

UNIVERSIDADE FEDERAL DE MINAS GERAIS
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WEB WARS: MALES OF THE GOLDEN ORB-WEB SPIDER *TRICHONEPHILA CLAVIPES* ESCALATE MORE IN CONTESTS FOR MATED FEMALES AND WHEN ACCESS TO FEMALES IS EASIER

Belo Horizonte

2020

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Dissertação apresentada ao Programa de Pós Graduação em Ecologia, Conservação e Manejo da Vida Silvestre da Universidade Federal de Minas Gerais como requisito parcial para obtenção do título de Mestre em Ecologia, Conservação e Manejo da Vida Silvestre.

Orientador: Paulo Enrique Cardoso Peixoto

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Belo Horizonte, 19 de fevereiro de 2020

***DECLARAÇÃO**

DECLARAMOS, para os devidos fins, que o aluno deste Programa, Amanda Vieira da Silva, cumpriu todos os créditos e atividades acadêmicas obrigatórias e obteve o título de Mestre em Ecologia, Conservação e Manejo da Vida Silvestre, em virtude de aprovação em defesa de Dissertação, a saber:

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Doutor(a) Glauco Machdo - Membro Titular

Doutor(a) Adalberto José dos Santos - Membro Titular

Título: “Web wars: males of the golden orb-web spider *Trichonephila clavipes* show more escalated contests for adult females in aggregated webs”.

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*Declaração válida por até 30 dias após à defesa

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Coordenador PPG-ECMV
ICB / UFMG



ACKNOWLEDGMENTS

Instead of citing names, I will tell a little story about me and, as the story continues, I will thank. But first I want to highlight that these acknowledgments were not revised, so English may be not so correct. When I finished my undergrad, I wanted to change to another city. I was sick of São Paulo and its traffic, its mess, its people. But I was afraid of moving to a place where I did know anybody, so I tried to stay at UNIFESP. After a rejection of an advisor (my future doc advisor – Laura Leal), she kindly recommend me UFMG. I did the exam and, for my surprise, I passed but I did not have an advisor. Again, Laura and my friend Vivian (and ex-student of Peixoto), nicely, recommend me Paulo Peixoto. I had no other option than knocking on his door. And I did. He asked me what I wanted to do and I just say: I really like plant-insect interactions and I WANNA GO TO THE FIEEEELD, with real emphasis on “go to the field” (my honors’ study was in the laboratory). I think he took advantage from my “field wish” and accepted me. He also gave me some articles about animal behavior, mainly about animal contests and sexual selection. I read all of them, and in the first meeting he asked me (as an ask that there is only one possible answer) if I wanted to study spiders. Well, it was in field, so I said yes. This yes was just the beginning of an advisor-Jedi and a student-Padawan relationship that did not end here (although I am no longer a present part of the @lasexia). So, after these charismatic words, I want to thank you for being my advisor (sometimes, including personal advisor). I really learned a lot (from science to English language), but most importantly, I learned to be confident as a scientist (my potential as scientist cannot be measured by other rulers than mine). I also want to thank Laura Leal for putting me in @lasexia and helping me in whenever I need.

So, I had an advisor, but I still had no friends (except from Vivian, that I knew from UNIFESP). The first friend I made was João Gabriel, popularly known as Bathroom. He was

at the laboratory all the time (and he was weird). Now, he send me memes (mostly not funny) all the time (and he also asks me a lot of things, like “do you remember my tomato plant?”). Then, I made friends in my rep (Amanda, Karine, Sarah, Samara) and we usually watched heroes’ movies and Masterchef (thanks to you, I watched all Avengers, Guardians of the Galaxy, Thor). We even saw The Lion King in a projector (that did not work after this movie). After that, I made friends in the pos-grad program (Lucas, Aninha, Laix, Karen, Carlos – especially Karen and Carlos, who helped me a lot to build a cage – that did not work – for my spiders – that escaped because of holes made by grasshoppers). These pos-grad friends were the hardest one to do to me, because I am shy, and I usually don’t feel comfortable with people that I don’t know. At some moment, Daniel got in @lasexia (to work with spiders too) and, later, Fani. I did not have much contact with Daniel because he did not go to the laboratory every day, but Fani was there (so early, almost dawn). At first, I did not like Fani (and, for several days, I doubted that she studied lizards – she did not seem the person that love catching lizards. But she was insistent, and I had no other choice than be her friend (she also pisses me off because she can’t understand, among other things, that the best hot dog ever has mashed potatoes). At the middle of 2019, Apu came to the laboratory and he always gave me a ride to home (actually, he was more like a company). I really want to thank each of you for being so lovely and patient with me. I hope to continue these friendships for years. And I want to thank all the people that helped me in the field (Bathroom, Fani, Daniel, Guilherme, Bathroom’ friends, Apu, Fernando).

I had an advisor and friends, what more could I want? A co-advisor, of course. I don’t exactly remember when, but Paulo asked Reisla what she thought about my project (I remember that my project still was about sexual cannibalism). She had so many great ideas. Thus, one day, Paulo came to me with a question (the same type of question that I said above): what I thought about her as my co-advisor? She was so great, and I obviously couldn’t say no. Reisla

is, for me, an example of a woman scientist. And I hope to be like you one day (except that I enjoy technologies, even if I don't know how to use). Thanks, Reislá, for correct my texts, my presentations and for give me a bracelet made by yourself. I would also like to thank profs. Adalberto Santos e Glauco Machado for accepting my invitation.

In these two years, I missed home a lot – more than I thought, and every time I could, I came back to home, to my “known and safe world”. I want to thank to all my friends from São Paulo (especially Karine, Jubi, Sarah, and Erika). I had funny times with you every time I went to SP. I want to thank to my boyfriend, Fernando, for encourage me all the time and never doubt of my capacities (even if I doubt). And, of course, I really need to thank my family for all support: mom (Adriana), grandmom (Donília), uncle (Willians) and aunt (Renata), sister (Brendha) and brother-in-law (Natã), brother (Gabriel), grand-uncle (Gelson), cousin (Cadu). If it wasn't for you, I wouldn't be here.

Finally, these two years were difficult but since 2019 (when #Elenão happened), things got worse every day for women, LGBTQIA+, black people, education, science, nature. I was one of the people that made the exam and was awarded a CNPq scholarship. But the number of students receiving scholarships from CAPES, CNPq and FAPEMIG decreases every day. Recently, I did the exam for doctorate degree and I passed, but I don't know if I will do the course, because my family isn't rich enough to support me financially for four years. And I know that many other students are at the same situation. I don't want to end these acknowledgements in a sad way, by contrary. I want to end believing that a better world is possible, because, just like males' spiders fight for access to females, we need to fight for science, education and respect. So, when someone says to us that “the resistance is dead. The war is over. And when I kill you, I will have killed the last Jedi”, we will have the force to say “The Rebellion is reborn today. The war is just beginning. And I will not be the last Jedi”.

May the force be with you!

Resumo

Durante as brigas por acesso às fêmeas, machos podem pagar diversos custos que mudam de acordo com o tempo investido na interação e com os comportamentos adotados durante a briga. Por essa razão, é possível que os machos aumentem o investimento em brigas quando os benefícios potenciais a serem obtidos com a vitória forem altos. Os benefícios podem variar de acordo com características das fêmeas ou com o custo que os machos pagam para encontrar fêmeas. Neste estudo, testamos as hipóteses de que os machos da aranha de teia dourada, *Triconephila clavipes*, investem mais em brigas por acesso a fêmeas virgens e mais fecundas e quando o acesso às fêmeas mais é difícil. Para testar essas hipóteses, gravamos a ocorrência, duração e adoção de comportamentos mais agressivos (*i.e.*, escalonamento) durante as brigas entre machos para acessar fêmeas virgens e acasaladas. Em cada briga, também estimamos a fecundidade da fêmea e gravamos se a teia da fêmea ocorria de maneira isolada ou agregada com as teias de outras fêmeas (informação usada como um estimador para o custo que os machos perdedores deveriam pagar para encontrar novas fêmeas). Encontramos que a ocorrência e duração das brigas não foram relacionadas com atributos das fêmeas. Entretanto, a probabilidade de escalonamento durante as brigas foi maior em duas situações: 1) quando as fêmeas eram adultas (e provavelmente acasaladas) e 2) quando as fêmeas estavam agregadas. Esses resultados indicam que machos de *T. clavipes* ajustam o investimento em brigas, mas ao contrário do esperado. Sugerimos que o investimento em brigas é maior por fêmeas adultas do que por juvenis porque os machos estão defendendo fêmeas acasaladas para evitar competição espermática e em fêmeas agregadas devido à alta troca de machos rivais, o que impede o estabelecimento de uma hierarquia de dominância.

Palavras-chave: interações agonísticas; briga entre machos; valor do recurso; características da fêmea; procura de parceiros; seleção intrasexual

Abstract

During contests for access to females, males may pay several costs that change according to the time invested in the interaction or the behaviors adopted during contest. For this reason, it may be that males increase their investment in contests when the expected benefits obtained with victory are higher. The benefits could vary according to female traits or to the cost that males have to pay to find females. In this study, we tested the hypotheses that males of the golden orb-weaver spider, *Triconephila clavipes*, invest more in contests for access to virgin and more fecund females and when access to females is harder. To test these hypotheses, we recorded the occurrence, duration and adoption of more aggressive behaviors (*i.e.*, escalation) during the contests between males for access to mated and virgin females. In each contest, we also estimated female fecundity and recorded if the female web occurred isolated or in aggregation with other female webs (information used as a proxy for the cost that loser males would have to pay to find new females). We found that the occurrence and duration of contests were unrelated to female traits. However, the probability of escalation during the contests was higher in two situations: 1) when females were adults (and probably mated) and 2) when females were aggregated. These results indicate that males of *T. clavipes* adjust investment in contests, but contrarily to our predictions. We suggest that the investment in contests is higher for adult than juvenile females because males are defending mated females to avoid sperm competition, and in female aggregations due to the high turnover of rival males, which prevents an establishment of dominance hierarchy.

Keywords: agonistic interactions; male-male contests; resource value; female traits; mate searching; intrasexual selection

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1 **Presentation**

2 Dear reader, although the title is like a movie, this dissertation was not funded by Disney
3 (although the story I will tell you will be as exciting as Rey killing Chewbacca – sorry, spoiler
4 alert). I began my dissertation two years ago and I had one idea in mind: I wanted to understand
5 why males in some species let females eat them during copulation. Ok, maybe it is not a big
6 new idea, but I was excited to discover more about sexual cannibalism and all the environmental
7 (or extrinsic) factors involved. So, I read about it and *Triconephila clavipes*, according to some
8 old articles, was a good model species to do my research. I wrote a project; I did videos on
9 Youtube and posts on Instagram and I felt prepared for doing the study.

10 First day on the field: the spiders were babies and I did not find any male. We (me and my
11 lab friends) decided to wait a little more (it was Christmas) and, after New Year, we planned to
12 go to the field every day. Now, we found adult males and females, we observed them, marked
13 them day after day... Two weeks in the field and no sexual cannibalism. One month in the field
14 and nothing. Two months... The females of *T. clavipes* did not eat one single male! My world
15 fell apart. After almost five months in the field, we saw three females eating one male each (and
16 one of these females was parasitized). So, sexual cannibalism in this species is not frequent and
17 it's not possible to do inferences about that issue with the time I had to do my research. No
18 sexual cannibalism then, but even so, I had a dissertation to make. I had one year to finish my
19 masters and no project. Because I am an anxious person, I just thought about how to return the
20 money CNPq gave me and started to watch YouTube videos about how to sell arts on beaches.
21 But, not surprisingly, after two months observing spiders, we had some data about contests and
22 mating, and it was enough to make at least one-chapter for the dissertation (the one I hope you
23 will read after this presentation!). So, it was time to change ideas and read other articles about
24 other things.

25 What I knew about male contests: 1) females are considered limited resources to males,
26 then males fight against other males for access to females and 2) because female quality varies,
27 contests may vary too according to female quality. One thing that I supposed could happen: if
28 female density varies in the field, maybe males' fight also varies according to female density.
29 And, in *T. clavipes*, density vary according to aggregations: males in female aggregations have
30 more options to mate. Obviously, it was not enough to write a dissertation, but it was a
31 beginning. I read about contests, male searching abilities, female value and I questioned: is it
32 possible that males of *T. clavipes* adjust contests according to female value and density? For
33 reasons I do not remember now, we (me and my advisors) decided not to focus on female
34 density, but on male costs in searching for females that may be adjusted by female density.
35 Many males of *T. clavipes* cannot arrive at females' webs because they are so delicious that
36 predators eat them before they arrive at females' webs (again, according to very old articles).
37 This pattern (being eaten before arriving at a crush female or in biological scientific terms
38 "predation risk while searching") is also observed in other species and groups (such as fishes,
39 insects, and mammals). Because some females of *T. clavipes* occur spatially aggregated, the
40 search costs of males could be reduced. Because search cost was reduced, we expected that
41 males would not fight so much for females when they were spatially aggregated. Is this what
42 we found? Well, if you read the title you know my main finding. But how I explain this finding
43 considering that it was unexpected, you don't know yet. For this reason, I hope you will be
44 curious enough to read my dissertation!

45 In summary, two years have passed so fast and so many things changed... But one thing I
46 can assure you, during all the time I had curiosity for knowing more about ecological processes
47 and this curiosity brought me (and maybe you) here. I hope you enjoy this read as much I
48 enjoyed writing it!

49

50 **Introduction**

51 In many species, males fight other males for access to females (Hardy & Briffa, 2013).
52 These contests have costs to males, such as injuries (*e.g.*, Austad, 1983; Lane & Briffa, 2017;
53 Davis *et al.*, 2018), energetic expenditure (*e.g.*, DeCarvalho *et al.*, 2004), reduced survival (*e.g.*,
54 Guo & Dukas, 2020) and, more rarely, death (*e.g.*, Crespi, 1988; Piper *et al.*, 2008). However,
55 males can adjust the costs accrued during the contest by varying contest duration (*e.g.*, Bergman
56 *et al.*, 2010) or by changing agonistic behaviors while contest progresses (*i.e.*, escalation; *e.g.*,
57 Tachon *et al.*, 1999). The potential benefits obtained by males in the contests also vary, but this
58 variation is mainly determined by female traits (Sand, 1996; Kraak & Bakker, 1998) or by
59 environmental conditions (Palaoro *et al.*, 2017). Both female traits and environmental
60 conditions contribute to determining the perceived value of females to males. Due to the
61 existence of variation in the potential benefits, males that adjust how much they are willing to
62 invest in a contest in a way to optimize the cost-benefit ratio according to the chance of victory
63 should be favored. Thus, it is expected that males should invest more (*i.e.*, pay higher costs) in
64 contests when the value of female to males is higher.

65 The value of females to males may be related to traits that increase male fitness (*e.g.*,
66 Verrell, 1986; Liu & Hao, 2019). When the value of female varies, and male can perceive this
67 variation, it is possible that males should be selective (Edward & Chapman, 2011). This should
68 be particularly important for males that are limited in relation to the number of females that
69 they can fertilize, because males either suffer from sperm depletion (Damiens & Boivin, 2006;
70 Boivin, 2013) or may be predated by females during copulation (Prenter *et al.*, 2006; Schneider
71 & Elgar, 2002). For these males, a higher investment in fights for more fecund females may be
72 favored because these males are unable to increase their reproductive success by increasing the
73 number of fertilized eggs. In addition, in some species is more beneficial for males to fight for
74 access to virgin than mated females, regardless of female fecundity (*e.g.*, Hoefler *et al.*, 2009).

75 This is especially true for species that have first male sperm precedence (*e.g.*, Jones *et al.*, 2002;
76 Jones & Parker, 2008; Norton & Wright, 2019). In these species, males should invest more in
77 contests for access to virgin than mated females.

78 Environmental conditions could also affect male-male contests (Palaoro *et al.*, 2017). In
79 some cases, environmental conditions might affect the chance of males to access females (*e.g.*,
80 Andrade, 2003). While searching for mates, males of some species may have a high predation
81 risk (Magnhagen, 1991; Foley *et al.*, 2018). For example, on average, 76% of the males of
82 *Triconephila plumipes* (Kasumovic *et al.*, 2007) and 80% of the males in *Latrodectus hasselti*
83 (Andrade, 2003) die while searching for females. In addition, some males do not feed while
84 searching (*e.g.*, Proctor, 1992) and may suffer energy depletion (Foellmer & Fairbairn, 2005).
85 Such costs may be particularly affected by female spatial distribution (Rao *et al.*, 2009). If
86 females are highly dispersed and are hard to find, males have to travel higher distances to find
87 females. Thus, the energy spent by males and the predation risk during mate search may be
88 higher when females are spatially dispersed. In species in which males fight for access to
89 females, males probably pay higher costs to find a new female if they lose a fight when females
90 are spatially dispersed. Therefore, males may invest more in contests when females are isolated
91 (*i.e.*, access to female is harder) than when females are aggregated (*i.e.*, access to female is
92 easier).

93 How males adjust their efforts on contests according to the value of a female can be
94 measured under laboratory conditions (*e.g.*, Hoefler *et al.*, 2009). However, for many species,
95 laboratory experiments could not reflect real environmental pressures because they are unable
96 to precisely simulate the conditions and costs that males experience under natural circumstances
97 (Chapin *et al.*, 2019). For example, the cost of accessing females can be hard to estimate under
98 laboratory, since predation risks and patterns of spatial distribution of females are difficult to
99 simulate. This is especially true for species in which individuals have large living areas. Thus,

100 field evaluations are necessary to measure some of the real pressures that act on males and may
101 affect their behavior during agonistic interactions.

102 The Golden orb-web spider *Triconephila clavipes* Linnaeus 1767 (Araneae: Araneidae)
103 is one species in which male investment in contests for access to females may depend on value
104 of female and on the costs of mate searching. Males of *T. clavipes* search for and establish on
105 female webs (Christenson & Goist, 1979). If the male finds another male on the female web,
106 they fight for access to the female (Constant *et al.*, 2011). Male-male contests start when males
107 produce vibrations on the female web (Constant *et al.*, 2011) and can end during the vibration
108 phase or escalate. In escalated contests, males bite each other, sometimes ripping out the rival
109 legs or killing it (Constant *et al.*, 2011). Because escalated contest involves physical aggression
110 and may incur injuries and death, escalated contests have higher costs to males than non-
111 escalated ones. Thus, males should be able to adjust their investment in contests by deciding
112 whether they fight more time or escalate according to female value. As occurs in most spider
113 species, females of greater size are more fecund (Marshall & Gittleman, 1994; Prenter *et al.*,
114 1994). In *T. clavipes*, larger females are preferred by larger males, while smaller females are
115 preferred by smaller males (Pollo *et al.*, 2019). In addition, paternity is higher for the first male
116 that copulates with a female (Vollrath, 1980; Christenson & Cohn, 1988; Rittschof, 2010).
117 Therefore, males may invest more in contests for access to virgin and larger than mated and
118 smaller females. Since most females mate until 48 h after their last molt (Christenson & Goist,
119 1979), and because males establish in webs of immature females (Hill & Christenson, 1988),
120 males may invest more in contests for juvenile and penultimate females because they are virgin.
121 This male preference for more fecund and virgin females could be selected in *T. clavipes*
122 because males of this species are able to copulate only two times during their life (Christenson,
123 1989; Michalik & Rittschof, 2011). Males of *T. clavipes* also pay costs when searching for
124 females because they are highly predated during mate searching (Christenson & Goist, 1979;

125 Vollrath & Parker, 1992). However, these costs should vary according to the spatial distribution
126 of the females: some females occur in aggregations where many webs are physically connected
127 by one or more threads, while other females occur in isolated webs with no contact to any other
128 female web (Rypstra, 1985). When females are aggregated, males do not need to walk on the
129 vegetation to search for females in case they lose a fight. Therefore, males that lose a fight in
130 an aggregation should pay a lower cost of finding a new female than males that lose a fight in
131 an isolated web. Because the cost of finding a new female when a female is spatially isolated is
132 higher than the cost of finding a new female in aggregation, males should invest more in
133 contests for access to females in isolated webs.

134 In this study, we investigated if males of *T. clavipes* adjust their investment in contests
135 for access to females according to the female value and to the costs of mate search. For this, we
136 tested the hypotheses that males are more likely to engage in a contest and to invest more in
137 contests for access to 1) more fecund females; 2) virgin than mated females; and 3) when the
138 cost of finding a new female is higher. If these hypotheses are true, we expected that the
139 probability of contest occurrence, duration of contests and probability of contest escalation
140 should be higher in 1) webs of females with higher abdominal area; 2) webs of juvenile and
141 penultimate females when compared to webs of adult females; and 3) isolated webs when
142 compared to aggregated webs.

143

144 **Methods**

145 *Study area*

146 We developed this study in the reserve Ecological Station located in the campus
147 Pampulha at Federal University of Minas Gerais, Minas Gerais, Brazil (19° 52' S, 43° 58' W).
148 The Ecological Station is covered by semi-deciduous forest and savanna (*i.e.*, Brazilian

149 Cerrado) vegetation, with areas dominated by invasive plants (Antonini & Martins, 2003). In
150 the study area, the reproductive season of *T. clavipes* begins in December and ends in June, but
151 most adult males die until March (as in Pollo *et al.*, 2019). We conducted our field observations
152 between December 2018 and March 2019.

153

154 *Female sampling*

155 To find females, we searched for their webs in three trails of the reserve between 09:00
156 h and 17:00 h. To make observations, we choose webs located until 2 m from the ground,
157 allowing us to mark the spiders. Whenever we found a web with a female and two or more
158 males, we photographed the female with a ruler positioned nearby and measured the abdominal
159 area in the software Image J (version 1.8.0). We used abdominal area to estimate female
160 fecundity (following Elwood & Prenter, 2013). Then, we distinguished female life stage in
161 juvenile, penultimate and adult to estimate female reproductive status. We considered juvenile
162 and penultimate females as virgins because they cannot reproduce, and adult females as mated
163 because they usually copulate until 48 h after their final molt (Christenson & Goist, 1979).
164 Adult females can be distinguished from juveniles due to the presence of a swollen and
165 sclerotized epigynum with two distinct openings (Higgins, 2000). This distinction can be made
166 with naked eye or with the aid of a camera attached to a macro lens. Penultimate females can
167 be identified according to the web structure because, during molting, females remove the sticky
168 spirals from the web but maintain the radii lines (Robinson & Robinson, 1973). We also
169 recorded if the female was molting to the last instar by observing females until the end of this
170 study. We used female size in molting as a proxy to last instar (Moore, 1977; Cohn *et al.*, 1988)
171 and we did not see any observed molting female making another molt during the period of this
172 study.

173 We also searched for females located in isolated and aggregated webs. For aggregated
174 webs, we choose to observe the females that we could mark without destroying the aggregation.
175 We used female spatial distribution to estimate facility of males to access other females after
176 losing a contest. We considered isolated webs as difficult to males to get access to other females
177 and aggregated webs as suitable to males to get access to other females because if a male loses
178 a contest in an isolated web and decides to search for another female, he will go on vegetation,
179 where the predation risk is high. On the opposite, if a male loses a contest in an aggregated web
180 and decides to search for another female, he could go to other female web that has a connection
181 to the web in which the male was located and, because he did not need to go on vegetation, the
182 predation risk is low. Isolated webs were at least 1 m distant from other webs and aggregated
183 webs were connected to another by one or more threads (Rypstra, 1985). Even aggregated,
184 females usually stay in their own webs, while adult males walk among them (Rypstra, 1985).

185

186 *Male contests*

187 Males can stay in two different positions in female webs: peripheral or central
188 (Christenson & Goist, 1979). Males in peripheral position stay away from female, close to the
189 boundary of the web while males in central position stay close (*ca.* 5 cm above) to female, in
190 barrier strands (Christenson & Goist, 1979). Males located in peripheral position start the
191 contest by approaching the male in central position (Christenson & Goist, 1979). Both the
192 approach and the contest often occur when females are feeding (Farr, 1977). Therefore, to
193 increase the propensity of males to fight for females, we placed a grasshopper as a prey to the
194 female on her web. If the female did not manipulate the grasshopper in 10 min, we finished the
195 trial with that female in that day. If the female started to manipulate the grasshopper, we
196 observed the web for additional 30 min. If the female abandoned the grasshopper before 30

197 min, we finished the observation at the moment the female stopped feeding. For penultimate
198 females, we tried to place a grasshopper on the radii lines, but sometimes the grasshopper did
199 not glue on the web. In these cases, we continued the observations for 30 min, even without the
200 grasshopper. We also recorded the number of males on the web of each female, since the
201 number of males could affect the probability of contest occurrence (Rittschof, 2010). After
202 placing the grasshopper, we recorded if males started to fight. When a contest occurred, we
203 recorded its duration and if the contest escalated. We consider that a contest escalated when
204 there was physical aggression. At the end of each observation, we marked males and females,
205 with individual codes, using acrylic non-toxic paint applied to their prosoma (Acrilex Inc.,
206 Brazil). With the markings, we could identify males that have moved to other webs after the
207 contest.

208

209 *Statistical analyses*

210 We separately considered the probability of contest occurrence, contest duration, and
211 probability of contest escalation as response variables in the models. As explanatory fixed
212 variables, we used the female life stage, female abdominal area, and female spatial distribution.
213 Because the number of males could affect the male propensity to engage in contests (Rittschof,
214 2010), we used this data as a covariate in all analyses. As explanatory random variables, we
215 used female identity, hour of observation, day of observation and trail identity in all models.
216 We used “female identity” as a random factor because we observed the same female in different
217 days; “hour of observation” because the behavior of spiders could change among different
218 periods of the day; “day of observation” due to environmental variation among days; and “trail
219 identity” because trail width varies, so that more open trails could have more prey and perhaps
220 more activity of spiders. We started all models with all four explanatory random variables

221 included. However, when the variance associated with an explanatory random variable was
222 zero, we removed that variable from the final model (Table 1). For models that considered the
223 probability of contest occurrence and probability of contest escalation, we used binomial
224 distributions of errors. Because there were only two penultimate females in models considering
225 probability of contest escalation, we removed these females from the analysis. For models that
226 considered the duration of contests, we log-transformed the data to adjust data for a normal
227 distribution.

228 To test if the probability of contest occurrence, contest duration, and probability of
229 contest escalation change according to female life stage, female abdominal area and female
230 spatial distribution, we build nine generalized linear mixed models, each one with one
231 explanatory fixed and one response variable (Table 1). We opted to build one model for each
232 explanatory fixed variable because, in many instances, we had only information on fecundity
233 or on female reproductive status. Thus, a model considering only females for which we had
234 information for all three explanatory fixed variables simultaneously would erode our sample
235 sizes. To calculate p values, we used maximum likelihood ratio tests comparing a null model
236 without a fixed explanatory variable and a model with the same variable inserted. We did
237 graphical inspections for heteroscedasticity. We did all statistical analyses in software R (R
238 Core Team, 2018), using the package lme4 (Bates *et al*, 2015). For figures, we used the package
239 sciplot (Morales, 2017).

240 **Table 1.** Final models included in all generalized linear mixed models with their respective
 241 response, explanatory fixed, and explanatory random variables. Our goal was to test if female
 242 traits (*e.g.*, female life stage and abdominal area) and female spatial distribution (*e.g.*, webs
 243 spatially aggregated or isolated) of the Golden orb-web spider *Trichonephila clavipes* could
 244 increase male investment in contests for access to females. To estimate male investment in
 245 contest, we recorded the probability of contest occurrence, duration of contest and probability
 246 of contest escalation. In all models, we used four random factors (day and hour of observation,
 247 female identity and trail identity). When the variance associated with a random factor was zero,
 248 we removed that random variable from the model. We used the number of males in each web
 249 as a covariate in all models. N refers to number of observations for each model.

250

Response variable	Explanatory fixed variable	Explanatory random variable	N	χ^2(df)	p
Probability of contest occurrence	Female life stage	Female identity and trail	139	1.23 ₍₂₎	0.54
Probability of contest occurrence	Abdominal area	Day, hour, female identity and trail	100	0.23 ₍₁₎	0.63
Probability of contest occurrence	Female spatial distribution	Day, hour, female identity and trail	153	1.62 ₍₁₎	0.20
Duration of contest	Female life stage	Day and female identity	33	1.66 ₍₁₎	0.20
Duration of contest	Abdominal area	Female identity	40	2.08 ₍₁₎	0.15
Duration of contest	Female spatial distribution	Female identity	45	0.04 ₍₁₎	0.83
Probability of escalation	Female life stage	Day, hour, female identity and trail	21	5.74 ₍₁₎	0.02
Probability of escalation	Abdominal area	Day and female identity	19	0.03 ₍₁₎	0.86
Probability of escalation	Female spatial distribution	Day, hour, female identity and trail	25	9.22 ₍₁₎	0.002

251

252 **Results**

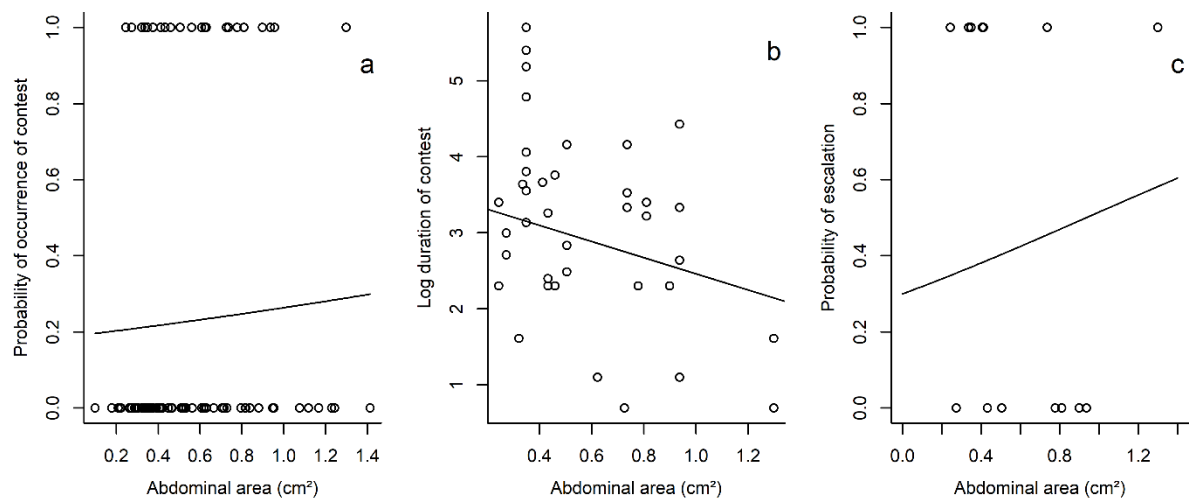
253 We did 156 observations on webs of 128 female webs of *T. clavipes*. After placing the
254 grasshopper on the web, the females usually (92.9% of the times) went toward the prey, even
255 penultimate females. Then, the female manipulated a grasshopper and went to the central
256 position on the web. At this moment, peripheral and central males started to move on the web.
257 Whenever a peripheral male approached the female, the central male started the contest by
258 moving toward the peripheral male and vibrating the web. We recorded contests between
259 males in 30 webs (23.4% of the 128 female webs sampled). Sometimes, after the end of the
260 contest, the peripheral male returned to the web and started the contest again. Then, in total,
261 we observed 56 contests (35.9% of the observations). When more than one contest was
262 observed on the same web, the contests occurred mostly (92.9%) between the same males. Half
263 of the observed contests escalated. After the contest, the loser usually stayed on the periphery
264 of the web or on the nearby vegetation or moved to another web (if the male was on an
265 aggregated web). The winner often went to the central position and tried to copulate with the
266 female while she was manipulating or consuming the prey. We never observed a male dying or
267 losing his legs during contests. We observed 15 females more than once and we saw a turnover
268 of central males in 66.7% of these females.

269 We did 59 observations in isolated webs and 94 observations in aggregated webs.
270 Aggregated webs had, on average, 4.93 ± 3.86 (mean \pm standard deviation) female webs and
271 7.10 ± 5.45 males in total. Isolated webs had 2.72 ± 0.90 males per web while aggregated webs
272 had 2.67 ± 1.32 males per web. In only five of 128 female webs we observed more than four
273 males: three webs with five males, one web with six males and one web with ten males.

274 The probability of occurrence of a contest ($b = 0.43$; fig. 1a; Table 1), the duration of
275 the contest ($b = -1.06$; fig. 1b; table 1) and the probability of escalation during the contest ($b =$
276 -67.41 ; fig. 1c; Table 1) were not related to female abdominal area. Similarly, neither the

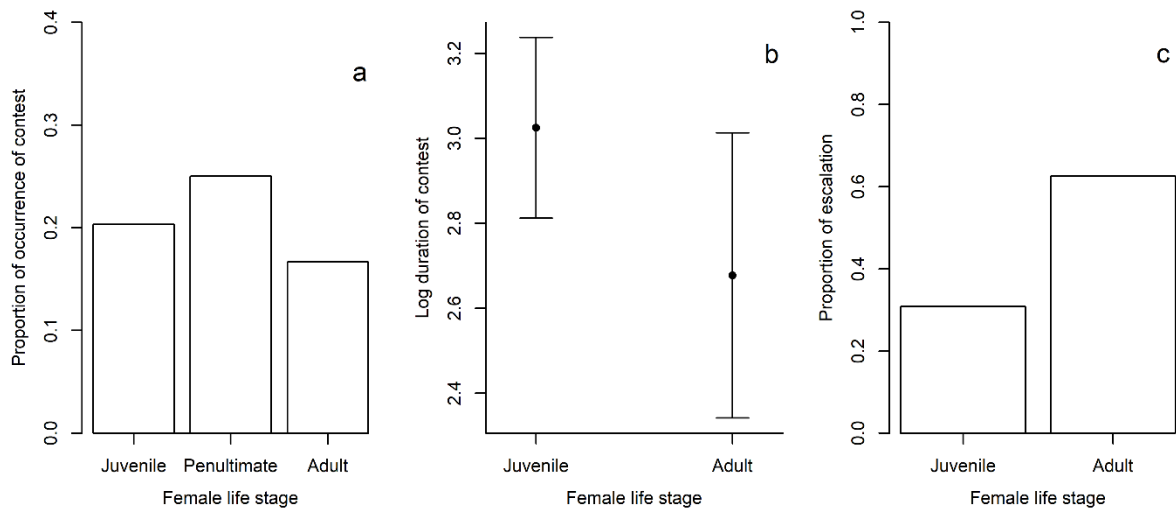
277 probability of occurrence of a contest (fig. 2a; table 1) nor the duration of the contest (fig. 2b;
 278 table 1) were related to female reproductive status. However, the probability of escalation (fig.
 279 2c; table 1) was higher when males were fighting for access to adult than to juvenile females.
 280 Neither the probability of occurrence of a contest (fig. 3a; table 1) nor the duration of contest
 281 (fig. 3b; table 1) were higher in aggregated than in isolated webs. However, the probability of
 282 contest escalation was higher in aggregated than in isolated webs (fig. 3c; table 1).

283



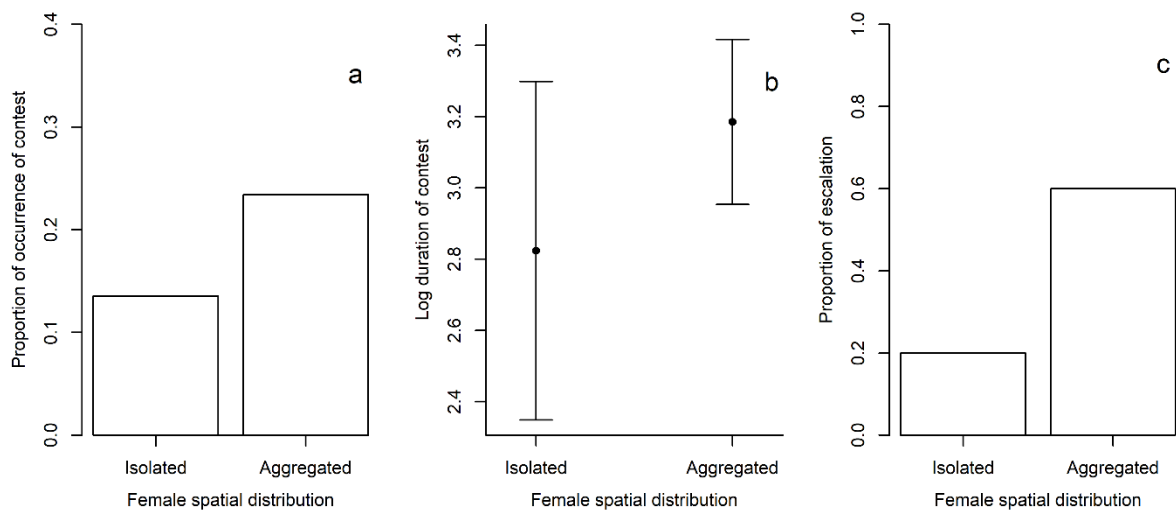
284

285 **Fig. 1.** Effect of female abdominal area on the probability of contest occurrence (a); duration
 286 of contests (b) and probability of escalation during the contest (c) for interactions between males
 287 of the golden orb-web spider *Trichonephila clavipes*. Contests were observed at the reserve
 288 Ecological Station of Federal University of Minas Gerais, Minas Gerais State, Brazil.



290

291 **Fig. 2.** Effect of female life stage on the proportion of contest occurrence (a), mean duration of
 292 contest and standard deviation (b), and proportion of escalation during the contest (c) for
 293 interactions between males of the golden orb-web spider *Triconephila clavipes*. Each
 294 proportion was calculated by dividing the number of occurrence of contests or the number of
 295 escalations by the total number of occurrence contests or number of escalations, respectively.
 296 Contests was observed at the reserve Ecological Station of Federal University of Minas Gerais,
 297 Minas Gerais State, Brazil.



298

299 **Fig. 3.** Effect of female spatial distribution on the proportion of contest occurrence (a), mean
 300 duration of contest and standard deviation (b), and proportion of escalation during the contest
 301 (c) for interactions between males of the golden orb-web spider *Triconephila clavipes*. Each
 302 proportion was calculated by dividing the number of occurrence of contests or the number of

303 escalations by the total number of occurrence contests or number of escalations, respectively.
304 Contests was observed at the reserve Ecological Station of Federal University of Minas Gerais,
305 Minas Gerais State, Brazil.

306

307 **Discussion**

308 We hypothesized that males of *T. clavipes* should increase their propensity to engage and
309 increase their investment in contests for access to virgin and more fecund females, and also
310 when access to females is harder. However, for most agonistic behaviors, we did not find any
311 relationship between male responses and female reproductive status, female fecundity, or when
312 accessibility to females was harder. However, we did find a male response in escalation contests
313 in relation to female reproductive status and accessibility to females. Surprisingly, the contests
314 were more probable to escalate when males were fighting for adult instead of juvenile females
315 and when female webs were aggregated in comparison to isolated webs. This indicates that
316 males of *T. clavipes* increase their investment in contests for access to females that are probably
317 already mated and when there are other females easily accessible nearby.

318 Regarding the propensity of males of *T. clavipes* to initiate contests, it is important to
319 highlight that males engaged in contests in approximately one-third of the observations. This
320 indicates that the propensity of males to begin a contest is generally low. Since fights only
321 occurred when peripheral males tried to reach the female, it is unclear why peripheral males
322 would not try to reach the female in most trials. Two possibilities may explain such pattern: 1)
323 peripheral males may avoid reaching the females because they lost previous contests against
324 the central male and established a dominance hierarchy or 2) peripheral males exhibit
325 alternative opportunities that provide access to females without contesting the central male
326 (Farr, 1977). If males establish a dominance hierarchy, it would be expected that peripheral
327 (losers) males would avoid fighting the central winner males and that the central position is

328 constantly maintained by the same winner male (*e.g.*, Issa *et al.*, 1999). However, because we
329 observed changes in the identity of the central males in 66.7% of the webs observed more than
330 once, we argue that it is improbable that males establish a dominance hierarchy. The other
331 possible explanation for why males do not engage in contests is because they had a short life
332 (Brown, 1985). Therefore, central males may be unable to defend females for a long time and
333 peripheral males may represent younger individuals waiting for the moments when the central
334 position will be available. The contests we observed may have occurred when peripheral
335 males were more motivated individuals, such as older males (*e.g.*, Kemp, 2006; Tsai *et al.*,
336 2014; Piper *et al.*, 2018) or males that had previously copulations with the female and are
337 preventing female access to other males (Switzer, 1997; Fromhage & Schneider, 2005; Zhang
338 *et al.*, 2019). In both cases, the residual reproductive value of such males is low and therefore
339 males should increase their investment in contests (Piper *et al.*, 2018).

340 Among the female traits we investigated, fecundity was the only one that did not affect
341 any of the male responses (*i.e.*, male propensity to engage in a contests or male investment in a
342 contest). A possible explanation for why males do not engage and do not adjust investment in
343 contests according to female fecundity is because males may pay a high cost to find a female
344 (*i.e.*, the mortality while searching for female is very high). Although there is a variation in
345 female fecundity, the searching costs that males pay until reaching a female web could be so
346 high that a variation in quantity of eggs among females may be not pay off search for another
347 female. This seems contradictory with a recent study made with *T. clavipes* that demonstrated
348 that very large and very small males show preferences for large and small females, respectively
349 (Pollo *et al.*, 2019). However, this study was made under experimental conditions in which the
350 costs of mate searching were reduced (Pollo *et al.*, 2019). Because the costs of finding a female
351 is high, it is probable that males stay in the web of the first female they find (Meraz *et al.*, 2012),

352 considering that *T. clavipes* males may not survive enough to search for another female
353 (Christenson & Goist, 1979; Vollrath & Parker, 1982).

354 Male responses related to the investment in each contest (*i.e.*, duration and escalation
355 probability) showed different patterns. While individuals changed their propensity to escalate
356 during the contest in response to female reproductive status and female spatial distribution,
357 there was no correspondent variation in contest duration. This is somewhat strange because in
358 many species escalated contests are also longer than non-escalated ones (*e.g.*, Taylor *et al.*,
359 2001; Pinto *et al.*, 2019). When escalated contest are longer, there is more energy invested by
360 males and more risk of occurrence of injuries if escalation is related to more aggressive
361 behaviors (which is often the case; Hardy & Briffa, 2013). However, in some species, mainly
362 spiders, there is no correlation between contest duration and the probability of escalation (*e.g.*,
363 Constant *et al.*, 2011; Keil & Watson, 2010). Because duration of contest is more related to
364 energy spent in a contest and escalation is more related to injury risk, it seems that the main
365 cost of contests to males of *T. clavipes* is injury risk and not necessarily energy spent.

366 An interesting result is that the probability of escalation is higher for adult (and probably
367 mated) than juvenile (and virgin) females. Considering that males of *T. clavipes* can copulate
368 only two times during their life (Christenson, 1989; Michalik & Rittschof, 2011), the
369 reproductive success of a male does not necessarily increase with the number of females he
370 fertilizes. If a male copulates with two virgin females, his reproductive success could be higher
371 than if he copulates with one virgin female. However, to find two virgin females is improbable
372 because the chance to find a second virgin female may be low (Brown, 1985). In addition, if a
373 male leaves a female that he fertilized and a second male fertilizes her, the first male will have
374 a reduction in his fitness, because he will lose a small fraction of paternity of the eggs
375 (Christenson & Cohn, 1988). Because of the limited number of inseminations and the risk of
376 losing fertilizations to another male, it could be more beneficial for a male to stay on the web

377 after a copulation and invest in contests for defense of a fertilized female than to search for
378 another female. In fact, males of some species prefer to stay in a location where they had prior
379 matings than to search for another location (Switzer, 1997). On the other hand, we asked: if
380 virgin females are more valuable, since they are not fertilized, why males do not escalate
381 contests for virgins too? This may be because a fight for access a female that is still not mature
382 may be costly if males can suffer injuries in contest that may prevent them to fight for that
383 female when she is receptive to mating.

384 Similar to the responses observed for the relationship between escalation probability and
385 female reproductive status, males exhibited a higher chance of escalating in contests when they
386 were located in aggregated webs. This indicates that males are more prone to increase their
387 investment in contests when the costs of mate search are lower. Therefore, there must be an
388 additional effect in aggregated webs that increases male investment in contests. In spiders that
389 build webs in aggregations, it is common that vibrations in one web trigger responses in
390 individuals located in nearby webs (Campón, 2007; Souza *et al.*, 2007). Considering that males
391 move more between female webs in aggregations, then, any event in one web may attract males
392 from nearby webs. If such events involve mating opportunities, it may be the case that whenever
393 a female is sexually receptive or a central position in the web is vacated, the number of males
394 that move to dispute these opportunities may be higher in comparison to the number of males
395 disputing the same opportunities in isolated webs. This would increase the competition among
396 males during the mating opportunity events. If the competition among males is higher, males
397 may become more prone to invest in fights because the chance of reproduction for each one is
398 lower.

399 In summary, our results show that males of *T. clavipes* increase their investment in
400 contests for adult (and probably mated) females and for females in web aggregations (easier to
401 find), by increasing escalation propensity during the contest. It may be that the cost of mate

402 search and male reproductive status (*i.e.*, if the male had mated with a female) interact in a way
403 that males stop searching and defend the female after mating. It is possible that paternity
404 assurance may be more important to determine males investment in contests than expectation
405 of access to female. In this way, if mate search is costly (*e.g.*, males located in isolated webs),
406 males that did not mate may stay in his location after finding a female and wait for an
407 opportunity to mate without fighting. On the other hand, if the cost of mate searching is low
408 (*e.g.*, males located in aggregate webs), males may increase the movement between webs and
409 their propensity to fight for a female. Because mate searching cost can only be measured in
410 field, studies like ours are important because we considered the real pressures to which
411 individuals are submitted. Thus, investigating the pattern of male contests under natural
412 conditions, we were able to show that environmental characteristics, such as female spatial
413 distribution, are important factors that contribute to the understanding of male decisions during
414 contests.

415

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