



Me against who? Male guppies adjust mating behaviour according to their rival's presence and attractiveness.

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1 **Me against who? Male guppies adjust mating behaviour according to their**
2 **rival's presence and attractiveness.**

3 **Short running title:** Male mating behaviour and rival traits

4

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32 **Abstract**

33 Sexual selection theory suggests that males need to constantly reappraise their mating decisions
34 to take account of the presence and the phenotype of their rivals. Here we examine this
35 expectation by asking: 1) If the presence of a rival influence male mating behaviour; 2) How
36 important is the attractiveness of the rival in shaping a male behaviour; and 3) How does a male
37 attractiveness in comparison to his rival influence a male's mating decisions. Using the
38 Trinidadian guppy, a species in which females choose males based on their attractive
39 phenotypes, playing an important role in male mating outcomes, we recorded the frequency of
40 mating tactics (courtship displays and unsolicited attempts) by focal males. First, we quantified
41 focal male mating behaviour with and without a rival. Since the probability of a successful
42 mating is, on average, halved by the presence of a rival, we predicted that under competition
43 the focal male would invest more in less costly unsolicited mating attempts. Second, we
44 examined how the rival's phenotype (standard length and area of orange colouration) mediated
45 focal male mating behaviour. We found that rival presence influenced how focal males
46 responded to females, in terms of both mating tactics. However, the rival attractiveness elicited
47 changes only in male courtship display. Focal males increased courtship display rate if his rival
48 was small or if possessed large amounts of orange, regardless of considering rival absolute or
49 relative attractiveness. Our results show that males invest in the costlier mating tactic when
50 there is no rival, or in the presence of a smaller rival. Interestingly, they make a similar
51 investment in the presence of an attractive orange rival. Overall, this study highlights the
52 importance of fine-grained male decisions in mating encounters and shows that mating tactics
53 are differentially shaped by multiple competition risk cues.

54

55

56 **KEYWORDS:** intrasexual competition; female mate choice; behavioural flexibility; social
57 information; body size, body colouration.

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58 1 | INTRODUCTION

59 Sexual selection studies have long focused on the fitness impacts of male-male competition for
60 access to females (Andersson, 1994; Darwin, 1871). A key question in this debate is how males
61 adjust their mating interactions with females in response to: 1) the presence of a rival; 2) the
62 overall attractiveness of the rival; and 3) the relative difference in attractiveness between a male
63 and his rival. These cues of competition risk have mostly been considered independently (e.g.
64 Chaudhary, Mishra, & Omkar, 2017; Leonard & Hedrick, 2009; Martin Plath & Bierbach,
65 2011; Tuni, Weber, Bilde, & Uhl, 2017). As such, their integrated impact on male mating
66 behaviour adjustment remains poorly understood.

67 Male reproductive success is determined by the presence of rival males during mating.
68 First, a rival can decrease another male's mating success through sexual interference or
69 sneaking (Andersson, 1994; Darwin, 1871). Second, given female multiple mating, a rival can
70 decrease a male's fertilization success by limiting sperm transfer, or through sperm competition
71 (Parker, Lessells, & Simmons, 2013). Selection should, therefore, favour males that adjust their
72 mating behaviour whenever a rival is present. Numerous studies have examined how male
73 mating behaviour changes in response to the presence of rival males. For example, leafhopper
74 *Aphrodes makarovi* males achieve mating success over a rival male by approaching the female
75 in silence, or, alternatively, by making an advertisement call that blocks the female reply to a
76 rival male call (Kuhelj, Groot, Blejec, & Virant-Doberlet, 2015; Kuhelj & Virant-Doberlet,
77 2017). Furthermore, in the wax moth *Achroia grisella*, males decrease mating latency and
78 increase copulation duration under the playback of a courtship song to prevent rival males from
79 mating first (Jarrige, Kassis, Schmoll, & Goubault, 2016). Males of different species have also
80 been shown to change their behaviour towards an initially preferred female when a male
81 audience is present, by reducing courtship activity and/or the time spent with the female
82 (Setoguchi, Kudo, Takanashi, Ishikawa, & Matsuo, 2015; Westerman, Drucker, & Monteiro,

83 2014; Wronski et al., 2012; Ziege et al., 2009; but see Dzieweczynski, Lyman, & Poor, 2009;
84 Fisher & Rosenthal, 2007). This response may serve to reduce sperm competition (by giving
85 up on the female) or, potentially, by directing the rival's interest towards less preferred females
86 (Plath et al., 2008; Plath & Schlupp, 2008; Ziege et al., 2009).

87 Male reproductive success is also determined by the attractiveness of rival males.
88 Firstly, females prefer to mate with the higher quality male (Andersson, 1994; Darwin, 1871).
89 Secondly, in species with female multiple mating, females can also retain sperm of rival males
90 if they are attractive or more attractive than focal males (Eberhard, 1996). While the role of
91 male attractive traits in driving female mate choice is well-studied (Hill, 2015; Hunt, Breuker,
92 Sadowski, & Moore, 2009), less is known about its effect in driving male mating decisions.
93 Even so, some studies have considered how males adjust their mating behaviour to the absolute
94 attractiveness of rival males (e.g. Plath & Bierbach, 2011; Tuni, Weber, Bilde, & Uhl, 2017),
95 while others have studied how the relative difference in quality between rival males modulate
96 male mating decisions (e.g. Leonard & Hedrick, 2009; Mautz & Jennions, 2011). While rival
97 absolute attractiveness informs about the rival's chances of reproductive success, rival relative
98 attractiveness informs about the focal male's chances of reproductive success in relation to the
99 rival. Therefore, to fully understand the underlying factors shaping male mating behaviour, we
100 need to study whether and how focal males respond to both rival absolute and relative
101 attractiveness.

102 Here, using the Trinidadian guppy (*Poecilia reticulata*), we address this gap by taking
103 an integrated approach and evaluating the effect of rival presence, of rival absolute and relative
104 attractiveness in the process of male mating decisions. Specifically, we investigate the effect
105 of two fitness correlated male traits: body length and orange colouration. We ask how focal
106 males adjust their mating behaviours to the: 1) presence of a rival, 2) to the presence of a

107 large/colourful rival (rival absolute attractiveness), and 3) to the presence of a rival that is
108 larger/more colourful than the focal male (rival relative attractiveness).

109 In guppies, females mate multiply and males face sperm competition (Houde, 1997;
110 Magurran, 2005). Males with larger and brighter spots of carotenoid-based colouration are
111 generally favoured by female choice (Auld, Pusiak, & Godin, 2016; Barbosa, Dornelas, &
112 Magurran, 2010; Endler & Houde, 1995; Evans, Bisazza, & Pilastro, 2004; Pitcher, Neff, Rodd,
113 & Rowe, 2003). Additionally, females tend to prefer larger body size males (Endler & Houde,
114 1995; Karino & Matsunaga, 2002; Magellan, Pettersson, & Magurran, 2005; Reynolds &
115 Gross, 1992). This does not mean that females ignore other traits, but that, on average, female
116 guppies select larger and more colourful males. Male guppies perform two mating tactics:
117 consensual courtship displays and unsolicited attempts (Magurran, 2005). Males that perform
118 courtship displays transfer more sperm (Pilastro, Mandelli, Gasparini, Dadda, & Bisazza, 2007;
119 Pilastro & Bisazza, 1999), and are preferred by females (Bischoff, Gould, & Rubenstein, 1985;
120 Farr, 1980; Houde, 1997; Nicoletto & Kodric-Brown, 1999; Pilastro et al., 2007). This may, in
121 some circumstances, lead to greater paternity success of courting males (Evans & Magurran,
122 2001). On the other hand, unsolicited mating attempts allow males to approach females without
123 being noticed and to transfer some sperm even without female consent, leading to some
124 paternity success (Matthews & Magurran, 2000; Pilastro & Bisazza, 1999; Pilastro et al., 2007).
125 Therefore, male guppies may adjust the frequency of mating tactics to the degree of
126 competition.

127 There is evidence that male guppies respond to the presence of a rival male by
128 performing more unsolicited mating attempts (Auld, Jeswiet, & Godin, 2015; Farr &
129 Herrnkind, 1974; Magellan et al., 2005). Additionally, male guppies engage less in mating
130 behaviours when in the presence of an overall colourful rival (Dugatkin & Sargent, 1994;
131 Gasparini, Serena, & Pilastro, 2013). Also, males reduce mating activity if the rival is relatively

132 more colourful or larger (Auld, Ramnarine, & Godin, 2017; Yoshikawa, Ohkubo, Karino, &
133 Hasegawa, 2016). Because most studies of mate choice focus on just one trait, less is known
134 about the combined effect of the presence of a rival and his absolute and relative attractiveness
135 in shaping focal male guppy mating interactions. We tested these combined effects by
136 recording the frequency of courtship displays and of unsolicited mating attempts performed by
137 focal males both in no-competition and competition scenarios and by investigating the
138 relationship between the frequency of each mating tactic with rival size and area of orange.

139 We predict that, first, focal males should employ more courtship display in the absence
140 of a rival, given that male reproductive success is, in general, higher than with unsolicited
141 attempts (Evans & Magurran, 2001). However, since the probability of mating is reduced in
142 the presence of a rival, and because unsolicited mating attempts are less costly, we predict that
143 focal males should, instead, employ this 'sneaky' mating tactic more frequently whenever they
144 find themselves in this competitive scenario. Second, we expect that focal males adjust their
145 mating behaviours to rival absolute attractiveness (i.e. according to rival's chances of
146 reproductive success). We predict the focal male will perform more unsolicited attempts when
147 rival has high rather than low attractiveness, to minimize the cost of sperm competition. On the
148 other hand, a focal male is less likely to lose a mating opportunity when his rival is less
149 attractive. Consequently, under this second competitive scenario the focal male is predicted to
150 employ courtship displays (which females can respond to). Third, we also expect that focal
151 male mating strategies are related to rival relative attractiveness (i.e. according to focal male
152 chances of reproductive success in relation to his rival). We predict the focal male will try to
153 enhance his reproductive success by performing more courtship displays when his rival is more
154 rather than less attractive than himself.

155

156 **2 | METHODS**

157 **2.1. | Experimental setup and design**

158 Experiments were conducted at the University of St Andrews using descendants from wild
159 guppies from the Lower Tacarigua River, in Trinidad. We used a focal sampling procedure to
160 record male mating behaviour (Altmann, 1974). Each focal male was observed in a tank
161 containing two females, and in two scenarios: 1) alone with the females (no-competition
162 scenario), and 2) with another male (competition scenario). The order in which scenarios were
163 presented to each focal male was randomised. Each trial lasted 15 minutes and the frequency
164 of courtship displays and unsolicited attempts by focal males was tallied using JWatcher v1.0
165 (Blumstein & Daniel, 2007).

166 Prior to observations, all individuals were kept in mixed-sex stock tanks, with similar
167 densities. In the afternoon before observations, males and females were haphazardly chosen
168 from stock tanks. Males were transferred to a holding tank (59 x 29 cm and 35 cm deep) and
169 pairs of females were transferred to three experimental tanks (59 x 29 cm and 35 cm deep).
170 Familiarity between males and females reduces male sexual interest (Kelley, Graves, &
171 Magurran, 1999; Mariette, Zajitschek, Garcia, & Brooks, 2010; but see Zajitschek, Evans, &
172 Brooks, 2006). Because of this, females and males were chosen from different stock tanks.
173 Females remained in the same experimental tank until the end of the observation day. The same
174 female pairs were observed with different focal males (two to three per day). This protocol
175 minimized stress in females, without substantially affecting females' responsiveness (females
176 were previously kept in mixed-sex stock tanks; Liley, 1966; Liley & Wishlow, 1974). All
177 individuals were fed with flake food one hour before the experiments started.

178 Observations were performed between 0900 and 1300 hours. If, for a given focal male,
179 the first scenario tested was the no-competition scenario, the focal male was randomly selected

180 from the holding tank and transferred to the experimental tank where it was kept in a transparent
181 container at least for 15 minutes to acclimate to the new conditions. The experiment started
182 when the container was removed, and the focal male could swim freely. After the trial, the
183 focal male was moved back to the transparent container, and a rival male, randomly selected
184 from the holding tank, was added to it. They remained together in the container for at least
185 another 15 minutes to acclimate and the experiment started when the container was removed,
186 and both males could swim freely. After the two treatments, both focal and rival males were
187 removed from the test tanks and replaced by new ones. If the first scenario to be tested was the
188 competition one, the trials started with both focal and rival males in the transparent container,
189 and at the end of the first trial, the rival male was removed and the focal was transferred alone
190 to the transparent container for another 15 minutes and then released alone in the test tank. At
191 the end of each day, females were also removed from the experimental tanks and replaced by
192 new ones and new males were transferred to the holding tank. We tested a total of 36 focal
193 males, 36 rival males (being distinguished by their unique colour patterns – Magurran, 2005)
194 and 17 female pairs.

195 The experimental tanks had an aerating system and the bottom was covered with gravel.
196 The water temperature was maintained with a thermostat heater (average (\pm SD) of $25.7 \pm$
197 0.8°C), a range like what is found in the wild rivers of Trinidad (24.1°C to 27.0°C ; Reeve et al.,
198 2014). To prevent individuals of different experimental and holding tanks from seeing each
199 other, opaque divisions were added to all sides of the tanks, except one side of the experimental
200 tanks to allow observations.

201

202

203

204 2.2 | Estimates of male attractiveness

205 We used body area (main body and tail) covered by orange and standard body length as proxies
206 of male attractiveness. The area covered by orange spots is a strong predictor of female mate
207 choice and of male fitness, while the effect of black and iridescent areas in explaining female
208 choice are less clear (Endler & Houde, 1995; Karino & Matsunaga, 2002; Magellan et al., 2005;
209 Reynolds & Gross, 1992). We recorded individual orange colouration by direct visual
210 evaluation and using a standardized scheme, following Deacon, Barbosa, & Magurran (2014).
211 This scheme divides the body into 12 sections and considers that a male has a smaller coloured
212 area compared to other males when his colour spots cover fewer body sections (Figure 1).
213 Quantification of orange area of a subsample of 20 males by direct visual evaluation *versus* by
214 photo evaluation demonstrates that these two methods are comparable (Welch Two Sample t-
215 test: $t = -0.3$, $N = 20$, $P = 0.744$; see supplementary information). Quantifying orange area by
216 direct visual evaluation allowed us to obtain an accurate estimate of male coloured area without
217 using an invasive method in which males are anaesthetized (Auld et al., 2016; Barrett, Evans,
218 & Gasparini, 2014; Brooks & Endler, 2001). Orange area was recorded prior to mating
219 interactions. Males were allocated to a tank with visual access to females, and because of this
220 sexual stimulation the appearance of their colour patterns was consistent with that seen during
221 mating encounters (Baerends, Brouwer, & Waterbolk, 1955). Although studies usually
222 consider the absolute or the proportion of coloured body area of one of the male's side chosen
223 *a priori* (e.g. Auld et al., 2016; Barrett et al., 2014; Brooks & Endler, 2001), our method
224 allowed to measure the most colourful side of each male since males preferentially expose this
225 side when courting (Gross, Suk, & Robertson, 2007).

226 As there are inconsistent results on the effect of male tail length on female preference
227 (Endler & Houde, 1995; Karino & Matsunaga, 2002), we chose to measure standard length
228 only. The standard length of a guppy is defined as the length from the tip of the mouth to the

229 base of the tail. To measure male standard length, each male was transferred to a Petri dish
230 with water after observations. Males were photographed from above with a ruler below
231 (quickly performed and without anaesthesia), and their standard length was measured using
232 ImageJ software (Schneider, Rasband, & Eliceiri, 2012).

233 Standard length and area of orange were used to evaluate rivals' absolute and relative
234 attractiveness (Table 1). For absolute attractiveness, we used the direct measurements of rivals'
235 standard length and area of orange. For relative attractiveness, we calculated the ratio of each
236 trait as the difference between the rival and the focal males' traits, divided by the average of
237 the rival and the focal males' traits. When the ratio was positive, the rival was more attractive
238 for that trait. Additionally, ratio amplitude represents the degree of difference between rival
239 and focal males' attractiveness.

240

241 **2.3 | Statistical analyses**

242 To evaluate if the frequency of courtship displays and unsolicited attempts employed by focal
243 males was affected by the presence and/or absolute and relative attractiveness of the rival, we
244 used Generalized Linear Mixed Effects models (GLMM), with the GLMER package from the
245 library lme4 (Bates, 2011).

246 First, we examined the effect of presence/absence of a rival on male mating behaviours.
247 The full model included the competitive scenario (two levels: no-competition and competition)
248 as fixed effect. Additionally, to account for a possible effect of the order in which the scenarios
249 were presented (order of arrival to the female), we included this variable in the fixed effects
250 (two levels: "first", when the focal male is first alone with the female and the rival is introduced
251 later, and "second", when the focal male is introduced with the rival and the rival is removed

252 later). The model also included focal male's identity as a random factor to account for the
253 replication effect. Second, we tested the effect of rival absolute attractiveness in shaping the
254 frequency of courtship displays and unsolicited mating attempts of the focal male. The model
255 included the rival's absolute standard length and absolute area of orange spots as explanatory
256 variables. Third, to examine how the relative difference in attractiveness between the rival and
257 focal male affects the frequency of mating behaviour, we used the ratio of standard length and
258 area of orange spots as explanatory variables in the full model. In the second and third cases
259 we no longer considered replicates (only the competitive scenario was included), so the focal
260 male's identity was not included as a random factor in the models.

261 The frequencies of courtship display and unsolicited attempts were analysed separately,
262 being included as the response variables in models with a Poisson distribution. Additionally,
263 all full models included the female pair nested within the experimental tank as random factors.
264 Model selection was based on a backward step-wise procedure with analysis of variance. The
265 significance was set at $P = 0.05$. All statistical analyses were performed using R 3.4.1 (R Core
266 Team, 2017).

267

268 **3 | RESULTS**

269 **3.1 | Rival presence**

270 The presence of a rival during mating influenced the focal male mating behaviours (courtship
271 display: $\chi^2_1 = 25.5$, $N = 72$, $P < 0.001$; unsolicited attempts: $\chi^2_1 = 42.9$, $N = 72$, $P < 0.001$).
272 Focal males performed fewer courtship displays ($z = -5.0$, $P < 0.001$; Figure 2a), but more
273 unsolicited attempts ($z = 6.5$, $P < 0.001$; Figure 2b) when a rival was present. The order of the

274 competitive scenario had no effect on the frequency of courtship displays ($\chi^2_1 = 0.1$, $N = 72$,
275 $P = 0.760$), nor on the frequency of unsolicited attempts ($\chi^2_1 = 0.4$, $N = 72$, $P = 0.504$).

276 The frequency of courtship display performed by each focal male was positively
277 correlated between the two scenarios (Pearson's correlation test: $r = 0.9$, $N = 36$, $P < 0.001$).
278 Such correlation was also positive for unsolicited attempts, but not so evident ($r = 0.4$, $N = 36$,
279 $P = 0.017$).

280

281 **3.2 | Rival absolute attractiveness**

282 Both the absolute area of orange and the absolute standard length of rival males produced a
283 significant effect in the frequency of courtship displays, but not in the frequency of unsolicited
284 mating attempts (Figure 3, Table 2). Focal males performed significantly more courtship
285 displays in the presence of a small rival, or in the presence of a rival with large area of orange.

286

287 **3.3 | Rival relative attractiveness**

288 The relative difference in attractiveness between the rival and the focal male affected
289 significantly the frequency of courtship displays but did not affect the frequency of unsolicited
290 attempts (Figure 3, Table 2). Focal males performed significantly more courtship displays
291 when the rival was smaller, or when the rival had a larger area of orange.

292

293 **4 | DISCUSSION**

294 Here, we asked whether male-female interactions are shaped by the presence of rival males,
295 and by rival overall and relative attractiveness. Our results provide strong experimental

296 evidence that male guppies adjust the frequency of courtship displays and unsolicited mating
297 attempts in response to the presence of a rival male in the mating arena. We found that focal
298 males responded to the presence of a rival by increasing the frequency of unsolicited attempts
299 and by performing fewer courtship displays than they would do if they were alone with the
300 female. Importantly, our results also indicate that the quality of the rival (in absolute and in
301 relative terms) mediates changes in courtship display by the focal male. Male guppies increase
302 the frequency of courtship displays if competing against a small (unattractive) rival, or if
303 competing against a colourful (attractive) rival – both in absolute and in relative terms. Overall,
304 our results are consistent with the general prediction that mating competition favours the
305 evolution of flexibility in mating behaviours. Furthermore, our results demonstrate that the
306 costlier mating tactic is dynamically and equally shaped by the overall quality as well as the
307 relative quality of the rival. This study emphasises the importance of incorporating more than
308 one male fitness-correlated trait in future studies to better understand the underlying factors
309 that shape male mating behaviours.

310

311 **4.1 | Rival presence**

312 As expected, we found that male guppies perform more unsolicited attempts and fewer
313 courtship displays in the presence of a rival male. This lends support to a strategy by the males
314 to outcompete the rival by mating faster, and thus minimize the reproductive costs of
315 competition. Our result is in agreement with other studies that found that male guppies increase
316 the frequency of unsolicited attempts when there is another male present in the mating arena
317 (Auld et al., 2015; Farr, 1980; Farr & Herrnkind, 1974; Magellan et al., 2005). Unsolicited
318 mating attempts allow males to successfully transfer sperm, albeit at mostly low levels
319 (Matthews & Magurran, 2000; Pilastro & Bisazza, 1999), and to mate more rapidly without

320 female consent (Houde, 1988; Magurran, 2005). Because of these reduced costs, the use of
321 unsolicited mating attempts is likely to be favoured in contexts of mating competition.

322 We also found that the focal male decreased the frequency of his courtship displays
323 when in the presence of a rival. Previous studies that used wild guppies also have reported a
324 decrease in courtship behaviour in response to the presence of a rival (Auld et al., 2015;
325 Magurran, 2005). A decrease in courtship display in response to high male-mating competition
326 risk have also been shown for other species. In the threespine stickleback *Gasterosteus*
327 *aculeatus*, males decreased the frequency of courtship display when a single rival is joined in
328 the mating arena (Candolin & Vlieger, 2013). Similarly, in the two-spotted spider mite
329 *Tetranychus urticae*, and in the Japanese medaka *Oryzias latipes*, male-biased sex ratio
330 environments caused a reduction in male courtship displays (Sato, Sabelis, & Egas, 2014; Weir,
331 2013).

332

333 4.2 | Rival absolute attractiveness

334 We expected focal males to perform more unsolicited attempts when in the presence of a highly
335 attractive rival, as a way of investing in a less costly tactic under a context where rival chances
336 of mating is very high. What we found is that only the frequency of courtship displays varied
337 with rival males' absolute attractiveness.

338 Males invested more in courtship displays when competing with a small rival, which is
339 advantageous given the higher chances of focal males being preferred by female guppies
340 (Karino & Matsunaga, 2002; Magellan et al., 2005; Reynolds & Gross, 1992). Additionally,
341 we expected males to reduce courtship display when competing with attractive rivals since
342 their chances of being chosen by females are, on average, halved. Surprisingly, we found that

343 focal males employed significantly more courtship displays in the presence of a higher quality
344 opponent with a large orange area. A potential explanation for this behaviour is that courtship
345 displays enhance the brightness of orange colouration (Houde, 1997). It is then plausible to
346 assume that males increase the frequency of courtship displays in response to a better-quality
347 male to circumvent the attractiveness handicap. This strategy has been described in the three-
348 spined stickleback, with males enhancing their carotenoid colours by performing more
349 courtship when competing with colourful males (Kim & Velando, 2014). Similarly, male
350 common eggfly butterflies *Hypolimnas bolina* adjust their courtship behaviour to enhance their
351 attractive colours (White, Zeil, & Kemp, 2015). In guppies, it has also been found that males
352 invest more in courtship and interfere more with other courting males when competing against
353 rivals with large orange areas (Jirotkul, 2000). Together, these findings indicate that courtship
354 displays may be used as a mechanism to enhance an attractive trait.

355

356 **4.3 | Rival relative attractiveness**

357 We expected focal males to perform more courtship displays when in the presence of a more
358 attractive rival to compensate for their reduced chances of reproductive success when
359 compared to the rival chances of reproductive success. We found evidence supporting this
360 prediction. Focal males engaged in more courtship displays when the rival was relatively
361 smaller or had a greater area of orange. Here, our findings complement other studies. For
362 instance, it has been found that male guppies give up more quickly from following a female
363 when a larger rival is nearby, or reduce their mating preference for an initially preferred female
364 if in the presence of a larger rival (Auld et al., 2017). Another study found that only males
365 guppies that are less colourful than their rivals decide to stop following females (Yoshikawa et
366 al., 2016).

367 As for rival absolute attractiveness, we also found that rival relative length and area of
368 orange colouration had no effect in the frequency of focal male's unsolicited attempts.
369 Unsolicited attempts have the advantage of permitting males to transfer some sperm without
370 female sexual interest (Houde, 1988; Magurran, 2005). Focal males could minimize
371 reproductive costs by employing this less costly mating tactic when facing an attractive or a
372 more attractive rival. Since unsolicited attempts was not used by most males, only the higher
373 costly courtship display seems to be under selection by rival attractiveness.

374

375 Male body length does not vary over the duration of courtship interactions, so males
376 tend to invest more only when they are already in advantage over their rivals. Contrariwise,
377 males can enhance their orange conspicuousness by increasing its brightness when performing
378 courtship displays, so they tend to invest more when they need to compensate. Orange
379 colouration is used by females during mate choice as proxy of male ability to find food rich in
380 carotenoids (Grether, 2000). Also, female guppies favour sperm from males with greater areas
381 of orange pigmentation (Barbosa, Dornelas, & Magurran, 2010; Pilastro et al., 2004). Allied to
382 this, studies have also shown that cryptic female choice impacts male fertilization success
383 (Magris, Cardozo, Santi, Devigili, & Pilastro, 2017). Courtship displays can also be used to
384 enhance male vigour. For example, using dichotomous-choice tests and computer-modified
385 videos, female guppies have been found to prefer male animations with orange colouration
386 rather than without if both animations showed low display rates, but this was no longer the case
387 when both animations displayed at a high rate (Kodric-Brown & Nicoletto 2001). This suggests
388 that males with fewer orange spots than their rivals can compensate by displaying more. Thus,
389 when faced with a better rival, the focal male may overcome his reduced chances of
390 reproductive success by investing in the mating tactic that maximizes his attractiveness, and
391 potentially increase his fitness. Moreover, male guppies tend to invest more in mating

392 behaviours when their risks of losing both mate and fertilization success are high (Órfão et al.,
393 2018). All this lends support for the hypothesis that coevolution between male mating
394 behaviours and male attractive traits is shaped by both female choice and male-male
395 competition (Kuijper, Pen, & Weissing, 2012).

396

397 **4.4 | Concluding remarks**

398 We showed that male guppies adjust their mating interactions in response to the presence and
399 quality of a male in several ways: 1) by using a mating tactic that allows to mate quickly
400 (unsolicited attempts) if a rival male is present in order to minimize reproductive costs; 2) by
401 investing more in a costlier and riskier mating tactic (courtship display) if a small or smaller
402 (hence, both less attractive to females and less competitive in male-male aggressive
403 interactions) rival male is present, possibly to avoid compromising a competitive advantage;
404 and also 3) by investing more in courtship display to enhance orange brightness if in the
405 presence of a colourful or more colourful male, possibly to increase his apparent attractiveness
406 to the female. Our results also show that male guppies adjust their mating tactics differently
407 according to rival traits. This suggests that multiple information collected from the social
408 environment shapes male mating decisions. It also supports the idea that such information is
409 provided not only by male-female interactions and modulated by female mate choice
410 (Candolin, 2003; Lozano, 2009), but also by the presence and attractiveness of rival males. It
411 is noteworthy that males have shown to make the same mating decisions based on rival absolute
412 and relative attractiveness. This suggests that both types of information shaped equally the
413 evolution of males mating decisions. What appears to be determining variation in male mating
414 behaviour is the flexibility of males' attractive traits: when the trait is not flexible, like body
415 size, focal males invest more when they are already in advantage over their rivals; when it is

416 flexible, like orange brightness, focal males invest more both as a way to interfere with rival
417 males' mating attempts and to compensate for a lack of attractiveness.

418 Future studies could now consider the effect of other more or less flexible male sexual
419 traits, such as structural colours, colour brightness, chroma, as well as other less studied
420 variables, such as olfactory cues (Endler & Houde, 1995; Magurran, 2005; but see Partridge,
421 van Oosterhout, Archard, & Cuthill, 2008). Future studies could also evaluate how both rival
422 absolute and relative attractiveness affect a male's mating and fertilization success and whether
423 this is linked to female mate and cryptic choice or to a correlation between male traits (e.g.
424 attractive traits and sperm production). Moreover, it would be informative to investigate the
425 contribution of rival behaviour to focal male mating behaviours, particularly considering that
426 some male traits are correlated with the propensity to perform one of the two tactics – e.g.
427 colourful males have been shown to perform more courtship displays and fewer unsolicited
428 attempts (Evans, 2010; Jirotkul, 2000; Kiritome, Sato, & Karino, 2012), while smaller males
429 perform more unsolicited attempts than larger ones (Becher & Magurran, 2004; Magellan et
430 al., 2005). An interesting next step would be to perform multivariate tests to study the combined
431 effect of different traits (Cohen, West, & Aiken, 1983). For instance, researchers could test
432 whether different sexual traits reinforce the same information or transmit different information
433 about male quality to females and to rivals (Bro-Jørgensen, 2010). This future work will
434 advance understanding of the co-evolution of secondary sexual characters.

435 Finally, our results reinforce previous studies in demonstrating that male guppies can
436 evaluate other males' attractiveness in relation to themselves (Auld et al., 2017; Yoshikawa et
437 al., 2016). This raises a compelling question: how are males aware of their own traits,
438 particularly their ornamental colours? "Of what an animal is aware" has been proposed as one
439 of the fundamental questions in biology (Bateson & Laland, 2013; Shettleworth, 2010). In the
440 present context this means investigating how social ecology mediates a male's ability to

441 synthesise information about attractiveness, not just his own attractiveness in objective terms
442 but also in relation to the other males around him. This type of information could be innate, or
443 learned from conspecific behaviours, either from other males, or from female responses,
444 namely through behaviours that demonstrate sexual interest. Which mechanism has evolved,
445 and the underlying neural structures it depends on, are questions that deserve future
446 investigation.

For Peer Review

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687

688 **FIGURE LEGENDS**

689

690 **Figure 1.** Scheme of male guppy body sections (main body and tail) used to estimate areas of
691 orange (adapted from Deacon et al. 2014). The main body area was divided evenly. The tail
692 area was divided considering the maximum variation in colour spots. The ratings of the total
693 areas of orange spots for each male were obtained by counting the number of body sections
694 with pigmentation.

695

696 **Figure 2.** Frequency of male mating tactics performed in the absence or presence of a rival
697 male. Mating tactics were: a) courtship displays, and b) unsolicited mating attempts. Focal
698 males ($N = 36$) were observed in two scenarios: with two females (no-competition), and with
699 two females and a rival male (competition). Pairwise comparisons between competitive
700 scenarios of the frequency of each mating tactic were obtained from the best-fit GLMER
701 models. Asterisks and lines above the plots show significant differences ($*P < 0.05$). In each
702 boxplot, the internal line represents the median. Lower and upper edges represent the 25% and
703 the 75% quantiles, respectively. Whiskers below and above the box edges represent,
704 respectively, the minimum and the maximum points within the 1.5 interquartile range. Circles
705 represent outliers.

706

707 **Figure 3.** Frequency of mating tactics performed in relation to rival attractiveness. Mating
708 tactics were (from the top to the bottom): courtship displays and unsolicited mating attempts.
709 Two traits of rival attractiveness were considered (from the left to the right): standard length
710 (cm) and area of orange spots (number of body sections with carotenoid-based pigmentation).
711 Rival attractiveness was considered either in absolute terms or relative to focal male's

712 attractiveness. In the x axis of each plot values at the left represent rival males of lower
713 attractiveness (unattractive while considering absolute attractiveness or less attractive than the
714 focal male while considering relative attractiveness). The lines in each graph represent the
715 predicted effect of each rival male trait on the frequency of focal males' mating tactics,
716 assuming the mean values for all the traits in the GLMER model. Plots with no line represent
717 no significant effects of rival trait on focal males' mating behaviours.

718

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719 **TABLES**720 **Table 1.** Mean and standard error of focal and rival males' traits.

Trait	Male	Mean ± SE	
Orange area	Focal	4.94	1.19
	Rival	4.86	1.25
Black area	Focal	2.86	1.13
	Rival	2.42	0.97
Standard length (cm)	Focal	1.50	0.13
	Rival	1.43	0.10

721

722 **Table 2.** Summary table of the best GLMER models for the effect of rival males' attractiveness
723 on the frequency of focal males' mating tactics.

Mating tactic	Rival attractiveness	Rival trait	χ^2	P-value	Z-value	P-value
Courtship display	Absolute	Orange area	8.3	0.004	2.9	0.004
		Standard length	20.5	<0.001	-4.3	<0.001
	Relative	Orange area	21.7	<0.001	4.5	<0.001
		Standard length	51.1	<0.001	-6.3	<0.001
Unsolicited attempt	Absolute	Orange area	1.1	0.303	-	-
		Standard length	1.3	0.256	-	-
	Relative	Orange area	2.1	0.148	-	-
		Standard length	0.6	0.429	-	-

724 Analyses were conducted separately for each mating tactic, and for rival absolute and relative
725 attractiveness ($N = 36$; except for standard length, where $N = 35$, since one of the rival males
726 was not measured).

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1 **Me against who? Male guppies adjust mating behaviour according to their**
2 **rival's presence and attractiveness.**

3 **Short running title:** Male mating behaviour and rival traits

4

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