



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



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Compared with female mate choice, male mate choice has been an important but minor topic in the past two decades. In the guppy, *Poecilia 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 of 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 decided their behaviours on the basis of their own appearance, not on their genotypes, because artificially induced brightly and dull-coloured brothers differed in their behaviour. Our results show that male mate choice behaviour is finely tuned to maximize the probability of acceptance by the approached female.

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Male mate choice is expected to evolve when the benefit of an adequate choice overcomes its cost (Cornwallis & Uller, 2010; Edward & Chapman, 2011). For example, males prefer virgin females because of their low preference (King, Saporito, Ellison, & Bratzke, 2005), high probability of fertilization (Bonduriansky, 2001) and advantage in sperm competition on the first copulation in some cases (Engqvist & Reinhold, 2006; Wedell, Gage & Parker, 2002). Males also prefer large females (Dosen & Montgomerie, 2004) because they 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 females, and thus a good female prefers such a male, who then gains high fitness. However, a male that continues to approach one female loses his chance to mate with another (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 & Chapman, 2011), including several kinds of fishes (Grant, Casey, & Shahsavarani, 1995; Rowland, 1982; Sargent, Gross, &

van den Berghe, 1986). 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 success is dependent on the two males' relative attractiveness to the female. Whether the focal male abandons his approach should be determined by the relative attractiveness of the two males. In addition, the presence of a rival male increases the risk that the focal male faces sperm competition in which he must compete with the rival even if he succeeds in mating with the focal female (Parker, Lessells, & Simmons, 2013). In this situation, a male approaching a female must decide whether or not to withdraw from the courtship. This decision will have a major effect on his fitness (Seymour & Suzou, 2009).

Jeswiet et al. (2011) showed that in guppies, *Poecilia reticulata*, an approaching male abandons his courtship when a rival male appears next to the female. However, his study did not examine the effect of the types of focal males and rivals. Mate choice behaviour may differ between bright and dull males in the presence of a rival. In addition, a male's acceptability is affected by the response of the focal female to the male's appearance (Karino & Matsunaga, 2002; Karino & Shinjo, 2004; Karino & Urano, 2008; Kodric-Brown, 1985). Female preference and male appearance have been thought to be genetically based (Chenoweth & McGuigan, 2010; Karino & Haijima, 2001), but Kodric-Brown (1989) indicated that females showed a preference for males with bright coloration artificially induced by dietary manipulations. Thus, a possibility exists that

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males change mate choice behaviours 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 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 orange spot sizes, that are similar to those of males of some native populations (Karino & Hajjima, 2001). We prepared four tanks (60 × 30 cm and 36 cm high), with two tanks assigned to each population (H or T). Approximately 50 fish were introduced into each tank. All rearing and experiments were conducted at 24–26 °C and 12:12 h light:dark. We prepared seven sets of six individuals (two bright males, two 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 cm and 36 cm high), and a large and small female were selected to make a set. For population T, the males in each set consisted of four brothers from a mother that had mated only once (full siblings), while the males in the seven different sets came from different broods with different mothers. These males were segregated into two groups in a small tank (30 × 20 cm and 17 cm high) that was separated into two areas by an acrylic board. Carotenoid-rich diets (Tetramin, Tetra, Malle, Germany) were fed to one group, and carotenoid-poor diets (CE-2, Clea, Tokyo, 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 Appendix Table A1). Both groups were reared under these conditions for approximately 11 weeks 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 a free software (ImageJ; <https://imagej.nih.gov/ij/>). We measured the body length (from the tip of the mouth to the tail) of all individuals used. We also measured the proportion of the orange areas on both sides 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 six randomly selected points (three 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 (60 × 30 cm and 36 cm high) was divided into three equal areas by two acrylic boards. Spaces between the tank wall and the acrylic boards were sealed to prevent the transport of chemical substances across the areas. The central area was further divided into three 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 for H; 19.01–26.21 mm for T) was released into the central area. The size difference between the females was more than 1.3 mm to induce a clear preference in the male (Dosen & Montgomerie, 2004; Herdman, Kelly, & Godin, 2004). We also paid close attention to the fact that a male hesitates to court a female when she is much larger (by more than two times) than he is (Houde, 1997). Thus, we did not use such big females. We allowed the fish to acclimate for 10 min; the behaviour of the male was then observed for the following 10 min. If the male turned his head towards a female and more than half of his body crossed either of the two separation lines (6.7 cm from a divider wall), we judged that the male preferred a female. Male preference was determined by the proportion of time he spent associating with one female (preferred time; Godin & Briggs, 1996) out of the total time spent associating with either of the females. This value was defined as the preference score (PS). To confirm the consistent preference of a male, we conducted the same observations by exchanging female positions, but no male changed his preference.

### Experiment 2

Following experiment 1, a rival male was introduced into the area of the preferred female. We observed the behaviour of the introduced male at the start of the experiment. Because a virgin female that has never met with males copulates indiscriminately (Houde, 1997), the females we used were experienced and had met with males in a small net (10 × 15 cm) in an aquarium tank where males were reared. This procedure was conducted to prevent the resident female from immediately copulating with the introduced male. The introduced male occasionally approached the resident female, but we did not confirm any copulation between the introduced male and the resident female. After 10 min, we observed the focal male for an additional 10 min and again calculated the PS. We calculated a PS for each of the four males in a set by using another male from the set as the rival male. We presented a bright rival and a dull rival to each male, thus obtaining four data points (two for bright and two for dull focal males). Each male in a set was used just once as a focal male. When a specific type (bright or dull) of male was used as a focal male, the rival male was selected randomly from the two males of the other type. When the total preferred time (the time spent approaching either of the two females in the presence of a rival) was less than 5 min, the data for these males were removed from the experiments because such a male seemed to be sexually inactive. Each of the seven sets was examined for each population (14 measurements for each type).

### Ethical Note

Laboratory raised fish were reared in several mother tanks (60 × 30 cm and 36 cm high) at a density of approximately 100 individuals/tank and a temperature of 24–26 °C. The individuals used in the experiments were selected from the mother tanks and were reared in a separate tank under the same conditions as the

mother tanks. We photographed the fish as quickly as possible to prevent them from becoming weakened. The fish that completed the experimental procedure were immediately returned to the mother tanks.

### Statistics

A paired *t* test was used to compare the differences in the body sizes of the males. Similarly, the Wilcoxon signed-ranks test was used to compare the differences in the proportions of the orange area and the colour saturation of the two groups of males. Because we artificially chose the differences in the body sizes between the two females in a set, we confirmed that the size ratio between the two females among the sets did not differ significantly by using a generalized linear model (GLM) with a binomial distribution. In the behavioural experiments, changes in the PS after the addition of a rival were examined using a GLM with a binomial distribution. In the GLM, the changes following the addition of a rival were set as the dependent variable with the following three independent variables: (1) the appearance of the focal male (bright or dull), (2) whether the males are naturally or artificially coloured and (3) the set. We conducted two GLM analyses, one for bright rivals and one for dull rivals. 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 was absent. In both populations, most males of both types preferred the large female (64.3–92.9%). The preference time for the preferred female was significantly longer than that for the less preferred female (population H: for bright males:  $t_{12} = 5.625$ ,  $P < 0.001$ ; for dull males:  $t_{12} = 5.935$ ,  $P < 0.001$ ; population T: for bright males:  $t_{12} = 5.928$ ,  $P < 0.001$ ; for dull males:  $t_{12} = 8.898$ ,  $P < 0.001$ ). These results confirmed the results of a previous study (Dosen & Montgomerie, 2004). In addition, in both populations, the total preferred time did not differ between the bright and the dull males (for population H: paired *t* test:  $t_{12} = 0.281$ ,  $P = 0.783$ ; for population T: paired *t* test:  $t_{12} = 0.351$ ,  $P = 0.08$ ), suggesting that the dull males were not sexually inactive.

### 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:  $23.57 \pm 1.16$  mm; dull:  $23.17 \pm 0.92$ ; paired *t* test:  $t_{13} = 1.757$ ,  $P = 0.102$ ). The bright males had a larger orange spot ( $8.60 \pm 2.95\%$ ) on their sides than the dull males ( $2.27 \pm 1.07\%$ ; Wilcoxon signed-ranks test:  $Z = 3.148$ ,  $N = 14$ ,  $P = 0.0002$ ).

The GLM showed that when the rival was bright, the appearance of the focal male significantly affected his decision (GLM:  $z = 2.958$ ,  $N = 13$ ,  $P = 0.003$ ). The other two independent variables (whether

naturally or artificially coloured and the set) had no effect on the behavioural changes in the focal males. When the rival was dull, no independent variable showed a significant effect on the decision of the focal males. These results indicate that the bright males did not change their PS for the approached female regardless of the brightness or dullness of the rival male (Fig. 1a). In contrast, dull males significantly decreased their PS for the approached female regardless of the rival male's type (Fig. 1b). However, the dull males did not decrease their total preference activity after the rival's introduction (Table 2), meaning that a dull male changed his courtship partner from the preferred female to the less preferred female after the appearance of the rival male. These results indicate that dull males changed their preference in the presence of the rival male, whereas bright males did not.

### Mate Choice Behaviour in Artificially Manipulated Males

Third, the preference behaviour was compared between artificially induced bright and dull brothers. Body size at the beginning of the experiments did not differ between the two types (bright:  $21.95 \pm 2.31$  mm; dull:  $22.11 \pm 2.22$  mm; paired *t* test:  $t_{12} = -0.866$ ,  $P = 0.402$ ), suggesting that the diet had no effect on growth (for the nutritional content of both diets, see Appendix Table A1). The size of the orange spot also did not differ between the bright ( $5.21 \pm 0.83\%$ ) and dull males ( $5.49 \pm 0.72\%$ ; Wilcoxon signed-ranks test:  $Z = 1.300$ ,  $N = 14$ ,  $P = 0.194$ ), but the colour saturation of the spot differed significantly (bright:  $68.39 \pm 6.23\%$ ; dull:  $47.02 \pm 2.96\%$ ; Wilcoxon signed-ranks test:  $Z = 3.148$ ,  $N = 14$ ,  $P < 0.001$ ). For the results of the GLM analyses, see above. The bright males did not change their PS to the approached female after the introduction of either type of rival (bright or dull), but the dull males decreased their PS in the presence of the bright rival (Fig. 1c,d). These results indicate that the preference behaviours of genetically controlled brothers were affected by the relative saturation between themselves and the rival male.

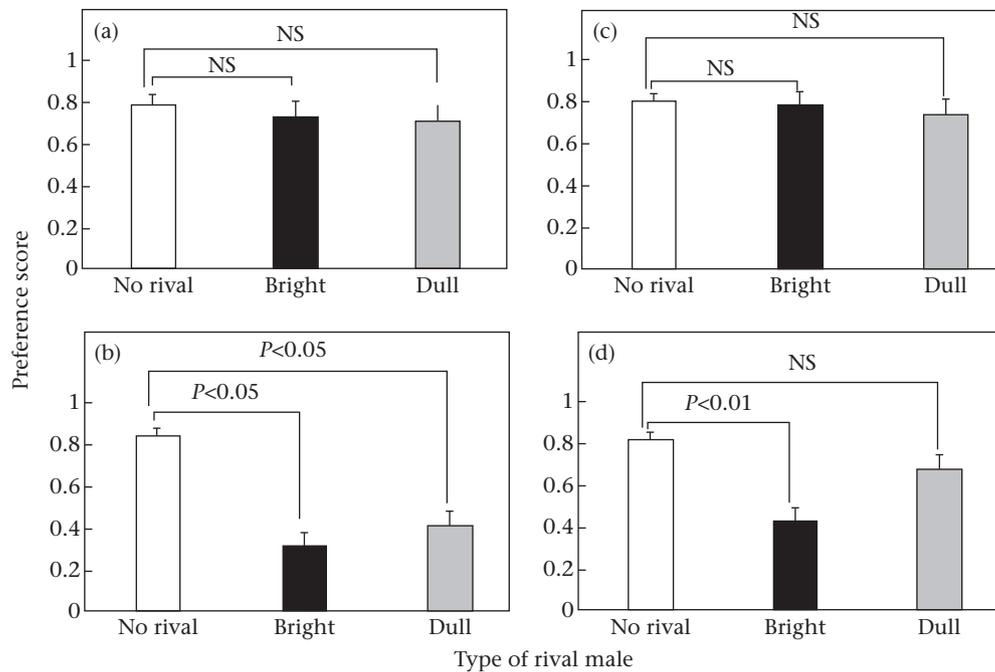
## DISCUSSION

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. A previous study found that approaching males abandon their courtship when rival males appeared in close proximity to the preferred female (Jeswiet et al., 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 the likelihood of acceptance by the female (Guevara-Fiore, Skinner, & Watt, 2009; Guevara-Fiore, Stapley, Krause, Ramnarine, & Watt, 2010; Herdman et al., 2004). The probability of accepting an approaching male is high for solitary females but low for attended females. Males should thus change their courtship from attended to solitary females to increase their probability of acceptance (Auld, Leswiet & Godin, 2015; Jeswiet et al., 2011; Lane et al., 2015).

**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 (mean±SD)	Preference time to the less preferred female (mean±SD)	Total preference time (mean±SD)
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



**Figure 1.** Preference scores (the proportion of time a male spent associating with one female out of the total time spent associating with either of the two females) of (a) naturally bright (=large orange spot) males, (b) naturally dull (=small orange spot) males, (c) induced bright (=high colour-saturated spot) males and (d) induced dull (=low colour-saturated spot) males in the presence of two different types of rivals. Bars and whiskers show the mean + SE.

**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 (s) (Mean±SD)	df	t	P
Population H	Bright	Bright	431.00±84.95	26	0.486	0.631
	Bright	Dull	415.86±79.98			
	Dull	Bright	445.07±90.90			
	Dull	Dull	433.14±96.56			
Population T	Bright	Bright	463.29±83.30	26	0.802	0.430
	Bright	Dull	439.64±72.22			
	Dull	Bright	488.14±63.42			
	Dull	Dull	490.36±64.18			

However, as female guppies prefer males with large and bright orange spots on their sides (Karino & Matsunaga, 2002; Karino, Shimada, Kudo, & Sato, 2010; Karino & Urano, 2008; Kodric-Brown, 1985), an approaching male and a rival may differ in quality. 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 of male guppies are finely tuned to maximize their probability of acceptance.

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

Artificially induced dull males changed their behaviour, but their bright brothers did not. These results indicate that decision

making is not genetically determined, that is, males changed their behaviour in response to their appearance or condition. A previous study that induced artificial dietary differences in brightness between brothers also showed that diet did not affect the male's size, the location or size of the red and orange pigment spots, or the intensity of courtship behaviour (Kodric-Brown, 1989). Also, in our study, the body size and size of the orange spots did not differ between the induced bright and dull brothers. In addition, neither type of male sibling differed in total preference time (Table 1). The dull males did not become inactive to approached females; however, they changed their partner from a preferred female with a rival to the other female (for the males in population T; see Table 1). The nutritional content of both diets is similar other than that Tetramin is carotenoid enhanced (see Appendix Table A1). Thus, the hypothesis that dull males are sexually inactive due to inferior body condition seems unlikely. However, our experiments could not completely remove the effects of the different diets on body condition. Further studies are needed with more careful manipulations (e.g. colour differences between bright and dull males induced by using the same diet without one group receiving additional carotenoids).

The males in our study made decisions according to their relative attractiveness compared to the rival. The observed withdrawal

from the female is adaptive, as such males do not pay additional costs for a fruitless approach. Therefore, a male guppy chooses a female that offers a high probability of acceptance. How does a male know his own appearance? Several hypotheses have been suggested concerning this issue: a male knows his appearance from (1) the responses of the focal females, (2) his past experiences and (3) the responses of rival males. Although some of these hypotheses have been tested (De Gasperin & Garcia, 2014; Gonzalez-Zuarth, Vallarino, & Garcia, 2011), more studies are needed to answer this question.

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, & Goldstein, 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 for common male phenotypes (Olendorf et al., 2006), our results will shed light on the mechanism for the maintenance of male ornamentation.

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## Appendix

**Table A1**  
Nutritional contents of the two diets

	Tetramin	CE-2
Crude protein (%)	45	25.13
Crude fat (%)	12	4.92
Crude fibre (%)	3	4.42
Moisture (%)	9	8.83
Phosphorus (%)	1.2	No data
Niacin (mg/100 g)	55	18.1
Choline (mg/100 g)	200	190
Inositol (mg/100 g)	100	613
l-carnitine (mg/100 g)	12.3	No data
Vitamin C (mg/100 g)	42.5	25
Canthaxanthin (carotenoids)	Included (amount is unknown)	Not included