. H., & Mirick, H. (1971). The predatory behavior of the golden-web spider *Nephila clavipes* (Araneae: Araneidae). *Psyche*, *78*(3), 123–139. https://doi.org/10.1155/1971/57182

Rowe, C. (1999). Receiver psychology and the evolution of multicomponent signals. *Animal Behaviour*, *58*, 921–931. https://doi.org/10.1006/anbe.1999.1242

Schneider, J. M., Lucass, C., Brandler, W., & Fromhage, L. (2011). Spider males adjust mate choice but not sperm allocation to cues of a rival. *Ethology*, *117*(11), 970–978.

https://doi.org/10.1111/j.1439-0310.2011.01960.x

Schneider, J. M., Zimmer, S. M., Gatz, A. L., & Sauerland, K. (2016). Context- and state-dependent male mate choice in a sexually cannibalistic spider. *Ethology*, *122*(3), 257–266. https://doi.org/10.1111/eth.12466

Schwagmeyer, P. L., & Parker, G. A. (1990). Male mate choice as predicted by sperm competition in thirteen-lined ground squirrels. *Nature*. https://doi.org/10.1038/348062a0

Stan Development Team. (2018). RStan: the R interface to Stan. Retrieved from http://mc-stan.org/

Tang-Martinez, Z. (2016). Rethinking Bateman's principles: challenging persistent myths of sexually reluctant females and promiscuous males. *The Journal of Sex Research*, *53*(4–5), 532–559. https://doi.org/10.1080/00224499.2016.1150938

Tang-Martinez, Z., & Ryder, T. B. (2005). The problem with paradigms: Bateman's worldview as a case study. *Integrative and Comparative Biology*, *45*(5), 821–830. https://doi.org/10.1093/icb/45.5.821

Venner, S., Bernstein, C., Dray, S., & Bel-Venner, M.-C. (2010). Make love not war: when should less competitive males choose low-quality but defendable females? *The American Naturalist*, *175*(6), 650–661. https://doi.org/10.1086/652432

Vollrath, F. (1980). Male body size and fitness in the web-building spider *Nephila clavipes*. *Zeitschrift Für Tierpsychologie*, *53*(1), 61–78. https://doi.org/10.1111/j.1439-0310.1980.tb00733.x

Vollrath, F., & Parker, G. A. (1992). Sexual dimorphism and distorted sex-ratios in spiders. *Nature*, *360*(6400), 156–159. https://doi.org/10.1038/360156a0

Wada, S., Arashiro, Y., Takeshita, F., & Shibata, Y. (2010). Male mate choice in hermit crabs: prudence by inferior males and simple preference by superior males. *Behavioral Ecology*, *22*(1), 114–119. https://doi.org/10.1093/beheco/arq183

Wedell, N., Gage, M. J. G., & Parker, G. A. (2002). Sperm competition, male prudence and sperm-limited females. *Trends in Ecology & Evolution*, *17*(7), 313–320. https://doi.org/10.1016/S0169-5347(02)02533-8