

## **Selection for relative brain size affects context-dependent male preferences, but not discrimination, of female body size in guppies**

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Keywords: Guppy, *Poecilia reticulata*, decision-making, context-dependent choice

## ABSTRACT

Understanding what drives animal decisions is fundamental in evolutionary biology, and mate choice decisions are arguably some of the most important decisions in any individual's life. As cognitive ability can impact decision-making, elucidating the link between mate choice and cognitive ability is necessary to fully understand mate choice. To experimentally study this link, we used guppies (*Poecilia reticulata*) artificially selected for divergence in relative brain size and with previously demonstrated differences in cognitive ability. A previous test in our female guppy selection lines demonstrated the impact of brain size and cognitive ability on information processing during female mate choice decisions. Here we evaluated the effect of brain size and cognitive ability on male mate choice decisions. Specifically, we investigated the preferences of large-brained, small-brained, and non-selected guppy males for female body size, a key indicator of female fecundity in this species. For this, male preferences were quantified in dichotomous choice tests when presented to dyads of females with small, medium and large body size differences. All types of males showed preference for larger females but no effect of brain size was found in the ability to discriminate between differently sized females. However, we found that non-selected and large-brained males, but not small-brained males, showed context-dependent preferences for larger females depending on the difference in female size. Our results have two important implications. First, they provide further evidence that male mate choice occurs also in a species in which secondary sexual ornamentation occurs only in males. Second, they show that brain size and cognitive ability have important effects on individual variation in mating preferences and sexually selected traits.

## INTRODUCTION

Animal decisions are central in the study of animal behavior. However, little is known about the underlying mechanisms of animal decision-making. Only recently, the theoretical framework of human decision-making has been suggested as a model for animal behavior research, defining the decision-making process as how individuals use information to judge a situation and behave in a particular way (Mendelson et al., 2016). This framework identifies *preference*, ranking certain options over others, and *choice*, selecting an option given a set of alternative options, as the two major components of animal decisions (Mendelson et al., 2016).

Despite the important conceptual difference between the two components of decision-making (preference and choice), dissimilarities between the two are seldom recognized in classic sexual selection theory (Kirkpatrick et al., 2006). Commonly, selective pressures are believed to drive positive reinforcement between preferences for a particular trait and choice for mates bearing those traits (Andersson, 1994). However, current sexual selection theory acknowledges that mate choice can be context-dependent and therefore does not necessarily correlate with absolute preference functions (Jennions and Petrie, 1997; Widemo and Sæther, 1999). Indeed, empirical studies across different taxa have demonstrated that for instance social context can shift mate preference and mate choice (Royle et al., 2008; Reaney, 2009; Lea and Ryan, 2015; Locatello et al., 2015; Griggio et al., 2016). Intrinsic condition and acquired experience have been further suggested to be important factors shifting innate preference functions and generating variability in mate choice (Ryan et al., 2007; Verzijden et al., 2012; Gilman and Kozak, 2015). Cognitive abilities are central for decision-making (Shettleworth, 2010), and likewise an important modulator of mating decisions (Akre and Johnsen, 2014; Cummings and Ramsey, 2015; Corral-López et al., 2017a). Yet, the impact of cognitive ability on mating decisions has mostly been assessed through studies of sensory perception and their effects on information acquiring processes (Kirkpatrick et al., 2006; Ryan et al., 2007; Ryan et al., 2009)

The influence of cognitive ability is seldom considered in sexual selection theory, as it is often assumed that selective pressures lead to mating with individuals who maximize fitness (Bateson and Healy, 2005; Castellano et al., 2012). However, cognitive ability likely plays a fundamental role in many aspects of sexual selection, as the ability to acquire information, and make judgments based on such information can influence the outcome of an individual's

behavior prior to, during and after mating. Yet, our understanding of the proximate factors that affect animal decision-making during mate choice is still incomplete. Based on the economic concept of rationality, where decision-making is based on maximizing benefits (Hurley and Nudds, 2006), it has been suggested that animals are adapted to perform optimal mate choices. Such rationality in mate choice implies that individuals use absolute preference decision rules to maximize fitness in their choice based on current information. Recent studies have shown optimal choice in diverse taxa such as cichlid fish (Dechaume-Moncharmont et al., 2013), fruit flies (Arbuthnott et al., 2017) and bats (Hemingway et al., 2017). However, these patterns remain inconsistent in the literature since irrational mate choice has also been reported (Royle et al., 2008; Reaney, 2009; Lea and Ryan, 2015; Locatello et al., 2015; Griggio et al., 2016). An alternative explanation to these inconsistencies is that rationality is context-dependent. Individual optima may vary and can lead to individual variation in mate choice (Fawcett et al. 2014). Indeed, studies applying theoretical modelling demonstrate an evolutionary advantage of not using absolute preference rules when incorporating environmental heterogeneity to animal decisions (Trimmer, 2013; McNamara et al., 2014). In line with this, constraints in cognitive resources can likewise affect mate choice decisions (Sasaki and Pratt, 2011). Development and maintenance of neural tissue is energetically demanding (Aiello and Wheeler, 1995; Isler and van Schaik, 2009; Kotrschal et al., 2013; Tsuboi et al., 2015). Hence, simplification of information processing resulting in lower neural investment can be beneficial from an evolutionary point of view. For instance, instead of the use of absolute functions, proportional processing of stimuli mainly influence decision-making in animals (Bateson and Healy, 2005; Akre and Johnsen, 2014), a neuronal process previously demonstrated to require less neuronal coding (Dehaene, 2003; Nieder and Miller, 2003).

The brain, as the central organ in the neural network, is essential in animal decision-making. Indeed, brain size has been shown to impact cognitive ability (Kotrschal et al., 2013, 2015; Benson-Amram et al., 2016), which in turn might influence animal decisions related to mate choice and mate preferences (Ryan et al., 2007). This view has recently been empirically demonstrated in female guppies artificially selected for relative brain size. Cognitive differences between large-brained and small-brained females (Kotrschal et al., 2013, Buechel et al., 2018), impact the judgment of attractiveness of male guppies (Corral-López et al., 2017a). Yet, better mate quality assessment should not only present an advantage for female choice, as variation in

female quality could potentially also drive the evolution of male preferences and their choices for specific traits (Edward and Chapman, 2011). Female body size is commonly positively correlated with female fecundity (Houde, 1997; Edward and Chapman, 2011). Hence, under classic sexual selection theory it would be expected that males develop preferences for larger females when such preferences yield fitness benefits. Previous studies documented a clear relationship between female fecundity and female size in the guppy (Reznick, 1983). In situations with no male-male competition, male guppies prefer to mate with larger females (Dosen and Montgomerie, 2004; Jeswiet et al., 2012; Auld and Godin, 2015; Auld et al., 2016). Yet, in previous studies such male preferences are often only observed when the difference in body length between the females presented in previous tests exceeded certain thresholds, commonly around 10 % difference in body size (Dosen and Montgomerie, 2004; Jeswiet and Godin, 2011). It is thus evident that better judgment of mate quality can play a major role in maximizing reproductive fitness not only in terms of female choice, but also for male mate choice in this species. Hence, male guppies artificially selected for divergence in relative brain size present a suitable model system to study the impact of brain size and cognitive ability in adaptive mating decisions.

In this study, we expose large-brained, small-brained, and non-selected males to three different female pairs with small, medium and large differences in body size. Previous tests in these fish showed that large-brained males outperformed small-brained males in cognitive tests (Kotrschal et al., 2015), but large-brained and small-brained males did not show differences in body condition or visual abilities (Kotrschal et al., 2013; Corral-López et al. 2017b). Likewise, cognitive differences between large-brained and small-brained males are unlikely attributable to hitchhiking of deleterious alleles in the selection process, as several assays have shown that the lines do not differ in a range of physiological and behavioral traits (Kotrschal et al., 2013; Kotrschal et al., 2014; Corral-López et al., 2015); the small-brained lines have even presented some immunological and life-history advantages (Kotrschal et al., 2013; Kotrschal et al., 2016). Here we assess innate preferences for larger females, as experimental males did not have previous mating experiences in our set-up. Our experimental approach tests the role of brain size and cognitive ability in sexual preferences of male guppies for female body size. We performed dichotomous choice tests with no male-male competition based only on visual cues and in male guppies of similar age and experience. Given the previous validation of the correlation between

preference and choice in a similar setup for this species (Houde, 1997, Jeswiet and Godin, 2011), our study allows us to focus on evaluating the ability of males to judge female quality to make adaptive mating decisions, i.e. preferring a larger female. In particular, if relative brain size affects the ability of male guppies to judge between differences in female body size, we predict stronger preferences for larger females in large-brained males regardless of the size difference between the females presented in dichotomous choice tests. In addition, if judgment of difference in female body size becomes more difficult when the difference is smaller than 10%, we predict that only large-brained males will prefer larger females when presented to small size differences between female pairs in our test.

## MATERIALS AND METHODS

**Study system.** We studied the preference for female body size in male guppies from laboratory-reared descendants of Trinidadian guppies from high predation areas of the Quare River. We used non-selected wild-type female and male guppies from this laboratory population, together with males artificially selected for small and large relative brain. Briefly, the artificial selection experiment was based on indirect selection for parental brain weight data corrected for body size, which was used to generate replicated lines with large and small relative brain size (three replicates for large- and three replicates for small-brained, six populations in total). See Kotrschal et al. (2013) for full details on the selection experiment. These selection lines have up to 13.6 % difference in the fourth generation (Kotrschal et al., 2015). After the fourth generation, 30 non-related males and females from each population were paired to generate a fifth generation of brain size selected offspring. All offspring were removed from their parental tanks after birth. Males were isolated from females and grouped in 12 liter tanks before they were sexually mature. We allowed visual contact between tanks to avoid social stress from isolation. However, males did not have visual contact with any female prior to the experiment. All males were approximately 6 months old when tested and had been kept in similar conditions in the laboratory. The laboratory was maintained at 26 °C with a 12:12 light:dark schedule, which resulted in 25 °C water temperature. Fish were fed an alternating daily diet of flake food and live artemia.

**Selection of female pairs.** To form female pairs with differences in body size, we measured standard body length in 60 lab-descendant wild-type female guppies that ranged between 7 and 9 months of age. All females had no prior experience with males, as they had been isolated from males prior to sexual maturity. Based on these measurements, we used 18 females to create 3 female pairs for each of the three experimental treatments: i) large difference in body size (7 mm; 25 % difference), ii) medium difference in body size (3.4 mm; 12.5 % difference), and iii) small difference in body size (1.6 mm; 6.25 % difference). To avoid potential differences in motivation to mate in the males across the three treatments and because absolute female size in the test can drive preferences for larger females (Dosen and Montgomerie, 2004), the combined value of body length of the two females was kept equal to the sum of 50 mm body length in every pair (large difference: 28.5 mm versus 21.5 mm; medium difference: 26.7 mm versus 23.3 mm; small difference: 25.8 mm versus 24.2 mm).

**Preference test.** Using a dichotomous choice test, we assessed the preference for larger females of male guppies when exposed to the three different treatment conditions. Our set-up consisted of eight plain glass tanks of 42x20x20 cm where males were allowed to observe both females in adjoining plain glass tanks of 11x10x20 cm during 10 minutes. Following the method used in Corral-López et al. (2017a), the male experimental tank was divided into three zones to determine female position: (i) left choice zone, the area adjacent to the left male tank up to a maximum distance of 10 cm from it; (ii) right choice zone, the area adjacent to the right male tank up to a maximum distance of 10 cm from it; and (iii) no choice zone, the area between the left and right choice zones and all areas further away than 10 cm from the female tanks (Figure S1). Females did not see each other during trials to avoid female-female competition effects. During 12 consecutive days, we measured for every male the time spent associating with the larger and the smaller female when exposed to a large, medium and small difference in female body size. We measured eight males each day for a total of 24 trials daily. Males and female pairs were placed in their respective experimental tank 24 hours prior to the test to allow for acclimation. The right and left position of the larger female in each trial, and the daily order of presentation of the female difference treatment were randomized. Three large-brained, three small-brained, and two non-selected males were tested every day. The impossibility to test nine

individuals daily in our experimental set-up resulted in a lower sample size of non-selected males tested. In total, we used 22 non-selected males, and 36 large-brained and 36 small-brained males from the fifth generation of the brain size selection lines (12 individuals from each of the three up- and down-selected lines). We selected this methodology to maximize the number of large-brained and small-brained males tested, and to ensure a balanced design between non-selected and brain size treatments in the female pairs that they interacted with, and in the order of presentation of the female difference treatments. Every trial was broadcasted live using a Logitech HD webcam C615 from a top position and viewed from a distance on a laptop to avoid disturbance. Following Corral-López et al. (2017a), the position of the male was scored by a single observer using the live observation mode in BORIS v 2.72 (Friard and Gamba, 2016). Quantification of behaviors was performed blind to the treatment since only running numbers identified the males. A preference ratio was obtained as the difference in time spent with each female, standardized by the total amount of time in any of the choice areas. Trials in which males did not move in the experimental tank (n=42) or in which males did not visit both female choice areas (n=19) were excluded from the analyses (Houde 1997). Final analyses included a total of 221 trials corresponding to 19 non-selected, 26 small-brained and 29 large-brained males.

Every day, after trials were completed, we measured the body size (standard length) of the tested male guppies using a vernier caliper to the nearest 0.1 mm. We found no differences in body size between large-brained and small-brained males, but non-selected males were slightly larger than selected males (Average body size (mm)  $\pm$  SE; small-brained:  $17.95 \pm 0.10$ ; large-brained:  $17.78 \pm 0.10$ ; non-selected:  $18.26 \pm 0.12$ ,  $t_{\text{small-brained vs large-brained}} = 1.17$ ,  $p = 0.244$ ;  $t_{\text{small-brained vs non-selected}} = -2.96$ ,  $p = 0.065$ ;  $t_{\text{large-brained vs non-selected}} = -1.85$ ,  $p = 0.003$ ). In order to control for the potential effect of body size differences between treatments in the time associating with larger females, we statistically controlled for male body size in our statistical analyses (see below).

**Statistical analyses.** We assessed potential behavioral differences between large-brained, small-brained and non-selected males in our study using a Linear Mixed Model (LMM) approach. To study male mating preferences for larger females, we used a LMM that included the preference ratio as the dependent variable. Brain size and the difference in size between females were used

as fixed effects. The full model included two covariates, male body size and the number of tests previously performed by the same male. Likewise, all interactions between fixed effects and the female pair difference covariate were included in the full model. In addition, the full model included female pair identity and male identity as random factors, as well as a random intercept for each replicate selection line and a random slope for brain size within each replicate. We performed a backward elimination of non-significant fixed factors of LMM by means of Satterthwaite's approximation for degrees of freedom (Kuznetsova et al., 2015; Appendix 1). Model diagnostics showed that residual distributions were roughly normal with no signs of heteroscedasticity. All analyses were performed in R version 3.3 (R Core Team, 2015). To evaluate whether large-brained, small-brained and non-selected males independently preferred larger females regardless of female body size differences, we obtained post-hoc contrasts of the best fitted preference model using the lsmeans package (Lenth et al., 2016). To reduce Type I error, post-hoc analyses included multiple testing correction applying the false discovery rate procedure (Benjamini and Hochberg, 1995).

To assess whether large brained, small-brained, and non-selected males differed in their ability to judge female body size differences, we used three independent LMM's for data on small, medium and large body size differences following the abovementioned procedure. Full models included brain size as a fixed effect and male body size and the number of tests previously performed by the same male as covariates. We included female pair identity as a random factor, as well as a random intercept for each replicate selection line and a random slope for brain size within each replicate. This statistical procedure was also followed to analyze five other behavioral patterns of the males during the test: side bias, time of no choice, total movement, number of visits and visit ratio (see Appendix 2).

## RESULTS

**Preference for larger females.** The quantification of male preference in a standard dichotomous choice set-up regardless of the percentage of difference between females showed that small-brained, large-brained and non-selected guppy males showed a significant preference for larger females (Means  $\pm$  SE: small-brained:  $0.20 \pm 0.07$ ,  $t = 2.82$ ,  $p = 0.02$ ; large-brained:  $0.15 \pm 0.07$ ,  $t = 2.22$ ,  $p = 0.04$ ; non-selected:  $0.25 \pm 0.08$ ,  $t = 3.14$ ,  $p = 0.01$ ; Figure 1). No significant

differences in the preference for larger females were observed between small-brained, large-brained and non-selected males (LMM<sub>preference: brain size</sub>:  $F_{2, 68.53} = 0.60$ ,  $p = 0.552$ ). Males reduced their preference for larger females in their second test. However, the number of tests performed did not affect preference across the different male types (Means  $\pm$  SE: first test:  $0.27 \pm 0.08$ ,  $t = 2.82$ ; second test:  $0.06 \pm 0.07$ ; third test:  $0.25 \pm 0.08$ ; LMM<sub>preference: test number \* brain size</sub>:  $F_{4, 137.7} = 1.38$ ,  $p = 0.248$ ).

We did not find any effect of brain size in the preference for larger females when the body size difference between females presented in the test was small (Figure 1B; LMM<sub>preference: brain size</sub>:  $F_{2, 72} = 1.89$ ,  $p = 0.317$ ). We also did not find any differences in preference for larger females between large-brained, small-brained and non-selected males for any of the female body size difference treatments (Figure 1B; medium difference: LMM<sub>preference: brain size</sub>:  $F_{2, 72} = 0.97$ ,  $p = 0.495$ ; large difference: LMM<sub>preference: brain size</sub>:  $F_{2, 72} = 0.65$ ,  $p = 0.522$ ). Likewise, we found no effect of the percentage of difference between the females presented in the dichotomous choice on the preference for larger females (LMM<sub>preference: female pair difference</sub>:  $F_{2, 5.91} = 1.25$ ,  $p = 0.352$ ). However, analyses of the preference for larger females showed a significant interaction between the percentage of difference between the females and male brain size (LMM<sub>preference: female pair difference \* brain size</sub>:  $F_{4, 138.70} = 2.53$ ,  $p = 0.043$ ). We observed large individual variation in context-dependent preferences for all male groups (see Figure S2). However, visual inspection of the results suggests that the significant interaction effect was due to two factors. First, average preferences in non-selected males for larger females increased when the difference between females presented was intermediate (12.5%). Second, average preferences for larger females in large-brained males increased as the difference between females increased, whereas the level of preference for larger females in small-brained males remained in average similar across female size difference treatments (Figure 1B).

**Behavioral patterns during preference test.** Analyses of how individuals behaved during the preference tests showed no differences in how males from different brain size selection regimes appraised information of the females offered (Appendix 2). In particular, we found no significant side bias in preference between the non-selected, large-brained, and small-brained fish (LMM<sub>side preference: brain size</sub>:  $F_{2, 2.76} = 0.21$ ,  $p = 0.825$ ), or in their motivation to mate, as there were no

differences between brain size treatments in the time spent outside the choice areas (LMM<sub>time no choice: brain size</sub>:  $F_{2, 3.46} = 0.53$ ,  $p = 0.631$ ). We did find a significant increase in time outside the choice areas in the second and third test on each male, although this pattern was not influenced by brain size of the males and on average males did not spend more than 16% of the time outside the left or right choice area (Means (sec)  $\pm$  SE: first test:  $70.7 \pm 13.3$ ; second test:  $94.2 \pm 13.4$ ; third test:  $99.0 \pm 13.3$ ; LMM<sub>time no choice: test number \* brain size</sub>:  $F_{4, 128.6} = 0.95$ ,  $p = 0.433$ ).

Furthermore, we found no evidence that non-selected, large-brained and small-brained males gather more information from one particular type of female since the ratio of visits between choice areas was not different between brain sizes (LMM<sub>visits ratio: brain size</sub>:  $F_{2, 3.11} = 0.15$ ,  $p = 0.870$ ). The difference in size of the females offered to the males did likewise not influence any of the abovementioned male behaviors (Appendix 2). On the contrary, female body size difference of the dyad influenced two activity proxy measurements, the total number of crossings between defined choice and no choice areas (total movement), and the total number of visits that males paid to both female choice areas (total visits; Appendix 2). In particular, we found that males paid significantly more visits to both female choice areas when the difference between females was small (LMM<sub>visits: female difference</sub>:  $F_{2, 3.53} = 8.00$ ,  $p = 0.048$ ; Figure 2).

## DISCUSSION

Our results show that brain size does not affect the discrimination of female body size in male guppies when associating with differently sized females using visual cues. In particular, no effect of brain size was observed in the preference for larger females when female body size differences presented were small (6.25%). Analyses combining the results for the different dyads of females while controlling for repeated measurements of each individual showed that all male groups, large-brained, small-brained and non-selected males, presented an overall preference for larger females. However, unlike small-brained males, large-brained males, and to some extent also non-selected males, biased their preference towards larger females as the difference in size between the females increased. Therefore, our results suggest that brain size and cognitive ability shape context-dependent preferences in male mate choice.

The observed overall preferences for larger females (when combining all results) are in concordance with what has been found in previous studies of visual male mate choice in guppies (Dosen and Montgomerie, 2004; Herdman et al., 2004; Godin and Auld, 2013; Auld and Godin, 2015; Auld et al., 2016). Furthermore, our results are consistent with these studies in that we find large individual variation in male preference for differently sized females. Female body size is a cue that conveys multiple information in this species; in addition to being more fecund, larger female guppies are often inseminated by more males, increasing the risk for sperm competition and reducing fitness benefits for individual males (Herdman et al., 2004). Yet, in our study, we tested males in a setup without male-male competition and all males experienced similar social environments, with no previous experience with females. Under such circumstances the overall preferences observed for larger females in all male groups should provide important benefits to maximize reproductive success.

Which information processing rules individuals follow to develop mating preferences remains a key question. Following the conceptual framework of animal decision-making (Mendelson et al., 2016), our results suggest that brain size does not affect judgment based on the ability of males to discriminate between female body size. These results are in agreement with the previously demonstrated similar ability of large-brained and small-brained males to resolve spatial detail (Corral-López et al., 2017b). Taken together, these findings suggest that discriminating larger females in this species is a relatively easy task for which demanding cognitive processes might not be required, as previously demonstrated for proportional processing rules (Akre et al., 2011; Akre and Johnsen, 2014). Yet, we observe differences between large-brained, small-brained and non-selected individuals in their level of preference when exposed to small, medium and large female body length differences. Surprisingly, it is in large-brained males where we find lower levels of preference for larger females when the task to discriminate between females was more difficult (small difference: 6.25%). However, preference levels of large-brained males in any of the female body size difference treatments do not differ from those of the small-brained and non-selected males. On the contrary, unlike small-brained males, large-brained males seem to bias their time more towards larger females as we increased the difference in female body size in the dyads. In our study, only 3 dyads of females were offered in binary choices for each treatment. Hence, we cannot rule out that preferences for particular females regardless of their body size might explain biases on the observed lack of

preferences for larger females in certain treatments. Another potential explanation to this pattern might be that large-brained males relied more on multiple traits to evaluate the quality of the females offered. Although females shown to males in this study were of equal age and raised in similar conditions to minimize differences among them (except for body size differences), it is possible that uncontrolled traits in our experiment, such as female condition and female response towards males, interacted with body size to shape male preferences. In this scenario, our results would suggest that while preferences for larger females of small-brained males might be based on a single trait (female body size), large-brained males seemed to incorporate information from these other traits to their mating decisions but the relative importance of other traits are much lower when the difference in size becomes larger.

Even though our test was not designed to test for evaluation and decision rules (Bateson and Healy, 2005; Kirkpatrick et al., 2006) in the preference for larger females, our results might be explained by differences in decision rules between small-brained and large-brained males. The fact that large-brained males associated most with the largest of all female stimuli offered across female dyads (i.e. with the largest female of 28.5 mm SL) might be explained by large-brained males being more sensitive when making their decisions based on the absolute size of the larger females. Indeed, absolute decision rules have previously been suggested to be more cognitively demanding than simple proportional processing based on comparative evaluation between stimuli (Bateson and Healy, 2005). Our results suggest that incorporating the role of cognitive ability in future studies of mating decision rules might be important to solve inconsistencies in recent literature on rational and irrational mating decisions (e.g. Lea and Ryan, 2015; Hemingway et al., 2017).

Non-selected males likewise are more flexible in their preferences towards larger females when presented with an intermediate difference in body size between them. But unlike in large-brained males, such preference for larger females was not observed in non-selected males for the largest of all female stimuli. Previous studies have shown that male guppies decrease their preferences toward larger females in situations of higher competition risk (Jeswiet et al., 2012; Auld and Godin, 2015; Auld et al., 2016). Our experimental design did not incorporate such environmental cues. Hence innate behavioral differences in mating preferences between selected and non-selected males potentially drove the strong reduction in preference for the largest of all

female stimuli in the non-selected males. Often, the largest females attract the highest number of mates in the guppy (Houde, 1997; Dosen and Montgomerie, 2004) and postcopulatory competition might decrease fitness benefits of preferring such females (Herdman et al., 2004). How such social cues affect context-dependence preferences in our selection lines remains to be investigated. However, the similarities that we observe between large-brained and non-selected males in relation to small-brained-males point mainly towards a decrease in the cognitive ability of small-brained males rather than an increase in cognitive ability in the large-brained lines. Interestingly, this finding match our previous results on female choice where small-brained females decreased their preference towards colorful males while we found no increase in the preference of large-brained females in relation to non-selected females (Corral-López et al., 2017a).

Are intrinsic differences between the brain size selection regimes driving our results? Previous assays in these selection lines showed physiological, personality and behavioral differences between large-brained and small-brained individuals (Kotrschal et al., 2013; Kotrschal et al., 2014; van der Bijl et al., 2015). In the present study, such differences are unlikely to alter how much information males could gather on the presented females. This is so because the time spent out of the choice areas and the ratio of visits between females did not differ between large-brained, small-brained and non-selected males. The fact that we did not find differences in the time spent out of the choice areas also suggests that there are no differences in motivation between brain size selection regimes. We however found a significant effect of the female pair offered on the activity of the males. But this effect was not affected by brain size selection. Hence, we find it unlikely that personality differences drove the preference patterns, as male activity levels varied mostly depending on the female pair offered in the tests. As such, we think that these findings support the possibility that preference patterns were driven by mate quality assessment rather than by male ability to discriminate female body size across dyads.

The observed context-dependent mate choice for female size in large-brained males could have important evolutionary implications and potentially counteract the costs of evolving a larger brain under certain ecological circumstances. Previous studies have demonstrated the influence of the quality of potential mates encountered in shaping flexible mating decisions (e.g. Kvarnemo and Simmons, 1999; Shine et al., 2006). In addition, the ability to adapt to

heterogeneous environments have been suggested as a key benefit of evolving a larger brain (Ratcliffe et al., 2006; Sol et al., 2008; Overington et al., 2009). In line with this, we speculate that our results provide experimental evidence for that lower investment into the brain might be sufficient under more homogeneous social and physical environments where consistent preferences for higher quality mates might be adequate. Indeed, previous studies found correlations between neural substrate development and increased social complexity in voles (Fowler et al., 2002), and birds (e. g Adar et al., 2008; see review by Gonda et al., 2013). In guppies, similar patterns might be expected as decreases in neural development have been found in captive-reared individuals, arguably a more homogeneous environment than natural habitats (Burns and Rodd, 2008; Burns et al., 2009). Likewise, our results stress the importance of incorporating intrinsic factors, such as cognitive ability, in context-dependent analyses of mate choice, as well as incorporating such intrinsic factors when evaluating optimal decision-making in mate choice tests (Fawcett et al., 2014). Moreover, our results complement previous findings of how cognitive ability affects female preferences when choosing between colorful and non-colorful males (Corral-López et al., 2017a). The combination of these findings highlights the role, *in both sexes*, that brain size and cognitive ability can play in the puzzling variation in mating preferences and sexually selected traits observed across individuals.

## ACKNOWLEDGEMENTS

We thank W. van der Bijl for help with graphical output, S. Buechel for laboratory work on the brain size selection lines, and A. Rennie for help with animal husbandry. We are grateful to two anonymous reviewers for their insightful comments on previous versions of the manuscript, and to R. Montgomerie and J-G.J. Godin for providing information on previous findings on male mate choice tests in the guppy.

## FUNDING

This work was funded by grants from the Swedish Research Council (2012-03624 and 2016-03435) and from the Knut and Alice Wallenberg Foundation (102 2013.0072) to N.K.

## COMPETING INTERESTS

The experiment complies with Swedish law and was performed in accordance with ethical applications approved by the Stockholm Ethical Board (Dnr: N173/13 and 223/15). No competing interests declared.

## DATA AVAILABILITY

Dataset is deposited in dryad repository (link pending acceptance)

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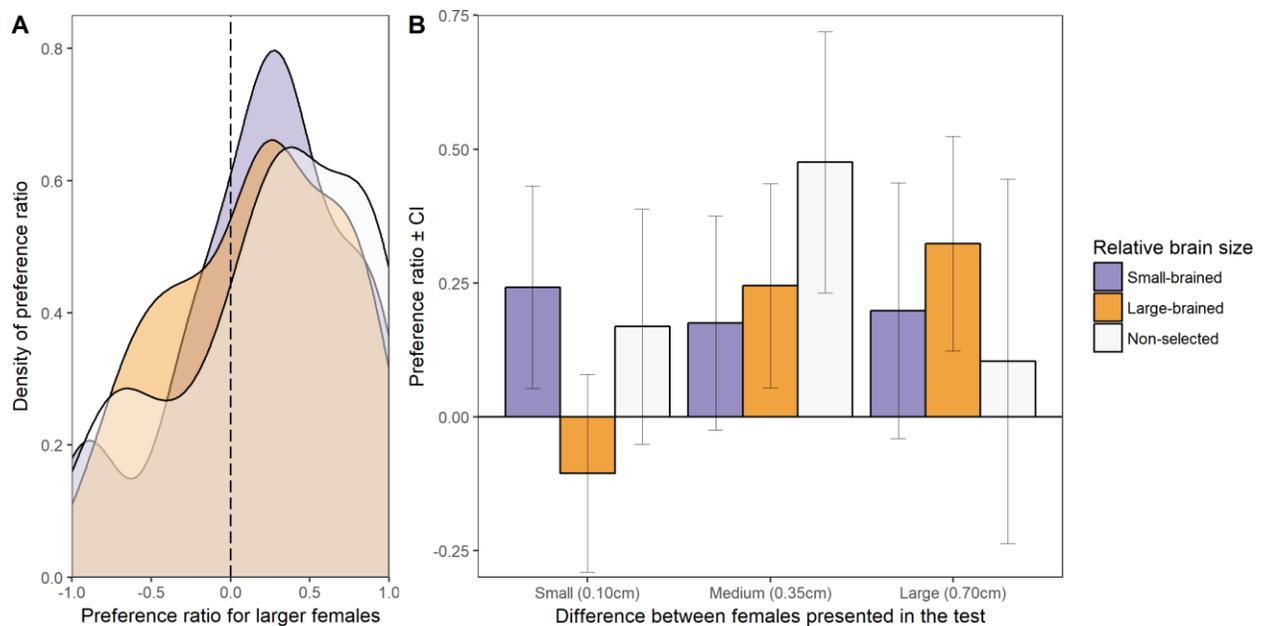
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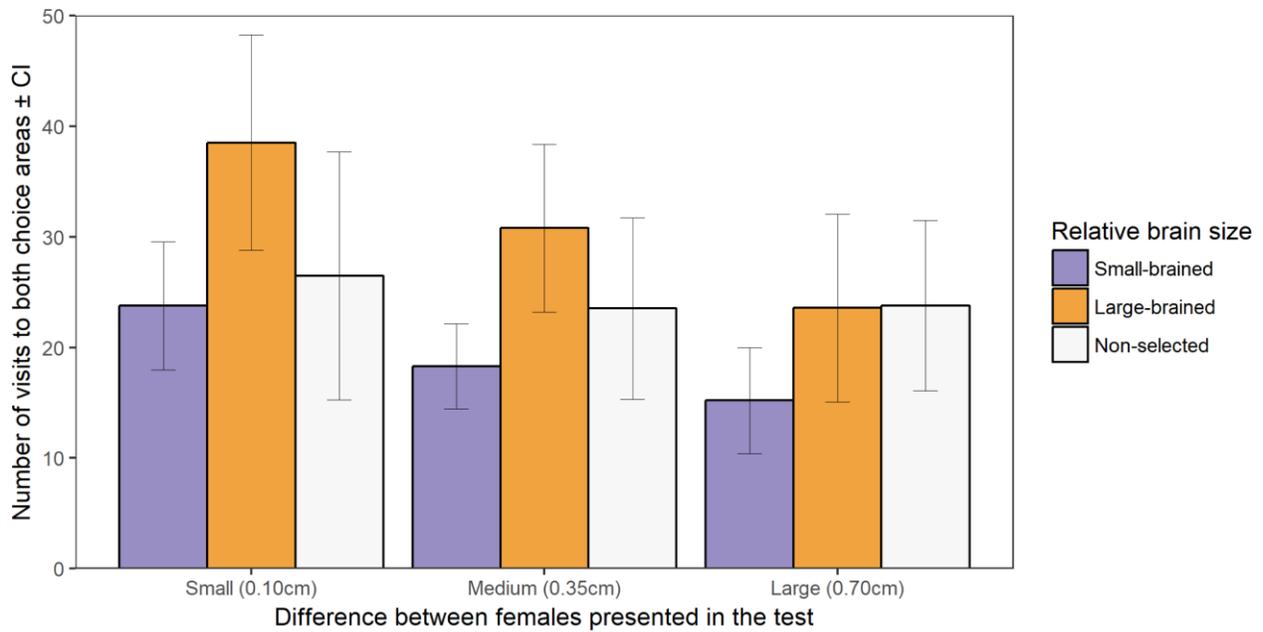
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## Figures



**Figure 1. Male preference for larger female guppies.** Preference ratio for larger females was calculated as the difference in time spent with each female, divided by the total amount of time in any of the choice areas. Preference ratio take values between -1 (all time spent with the smaller female) and 1 (all time spent with the larger female). **(A)** Kernel density plot showing the distribution of preference ratio for larger females of large-brained, small-brained and non-selected males regardless of female body length differences of dyads presented in dichotomous choice tests. **(B)** Mean values and confidence intervals of preference for larger females in large-brained, small-brained and non-selected males when presented with dyads of females that had small (6.25%), medium (12.5%) and large (25%) differences in body size. There were no differences between large-brained, small-brained and non-selected males in preference for larger females ( $p = 0.552$ ). However, we found a significant interaction between relative brain size and female pair difference treatments ( $p = 0.043$ ).



**Figure 2. Number of visits to choice areas during dichotomous choice preference tests.**

Average number of visits and confidence intervals of large-brained, small-brained and non-selected male guppies in dichotomous choice tests evaluating preferences for larger females.

There was no significant differences between brain size selection regimes in number of visits to both choice areas ( $p = 0.273$ ). Overall, males performed more visits when presented with female dyads with a smaller difference ( $p = 0.048$ ). Data was log-transformed prior to statistical analyses.

## APPENDICES

### Appendix 1

To study the effect of female difference in size and relative brain size in male mate choice, we used a LMM with preference for larger females as the dependent variable (pref). Full models included the difference in female size in the test (femalepairdiff) and brain size selection regime (bs) as fixed effects. We used the number of tests previously performed by the male (test number) and male body size (size) as covariates in the models. The full model included all interactions between fixed effects and the order of presentation. In addition, all models included the identity of the male and the female pair used as random factors. In addition, we included a random intercept for each replicate selection line and a random slope for brain size within each replicate (Table A1).

**Table A1.** Backward elimination procedure for the full LMM with the preference ratio for larger females as the dependent variable. Asterisks indicate significant effects.

Fixed effect	SumSq	NumDF	DenDF	F value	Stepwise elimination	Pr(>F)
size	0.069	1	73.07	0.271	1	0.604
femalepairdiff:bs:test number	1.949	8	168.86	0.960	2	0.469
femalepairdiff:test number	1.214	4	24.55	1.172	3	0.347
bs:test number	1.434	4	134.93	1.391	4	0.240
femalepairdiff	0.652	2	5.91	1.252	kept	0.352
bs	0.312	2	68.53	0.599	kept	0.552
test number	2.340	2	66.49	4.494	kept	0.015*
femalepairdiff:bs	2.633	4	138.70	2.528	kept	0.043*

## Appendix 2

Based on the frequency and duration of visits to left choice area, right choice area and no choice area, quantified during the dichotomous choice tests using BORIS software (Friard and Gamba 2016), we calculated the following behavioral responses for each male: i) side bias ratio, difference in time spent in left choice area and right choice area, standardized by the total amount of time in any of the choice areas; ii) time of no choice, total time in seconds spent outside any of the two choice areas of the experimental tank; iii) total movement, total number of crossings between choice areas and no choice areas or vice versa during the test; iv) number of visits, the total number of crossings from the no choice area to either left choice or right choice areas; v) visits ratio, the difference in number of visits to the larger female and the smaller female, standardized by the total amount of visits . We then assessed potential differences in behavior of large-brained, small-brained and wild-type males during the dichotomous choice tests. For this, we used each behavioral response as the dependent variable in the model. All models included the interaction between brain size selection and the difference in female size as fixed effects, and the order of presentation as a covariate. Models also included the identity of the male and the female pair used as random factors, as well as, a random intercept for each replicate selection line and a random slope for brain size within each replicate. When necessary the dependent variable was log-transformed to ensure that residual distributions were roughly normal with no signs of heteroscedasticity. All analyses were performed in R version 3.3 (R Core Team 2015). There were no significant differences in the response of large-brained, small-brained and wild-type males for any of the behaviors quantified (Table A2). On the contrary, the difference in body size between females offered in the dichotomous choice test had a significant

effect on two of the behaviors analyzed, the total movement of the males, as well as the total number of visits to both choice areas during the test (Table A2).

**Table A2. Results from LMM models used to quantify behavioral patterns.** Asterisks indicate significant effects.

### BEHAVIOR 1: SIDE BIAS

Response = Left/right choice area ratio

	NumDF	DenDF	F	p-value
femalepairdiff	2	5.024	0.515	0.626
bs	2	2.759	0.206	0.825
test number	2	110.639	2.355	0.099
femalepairdiff:bs	4	135.962	0.647	0.623

### BEHAVIOR 2: TIME OF NO CHOICE

Response = log (time outside choice areas)

	NumDF	DenDF	F	p-value
femalepairdiff	2	3.285	2.064	0.262
bs	2	3.466	0.526	0.631
test number	2	23.754	6.854	0.004*
femalepairdiff:bs	4	133.652	1.827	0.127

### BEHAVIOR 3: TOTAL MOVEMENT

Response = log (number of crossing between choice-nochoice areas)

	NumDF	DenDF	F	p-value
femalepairdiff	2	3.574	7.826	0.049*
bs	2	3.232	1.926	0.281
test number	2	30.119	3.306	0.051
femalepairdiff:bs	4	135.558	1.237	0.297

### BEHAVIOR 4: TOTAL VISITS

Response = log (number of visits to both choice areas)

	NumDF	DenDF	F	p-value
femalepairdiff	2	3.523	8.008	0.048*
bs	2	3.172	2.062	0.266
test number	2	28.146	3.293	0.051
femalepairdiff:bs	4	135.661	1.237	0.298

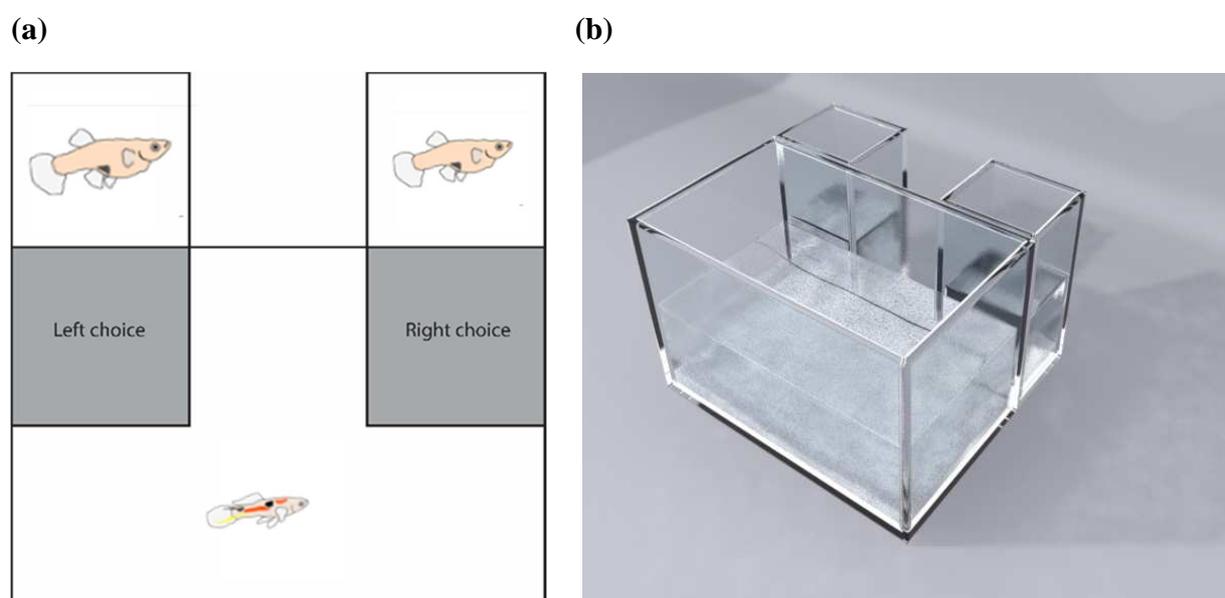
## BEHAVIOR 5: VISITS RATIO

Response = Ratio of visits to larger/smaller female

	NumDF	DenDF	F	p-value
femalepairdiff	2	3.523	0.650	0.559
bs	2	3.110	0.145	0.870
test number	2	100.741	1.852	0.162
femalepairdiff:bs	4	138.974	0.794	0.531

## Supplementary Information 1

**Figure S1. Experimental setup.** Top view (a) and 3d view (b) schematics of the experimental setup used with large-brained, small-brained and wild-type males for dichotomous choice tests between larger and smaller females. Grey shaded areas in (a) represent the area of the tank in which a female was considered to associate with each male.



## Supplementary Information 2

**Figure S2. Individual and average preference for larger females across contexts.** Individual preferences (lines) and mean values including confidence intervals (dots) of preference ratio for larger females in large-brained, small-brained and non-selected males when presented with dyads of females that had small (6.25%), medium (12.5%) and large (25%) differences in body size. Preference ratio for larger females was calculated as the difference in time spent with each female, divided by the total amount of time in any of the choice areas. Preference ratio take values between -1 (all time spent with the smaller female) and 1 (all time spent with the larger female).

