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

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Abstract

Males are expected to mate with as many females as possible, but can maximize their reproductive success through strategic mating decisions. For instance, males can increase their own fitness by mating with high quality females that produce more offspring. Additionally, males can adjust mating effort based on the relative distribution of females and male competitors. To test factors that influence male mate choice, we assessed male mating decisions in the golden-web spider, *Trichonephila clavipes* (Nephilidae), a species in which females are polyandrous and males guard females before and after copulation occurs. We tested the hypothesis that males spend more time guarding high quality females that are spatially isolated, and when the risk of sperm competition is higher. We assessed solitary and aggregated female webs in the field and quantified female quality (i.e. female body condition), the risk of sperm competition (i.e. number of males in each female web), and mate-guarding duration (i.e. number of days each male spent in each web). We found that mate-guarding behaviour is influenced by the presence of male competitors. In addition, the type of web seems to moderately influence male mating decisions, with males spending more time guarding a female when on solitary webs. Finally, female body condition seems to play a small role in mate-guarding behaviour. As mate-guarding duration considerably increased per each additional male competitor in the web, and guarding behaviour prevents males from seeking additional mates, it seems that males do benefit from guarding females. We conclude that failing to guard a sexual partner promotes high costs derived from sperm competition, and a male cannot recover his relative loss in fertilization success by seeking and fertilizing more females. In addition, the search for more sexual partners can be constrained by possible high costs imposed by predators and fights against other males, which may explain why the type of web only moderately influenced male mate choice. Following the same rationale, if high-quality females are not easy to find and/or mating with a high-quality female demands much effort, males may search females and guard them regardless of female quality. In conclusion, the factor that most influences male mate-guarding behaviour among *T. clavipes* in the field is the risk of sperm competition.

Keywords: body condition, guardian, mating effort, male mate choice, Nephilidae, reproductive effort, satellite, *Trichonephila clavipes*, sexual selection, web.

Introduction

The classical theory of sexual selection predicts that male reproductive success is positively influenced by the number of females a male copulates with (Darwin, 1871; Bateman, 1948). Therefore, following the Darwin-Bateman paradigm, males were initially predicted to mate with the maximum number of females they can have access to (Dewsbury, 2005). However, factors such as the quality of a potential mate, the difficulty to find additional matings, or the intensity of sperm competition should all influence the expected reproductive success of males (Scharf, Peter & Martin, 2013). Consequently, developments in sexual selection theory lead to predictions that males should adjust their mating investment based on expected reproductive success (Parker, 1998; Bonduriansky, 2001). This strategic adjustment of male mating investment should occur especially in species in which males face high costs of mating, or limited mating opportunities (Schneider, 2014). Thus, male reproductive success is not always maximized by copulating with as many females as possible.

Male strategic mating decisions are likely influenced by female quality (Bonduriansky, 2001; Edward & Chapman, 2011). For males, high quality females may be the females with the best body condition, given that they are more likely to survive until oviposition and provide resources for the eggs (Rittschof, 2011). Female body size can indicate female condition for males and it is also a predictor of female fecundity in arthropods (Honěk, 1993; Foellmer & Moya-Laraño, 2007). Thus, copulating with large females could increase a male's reproductive success as a result of a greater likelihood of female survival until oviposition and also as a result of an increased number of viable eggs a male can fertilize. In scenarios in which female condition is variable, males can optimize their reproductive success by choosing to mate with females that are in the best condition (Reinhold, Kurtz & Engqvist, 2002). Thus, mating with a few high-quality females may yield a greater reproductive success than mating with various low- or average-quality females (Bonduriansky, 2001).

Complementarily, male strategic mating decisions may be influenced by the spatial distribution of females and male competitors. For instance, whether females are scattered or aggregated around a habitat should influence how males encounter females. This spatial distribution dictates whether males encounter females in a more simultaneous or sequential manner, and male mate choice is expected to evolve under the former condition (Barry & Kokko, 2010). For males that have to search for females, finding more than one viable partner can be easier when females are "clumped", as there can be “hot-spots” of reproductive opportunity (Emlen & Oring, 1977). However, when the costs of finding females are low, the probability of facing male competitors and the risk of sperm competition tend to increase (Gage, 1995; Kokko & Rankin, 2006). Thus, when investigating aspects of male mate choice, one should take into account the distribution of potential mates as well as the distribution of competitors. Whenever different females can be easily found, males could optimize their reproductive success by investing in a mating strategy of searching for several mates (Kasumovic & Jordan, 2013). On the other hand, whenever several males compete for the same female, or females are hard to find, males could optimize their reproductive success by defending the access to the female(s) (Seibt & Wickler, 1979).

To test these ideas, we used the golden-web spider, *Trichonephila clavipes*, a species in which females can mate with multiple males (Rittschof et al., 2012) and males use a mate-guarding strategy (Christenson & Goist, 1979). Females construct webs that can be physically connected to the webs built by other females or can be completely isolated (Rypstra, 1985). Males are able to move to different webs and sample females, which may allow them to choose the best options among potential sexual partners (Pollo, Muniz & Santos, 2019). Additionally, males become sexually mature sooner in the breeding season than females, thus they can mate-guard juvenile females and wait to copulate when they become sexually mature (Christenson & Goist, 1979). Given that different males adopt different strategies (i.e., guarding behaviour vs. searching behaviour), our main interest was to answer the question: why do some *T. clavipes* males spend more time with the same female instead of searching for more females, while others do not? Our hypothesis is that males spend more time guarding high quality females that are spatially isolated, and when the risk of sperm competition is higher. Therefore, we predict that males will guard females for longer periods of time when (1) females are in solitary webs that are not physically connected to other female webs, (2) there are more satellite males in the female web, and (3) females are in a better body condition.

Methods

Data collection

We conducted our study in the gardens that surround the Zoology Department building at Instituto de Biociências (23.564°S, 46.729°W) on the main campus of the University of São Paulo, in São Paulo, Brazil. The garden is delimited by the Zoology Department building and by the access roads around it. *Trichonephila clavipes* individuals present in the area build their webs using trees, shrubs, lamp poles and walls as anchoring substrate. We made observations twice a day, at approximately 9 A.M. and at 2 P.M. between the 11th of February and the 23rd of May of 2015. We did not collect data (see below, for details) when there was heavy rain, because under these conditions females can eat up to half of their webs (LADM, personal observation), influencing the position of males, and, thus, our data collection.

We limited our data collection to those webs that were built between 0 and 2 m above the ground (measured from the hub of the web to the ground) in order to allow us to individually mark individuals and conduct accurate observations. During each visit to a web, we classified it as "solitary" or "aggregated". If the web did not share threads with other webs, we considered it as a solitary web. If the web shared threads with other webs, we considered it as an aggregated web. The type of web could change from day to day, with new females sharing threads with a previously solitary web or females becoming isolated because other females on the aggregation died. We identified females by combinations of colours painted in the dorsal side of their abdomens using acrylic paint (Testors). We conducted this procedure without removing the females from the web to avoid disturbances that may cause females to abandon their webs. In some aggregations, painting females' abdomen was not possible without removing threads or destroying adjacent webs. Therefore, some females were identified daily by their web position in the aggregation, as positions hardly changed from day to day (Vollrath, 1985; LADM, personal observation).

We also identified males with fine dots of acrylic paint on the dorsal side of their abdomen. Each male received a unique combination of two or three colour dots that allowed individual identification. In order to mark males, when monitoring the female webs, if we found a male that was not uniquely identified, we removed the male from the web with a fine paintbrush and placed him inside a Petri dish. We placed this

Petri dish on top of ice and kept it inside an ice-cooler for approximately two minutes. We used this cooling procedure to make males less active in order to facilitate individual colour-marking and measuring. Each male received a unique combination of two or three colour dots that allowed individual identification. We recorded males as "guardians" if they were occupying a central position on the web and were the closest male to the female. We identified males as "satellites" if they were on sustaining threads or on the periphery of the female web and were not the closest male to the female.

We photographed females at intervals of approximately 10 days. All photos contained a scale bar for posterior measuring. We measured cephalothorax width (mm), abdomen width (mm), and abdomen length (mm) of females using *Image J* (Schneider, Rasband & Eliceiri, 2012) to infer body condition. If more than one photo of a female was taken, the photos used for measurement were the ones that were taken closer to the period a male stayed with the female. Female abdomen volume was estimated based on abdomen length and width, and assuming its shape to be equivalent to the volume of a cylinder $V = \pi \times r^2 \times h$. We used residuals from a regression between abdomen volume (response variable) and cephalothorax width (predictor) to calculate a body condition index. This body condition index provides an estimate that is uncorrelated with body size, and is widely used in studies with spiders (Taylor, Price & Wedell, 2014; Macedo-Rego et al., 2016). Negative values represent females with smaller predicted abdomen volume given their cephalothorax width, whereas positive values represent females with larger predicted abdomen volume given their cephalothorax width.

Data analysis

We tested our predictions about the amount of time guarding males spent with females with generalized linear mixed models (GLMMs) with model selection and model averaging based on AIC (Burnham & Anderson, 2002). The response variable of the models was time spent (in days) by a guardian male with the same female. Thus, we fitted GLMMs with a Poisson error structure and a log link function. In all models, we used male identity as a random factor to account for the repeated observations made on individual males. Moreover, to account for overdispersion, we included an observation level random effect in the model. We included the type of web (aggregated or solitary) as a categorical binary predictor. The risk of sperm competition was included as a predictor variable and was coded as the maximum number of males present on the web during the time a guarding male spent with the same female. We also included female body condition index as a continuous predictor. We included an interaction between type of web and number of males, and another interaction between type of web and female condition in the global model. We standardized all input variables with the function *rescale* from the package *arm* (Gelman & Su, 2016) in order to be able to directly compare effect sizes from the predictors and to allow comparison of the effects when interactions are present.

We used model averaging (Burnham & Anderson, 2002; Grueber et al., 2011), with the functions available in the package *MuMIn* (Bartón, 2018), to determine what were the most important predictors in our GLMM. We built a global model with all predictors and interactions based on our predictions, and then derived a set of models with all combinations of explanatory variables. We defined our top model set as those models that fell within two AIC_C of the best model in the set. We used the natural-average method to conduct model averaging (Burnham & Anderson, 2002; Grueber et al., 2011; Nakagawa & Freckleton, 2011). Model averaging yields two outputs, the standardized coefficients (and their unconditional standard errors, which incorporate model-selection uncertainty) and the relative importance of each coefficient for explaining the variance in the response variable (Grueber et al., 2011). We present estimated parameters along with their 95% compatibility intervals (CIs) and discuss our findings interpreting the parameter point estimates, while at the same time acknowledging their uncertainty (Wasserstein, Schirm & Lazar, 2019). All GLMMs were built using the *lme4* package (Bates et al., 2015) in the *R* programming environment (R Core Team, 2017).

Results

We sampled a total of 40 guardian males (93 observations) and 31 females (22 aggregated and 14 solitary; type of web sums 36 webs because some females changed between types). The median number of males — including guarding and satellite males — in a web was 1 (range: 1 to 5; aggregated webs: 1 [1 to 3] male; solitary webs: 1 [1 to 5] male). We re-sighted 25 guardian males (62.5%) in at least another web, from a different female than where he was originally observed. Female body condition, as estimated by the residual of abdomen volume and cephalothorax width ranged from -492.14 to 516.46 (mean female body condition = -6.94, S.D. = 243.0).

We generated a model set (including the null model) from the global model that resulted in 13 models (Table 1). Including the best model, we had two models within two AIC_C top model set (Table 1). Both of these models contained the predictor number of males and one model contained the type of web so that we could estimate the effects of these variables on the number of days that males spent guarding a female. Males spent, on average, 2.54 days guarding females, as estimated by the model averaged parameter (Table 2).

The parameter estimate for the number of satellite males on the web was positive, indicating that the amount of time a guarding male spent with a female increased as the number of males on the web increased (Table 2, Fig. 1). The 95% CI range for the number of males on the web ranged from 0.315 to 1.105, suggesting that our data is compatible with small to large positive effects of the presence of satellite males on the time spent guarding (Table 2). The type of web parameter was also positive, indicating that guarding males spent more time with females on solitary webs (Table 2). The unconditional standard error (which incorporates model-selection uncertainty) of the effect size of type of web was large relative to the effect size, and the 95% CI range for this effect size ranged from a small negative effect to a moderate positive effect suggesting that these results are most compatible with no important effect (Table 2). The magnitude of the effect size of the number of males on the web was approximately 2.6 times larger than the effect of the type of web on the number of days that a male spent with a female, as can be observed by the relative importance of these parameters (Table 2).

Discussion

Here, in a population of *T. clavipes*, we showed that the factor that best explained the variation in male mate-guarding behaviour was the number of males cohabiting the female web. In other words, males seem to guard females for a longer time when the risk of sperm competition is higher, and sperm competition seems to be the factor that most influences male mating decisions. Additionally, we showed that the type of web (i.e. aggregated vs. isolated webs) may moderately influence male mating decisions, with males investing more time on guarding females when in solitary webs. However, it is important to highlight that, when contrasting the type of web and the number of competitors on the web, the number of males alone seemed to be the sole factor that imposed a biologically relevant effect on male guarding behaviour. Finally, we also showed that the intrinsic quality of the female seems to play a small role in the decision that males make to spend more or less time guarding on a web. Below, we discuss why the risk of sperm competition is more effective in shaping male mate-guarding behaviour than the web's connectivity and female quality.

As the number of satellite males on a web increases, it is possible that some of these males will manage to copulate with the female, leading to sperm competition for the fertilization of her eggs. This is a probable scenario given that it is now well-known that females mate multiply (Gowaty, 2006; Taylor, Price & Wedell, 2014), with several examples among spiders (*e.g.*, Prokop & Maxwell, 2009; González, Costa & Peretti, 2019), including *T. clavipes* (Rittschof et al., 2012). Males may avoid such risk of sperm competition by guarding females and, therefore, preventing competitors from mating with her, as widely documented for spider species (*e.g.*, Herberstein et al., 2005; Elias et al., 2014). And, the higher the number of competitors, the higher should be the effort employed by guarding males. Accordingly, in our study, mate-guarding time increased 0.71 day per each additional satellite male on the web. Given this, we can ask: if guarding females is so costly, why do *T. clavipes* males guard females? Probably because the fitness costs of seeking additional mates and facing sperm competition are higher than the costs of mate-guarding behaviour, and males have a higher fitness return when reducing their lifetime mating success and guarding their current sexual partners. In other words, a male that mates with a given female and does not guard her tends to face a reduction in his own fertilization success that cannot be recovered by fertilizing other females.

Combined with the number of male competitors, despite being a less important factor, the type of web also seems to influence mate-guarding behaviour in *T. clavipes*. Assuming that guarding males in aggregated webs are more exposed to competitors, one could argue that both factors (i.e. the type of web and the number of satellites on the web) indicate the risk of sperm competition and that this similarity could explain why the interaction between these factors seems to explain variation in male mate-guarding behaviour. However, it is known that males in *T. clavipes* arrive at aggregated and solitary webs in equal frequencies (Meraz, Hénaut & Elgar, 2012). Therefore, the type of web is not a good proxy for the risk of sperm competition, but solely indicates how easy it is for each male in a given web to find additional potential mates in other webs.

Surprisingly, the effect of web connectivity alone is moderate to nonexistent, which may indicate that despite de connectivity provided by aggregated webs, leaving a given web and mating multiply brings high costs for males regardless of web type. These costs of moving among webs and searching for additional mates may include weight loss (Meraz, Hénaut & Elgar, 2012) and fighting with competitors (Rittschof, 2010), which may explain why the type of web has little influence on mate-guarding behaviour.

The possible costs derived from looking for high-quality mates and fighting with competitors may also explain why males did not spend more time on the webs of high-quality females. If the search for females incurs severe survival costs for males (see Kasumovic et al., 2007; Berger-Tal & Lubin, 2011), the distribution of males among female webs may be little influenced by female quality, especially if high-quality females are not easy to find. In this scenario, and given that looking for a high-quality female probably means facing extreme intrasexual competition, males may avoid leaving a current sexual partner. Consequently, the decision to stay in a given web or leave it in the search for another female is based almost solely on the quantity of competitors in the web, regardless of female quality. However, one should not conclude from our study that males in *T. clavipes* do not express mate choice for female partners. Recent papers are showing that male mate choice is more common in nature than previously expected (Edward & Chapman, 2011), our study is restricted to mate-guarding behaviour (therefore, it does not cover all aspects of male mate choice in *T. clavipes*), and field experiments have shown that large *T. clavipes* males prefer large females (Pollo, Muniz & Santos, 2019). Additionally, Rittschof (2011) showed that *T. clavipes* males transfer more sperm to females that are close to oviposition. Interestingly, females close to oviposition are the ones that will have less opportunity to mate with additional mates, which means that their sexual partners will face lower sperm competition. Therefore, because we demonstrate that mate-guarding behaviour is mainly determined by the density of male competitors, our results and the results provided by Rittschof (2011) reinforce the idea that male reproductive effort in *T. clavipes* is mostly influenced by the risk of sperm competition.

We predicted that male mate-guarding effort in *T. clavipes* would be influenced by female quality (i.e. female body condition), type of female web (aggregated or isolated), and the risk of sperm competition (i.e. the number of satellite males in a given web). However, only the intrasexual competition promoted by satellite males effectively influenced guardian mating decisions, increasing the amount of time guardian males spent defending the access to females. The type of web only had a moderate effect and female quality had no influence on mate-guarding duration. Our results provide additional evidence that post-mating competition plays an important role in determining male mating and reproductive investments in animals (Parker & Pizzari, 2010). Importantly, our results do not demonstrate absence of male preference for high-quality females, but show that female body condition does not modulate the time spent by males in guarding (potential) sexual partners. Given (1) the main role played by the risk of sperm competition demonstrated here, (2) that strategies to outcompete post-mating competitors across animals include offence and defence mechanisms (Sivinski, 1980; Parker, 2020), and (3) that different forms of post-ejaculatory investment may be traded-off (Parker, 2020), future studies should evaluate how investment in mate-guarding and investment in ejaculate are related in *T. clavipes* and other species. Complementarily, given the relevance of post-mating events in male decisions and that spiders are good systems do study sperm competition and cryptic female choice (Eberhard, 2004), future studies should address how mate-guarding duration influence how females use the sperm of each sexual partner in *T. clavipes* and other spider species.

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Table 1. Total model set for the number of days a male *Triconephila clavipes* spent guarding a female (n = 93 observations).

All models include the random effects of the male identity and also observation level. Models are ranked by increasing order of their Akaike information criterion corrected for small sample size (AIC$_C$). The top model set (ΔAIC$_C$ < 2.0) is indicated in bold.

Fixed effect predictors	**AIC$_C$**	**K**	**ΔAIC$_C$**	**Weight**
Number of males	409.6	4	0.00	0.380
Type of web + number of males	410.5	5	0.99	0.232
Number of males + female condition	411.7	5	2.13	0.131
Type of web + number of males + type of web x number of males	412.1	6	2.52	0.108
Type of web + number of males + female condition	412.8	6	3.21	0.076
Type of web + number of males + female condition + type of web x number of males	414.4	7	4.86	0.033
Type of web + number of males + female condition + type of web x female condition	415.1	7	5.53	0.024
Type of web + number of males + female condition + type of web x number of males + type of web x female condition	416.7	8	7.13	0.011
Type of web	419.8	4	10.29	0.002
Null model	420.3	3	10.70	0.002
Type of web + female condition	422.0	5	12.45	0.001
Female condition	422.3	4	12.72	0.001
Type of web + female condition + type of web x female condition	423.3	6	13.78	0.000

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

Table 2. Standardized predictors, from the averaged model, of the number of days a male *Triconephila clavipes* spent guarding a female.

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

Parameter	**β**	**SE**	**95% CI**	**RI**
intercept	0.93	0.12	0.689 to 1.175	
Number of males	0.71	0.20	0.315 to 1.105	1.00
Type of web	0.27	0.24	-0.207 to 0.747	0.38

β, standardized coefficient for model predictors; SE, unconditional standard error; 95% CI, 95% compatibility interval; RI, relative importance of each parameter to the other parameters in the final model.

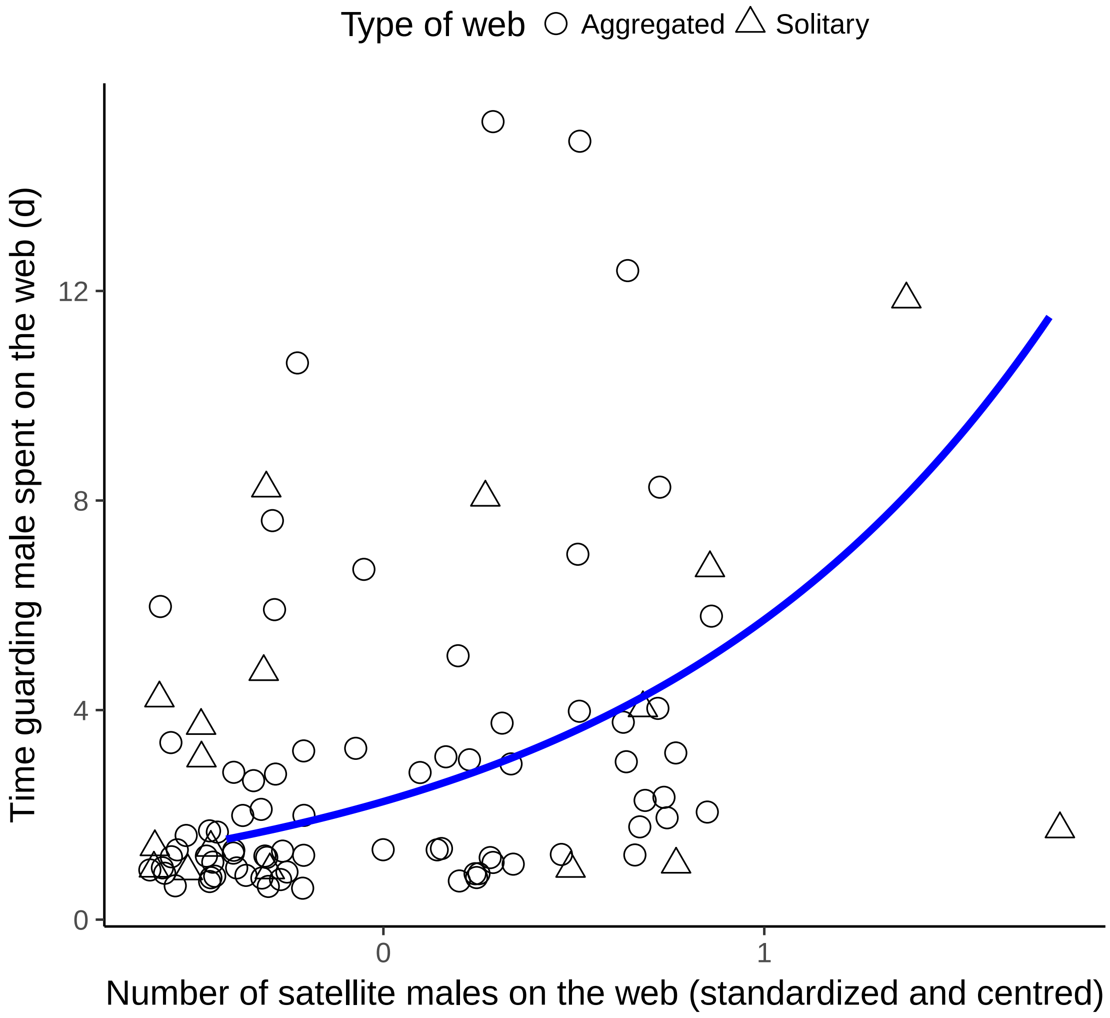

Figure 1. Relationship between the number of males (centred and scaled) on the female web and the time the guarding male spent (in hours) with a female on her web. Aggregated webs are represented by circles; solitary webs by triangles. Regression line (in blue) shown from coefficients of average model. Original points have been jittered horizontally to minimize overlap. One observation in which a male spent 32 days in a female web was removed from the plot for better visualization.