

Experience with mating receptivity cues affects sexual behaviour of male guppies, but not their strength of preference towards receptive females

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## Abstract

Females are traditionally presented as the choosier sex, selecting males based on the quality of their traits. Yet, there is increasing evidence that male mate choice is also important, even in species without male parental care. Social environment and learning are key factors in determining mate preference, and animals are able to use the information they gather from previous experience to potentially increase their odds of obtaining a high-quality mate. We examined how the social environment affects male mate choice in the guppy (*Poecilia reticulata*). We evaluated whether male guppies with previous social experience of female receptivity cues learn to prefer and adapt their behavioural repertoire towards females with higher receptiveness levels, as this represents an optimal use of time and energy and is more likely to result in insemination. For this, we measured sexual preference and behaviour for receptive females in no-choice and dichotomous choice tests using guppy males experienced or naïve to female receptivity cues. Experience with receptivity cues did not change the strength of preference towards receptive females. However, male guppies that had previous experience with female receptivity cues adapted their mating tactic compared to naïve males. The change in mating tactics but lack of preference towards receptive females shows that the influence of social learning is present but might be weaker than predicted in this species. Furthermore, these results provide further support to studies of female mate choice suggesting mating status is not a key factor driving the strength of sexual preferences in natural populations.

**Keywords:** mating status; alternative mating tactics; social learning; sexual selection; male choice

1 **Introduction**

2  
3 Mate choice is a fundamental evolutionary process, as it influences which individuals successfully  
4 pass their genes to the next generation (Darwin, 1871; Andersson, 1996). While females  
5 historically have been studied as the choosy sex, there is compelling evidence of male mate choice,  
6 suggesting that males can be choosy even in species without male parental care (Edward &  
7 Chapman, 2011; Herdman et al., 2004; Rosenqvist, 1990; Werner & Lotem, 2003; Wong &  
8 Jennions, 2003).

9

10 Previous experience can play an important role in forming mating preferences. The social  
11 environment can create opportunities for animals to collect information about their surroundings.  
12 Animals regularly use this information to adapt their behaviour and efficiently obtain and compete  
13 for mates, as well of for other resources that lead them to increase their fitness (Bailey & Moore,  
14 2012; Danchin et al., 2004; Fowler-Finn & Rodríguez, 2012; Valone & Templeton, 2002). In  
15 systems in which males perform sexual displays to solicit copulation consent, previous experience  
16 can help males to better assess female quality or to avoid soliciting females that signal  
17 unwillingness to mate (Akinyemi & Kirk 2019, Dukas 2005, Rather et al., 2022). Similarly, males  
18 can adapt their behavioural repertoire or shift to alternative mating tactics based on previous  
19 encounters with females (Bailey et al., 2010, Řežucha & Reichard, 2014). Considering the effect  
20 of social environment is therefore paramount when evaluating male mate choice patterns.

21

22 In fish, male mate choice has been documented in many species (Schlupp, 2018), with males  
23 selecting for female traits associated with higher fecundity. For instance, males exhibit preference  
24 for larger females with higher fecundity potential in species such as eastern  
25 mosquitofish, *Gambusia holbrooki* (Bisazza et al., 1989; Head et al., 2015, Hoysak & Godin,  
26 2007), sailfin mollies, *Poecilia latipinna* (Gumm & Gabor, 2005), and Atlantic mollies, *Poecilia*  
27 *mexicana* (Plath et al., 2006). In fish species with internal fertilization, preference for females  
28 showing higher receptiveness levels is theoretically expected, as it is more likely to result in  
29 insemination and therefore higher reproductive fitness per unit effort (Bondurianski, 2001, Jordan  
30 et al., 2014). However, more research effort is needed to understand pre-copulatory male mate  
31 choice in relation to female mating status.

32

33 Guppies, fish native to streams of north-eastern South America and the Caribbean, are a traditional  
34 model for sexual selection studies. The species is sexually dimorphic, with smaller and colorful  
35 males, and have a non-resource based promiscuous mating system (Houde, 1997). Studies of male  
36 mate choice in the guppy have revealed that males prefer larger, more fecund females (Corral-  
37 Lopez et al., 2018, Dosen and Montgomerie, 2004, Herdman et al., 2004., Jeswiet et al., 2012).  
38 Additionally, guppy males adapt their mating tactics and sexual effort in relation to female mating  
39 status (Guevara-Fiore et al., 2009; 2010). Studies in guppies have likewise been crucial for our  
40 understanding of the role of social environment in sexual selection, and have shown that females  
41 shift their preferences for male color traits depending on early social experiences (Rosenqvist &  
42 Houde, 1997, Macario et al., 2017). Similarly, rearing conditions and previous success in mating  
43 affect subsequent male mating behavioural tactics (Guevara-Fiore, 2012; Guevara-Fiore & Endler,  
44 2018).

45  
46 While the role the social environment in sexual selection and male mate choice for specific female  
47 traits have been studied in guppies, this system provides the additional opportunity to understand  
48 how prior social experiences can affect male preference for female mating status. Here we study  
49 whether previous social experience with female receptivity cues affect behaviour and strength of  
50 preference of male guppies in relation to female mating status. To empirically test this, we  
51 experimentally manipulated the social environment of male guppies and quantified their  
52 preference and behavioral repertoire in the presence of receptive and non-receptive females. To  
53 avoid potential biases introduced by choice of experimental design paradigm (Dougherty &  
54 Shuker, 2015), we assessed the role of previous experience in guppy male choice for receptive  
55 females using a combination of dichotomous choice and no choice tests. Given theoretical  
56 expectations of fitness maximization, we predicted that male guppies would use social learning to  
57 shift their preference levels to favour receptive females. Similarly, we predicted that the social  
58 environment would affect how males adapt their behavioural repertoire depending on whether they  
59 encounter receptive or non-receptive females.

60  
61 **Methods**

62 *Study system*

63  
64

65 All guppies used in this experiment originated from a laboratory-adapted stock population,  
66 originally collected from the high predation region of the Quaré River (Trinidad & Tobago).  
67 Aquaria contained gravel, water filters, and aquatic plants, and all experiments were approved by  
68 institutional animal ethics protocols. Fish were raised at a water temperature of 25 °C with a 12:12  
69 light:dark schedule, and fed a daily diet of flake food (Hikari Fancy Food) and live *Artemia* brine  
70 shrimp.

71

72 We collected newborn guppies from a stock aquarium and held them in nursery aquaria until they  
73 could be accurately sexed by the development of a gonopodium, a modified anal fin (Houde, 1997;  
74 Liley, 1966), at which point we removed males and held them in male-specific aquaria in groups  
75 of seven individuals. During this time, males were not allowed to visualize any females. Once  
76 males reached sexual maturity, as evidenced by the development of male colouration, we randomly  
77 allocated them into two experimental treatments, experienced and naïve. To ensure all males had  
78 similar age and social experience when tested in behavioural experiments and due to logistic  
79 reasons, we performed the experiment in two batches that account for half of the individuals each.

80

81 To study the preference and behaviour of naïve and experienced males towards females with  
82 different mating status, we exposed them to receptive and non-receptive females in dichotomous  
83 and no-choice tests (see details below). Sexual receptiveness towards males strongly correlates  
84 with the female guppy reproductive cycle. Levels of female receptiveness are highest following  
85 parturition of live offspring and for a period of approximately three days in which new ova are  
86 commonly fertilized. Receptiveness levels decrease linearly for the following days until they reach  
87 minimum levels approximately ten days post-parturition and are maintained in minimum levels  
88 until parturition of a new clutch of offspring (approximately 28 days; Liley, 1966, Houde, 1997).  
89 Receptiveness in virgin females presents a similar pattern during first reproductive cycle (Houde,  
90 1997). Following theoretical expectations of receptiveness levels towards males and methods in  
91 Guevara-Fiore et al. (2010), we housed small groups of virgin females with males in a 1:1 ratio  
92 and used them in behavioural tests the following day (receptive females) or 14 days after (non-  
93 receptive females).

94

95 *Dichotomous choice preference tests*

96

97 To assess potential differences in preference for receptive females between naïve and experienced  
98 males, we measured time associating with receptive and non-receptive females in dichotomous  
99 choice tests. We performed two dichotomous choice tests, with an intervening 45 days treatment  
100 exposure to females for experienced but not for naïve males. This testing protocol allowed us to  
101 determine whether experienced males acquire information about receptivity during their extended  
102 exposure to females.

103

104 First, to measure baseline preference for receptive females (pre-treatment test), we performed an  
105 initial dichotomous choice test on 62 reproductively mature males of similar age (approximately  
106 four months old). We photographed each male after behavioural testing using a Canon EOS Rebel  
107 T7i camera in a small glass aquarium (5 x 5 x 5 cm) with white walls and a scale for sizing. Camera  
108 colour calibration was performed daily with a Calibrite ColorChecker (X-Rite Inc.). Next, we  
109 transferred males to treatment 10L aquaria for a 45-day period. Specifically, we transferred each  
110 male tested in the pre-treatment test to a separate tank, with half of the males placed with two other  
111 virgin males, two virgin females and two non-virgin females of similar age (experienced  
112 condition), and the other half placed with two other virgin males of similar age (naïve condition).  
113 Naïve males had restricted visual access to tanks with females.

114

115 We used photographs to identify males from the pre-treatment tests following the 45 days of  
116 experimental treatment, and transferred them in a 3L aquarium three days prior to a second  
117 dichotomous choice test (post-treatment test) to allow for sperm replenishment. This avoids biases  
118 in preference measurements due to lack in motivation (Pilastro et al., 2002). While biases due to  
119 motivation were only expected for experienced males, naïve males were likewise transferred to 3L  
120 aquarium three days prior to their second behavioral test. Males were then presented for  
121 dichotomous choice post-treatment tests.

122

123 We performed all behavioral tests in a circular arena (diameter = 47 cm) sheltered to prevent  
124 disruption. We filmed the arena for 15-minute periods using a OBSBOT webcam (1080P at 30  
125 fps) after a five-minute acclimatization period. For accurate identification of fish with tracking  
126 software, we placed them in the experimental arena in 20 second intervals. We placed females first  
127 in the arena, randomizing the order of placing receptive and nonreceptive females. Additionally,

128 to account for any olfactory cues, we changed water in the arena between tests. To minimize stress,  
129 each fish was netted, placed in a glass bowl and transferred to the testing apparatus. For  
130 consistency, the tests were always conducted in the morning for a period of 4-6 hours.

131

132 We used idTracker to track the position of males and females in video recordings (Perez-Escudero  
133 et al., 2014) and to quantify the distance between the male and female for each video frame. To  
134 calculate preference for receptive females, we defined the time that a male associated with each  
135 female as the number of frames in the video recording that male was < 4 cm (less than two female  
136 guppy average body lengths) to each female. Preference ratio was calculated as:

137

$$138 \frac{(time\ spent\ with\ receptive - time\ spent\ with\ nonreceptive)}{total\ amount\ of\ time\ spent\ with\ both\ females}$$

139

140 To evaluate differences in preferences for receptive females between experienced and naïve males,  
141 we used a Linear Mixed Model with preference ratio as the dependent variable, and the time of  
142 testing, experience to mating receptivity cues and the interaction of these as fixed factors. We  
143 included the experimental batch as a random factor in the model. Given singularity issues caused  
144 by low variance in batch effects, we performed an analogous linear model including batch as a  
145 fixed effect. Significance tests were computed using a Wald t-distribution with Kenward-Roger  
146 approximation using the parameters package (Lüdecke et al., 2020). All analyses were performed  
147 in R (v. 4.1.3; R Core Team, 2022).

148

149 To assess potential differences in morphology or colouration patterns between fish used across  
150 treatments, we extracted measurements from male photographs taken right after pre-treatment  
151 tests. We quantified the number of pixels with carotenoid colouration, black melanistic colouration,  
152 body size (fish standard length) and tail size in the photographs using ImageJ (Schneider et al.,  
153 2012). We used a linear model with each measurement as the dependent variable and social  
154 treatments as fixed effect in R (v. 4.1.3; R Core Team, 2022).

155

156 *No choice preference tests*

157

158 To assess potential differences in preference for receptive females, as well as differences in the  
159 sexual behaviour repertoire of naïve and experienced males toward receptive females, we  
160 performed no-choice tests with receptive and non-receptive females on 122 males from the 31  
161 experienced and 31 naïve aquaria, described above. The males not used in the dichotomous choice  
162 tests were presented to either a receptive or nonreceptive female, with 62 experienced males (n=31  
163 with nonreceptive females and n= 31 with receptive females) and 60 naïve males (n=30 with  
164 nonreceptive females and n= 30 with receptive females). We excluded two naïve males due to  
165 uncertainty with fish labelling. All males were removed from treatment aquaria three days prior to  
166 experimentation for sperm replenishment, and to avoid biases in preference measurements due to  
167 lack in motivation (Pilstro et al., 2002).

168

169 Tests were conducted in a similar fashion to the dichotomous choice tests except only one female  
170 was present for each test, alongside a male, with the female placed first in the testing apparatus.  
171 Additionally, to account for any olfactory cues, we changed the water between each test. In order  
172 to minimize stress, each fish was netted, placed in a glass bowl and transferred to the testing  
173 apparatus. For consistency the tests were always conducted in the morning for 4 – 8 hours.

174

175 A single observer scored male sexual behaviour in video recordings in a random order and  
176 quantified the following behaviours as defined in Liley (1966): i) number of sigmoid displays,  
177 every time a male positioned himself in front of the female with an S-shaped posture soliciting  
178 copulation; ii) number of sneak attempts, unsolicited attempts of inseminating a female from  
179 behind by thrusting his gonopodium at the female's urogenital pore. We also calculated latency to  
180 initial sexual behaviour. Following the procedure described for dichotomous choice tests, we used  
181 idTracker software (Perez-Escudero et al., 2014), to calculate the distance between the male and  
182 female for each frame in the video recordings and extracted the time spent following a female for  
183 each trial (number of frames < 4 cm).

184

185 To compare the number of sigmoid displays in experienced and naïve males, we fit a statistical  
186 model using a Poisson distribution and a logit link function for the conditional mean in the package  
187 glmmTMB (Brooks et al., 2017). We used the mating status of the female, experience to mating  
188 receptivity cues and the interaction of these as fixed factors. We included the number of tank and

189 experimental batch as random factors in the model. For sneak attempts and latency to first sexual  
190 behaviour, we used analogous models including a zero inflation linear predictor. For time  
191 following the female, we used an analogous structure in a Linear Mixed Model fitted with lmer  
192 package (Bates et al., 2007). We evaluated the adequacy of our fitted models using scaled-residuals  
193 quantile-quantile plots, residual versus predicted values plots and a zero-inflation test in the  
194 DHARMa package (Hartig, 2018). We processed the parameters of our statistical models using  
195 Wald tests obtained via the parameters package (Lüdecke et al., 2020). We obtained post-hoc  
196 comparisons of the male response between female receptivity levels at pre-treatment and post-  
197 treatment time of testing in the previous models using the emmeans package with the tukey-  
198 adjustment method for multiple comparisons (Lenth et al., 2019). All analyses were performed in  
199 R (v. 4.1.3; R Core Team, 2022).

200

## 201 **Results**

202

### 203 *Male colour and morphology analyses*

204 Average proportion of orange or black coloration did not differ between males used for  
205 experienced or naïve treatments (mean  $\pm$  SE; orange coloration: naïve males  $7.33 \pm 3.47$ ,  
206 experienced males  $7.14 \pm 2.47$ ,  $F_{df=1} = 121$ ,  $p = 0.729$ ; black coloration: naïve males  $2.27 \pm 1.02$ ,  
207 experienced males  $2.20 \pm 0.86$ ,  $F_{df=1} = 0.154$ ,  $p = 0.695$ ; Supplementary Figure 1). Similarly, there  
208 was no significant overall difference between naïve and experienced males in morphological traits  
209 (body size: naïve males  $1.55 \pm 0.42$ , experienced males  $1.57 \pm 0.42$ ,  $F_{df=1} = 0.00$ ,  $p = 0.99$ ; tail  
210 size: naïve males  $0.51 \pm 0.08$ , experienced males  $0.48 \pm 0.09$ ,  $F_{df=1} = 0.75$ ,  $p = 0.38$ ; Supplementary  
211 Figure 1).

212

### 213 *Dichotomous Choice Preference Tests*

214 Neither experienced nor naïve males changed their strength of preference towards receptive  
215 females after 45 days of treatment (time of testing: estimate<sub>pre-treatment</sub>:  $-0.01 \pm 0.04$ ,  $t = -0.37$ ,  $p =$   
216  $0.72$ ; Figure 1; Table1). In addition, we found no overall differences in the strength of preference  
217 for receptive females between experienced and naïve males, or in the rate of change in preference  
218 between experienced and naïve males following the 45 days of treatment (male social treatment:

219 estimate<sub>experienced</sub>:  $0.01 \pm 0.04$ ,  $t = 0.45$ ,  $p = 0.65$ ; male social treatment x time of testing : estimate<sub>pre-  
220 treatment x experienced</sub> :  $0.04 \pm 0.06$ ,  $t = 0.72$ ,  $p = 0.48$ ; Figure 1; Table 1).

221

222 *No Choice Preference Tests*

223 There was no significant difference between naïve and experienced males in their levels of display  
224 behaviour or the number of displays that were performed towards receptive versus non-receptive  
225 females (Figure 2a; Table 2). However, we found that, unlike naïve males, experienced males  
226 significantly increased the number of displays towards receptive females compared to non-  
227 receptive females (female status x social treatment: estimate<sub>receptive x experienced</sub> :  $0.26 \pm 0.08$ ,  $t = 3.01$ ,  
228  $p = 0.002$ ; Figure 2a; Table 2).

229

230 Experienced males exhibited significantly more sneak attempts than naïve males (male social  
231 treatment: estimate<sub>experienced</sub>:  $0.49 \pm 0.22$ ,  $t = 2.19$ ,  $p = 0.028$ ; Figure 2b; Table 2), and this difference  
232 was due to greater frequency of sneak attempts by experienced males toward non-receptive  
233 females. We observed no difference in sneak frequency between experienced and naïve males in  
234 tests with receptive females ( $\log_{\text{mean}} \pm \text{SE}$ ; non-receptive females: naïve males  $1.16 \pm 0.18$ ,  
235 experienced males  $1.65 \pm 0.14$ ,  $t\text{-ratio}_{df=119} = -2.19$ ,  $p = 0.030$ ;  $\log_{\text{mean}} \pm \text{SE}$ ; receptive females: naïve  
236 males  $1.14 \pm 0.16$ , experienced males  $1.16 \pm 0.15$ ,  $t\text{-ratio}_{df=119} = -0.07$ ,  $p = 0.94$ ; Fig. 2b; Table 2).  
237 Additionally, naïve and experienced males showed no significant difference in the time spent  
238 following females in the trials, or in overall time spent following receptive and non-receptive  
239 females (Figure 2c; Table 2).

240

241 Experienced males showed decreased latency to perform sexual behavior compared to naïve males  
242 in tests with non-receptive and receptive females (male social treatment: estimate<sub>experienced</sub>:  $-1.51 \pm$   
243  $0.36$ ,  $z = -4.17$ ,  $p < 0.001$ ; Figure 2d; Table 2) However, post-hoc tests indicate that this decrease  
244 was significant just in tests with non-receptive females ( $\log_{\text{mean}} \pm \text{SE}$ ; non-receptive females: naïve  
245 males  $4.28 \pm 0.26$ , experienced males  $2.76 \pm 0.25$ ,  $t\text{-ratio}_{df=115} = 4.17$ ,  $p < 0.001$ ; mean  $\pm$  SE;  
246 receptive females: naïve males  $3.75 \pm 0.26$ , experienced males  $3.23 \pm 0.25$ ,  $t\text{-ratio}_{df=115} = 1.44$ ,  $p =$   
247  $0.15$ ; Fig. 2d; Table 2).

248

249 **Discussion**

250

251 We used dichotomous choice and no-choice tests to investigate how previous experience with  
252 female receptivity cues alter guppy male sexual behaviour and strength of preference for female  
253 mating status. Our results showed that males with previous access to female receptivity cues  
254 exhibited significantly greater frequency of coercive sexual behaviours and lower latency to first  
255 sexual behaviour to non-receptive females than naïve males. In addition, only experienced males  
256 significantly increased their number of displays towards receptive females compared to the number  
257 of displays performed with non-receptive females. However, previous experience with receptivity  
258 cues did not affect the strength of guppy male preference for female mating status.

259  
260 Previous studies evaluating how female mating status affect male mating behavior showed higher  
261 levels of coercive copulation attempts towards non-receptive females and higher levels of sigmoid  
262 displays towards receptive females (Guevara-Fiore et al., 2010a;2010b). Our results match these  
263 patterns only in males with previous experiences with female receptivity cues, suggesting a key  
264 role of social learning driving preferences for high quality females in this species. Our  
265 experimental design does not allow to disentangle which mechanism leads to changes in behaviour  
266 between experienced and naïve males. Prior experience with females might lead to males better  
267 recognizing which mating tactics provide higher success, as previously observed in species such  
268 as *Drosophila melanogaster* (Dukas, 2005, Saleem et al., 2014, Balaban-Feld & Valone., 2017), or  
269 eastern mosquitofish (Bisazza et al., 1996 but see Iglesias-Carrasco et al., 2019). Alternatively,  
270 changes in encounter rates of females and in mating success artificially created by the two social  
271 environments used in our experimental setup are known to affect male mating tactics (Cattelan et  
272 al., 2016; Devigili et al., 2015; Jordan & Brooks, 2012, Guevara-Fiore & Endler, 2018).  
273 Ultimately, the behavioural patterns observed in males with access to female receptivity cues  
274 correspond to theoretical predictions of fitness maximization, once accounting for the lower  
275 energetic requirements of sneak attempts of sperm insemination in relation to more costly sigmoid  
276 displays aiming to engage female with sexual consent (Devigli et al., 2013, Head et al., 2010).

277

278 Contrary to our prediction, males with social learning experience of female receptivity cues did  
279 not became choosier or increase their preference towards receptive females in dichotomous choice  
280 tests. It may be that that our experimental treatment might have changed the perception of naïve  
281 and experienced males in future reproductive opportunities, potentially biasing the investment of

282 naïve males in sexual behaviors that we observed in the first sexual encounter of their pre-treatment  
283 test (Fischer et al., 2008, Aich et al., 2021). Furthermore, determining the costs of sexual  
284 behaviours are challenging in benign lab environments where food is not a limiting factor. Future  
285 work incorporating resource limitation will be helpful to determine ecologically-relevant effects  
286 of social learning in male mating preferences. Yet, it is important to note that our results are  
287 concordant with a recent meta-analysis showing no evidence that mating status is an important  
288 factor for preference. Specifically, across species, virgin females are as choosy as mated females  
289 across reproductive isolation, inbreeding avoidance, and sexually transmitted disease scenarios  
290 (Richardson & Zuk, 2022). Our study here presents a similar finding, as males with no previous  
291 mating experience (naïve males) presented similar preferences to males with mating experience  
292 (experienced males). Hence, our study focusing on male mate choice on preference for higher  
293 quality females is in broad agreement to previous observations on the role of mating status for  
294 female preference across species.

295  
296 Using both dichotomous and no-choice approaches allowed for a broader picture of male  
297 preference in the context of social learning. It is possible that mating preferences may be stronger  
298 in choice tests compared to no-choice design, as males can select the female that is more likely to  
299 result in insemination (Dougherty, 2020). However, there is arguably an increased risk of being  
300 rejected by the only potential mate in a no-choice tests, and this could make males more careful in  
301 tuning their mate strategy to female receptivity cues (Dougherty & Shuker, 2015). Our tests using  
302 these two complementary experimental paradigms are concordant in that we did not observe  
303 changes in overall preference for receptive females or differences in overall sexual behaviour  
304 levels based on female receptivity status. However, our observations of changes in rates of sexual  
305 display and coercive copulations depending on female mating status suggest that future mate  
306 choice studies should incorporate both methodologies.

307  
308 The changes we observe in male guppies in behavioural repertoire and latency towards females  
309 based on previous experience with female receptivity cues add to the evidence suggesting that  
310 social environment and learning from previous experience can affect male sexual behaviour.  
311 Overall, our results are consistent with the idea that male guppies use social learning to efficiently

312 tune their mating tactics, soliciting copulation in higher rates to receptive females and performing  
313 higher coercive copulation attempts towards non-receptive females.

314

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323

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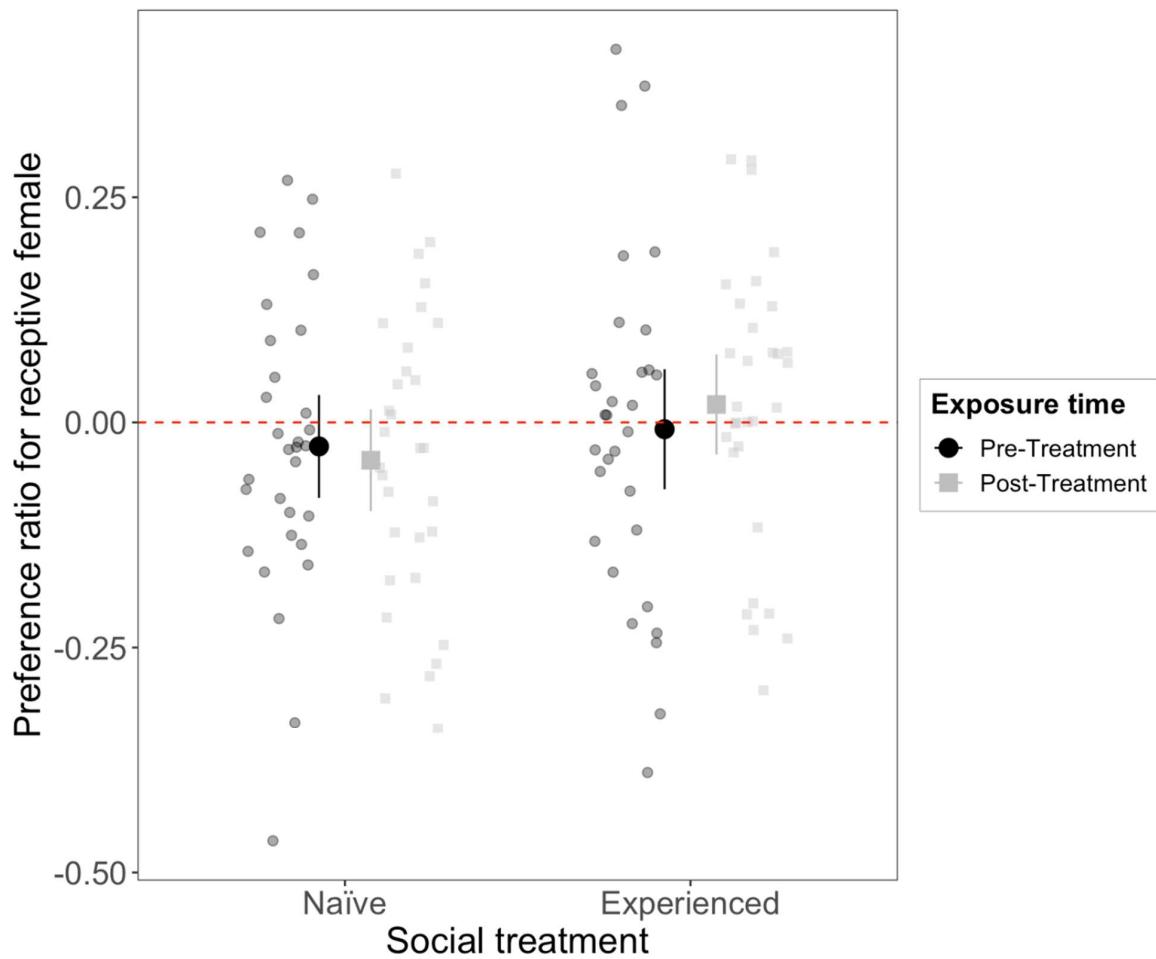
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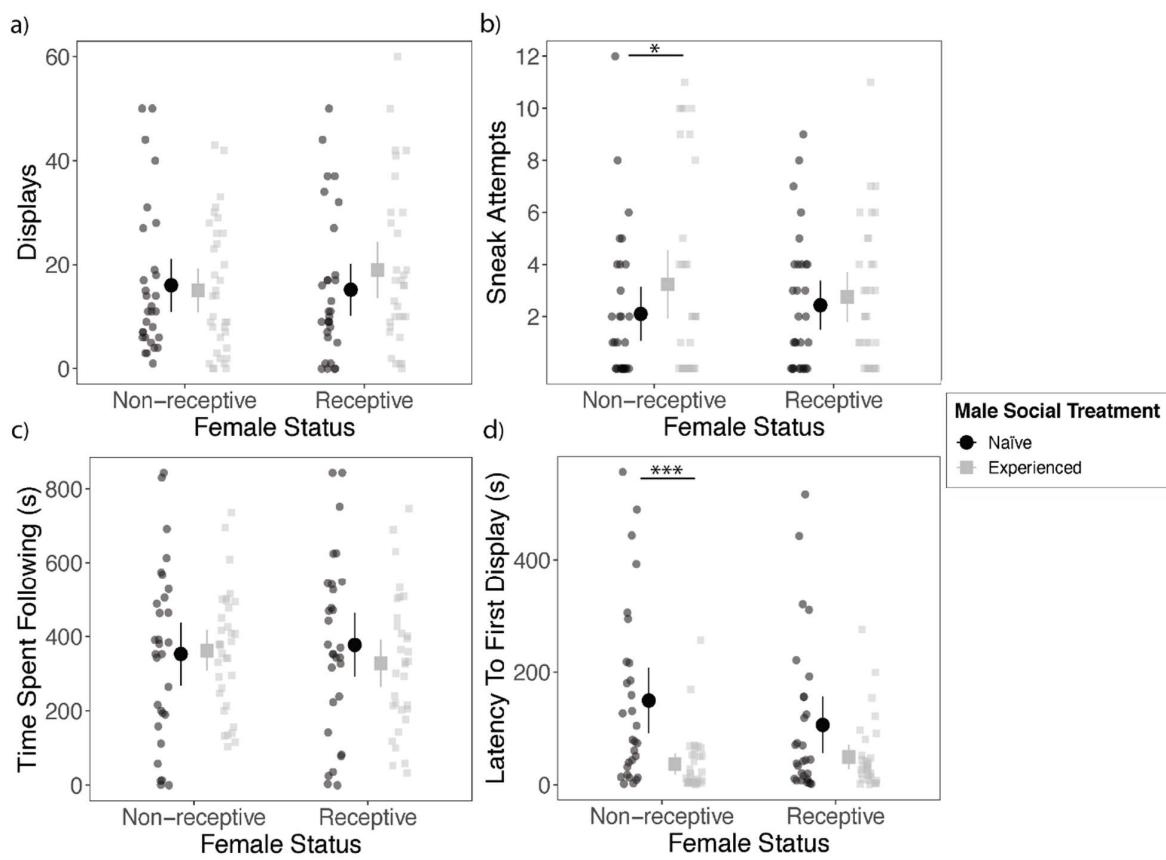
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454 **Figure 1. The effect of previous experience with female receptivity cues in guppy male**  
455 **preference for receptive females.** Preference ratios were calculated as total time spent associating  
456 with receptive females by the total time associating with receptive and non-receptive females in  
457 dichotomous choice tests. Tests were performed to males naïve and experienced with female  
458 receptivity cues before (pre-treatment, black circles) and after (post-treatment, gray squares) a 45-  
459 day treatment in their respective experimental condition. Larger circles and squares indicate  
460 average preference ratio for each treatment and time of testing with 95% CI bars. We found no  
461 differences in the amount spent with receptive or nonreceptive females between naïve and  
462 experienced males in pre-treatment or post-treatment tests (see Table 1).

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467 **Figure 2. The effect of previous experience with female receptivity cues in male sexual**  
468 **behaviour.** Quantification of (a) the number of sigmoid displays, (b) number of sneak attempts,  
469 (c) total time spent following females, and (d) latency to first sexual behaviour performed towards  
470 non-receptive and receptive females in guppy males naïve (black circles) and experienced (gray  
471 squares) with female receptivity cues. Larger circles and squares indicate average values of each  
472 behaviour with 95% CI bars. For number of displays, experienced males significantly increased  
473 the number of displays towards receptive females compared to non-receptive females (female  
474 status x social treatment:  $p = 0.002$ ; see Table 2). Stars indicate significance in post-hoc  
475 comparisons of the male response between female receptivity levels at pre-treatment and post-  
476 treatment time of testing ( $p < 0.001$  \*\*\*,  $p < 0.05$  \*).

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488    **Table 1.** Results for a Linear Mixed Model comparing preference for receptive females performed  
489    to male guppies naïve and experienced with female receptivity cues before and after a 45-day  
490    treatment in their respective experimental condition.  
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Parameter	Estimate	SE	t (df:119)	P – value
Intercept	-0.02	0.03	-0.88	0.60
Male social treatment (experienced)	0.01	0.04	0.45	0.65
Time of testing (pre-treatment)	-0.01	0.04	-0.37	0.72
Batch (2)	-0.02	0.03	-0.62	0.53
Male social treatment:time of testing	0.04	0.06	0.72	0.48

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521 **Table 2.** Statistical tests for models comparing potential differences in behaviour in no choice tests  
 522 with non-receptive and receptive females performed to male guppies naïve and experienced with  
 523 female receptivity cues. Parameters with significant differences in bold.  
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Behaviour	Parameter	Estimate	SE	Statistic <sub>df</sub>	P - value
<i>Display</i>	Intercept	2.57	0.20	z : 12.70	<b>&lt;0.001</b>
	Female status (receptive)	-0.05	0.06	z : -0.85	0.39
	Male social treatment (experienced)	-0.05	0.17	z : -0.28	0.77
	Female status:male social treatment	0.26	0.08	z : 3.01	<b>0.002</b>
<i>Sneak</i>	Intercept	1.15	0.17	z : 6.48	<b>&lt;0.001</b>
	Female status (receptive)	-0.01	0.20	z : -0.07	0.94
	Male social treatment (experienced)	0.49	0.22	z : 2.19	<b>0.028</b>
	Female status:male social treatment	-0.47	0.26	z : -1.79	0.07
	<i>Zero Inflation</i> : Intercept	0.49	0.10	z : -3.37	<b>&lt;0.001</b>
<i>Latency</i>	Intercept	4.27	0.26	z : 16.42	<b>&lt;0.001</b>
	Female status (receptive)	-0.52	0.36	z : -1.42	0.15
	Male social treatment (experienced)	-1.51	0.36	z : -4.17	<b>&lt;0.001</b>
	Female status:male social treatment	0.99	0.51	z: 1.93	0.053
	<i>Zero Inflation</i> : Intercept	0.00	0.00	z : -0.00	0.996
<i>Time spent following</i>	Intercept	351.27	44.13	t <sub>3,7</sub> : 7.95	<b>0.002</b>
	Female status (receptive)	24.51	42.85	t <sub>61.5</sub> : 0.57	0.56
	Male social treatment (experienced)	13.70	51.52	t <sub>105.5</sub> : 0.26	0.79
	Female status:male social treatment	-61.06	59.30	t <sub>61.8</sub> : -1.03	0.31

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## Supplementary Materials for:

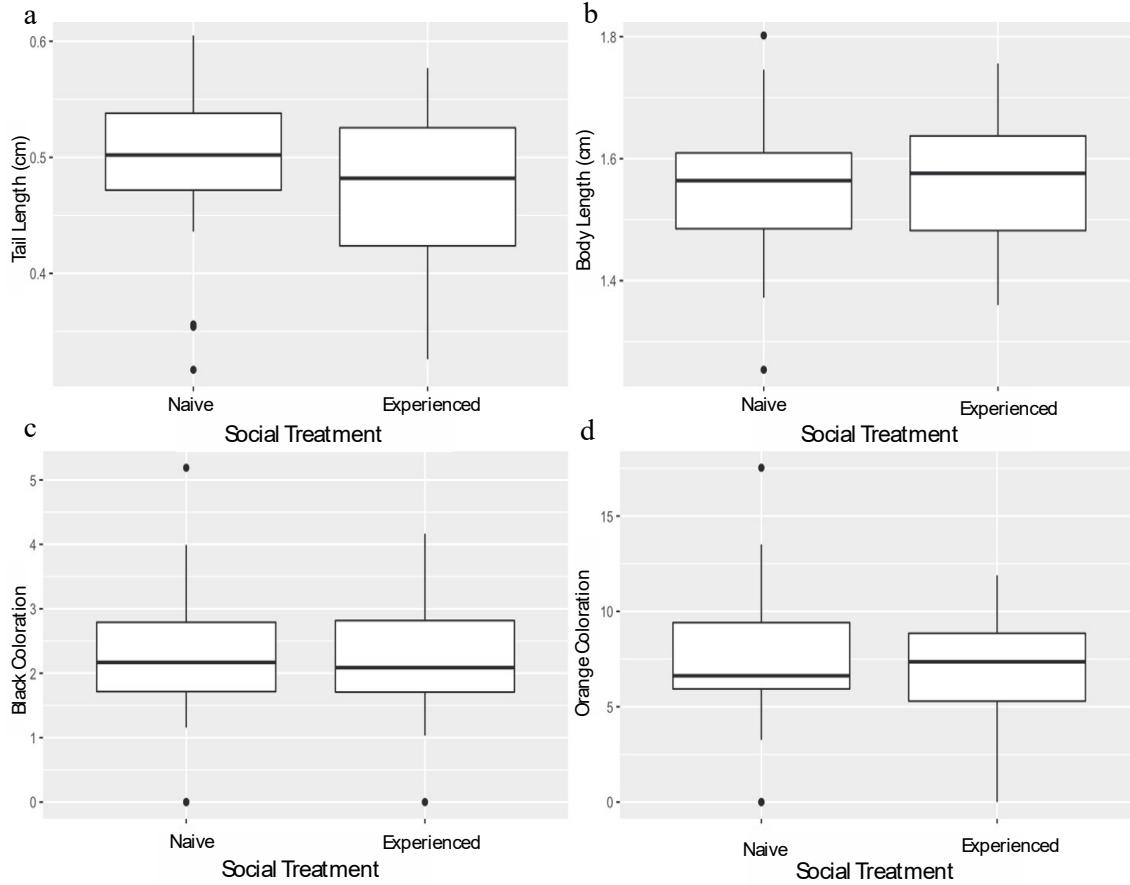
Male guppies (*Poecilia reticulata*) exposed to mating receptivity cues change their sexual behaviour, but not their preference, towards receptive females

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**Table S1.** Results of linear model comparing colouration and morphological traits in male guppies used for social experience experimental treatments

Trait	Sum Sq	Mean Sq	F value (df = 1)	P value
Orange Colouration	1.1	1.09	0.12	0.72
Black Colouration	0.14	0.13	0.15	0.69
Standard Length (cm)	0.00	0.00	0.00	0.93
Tail Length (cm)	0.012	0.01	3.08	0.08



**Figure. S1. Body morphology and colouration in experienced and naïve males.** No significant differences were found between randomly assigned males to naïve ( $n = 31$ ) and experienced ( $n = 31$ ) treatments for (a) tail length, (b) body length, (c) proportion of black, or (d) proportion of orange. For all boxplots, horizontal lines indicate medians, boxes indicate the interquartile range, and whiskers indicate all points within 1.5 times the interquartile range.