Recent theory predicts that in species where females tend to mate with the relatively most ornamented males, males may increase their attractiveness to females, and hence mating success, by preferentially associating with females that are surrounded by less ornamented competitors. Despite this prediction, we still lack explicit experimental evidence that males strategically prefer females surrounded by less attractive competitors to maximize their relative attractiveness. In this paper, we provide a comprehensive test of this hypothesis in the guppy (Poecilia reticulata), a species where a female’s perception of a male’s attractiveness depends on his coloration relative to that of surrounding males. We found that males preferentially associated with females that were surrounded by relatively drab competitors, and that the strength of an individual male’s preference was negatively correlated with his level of ornamentation. A series of control experiments made it possible to exclude the potentially confounding effects of male–male competition or social motivations when drawing these conclusions. The ability of males to choose social context to increase their relative attractiveness has important evolutionary consequences, for example, by contributing towards the maintenance of variability in male sexual ornamentation despite the strong directional selection exerted by female preferences.

1. Introduction

Male mate choice, although traditionally less studied when compared with female mate choice, may have far-reaching evolutionary consequences [1,2]. Male mate choice can evolve under a wide range of circumstances when the variation in the net fecundity-related benefit of mating with different females exists [1,3]. Male mate choice is expected to evolve, for example, when available females differ in their quality (fecundity or ability to provide parental care) [1]. Furthermore, when males encounter more than one female at the same time and courtship is costly, a male is likely to direct his mating efforts towards females that are most likely to accept his courtship and mate with him. Indeed, there is ample evidence that males preferentially direct their sexual interest towards sexually receptive females [4,5,6].

Male mating choice is also influenced by social context. For example, in species where male–male competition is strong, the ability to physically outcompete rival males is crucial, and males are expected to prefer females associated with fewer or less aggressive, competitors, even if this means targeting low-quality females [7,8]. For example, in the spider Zygiella x-notata, males with weak competitive ability prefer less fecund females which reduces local competition with stronger rivals, and increases mating opportunities [9]. Similarly, in the three-spine stickleback (Gasterosteus aculeatus), male choosiness for larger females is reduced when the intensity of male competition increases [10]. However, a recent study on the fish Gambusia holbrooki revealed no evidence that competitor size affects male mate choice [11].

In addition to the ability to outcompete rival males, a male’s reproductive success is influenced by his attractiveness to females, especially in species in which precopulatory male mating success is mainly influenced by female
choice. Accordingly, theory predicts that males benefit from choosing the social context that enhances their attractiveness to females through proximity to less attractive rivals [12]. Indeed, there is experimental evidence that social context influences female choice. For example, female guppies (Poecilia reticulata) show a 'cryptic' preference for the relatively more colourful male when given the choice of two potential mates, indicating that female perception of a male attractiveness is influenced by the degree of ornamentation of the surrounding males [13]. Similarly, in the fiddler crab (Uca mjoebergi), females exhibit heightened sexual preferences for males that are surrounded by relatively small neighbours, but do not show any preference when the same males have large neighbours [14]. Empirical evidence that males actively choose the context in which their relative attractiveness to females is maximized, however, is limited. In the house finch (Carpodacus mexicanus), less attractive males are more likely to change social group and males that frequently moved between social groups had greater pairing success than less social individuals with equivalent sexual ornamentation [15]. This suggests that males may be able to choose the most appropriate social context to increase their relative attractiveness, although these results could also be explained by male–male competition and an association between male condition, male movements and mating success. Dugatkin & Sargent [16] demonstrated that male guppies show a proximity preference for other males that were previously seen far from a female ('losers'), when compared with males that were seen close to a female ('winners'), although male–female association and not male mate choice was measured in this study. Therefore, while these studies suggest that males actively choose the social context in which their relative attractiveness is maximized, explicit support from studies in which the effect of direct male interactions, such as male–male competition, is controlled, is still lacking.

The guppy is an ideal species to test context-dependent male mate choice according to competitor attractiveness. Male mating success is strongly influenced by female choice [17–19]. In particular, female guppies prefer relatively colourful males [19,20] and importantly, female perception of male relative attractiveness influences the number of sperm transferred during copulation in favour of the more colourful male [13]. Female guppies are highly promiscuous [21], and sperm number is the most important predictor of sperm competition success in this species [22]. Since pursuing and courting females may result in significant energetic costs [23,24], and males cannot simultaneously court two different females, it can be expected that male guppies exhibit some degree of choosiness. Indeed, males have been shown to prefer receptive [4], larger [25] and unfamiliar females [26] as mates.

We tested the ability of male guppies to choose the social context that will increase their mating opportunity, by preferring females associated with less ornamented males. In particular, a focal male was given the opportunity to choose between two females surrounded by either a drab or a colourful pair of males. We focused on orange coloration as it has been well documented that females show a strong preference for males with large orange body spots (see [19,27] for a review, and [13,20,28] for evidence in the population used). We tested whether (i) male guppies prefer females that are associated (experiment I), or were previously seen in association (experiment II), with drab competitors; and (ii) the strength of a male’s mating preference is correlated with his own attractiveness. We also investigated the role of male experience on male mating preference by performing choice tests also using males which never interact with other sexually mature males (experiment I). Indeed, the ability to choose the appropriate mating context may be acquired by a male through experience, for example, by comparing his mating success in different occasions in which competitors varied for the degree of their ornamentation. To this aim a group of males was raised with females only, to experience normal sexual activity during development but without having the possibility to compare their mating success with that of other males. We conducted choice preference tests both in the presence and in the absence of females, with the latter used to control for possible social, non-sexual, preference for one of the stimulus male type (drab or colourful). Any preference for drab male stimuli may depend on differences in aggressiveness/dominance associated with the extension of colour spots. To further rule out the possibility that male–male competition played a role in male mate preference, we ran an experiment to test whether male coloration affects male–male aggressive behaviour when males physically interact in the presence of a female (experiment III).

2. Material and methods

(a) Experimental fish

Experimental fish were laboratory-reared descendants of wild-caught fish from the Lower Tacarigua River (Trinidad). Fish were fed twice daily, stock and experimental tanks were maintained at a constant temperature of 26 ± 1°C and with a cycle of 12 L:12 D h. All fish were sexually mature (four to six months old) when used for the experiments. Females used in experiments were chosen to be of similar size (standard length range: 23–25 mm).

(b) Selection of males and measurement of colour pattern

Male colour pattern was measured following an established procedure [29]. Briefly, each male was first anaesthetized in a water bath containing MS 222 (Sigma Aldrich, St. Louis, MO, USA). The sedated fish was then digitally photographed in lateral view using a Canon EOS v. 450d. We used IMAGEJ software to measure standard length and the relative area of orange colour spots (including orange, yellow and red). Following the criterion used in a previous experiment [13], we scored males for colour pattern in order to form two groups of stimulus males: drab males, with less than 9 per cent of body area covered by orange coloration (mean ± s.e.: 4.84 ± 0.43%, n = 46), and colourful males, with greater than 20 per cent of body area covered by orange coloration (mean ± s.e.: 25.13 ± 0.56%, n = 45). Drab and colourful males did not differ significantly in body size (standard length, t = 1.330, p = 0.194). Stimulus males were randomly taken from each group to form the stimulus dyads used in the choice tests. They did not take part in any other trials until all the males of their group were used once. As we ran 166 experimental trials (see below), each stimulus male was used 7.3 times on average, but the probability that the same drab or colourful male pair was used in two different trials is very low (p = 0.0012), and the probability of using the same quartet of stimulus males is consequently negligible (p < 0.001).
(c) Male mate choice experiments

For male choice experiments, we used dichotomous choice tanks, similar to those extensively used to determine mate preference (association time) in the guppy (e.g. [30,31]). This experimental design has been recently validated as reliable predictor of actual male mating preference in this species [32].

Experiment I. The experimental tank (48 × 20 × 30 cm) consisted of a central compartment and two lateral compartments (9 cm each), which were divided into three identical sectors (each stimulus sector: 9 × 6.6 cm) to accommodate the female, in the centre, and the two stimulus males (either drab or colourful) on her sides (figure 1). Stimulus males and females were visually and physically isolated one from each other. The two virgin females were allowed to settle in their sectors for 1 h. Stimulus males were then introduced into their dedicated sectors so that one female was surrounded by two colourful males and the other female, on the opposite side of the tank, by two drab males (left or right side was randomized across trials). The test male was positioned in a transparent Plexiglas cylinder in the middle of the central compartment and allowed to observe the two females and the four stimulus males for 10 min. The cylinder was then gently raised and the choice test started when the test male began to swim freely in the tank (usually within 10 s). Male position was recorded every 5 s for a 10 min period by an observer positioned behind a black curtain into which a small hole had been created (to avoid disturbance to the experimental fish). The same male was tested twice, 3 days apart, with and without females; testing order (with or without females) was randomized. Test males were either taken from stock tanks, in which groups of about 100 fish (sex ratio approx. 1:1) were allowed to freely interact (hereafter, ‘experienced’ males, n = 50), or they were raised in the presence of females (to maintain normal sexual activity during development) but without any visual and physical interaction with other males (hereafter, ‘naïve’ males, n = 23). ‘Choice zones’ consisted of the 5 cm (about two standard lengths) areas at the ends of the central tank facing the side in which stimulus fish (males and females) were placed (figure 1). Male preference was expressed as the number of times the male was in the choice zone. Male preference in the absence of females was used to control for a male preferential association with drab or colourful males irrespective of his mating preference.

Experiment II. This experiment was similar to experiment I, but after presenting the two females surrounded by two drab or two colourful males, the stimulus males were hidden (see below) before measuring preference. The experimental apparatus was slightly larger (68 × 68 × 36 cm, each stimulus sector: 11 × 11 cm) than the previous one to allow a differential illumination of each tank sector. Before each trial, two females were allowed to settle in their sectors for 1 h, after which the four stimulus males were introduced. As above, the side of drab and colourful males was randomized. The test male was positioned in a transparent Plexiglas cylinder in the middle of the central compartment and allowed to observe females and stimuli for 10 min. At the end of this period, the lights positioned above the stimulus males were turned off, allowing the test male to see only the two females. The cylinder was then raised and focal male behaviour was observed for 10 min (position of the focal male was recorded every 5 s). The preference was scored using videos collected by a video camera placed above the testing tank. Male preference was recorded as above. A total of 20 test males were used (that were different individuals from those used in experiment I). In both experiment I and II, the females could not see stimulus males, in order to exclude the possibility that the focal male behaviour was influenced by any female behaviour mediated by her proximity to drab or colourful stimulus males.

(d) Colour-dependent aggressive behaviour

Experiment III. We tested for a colour-dependent male aggressiveness in dyads of freely interacting males in presence of a female. Male dyads (n = 24) were formed with individuals from different stock tanks to avoid previous familiarity [33]. Two randomly chosen males were introduced in the experimental tank (24 × 20 × 30 cm) and left to acclimatize for 20 min. At the end of this period, a female was introduced into the tank and allowed to settle for further 20 min, and then the number of aggressive interactions (chasing, nipping and physical contact, see [33] for a complete description of behaviours) between the two males was recorded for 20 min.

(e) Statistical analyses

In experiment I, we first tested whether test males showed a preference for the drab males’ side using one sample t-tests (expected value for no choice = 0.50) both in the presence and in the absence of females. To test male mate choice according to the female presence and his own attractiveness, we used a repeated measure ANCOVA in which the paired dependent variable was the preference for the side with drab stimuli (over the total time spent in the choice zones) in the presence and the absence of females, and male
orange coloration was the covariate. We conducted separate analyses for experienced and naive males. In experiment II, females were always present, and therefore, we used the preference for the side with drab and colourful stimuli side as dependent paired variable, and the orange coloration of the focal male was entered as covariate. To analyse whether aggressive behaviour was associated with male coloration, we used a GLMM with a Poisson error distribution and a logarithm link function. Trial number (i.e. the pair of male) was included as random factor to account for the non-independence of data within each trial. Where required, all data were checked for normality and homogeneity of the variance. Male preference and coloration are proportions and angular transformation (arc sine of square root) was used when required. Statistical analyses were performed using P A W S 18 (S P S S Inc.) and G E N S T A T v. 15. Means are given with their standard error (± s.e.).

3. Results

(a) Male mate choice experiments

Experiment I. Experienced males spent 80 ± 1.9% of the total time in both ‘choice zones’ in the absence of females, and 86.7 ± 1.5% when females were present. Naive males spent 81.2 ± 2.3% of the total time in both ‘choice zones’ in the absence of females and 87.7 ± 1.9% when females were present. Experienced and naive males spent, on average, virtually the same proportion of their choice-time near the two types of stimulus males in the absence of females (% time spent near drab males: experienced = 51.7 ± 3.9%, t = 0.516, p = 0.608, n = 50; naive = 51.6 ± 5.7%, t = 0.433, p = 0.669, n = 23, one sample t-test; figure 2a). When females were introduced, males increased their preference for the drab stimuli side but only in experienced males the effect was significant (% time spent near drab males: experienced = 62.3 ± 4.0%, t = 3.025, p = 0.004; naive = 57.7 ± 6.0%, t = 1.292, p = 0.210, one sample t-test; figure 2b). Results from repeated measure ANCOVA (table 1) showed that experienced males significantly increased their preference for drab stimuli side when females were present and that this preference was negatively correlated with test male orange coloration (figure 2c and table 1).

Experiment II. Test males spent 77.8 ± 2.4% of the total time in the choice zones. Male preference depended on which stimulus males were previously seen surrounding the female (F_{1,18} = 10.194, p = 0.005). As in experiment I, the strength of male’s preference was influenced by his own coloration, with less colourful test males exhibiting a stronger preference (stimulus × orange coloration F_{1,18} = 5.802, p = 0.027).

(b) Colour-dependent aggressive behaviour

Experiment III. On average, males (n = 48) performed 0.52 ± 0.09 aggressive behaviours per minute. Aggressive behaviour was not related to coloration (orange: F_{1,17.6} = 0.21, p = 0.650, iridescent: F_{1,17.9} = 1.79, p = 0.198, and black: F_{1,17.1} = 0.79, p = 0.385) or body size (SL: F_{1,18.1} = 0.67, p = 0.425, GLMM). A model, including only orange coloration showed similar result (orange: F_{1,20.2} = 0.54, p = 0.470).

4. Discussion

We have shown that, according to predictions, male guppies actively choose females surrounded by drab (unattractive) males rather than the females surrounded by colourful (attractive) males. This proximity preference was significantly affected by the male’s own degree of ornamentation, as it was negatively correlated with the percentage of body area covered by orange spots. While there is growing evidence that social environment affects male mating decisions [34], this study is the first to demonstrate that males are able to select the most appropriate social context
in which to court a female in relation to their attractiveness relative to that of rival males, after experimentally controlling for the potential effects of direct male–male competition or other social motivations.

Indeed, to demonstrate that a male’s proximity preference is driven solely by his sexual attractiveness relative to that of competitors, it is necessary to control for other confounding factors, in particular male–male competition, but also other social motivations. Colourful males are exposed to higher predation risk [35], also increasing the predation risk for nearby fish [36], and the preference for drab males may indeed be explained as an avoidance of a more risky context. Experiment I revealed that, when no females were present, males did not show any preference for drab or colourful males, suggesting that there is no social motivation underlying the observed proximity preference for drab males when females were present. Male guppies may avoid colourful competitors because they are more aggressive in male–male interactions. In P. reticulata, male–male competition is thought to be of minor importance in natural conditions (reviewed in [17]). While results of experiment I suggest that males do not avoid colourful competitors when females are not present, it could be argued that male–male aggressive behaviour is expressed only when a potential mating partner is present. Results of experiments II and III excluded this possibility as they showed that the preference for the female which was close to drab stimuli was expressed also when competitors were hidden, and that male coloration is not associated with male aggressive behaviour in the presence of a females, respectively.

Altogether, these results indicate that neither male–male competition nor other social interactions are responsible for the observed male mating preference for the female surrounded by less colourful males.

The fitness consequences for males targeting females in close proximity to relatively unattractive males are twofold: males are expected to increase both their mating opportunity and their sperm competition success. When given the choice between two males, female guppies usually prefer to mate with the more colourful one [17,19]. Even when a female has already mated, her propensity to mate with another male depends on the attractiveness of the second male relative to the first [37]. Males choosing females that may have mated, or possibly will mate, with less colourful males are also expected to have a fitness return in terms of increased sperm competition success. Indeed, the number of sperm transferred during copulation, which is the main predictor of sperm competition success in this species [22], is positively influenced by the male’s perception of relative male coloration [13]. The male preference for females surrounded by drab males can be further strengthened by the fact that, on average, less colourful males produce sperm with inferior competitive ability [38,39] and have lower sperm competition success when the number of sperm inseminated is experimentally controlled [40]. The relative importance of these two levels of selection (expected increase in mating opportunities and sperm competition success) in shaping male preference is difficult to assess, as they are probably positively associated. Nevertheless, it is clear that choosing the appropriate social context is likely to significantly affect the reproductive fitness of male guppies.

The results of experiment II demonstrated that males prefer females that were seen surrounded by less attractive males even when competitors are out of sight, suggesting that males choose females on the basis on previous information about rival males, at least on a short time-scale (see also [41]). The results of the experiment with naive males, although far from being conclusive, suggest that the assessment of rivals’ and own attractiveness is acquired through experience. Elucidating how previous information is obtained, how long is retained and to what extent influences male mating preference, represents a challenge for future research on mating preferences [12].

Drab males are expected to have a low mating success rate when competing with more colourful males, and our finding that less attractive males exhibit a stronger preference for the females nearby relatively unattractive competitors (experiments I and II) corroborates the assumption that these males have more to gain from choosing the right social context. The stronger preference observed in drab males has potentially important evolutionary consequences as it may help explaining the maintenance of the variance in sexually selected traits subjected to directional female preference, the so-called lek paradox [42]. Although other non-directional sexual selection mechanisms have been shown to operate in guppies [43–45], our results suggest that the strength of directional selection generated by female preference for colourful males, may be further weakened by the ability of less ornamented males to increase their reproductive success by choosing the appropriate context in which courting a female.

In conclusion, our results provide experimental evidence that, in a species in which female perception of male attractiveness is influenced by that of competitors and has significant consequences for male reproductive success [13,22], males actively choose the social context that maximizes their relative attractiveness and that is expected to increase their reproductive success.

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Table 1. Results for male choice tests from repeated measures ANCOVA in which preference for drab stimuli side was compared in the presence and in the absence of females and test male coloration (orange) was included as covariate.

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<th>experienced males</th>
<th></th>
<th>naive males</th>
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<td>F</td>
<td>p-value</td>
<td>d.f.</td>
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<td>0.007</td>
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<td>0.740</td>
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<tr>
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References


