Title: Male guppies change courtship behaviour in response to their own quality relative to that of a rival male.

Authors: Takashi Yoshikawa[1], Yusaku Ohkubo[1], Kenji Karino[2] and Eisuke Hasegawa[1*]

Affiliations:

1: Laboratory of Animal Ecology, Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan.

2: Department of Biology, Tokyo Gakugei University, Tokyo 184-8501, Japan

*: To whom correspondence should be addressed.

Eisuke Hasegawa

Laboratory of Animal Ecology, Department of Ecology and Systematics, Graduate School of Agriculture, Hokkaido University, Sapporo 060-8589, Japan.

e-mail: ehase@res.agr.hokudai.ac.jp

Tel:+81-11-706-3690; Fax:+81-11-706-2494

Word count: 3510

Abstract

Male mate choice has been an important but minor topic compared with female mate choice in the past two decades. In the guppy, *Poelicia reticulata*, a male approaching a female abandons his courtship when a rival male appears next to the focal female; however, the effect of the relative quality between the males on this behavioural change is unknown. We show here that male guppies abandon their approach to a female only when the rival male is phenotypically superior. Both natural and artificially induced brightly-coloured males continued to approach a female even when the rival male was brightly coloured, but both natural and induced dull-coloured males abandoned their approach to a female when the rival was brightly coloured. Males decide their behaviours on the basis of their own appearance, not on their genotypes, because artificially induced brightly- and dull-coloured brothers differ in their behaviour. Our results showed that male mate-choice behaviour is finely tuned to maximize the probability of acceptance by the approached female.

Key Words: guppy, female preference, mate choice, male preference, sexual selection

INTRODUCTION

Male mate choice is expected to evolve when the benefit of an adequate choice overcomes its cost (Edward, & Chapman, 2011). For example, males prefer virgin females because of their low preference (King, Saportio, Ellison, and Bratzke, 2005), high probability of fertilization (Bonduriansky, 2001), and advantage in sperm competition on the first copulation in some cases (Wedell, Gage & Parker. 2002; Engqvist & Reinhold, 2006). Males also prefer large females (Dosen & Montogomerie, 2004) because such females produce more eggs than small females. In male mate choice, if a male can discriminate the quality of the females around him, he directs more effort to convince better female, and thus, a good female prefers such a male, resulting in high fitness of such a male. However, a male that continue to approach one female loses his chance to mate with another female (Andersson, 1994). Thus, the probability of obtaining a good mate is a function of both the timing of abandoning the current pursuit and the probability of finding an alternative partner.

Mate choice by males has been demonstrated in many animals (see Edward and Chapman 2011), including several kinds of fishes (Roland, 1982; Sargent, Gross & van den Berghe, 1986; Grant, Casey, & Shahsavarani, 1995). However, when females can decide which male to mate with, if a rival male appears near the female that is being approached by the focal male, the focal male's

52 success is dependent on the two males' relative attractiveness to the female. Whether the focal male
53 abandons his approach should be determined by the relative attractiveness of the two males. In
54 addition, the presence of a rival male increases the risk that the focal male faces sperm competition
55 in which the focal male must compete with the rival even if he succeeds in mating with the focal
56 female (Parker, Lessells, & Simmons, 2013). Under such a condition, a male approaching a female
57 must decide whether to withdraw from the courtship or not. This decision will have a major effect
58 on his fitness (Seymour, & Suzou, 2009).
59 Jeswiet (2011) showed that in guppies, an approaching male abandons his courtship when a rival
60 male appears next to the female. However, his study did not examine the effect of the types of focal
61 males and rivals. Mate choice behaviour may differ between bright and dull males in the presence
62 of a rival. In addition, a male's acceptability is affected by the response of the focal female to the
63 male's appearance (Kodric-Brown, 1985; Karino, & Matsunaga, 2002; Karino, & Shinjo, 2004;
64 Karino, & Urano, 2008). Female preference and male appearance have been thought to be
65 genetically based (Karino, & Haijima 2001; Chenoweth, & McGuigan, 2010), but Kodric-Brown
66 (1989) indicated that females showed a preference for bright males that were artificially induced by
67 dietary manipulations. Thus, a possibility exists that males change mate choice behaviours
68 depending on their appearance even when it is artificially induced.

In this study, we examined the mate-choice behaviour of male guppies to test whether in the presence of a rival male a focal male changes his courtship behaviour on the basis of his appearance. We conducted this experiment using both naturally coloured males and their artificially coloured brothers.

METHODS

Materials

We used laboratory-reared guppies, *Poelicia reticulata*, that were originally collected from a wild population at Hijigawa, Okinawa Pref., Japan. Population T was maintained in a laboratory at Tokyo Gakugei University from 2011, and population H was reared in a laboratory at Hokkaido University from 2012. They were reared in the Hokkaido University laboratory for approximately 1 year before the experiments. Because guppies were introduced into Okinawa in the 1970s (Kouchi 1997), they have been subjected to natural and sexual selection for a long time. In fact, these males have secondary sexual traits, such as similar orange spot sizes, to those of males of some native populations (Karino and Haijima 2001). We prepared 4 tanks (60 × 30 × 36 cm), with 2 tanks assigned to each population (H or T). Approximately 50 fish were introduced into each tank. All rearing and experiments were conducted under conditions of 24-26°C and 12 L:12 D. We prepared

7 sets of 6 individuals (2 bright males, 2 dull males, a large female, and a small female) for each population. For population H, we selected bright males that had recently matured (with a large orange area on the sides of the body) and dull males (a small orange area) from the stock aquaria and combined them randomly to make a set after they matured. Young unmated females were reared in a separate large fish tank (60 × 30 × 36 cm), and a large and small female were selected to make a set. For population T, the males in the set consisted of 4 brothers from a mother that had mated only once (full siblings). Each of two males in the 7 sets came from each different brood of different mothers. These males were segregated into two groups in a small tank (30 × 20 × 17 cm) that was separated into two areas by an acrylic board. Carotenoid-rich diets (Tetramin®, Tetra, Germany) were fed to one group, and carotenoid-poor diets (CE-2, Clea Japan) were fed to the other group to artificially induce bright and dull males, respectively (Kodric-Brown, 1989: for nutritional contents of each diet, see Table S1 in Supplementary Material). They were reared for approximately 11 weeks under these conditions to sexual maturity. One large and one small unmated female were selected to make a set. All the individuals used were sexually mature at the time of the experiments.

Measurements

All individuals used were photographed from both sides with a scale, and the photos were imported into a computer. All measurements were obtained using computer software (ImageJ). We measured the body length (from the tip of the mouth to the tail) of all individuals used. We measured the proportion of the orange areas on both sides of the bodies of the males, and the averaged value was used as the index of a male's appearance. For T males, a colour saturation of orange spots (%) was estimated by averaging 6 randomly selected points (3 on each side) of a photographed male.

Experiment 1

We conducted two sequential experiments using naturally bright and dull males (from population H); we then conducted the same sequential experiments with artificially induced bright and dull brothers (from population T). First, we established a male's preference for females when rivals were absent. A large tank was divided into 3 equal areas (20 × 30 × 36 cm) by two acrylic boards. Spaces between the tank wall and acrylic boards were sealed to prevent the transport of chemical substances across the areas. The central area was further divided into 3 equal parts by drawing two parallel lines (separation lines) on the floor of the tank. We placed a large (body length, 23.01–30.49 mm for H: 19.31–26.65 mm for T) and a small female (body length, 20.75–27.13 mm for H: 14.53–24.01 mm for T) into each of the side areas, and a focal male (body length, 22.02–25.86 mm

120 for H: 19.01–26.21 mm for T) was released into the central area. The size difference between the
121 females was more than 1.3 mm to induce a clear preference in the male (Dosen, & Montogomerie,
122 2004; Herdman, Kelly & Godin, 2004). We also paid close attention to the fact that a male hesitates
123 to court a female when she is much larger (by more than two times) than he is (Houde, 1997). Thus,
124 we did not use such big females. We allowed the fish to acclimate for 10 minutes; the behaviour of
125 the male was then observed for the following 10 minutes. If the male turned his head towards a
126 female and more than half of his body crossed beyond either of the two separation lines (6.7 cm
127 from a divider wall), we judged that the male preferred a female. Male preference was determined
128 by the proportion of time he spent associating with one female (preferred time; Godin & Briggs,
129 1996) out of the total time spent associating with either of the females. This value was defined as
130 the preference score (PS). To confirm the consistent preference of a male, we conducted the same
131 observations by exchanging female positions, but no male changed his preference.
132
133 ***Experiment 2***
134 Following experiment 1, a rival male was introduced into the area of the preferred female. We
135 observed the behaviour of the introduced male at the start of experiment. Because a virgin female
136 that has never met with males copulates indiscriminately (Houde, 1997), the females we used were

137 experienced and had met with males in a small net (10 cm × 15 cm) in an aquarium tank where
138 males are reared. This procedure was conducted to prevent the resident female from immediately
139 copulating with the introduced male. The introduced male occasionally approached the resident
140 female, but we did not confirm any copulation between the introduced male and the resident female.
141 After 10 minutes, we observed the focal male for an additional 10 minutes and again calculated the
142 PS. We calculated a PS for each of the 4 males in a set by using another male from the set as the
143 rival male. We presented a bright rival and a dull rival to each male, thus obtaining 4 data points (2
144 for bright and 2 for dull focal males). Each male in a set was used just once as a focal male. When a
145 specific type (bright or dull) of male was used as a focal male, the rival male was selected
146 randomly from the two males of the other type. When the total preferred time (the time spent
147 approaching either of the two females in the presence of a rival) was less than 5 minutes, the data
148 for these males were removed from the experiments because such a male seemed to be sexually
149 inactive. Each of the 7 sets was examined for each population (14 measurements for each type).
150
151 ***Ethical Note***
152 Laboratory raised fish were reared in several mother tanks (60 × 30 × 36 cm) under an adequate
153 density (approximately 100 individuals/tank) and temperature (24-26°C). The individuals used in

154 the experiments were selected from the mother tanks and were reared in a separate tank under the
155 same conditions as the mother tanks. We photographed the fish to prevent them from becoming
156 weakened. The fish that completed the experimental procedure were immediately returned to the
157 mother tanks.
158
159 ***Statistics***
160 A paired *t*-test was used to compare the differences in the body sizes of the males. Similarly, the
161 Wilcoxon sign rank test was used to compare the differences in the proportions of the orange area
162 and the colour saturation of the two groups of males. Because we artificially chose the differences
163 in the body sizes between the two females in a set, we confirmed that the size ratio between the two
164 females among the sets did not differ significantly by using a generalized linier model (GLM) with
165 a binomial distribution. In the behavioural experiments, changes in the PS after the addition of a
166 rival were examined using a GLM with a binomial distribution. In the GLM, the changes following
167 the addition of a rival were set as the dependent variable with the following three independent
168 variables: 1) the appearance of the focal male (bright or dull), 2) whether the males are naturally or
169 artificially coloured and 3) the set. We conducted two GLM analyses, one being the cases in which
170 the rival is bright, and the other the cases in which the rival is dull. All of the statistical analyses

were performed in R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Male preference when a rival is absent

Table 1 shows male preference for females when a rival is absent. In both populations, most males of both the types preferred the large female (64.29%~92.85%). The preference time for the preferred female was significantly longer than that for the less preferred female (for all the cases, Paired *t*-test: $t13$, $p<0.0001$). These results confirmed the results of a previous study (Dosen, & Montogomerie, 2004). In addition, in both the populations, the total preferred time is not different between the bright and the dull males (for population H; Paired *t*-test: $t12=0.281$, $P=0.783$: for population T; Paired *t*-test: $t13=0.351$, $p=0.0799$), suggesting that the dull males are not sexually inactive.

Male mate choice behaviour in naturally coloured males

Second, we examined behaviours between naturally bright and dull males. Body size did not differ between the two types (bright, 23.57±1.16 mm; dull, 23.17±0.92; paired *t*-test: $t13=1.7569$, $P=0.102$). The bright males had a larger orange spot (8.60±2.95%) on the sides of their bodies than

189 the dull males (2.27±1.07%; Wilcoxon signed-rank test: Z14=3.148, *P*=0.0002).

190 The GLM showed that when the rival is bright, the appearance of the focal male significantly
191 affects his decision (GLM: z13=2.958, *P*=0.0031). The other two independent variables (whether
192 naturally or artificially coloured and the set) had no effect on the behavioural changes in the focal
193 males. When the rival is dull, no independent variable shows a significant effect on the decision of
194 the focal males. These results indicate that the bright males did not change their preference score
195 (PS, the proportion of preferred time for a given female out of the total preference time during
196 which the male showed a preference for either of the two females) for the approached female
197 regardless of the brightness or dullness of the rival male (Fig. 1a). In contrast, dull males
198 significantly decreased their PS for the approached female regardless of the rival male's type (Fig.
199 1b). However, the dull males did not decrease their total preference activity after the rival's
200 introduction (Table 2), meaning that a dull male changed his courtship partner from the preferred
201 female to the less-preferred female after the appearance of the rival male. These results indicate that
202 dull males change their preference in the presence of the rival male, whereas bright males do not.

203

204 *Male mate choice behaviour in artificially manipulated males*

205 Third, the preference behaviour was compared between artificially induced bright and dull brothers.

206 Brothers from a once-mated mother were divided into two groups. One group was fed a
207 carotenoid-rich diet, and the other group was fed a carotenoid-poor diet. We induced these bright
208 and dull pairs from seven mothers. Body size at the beginning of the experiments did not differ
209 between the two types (bright, 21.95±2.31 mm; dull, 22.11±2.22 mm: paired-*t* test, $t12 = -0.8664$,
210 $P = 0.402$), suggesting that the diet had no effect on growth (for the nutritional content of both diets,
211 see Table S1 in the Supplementary Material). The size of the orange spot also did not differ
212 between the bright (5.21±0.83%) and dull males (5.49±0.72%: Wilcoxon signed-rank test:
213 Z14=1.300, P=0.194), but the colour saturation of the spot differed significantly (bright;
214 68.39±6.23%, dull; 47.02±2.96: Wilcoxon signed-rank test: Z14=3.148, P<0.001). For the results
215 of the GLM analyses, see the above section. The bright males did not change their PS to the
216 approached female after the introduction of either type of rivals (bright or dull), but the dull males
217 decreased their PS in the presence of the bright rival (Fig. 1c, d). These results indicate that the
218 preference behaviours of genetically controlled brothers are affected by the relative saturation
219 between themselves and the rival male.
220
221 **DISCUSSION**
222 Our results indicate that in *P. reticulata*, bright males do not hesitate to approach the preferred

females when a rival is present; however, dull males abandon their approach when a superior rival appears. Previous studies have shown that approaching males abandon their courtship when rival males appeared in close proximity to the preferred female (Jeswiet, 2011). However, this study did not consider the type of focal and rival males (bright or dull). Our study is the first to show a behavioural difference between bright and dull males when a rival male appears near the approached female.

Male guppies evaluate a female using several criteria to increase likelihood of acceptance by the female (Herdman et al., 2004; Guevara-Fiore, Skinner, & Watt, 2009; Guevara-Fiore, Stapley, Jrause, Ramnarine, & Watt, 2010). Solitary females will accept an approaching male with a high probability; however, a low probability of acceptance is observed with attended females. Males should thus change their courtship from attended to solitary females to increase their probability of acceptance (Jeswiet, 2011; Lane et al. 2015; Auld, Leswiet, & Godin, 2015). However, as female guppies prefer males with large and bright orange spots on their sides (Kodric-Brown 1985; Karino & Matsunaga, 2002; Karino & Urano, 2008; Karino, Shimada, Kudo, & Sato, 2010), a difference in quality will exist between an approaching male and a rival. For a male, the presence of an inferior rival does not lower his probability of acceptance; however, approaching a female in the presence of a superior rival is a waste of time. Our observations demonstrate that the preference behaviours

240 of male guppies are finely tuned to maximize their probability of acceptance.

241 The time to abandon an approach may be affected by the population sex ratio. Under male-biased
242 sex ratios, finding an accepting female is difficult when a male abandons his approach. The sex
243 ratios of *P. reticulata* populations fluctuate cyclically (Pettersson, Ramnarine, Becher, Mahabir, &
244 Magurran, 2004). In addition, *P. reticulata* females prefer rare males (Hughes, Houde, Price, &
245 Rodd, 2013), meaning that dull males are preferred when they are in the minority. This study might
246 show that females prefer rare 'colour patterns', but overall, they still preferred brightly-coloured
247 males even when they were the minority. We need to further investigate how a male makes
248 mate-choice decisions under such ecological conditions.

249 Artificially induced dull males changed their behaviour, but their bright brothers did not. These
250 results indicate that decision-making is not genetically determined, meaning that the males changed
251 their behaviour in response to their appearance or condition. A previous study that induced artificial
252 dietary differences in brightness between brothers also showed that diet did not affect the male's
253 size, the location or size of the red and orange pigment spots, or the intensity of courtship
254 behaviour (Koderic-Brown, 1989). Also, in this study, the body size and size of the orange spots
255 did not differ between the induced bright and dull brothers. In addition, neither type of male sibling
256 differed in total preference time (Table 1). The dull males did not become inactive to approached

257 females; however, they changed their partner from a preferred female with a rival to the other
258 female (for the males in population T; see Table 1). The nutritional content of both diets is similar
259 other than that Tetrain® is carotenoid enhanced (see Table S1 in Supplementary Material). Thus,
260 the hypothesis that dull males are sexually inactive due to inferior body condition seems unlikely.
261 However, our experiments could not completely remove the effects of the different diets on body
262 conditions. It is worth noting that further studies with more careful manipulations (e.g., colour
263 differences between bright and dull males induced by using the same diet without one group
264 receiving additional carotenoids) are needed.
265 The males made decisions according to their relative attractiveness compared to the rival. The
266 observed withdrawal is adaptive, as such males do not pay additional costs for a fruitless approach.
267 Therefore, a male guppy chooses a female that offers a high probability of acceptance. How does a
268 male know his own appearance? Several hypotheses have been suggested concerning this issue; a
269 male knows his appearance from 1) the responses of the focal females, 2) his experiences in the
270 past, and 3) the responses of rival males. Although some of these hypotheses have been tested
271 (Gonzalez-Zuarth, Vallarino, & Garcia, 2011; De Gasperin, & Garcia, 2014), more studies are
272 needed to answer this question.
273 Our results suggest that dull male guppies may increase their probability of mating with females by

changing mate decisions in the presence of relatively more attractive rivals. This finding may imply a possible mechanism for maintaining the polymorphism of male ornamentation within populations. The evolution and maintenance of polymorphisms are key issues in evolutionary biology (Mitchell-Olds, Willis, & Goldsteis 2007), and sexual ornamentation in male guppies is highly polymorphic (Houde 1997). Together with the rare-male mating advantage (Hughes et al. 2013) and high predation risk on common male phenotypes (Olendorf et al. 2006), our results will shed light on the mechanism for the maintenance of male ornamentation.

ACKNOWLEDGMENTS

This work was supported in parts by grants-in-aid from the Ministry of Education, Culture, Sports Science and Technology of Japan to E.H. (nos. 26440228, 15H04420) and to K.K. (no. 26440234).

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Table 1. Male preference without a rival (control treatment).

	Male Type	Percentage of males that prefer the large female	Preference time to the preferred female (Average±S.D.)	Preference time to the less preferred female (Average±S.D.)	Total preference time (Average±S.D.)
Population H	Bright	78.57%（11/14）	379.79±117.20	106.75±86.21	239.61±174.84
	Dull	71.43%（10/14）	369.14±115.69	83.00±62.66	226.07±169.81
Population T	Bright	92.85% (13/14)	364.00±114.66	87.62±44.10	213.68±159.45
	Dull	64.28% (9/14)	379.36±75.61	91.07±84.62	235.21±160.42

In both populations, males preferred the large females, and the total preference time did not differ between the bright and dull males.

Table 2. Difference in the total preference time between bright and dull males for different types of rivals in each population.

Population	Focal male	Rival male	Preference time (sec) (Average ± S.D.)	df	*t* value	*P*
Population H	Bright	Bright	431.00±84.95			
	Bright	Dull	415.86±79.98	26	0.486	0.631
	Dull	Bright	445.07±90.90			
	Dull	Dull	433.14±96.56	26	0.337	0.739
Population T	Bright	Bright	463.29±83.30			
	Bright	Dull	439.64±72.22	26	0.802	0.430
	Dull	Bright	488.14±63.42			
	Dull	Dull	490.36±64.18	26	-0.092	0.928

In both populations, no significant difference between bright and dull males was found for any of the possible combinations.

Figure Legends

Figure 1. Behaviours of naturally bright (= large orange spot) males (a) and naturally dull (= small orange spot) males (b) or to induced bright (= high colour-saturated spot) males (c) and induced dull (= low colour-saturated spot) males (d) to two different types of rivals. Bars and whiskers show the mean ± S. E.

Figure1