THE EFFECTS OF SOCIAL EXPERIENCE AND ORANGE COLOURATION ON MATING EFFORT IN MALE GUPPIES (POECILIA RETICULATA)

by

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The Effect of Social Experience and Orange Colouration on Mating Effort in Male Guppies (*Poecilia reticulata*)

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Abstract

Selection for increased fitness often results in changes to an organism’s behavioural repertoire, and sexual behaviour can be modified in a plethora of ways. Here, I used the Trinidadian guppy, *Poecilia reticulata*, as a model study species to investigate two different aspects of male sexual behaviour. First, I examined how social environment affects male mate choice. I evaluated whether male guppies with previous social experience of female receptivity cues learn to prefer and adjust their behavioural repertoire towards females with higher receptiveness levels, as this represents an optimal use of time and energy and is more likely to result in insemination. While males that previously experienced receptivity cues did not change the strength of preference towards receptive females (results from dichotomous choice test), these males adapted their mating tactic compared to naïve males (results from no choice tests). This change in mating tactics but lack of preference towards receptive females suggests that although learning from previous experience is important, it might be weaker than predicted in this species. Next, I tested the association between two crucial factors driving sexual selection in this species, male colouration and male mating behaviour. To experimentally test the causal link between these two factors, I used guppies artificially selected for a high or low proportion of orange and quantified their mating tactics. I found that male guppies with greater orange colour increased their mating effort in sexual behaviours directed to court females for insemination. Additionally, males with greater orange colour increased their mating effort in coercive mating tactics. My results suggest that this sexually-selected signal is directly correlated with overall sexual effort. These results support the idea that orange is associated with greater male vigour through higher overall levels of sexual behaviour.
Lay Summary

The study of how sexual selection operates is key to our understanding of processes shaping the evolution of traits and behaviours. Using guppies, a fish traditionally used in the study of sexual selection, I studied the role of social environment in shaping mating decisions and the association between colouration and mating effort in males of this species. I found that previous experience with mates is an important modulator of decision-making for mating tactics of male guppies. Also, my results provided empirical support for a causative link between orange colouration and overall mating effort of male guppies.
Preface

I conducted the research and data analysis presented in Chapters 2 and 3 alongside Dr. Alberto Corral-Lopez and under the supervision of Dr. Judith E. Mank. The general research question and initial experimental design in Chapter 2 was developed in collaboration with Drs. Corral-Lopez and Mank. The general research question and initial experimental design in Chapter 3 was developed in collaboration with Dr. Corral-Lopez, Dr. Wouter van der Bijl and Dr. Mank. All colour quantification presented in Chapter 3 was done by Dr. van der Bijl, with data analysis done by me. I wrote all three chapters of this thesis, with assistance from Drs. Mank and Corral-Lopez, and received editorial feedback from my committee members, Dr. Darren Irwin and Dr. Leticia Avilés.

All experiments performed for this thesis followed the guidelines of the Canada Council for Animal Care, under approval of the animal care committees at the University of British Columbia (animal care certificate number: A22-0239).
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Chapter 1: Introduction

1.1 General Introduction

Within a species, there is individual variation in behaviour, survival and reproduction. Those individuals best adapted to their environment will contribute more of their genes to the following generation (Darwin, 1871). This is the concept of natural selection, which links some behavioural patterns to reproductive output. In this thesis I evaluate how male guppies differ in sexual behaviours depending on two different factors, social learning during early development, and overall colouration, measured by the proportion of orange on the body and tail. Specifically, I am looking into how males cater their mating effort in a way to optimize their fitness.

The adaptive value of behaviour can reveal the behavioural basis of reproductive success; however, it is difficult to study under natural conditions and over long periods of time. For the purpose of my research here, I measured the frequency and duration of sexual behaviour as indicators of the behavioural repertoire of male guppies in a laboratory setting.

1.2 Mating Tactics in General

Mating tactics develop within specific environmental contexts. The contextualization of mating tactics from an evolutionary-theoretical framework come from the Life History Theory. This theory explains the coordinated allocation of bioenergetic and material resources to survival and reproduction across an individual’s life-cycle. The theory characterizing allocation of energy is based on whether the organism is a K-selected (offspring are few but long-lived) or r-selected (many offspring, with a short life span). Whereas all organisms need to invest in both their somatic and reproductive efforts to survive and reproduce, their relative effort in either category may differ. The theory characterizes K-selected species (e.g., humans, whales, elephants) as allocating bioenergetic resources to somatic effort (survival of the organism) over their reproductive effort (production of new organisms), whereas r-selected species (e.g., cockroaches, mice, guppies) preferentially allocate these same resources to reproductive effort over somatic maintenance. Moreover, the theory suggests that when allocating reproductive effort, K-selected species preferentially allocate resources to the survival of offspring and the survival of kin over obtaining and retaining a sexual partner) whereas r-selected species preferentially allocate
resources to mating effort over both parental and nepotistic effort (Figueroedo et al., 2007). Mating tactics need to be coordinated with a variety of related life-history traits for the allocation of resources among these fitness enhancing activities to be optimal (Figueroedo et al., 2007).

Males will often use more than one tactic to gain access to females. In general, there are two common mating tactics. Typically, males will have a territorial tactic and attempt to monopolize access to females; alternatively, males will use a sneaky tactic in which they try to mate with a female without defending a resource (Gross, 1996). When mating tactics are present, individuals can engage in either one or the other or they can have a mixed strategy (continuous variation) where they use both. The change in mating tactics tends to be related to maximizing fitness (Taborsky, 2001; Taborsky et al., 2008). Mating tactics might differ in between males of the same species when there are size dimorphism, colour polymorphism, and dimorphic morphological structures that are involved in securing resources, mates, etc. (Taborsky et al., 2008). These differences in mating tactics are found when there is fitness to be gained by pursuing different reproductive tactics (Taborsky et al., 2008). Investment to access mates bears costs. This investment includes, but is not limited to, the production of conspicuous signals that may not only attract mates but also predators and competitors (Andersson 1994). Since investment into mates does bear costs, males should also be optimizing fitness by choosing a mate that they are more likely to have a successful copulation with.

1.3 Mating Tactics in Guppies

The guppy is a classic system for the study of mating behaviour due to the coevolution of female preference and male colour. Female guppies use the elaborate colour displays of males as an indicator of good health. Males provide no resources during mating and do not defend territories against other males. Therefore, males do not offer other benefits beyond their genes. The mating system of guppies is promiscuous. Male guppies have two mating tactics, courtship and coercion. Courtship displays occur when males position their bodies into an arc or S-shape curve (sigmoid displays) and quiver in front of a females. This female can then accept or reject the male (Luyten & Liley 1985). Males can also use coercion when they attempt to inseminate a female without seeking consent (Godin, 1995). Liley (1966) defined coercion or sneak attempts, as unsolicited attempts of inseminating a female from behind by thrusting his gonopodium at the female’s
urogenital pore. The two mating tactics have different energetic costs, rates of success and contexts in which its usage may optimize fitness for the courter. Courtship requires more energy but is also more successful in terms of reproductive success than sneaking (Magurran, 2005). In guppies, males can use both tactics interchangeably, however there is evidence that males will prefer one tactic over the other (Kelley, Phillips, & Evans, 2013; Magellan & Magurran, 2007) depending on an individual’s age, size, morphology, or energy level (Sih et al., 2015). Males who possess the lesser picked trait, or for whom the costs do not outweigh the benefits, are predicted to prefer sneaking, as it is less energetically costly than engaging in courtship and would therefore offer greater fitness returns for those males. I might also expect males with lower energy level to minimize costs associated with the expression of costly sexual performances and adopt sneaky tactics to ensure their reproductive success (Gross 1996; Hunt et al., 2004; Smith & Blumstein, 2008).

1.4 Male Mate Choice and the Social Environment

Mate choice is an incredibly important evolutionary process because it influences which individuals successfully leave genes to the next generation leading to evolution via the process of sexual selection (Darwin, 1871; Andersson, 1994). Females are traditionally presented as the choosier sex, selecting males based on the quality of their traits. This is because females typically invest more in their offspring. Yet, there is increasing evidence that male mate choice is also important, even in species without male parental care (Edward & Chapman, 2011; Herdman et al., 2004; Rosenqvist, 1990; Werner & Lotem, 2003; Wong & Jennions, 2003). Social environment and learning are key factors in determining mate preference, and animals are able to use the information they gather from previous experience to potentially increase their odds of obtaining a high-quality mate.

The environment also has an influence throughout the life cycle of an organism. As organisms gather information, they may use it to increase their odds of securing a better-quality mate. In these terms, the individuals are learning (Hebets & Sullivan-Beckers, 2010). Early social experiences can have effects on social behaviour such as mate choice (Jonsson B & Jonsson N, 2014). Mate-choice learning can come from two different directions, personal experiences and observing others (Auld & Godin, 2015; Hebets & Sullivan-Beckers, 2010). For chapter 2 I
examined how the social environment affects male mate choice in the guppy *Poecilia reticulata*. Previous experience can play an important role in forming mating preferences. Animals regularly use the information they gain from their environment to adapt their behaviour and efficiently obtain and compete for mates, as well as for other resources that lead them to increase their fitness (Bailey & Moore, 2012; Danchin et al., 2004; Fowler-Finn & Rodríguez, 2012; Valone & Templeton, 2002).

In fish, male preference for females tends to be studied in terms of body size. This preference is often based on visual information. In theory larger females are likely more fecund (Arriaga et al., 2013; Edward & Chapman, 2011; Guevara-Fiore et al., 2010; Head et al., 2015; Herdman et al., 2004). Another potential determinant of male preference is receptivity, the likelihood of successful female insemination, which is based on the reproduction cycle of females. Choosing to display and mate with a receptive female is an optimal use of time and energy, as it is more likely to result in insemination. Previous research has shown that males can distinguish between virgin and mated females using olfactory cues over visual cues. In these regards, using the hormonal differences from olfactory cues seen between receptive or nonreceptive females may influence the mating choices male guppies make (Guevara-Fiore et al., 2010; Guevara-Fiore et al., 2009; Guevara-Fiore & Endler, 2018).

I evaluated whether male guppies with previous social experience of female receptivity cues learn to prefer and adapt their behavioural repertoire towards females with higher receptiveness levels, as this represents an optimal use of time and energy and is more likely to result in insemination. Males can adapt their behavioural repertoire or shift to alternative mating tactics based on previous encounters with females (Bailey et al., 2010, Řežucha & Reichard, 2014). To do so, I experimentally manipulated the social environment of male guppies and quantified their preference and behavioural repertoire in the presence of receptive and non-receptive females. I investigated if previous social experience with female receptivity cues affects behaviour and strength of preference of male guppies in relation to female mating status. I quantified their preference and behavioural repertoire in the presence of receptive and non-receptive females. Choice can be measured either using a choice test, in which subjects are presented with two or more options simultaneously, or a no-choice test, in which subjects are presented with a single
option and have to choose between this option or nothing (Wagner Jr, 1998). For our testing purposes, I measured sexual preference and behaviour for receptive females in no-choice and dichotomous choice tests using guppy males experienced or naïve to female receptivity cues.

Given theoretical expectations of fitness maximization, I predicted that male guppies would use learning from their social environment to shift their preference levels to favour receptive females. I also predicted that males would adapt their behavioural repertoire depending on whether they encounter receptive or non-receptive females. Specifically based on the energetic requirements of the two different mating tactics I predicted that experienced males (those with previous experience of receptive and non-receptive females) would preferentially allocate effort into displaying to receptive females during courtships and performing sneak attempts to nonreceptive females in comparison to naïve males (without experience of females).

Experience with receptivity cues did not change the strength of preference towards receptive females. However, male guppies that had previous experience with female receptivity cues adapted their mating tactic compared to naïve males. The change in mating tactics but lack of preference towards receptive females shows that the influence of social learning is present but might be weaker than predicted in this species. Furthermore, these results provide further support to studies of female mate choice suggesting mating status is not a key factor driving the strength of sexual preferences in natural populations.

1.5 Mating Effort and Colouration

Sexual dimorphism can affect many traits, including body size, colouration, shape, and behaviour. Many cases of sexual selection arise from the fitness differences that are associated with nonrandom success in the competition for access to gametes for fertilization (Shuker & Kvarnemo, 2021). Males commonly use multiple signals, including behavioural signals and colouration, as indications of their level of fitness. These levels of fitness have an influence over their mating tactics.
Colour is a key component of many aspects of animal ecology and behaviour. The guppy is an important model species to understand the evolution of colour in mate choice and sexual behaviour. Male guppies have vivid colour spots and females tend to prefer males with larger, highly saturated orange spots (Kawamoto et al., 2021). Previous studies have suggested that female guppies can recognize and evaluate orange spots based on the relative area and colour saturation, as potential indicators of male quality. The colour saturation of orange spots is determined by carotenoid uptake and is also affected by parasite load, implying that the saturation is reflective of male health. Additionally, the area of orange spots has been correlated with sperm competitiveness and the ability of offspring to avoid capture (Locatello et al., 2006; Evans et al., 2004) suggesting that orange spots may be an indicator of offspring fitness (“good genes” models) (Hamilton & Zuk, 1982; Zahavi, 1975). Carotenoids are thought to be an honest indicator of health because it solely comes from diet (Grether, 2000). Orange spots are also an indicator of offspring attractiveness, as described by the “Fisher’s runaway process” (Fisher, 1930), since the area of orange spots is heritable and partially genetically controlled (Houde, 1992; Morris et al., 2020; Sato & Kawata, 2020).

In Chapter 3, I describe a test of the association between a sexually selected signal, colour, and sexual behaviour. Sexual selection is a fundamental driver of phenotypic evolution (Darwin 1871; Wallace 1889), however the diversity of animal sexual signaling traits remains challenging to explain (Price 2017; Mason et al., 2017). Signaling traits, such as colour, are under strong selection because they facilitate effective communication between individuals; however benefits are balanced against costs such as those associated with signal production, maintenance or display, including predation (Weaver et al., 2017; Zahavi & Zahavi 2007).

Previous research has briefly looked at this concept. It has been shown that dull male guppies increased their probability of mating with females by changing their mate decisions, but this was done in terms of testing in the presence of a rival male where they could evaluate their quality (Yoshikawa et al., 2016). Herdegen-Radwan et al. (2021) selected male guppies bidirectionally for six generations for high and low carotenoid spot areas and looked to see if selection caused a significant correlated increase in male reproductive competitiveness. However they did not find an effect of artificial selection for orange colouration on courtship display (Herdegen-Radwan et
al. 2021). In contrast however, Jirotkul (2000) found that guppies with more orange colouration performed more displays. Our study will look to bring more clarity to how colour leads to different behavioural tactics and strategies but add in coercive behaviour. It is also important to look into coercive behaviours. This may show potential fitness consequences of evolving a larger orange colouration as the difference in fitness levels of high-orange and low-orange male guppies may alter the mating tactics they choose to perform. In a close species to the guppy, Poecilia parae, it was shown that the dull male typically did not court females but gained successful copulations exclusively through coercive behaviours. The colourful morphs (blue, red and yellow) used a combination of sneaker and courtship tactics. But within the different colour morphs different proportions of mating tactics were used. Blue males gained more successful copulations through courtship than through sneak attempts. Red males generally performed fewer courtships, suggesting that red males may simply rely on their body colouration instead of elaborate displays to attract females. Finally, yellow males were more successful in obtaining mating when they used the sneaker tactic than were the blue and red morphs (Hurtado-Gonzales & Uy 2009). For P. parae it seems that individuals optimized their fitness given their relative condition (Gross 1996).

In order to investigate the potential fitness consequences of evolving orange colouration I quantified male sexual behaviour of artificially selected guppy (Poecilia reticulata) lines that vary in the proportion of orange colouration on the body and tail. This experimental design allowed us to test the link between a specific sexually-selected colour signal and sexual behaviour. I found that males with higher proportions of orange performed more frequent and longer courting displays. Similarly, males with higher proportion of orange increased their mating effort in coercive behaviours, including a higher number of sneak attempts and longer time chasing females. These results support the idea that orange is associated with greater male vigour through higher overall levels of sexual behaviour.
Chapter 2: Experience with mating receptivity cues affects sexual behaviour of male guppies, but not their strength of preference towards receptive females

2.1 Introduction

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

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

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

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

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

2.2.1 Study system

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

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

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

2.2.2 Dichotomous choice preference tests

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

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

I used photographs to identify males from the pre-treatment tests following the 45 days of experimental treatment, and transferred them in a 3L aquarium three days prior to a second dichotomous choice test (post-treatment test) to allow for sperm replenishment. This avoids biases in preference measurements due to lack of motivation (Pilastro et al., 2002). While biases due to motivation were only expected for experienced males, naïve males were likewise transferred to 3L aquarium three days prior to their second behavioural test. Males were then presented for dichotomous choice post-treatment tests.
I performed all behavioural tests in a circular arena (diameter = 47 cm) sheltered to prevent disruption. I filmed the arena for 15-minute periods using a OBSBOT webcam (1080P at 30 fps) after a five-minute acclimatization period. For accurate identification of fish with tracking software, I placed them in the experimental arena in 20 second intervals. I placed females first in the arena, randomizing the order of placing receptive and nonreceptive females. Additionally, to account for any olfactory cues, I changed water in the arena between tests. To minimize stress, each fish was netted, placed in a glass bowl and transferred to the testing apparatus. For consistency, the tests were always conducted in the morning for a period of 4-6 hours.

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

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

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

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

2.2.3 No choice preference tests

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

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

A single observer scored male sexual behaviour in video recordings in a random order and quantified the following behaviours as defined in Liley (1966): i) number of sigmoid displays, every time a male positioned himself in front of the female with an S-shaped posture soliciting copulation; ii) number of sneak attempts, unsolicited attempts of inseminating a female from behind by thrusting his gonopodium at the female’s urogenital pore. I also calculated latency to initial sexual behaviour. Following the procedure described for dichotomous choice tests, I used idTracker software (Perez-Escudero et al., 2014), to calculate the distance between the male and female for each frame in the video recordings and extracted the time spent following a female for each trial (number of frames < 4 cm).
To compare the number of sigmoid displays in experienced and naïve males, I fit a statistical model using a Poisson distribution and a logit link function for the conditional mean in the package glmmTMB (Brooks et al., 2017). I used the mating status of the female, experience to mating receptivity cues and the interaction of these as fixed factors. I included the number of tank and experimental batch as random factors in the model. For sneak attempts and latency to first sexual behaviour, I used analogous models including a zero-inflation linear predictor. For time following the female, I used an analogous structure in a Linear Mixed Model fitted with lmer package (Bates et al., 2007). I evaluated the adequacy of our fitted models using scaled-residuals quantile-quantile plots, residual versus predicted values plots and a zero-inflation test in the DHARMa package (Hartig, 2018). I processed the parameters of our statistical models using Wald tests obtained via the parameters package (Lüdecke et al., 2020). I obtained post-hoc comparisons of the male response between female receptivity levels at pre-treatment and post-treatment time of testing in the previous models using the emmeans package with the tukey-adjustment method for multiple comparisons (Lenth et al., 2019). All analyses were performed in R (v. 4.1.3; R Core Team, 2022).

2.3 Results

2.3.1 Male colour and morphology analyses
Average proportion of orange or black colouration did not differ between males used for experienced or naïve treatments (mean ± SE; orange colouration: naïve males 7.33 ± 3.47, experienced males 7.14 ± 2.47, F_{df=1} = 121, p = 0.729; black colouration: naïve males 2.27 ± 1.02, experienced males 2.20 ± 0.86, F_{df=1} = 0.154, p = 0.695; Figure. A1). Similarly, there was no significant overall difference between naïve and experienced males in morphological traits (body size: naïve males 1.55 ± 0.42, experienced males 1.57 ± 0.42, F_{df=1} = 0.00, p = 0.99; tail size: naïve males 0.51 ± 0.08, experienced males 0.48 ± 0.09, F_{df=1} = 0.75, p = 0.38; Figure. A1).

2.3.2 Dichotomous Choice Preference Tests
Neither experienced nor naïve males changed their strength of preference towards receptive females after 45 days of treatment (time of testing: estimate_{pre-treatment} = -0.01 ± 0.04, t = -0.37, p = 0.72; Figure 2.1; Table 2.1). In addition, I found no overall differences in the strength of preference for receptive females between experienced and naïve males, or in the rate of change in preference
between experienced and naïve males following the 45 days of treatment (male social treatment: estimate\textsubscript{experienced} : 0.01 ± 0.04, t = 0.45, p = 0.65; male social treatment x time of testing : estimate\textsubscript{pre-treatment x experienced} : 0.04 ± 0.06, t = 0.72, p = 0.48; Figure 2.1; Table 2.1).

**Figure 2.1. The effect of previous experience with female receptivity cues in guppy male preference for receptive females.** Preference ratios were calculated as total time spent associating with receptive females by the total time associating with receptive and non-receptive females in dichotomous choice tests. Tests were performed to males naïve and experienced with female receptivity cues before (pre-treatment, black circles) and after (post-treatment, gray squares) a 45-day treatment in their respective experimental condition. Larger circles and squares indicate average preference ratio for each treatment and time of testing with 95% CI bars. I found no differences in the amount spent with receptive or nonreceptive females between naïve and experienced males in pre-treatment or post-treatment tests (see Table 2.1).
Table 2.1. Results for a Linear Mixed Model comparing preference for receptive females performed to male guppies naïve and experienced with female receptivity cues before and after a 45-day treatment in their respective experimental condition.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>t (df:119)</th>
<th>P – value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-0.02</td>
<td>0.03</td>
<td>-0.88</td>
<td>0.60</td>
</tr>
<tr>
<td>Male social treatment (experienced)</td>
<td>0.01</td>
<td>0.04</td>
<td>0.45</td>
<td>0.65</td>
</tr>
<tr>
<td>Time of testing (pre-treatment)</td>
<td>-0.01</td>
<td>0.04</td>
<td>-0.37</td>
<td>0.72</td>
</tr>
<tr>
<td>Batch (2)</td>
<td>-0.02</td>
<td>0.03</td>
<td>-0.62</td>
<td>0.53</td>
</tr>
<tr>
<td>Male social treatment:time of testing</td>
<td>0.04</td>
<td>0.06</td>
<td>0.72</td>
<td>0.48</td>
</tr>
</tbody>
</table>

2.3.3 No Choice Preference Tests

There was no significant difference between naïve and experienced males in their levels of display behaviour or the number of displays that were performed towards receptive versus non-receptive females (Figure 2.2a; Table 2.2). However, I found that, unlike naïve males, experienced males significantly increased the number of displays towards receptive females compared to non-receptive females (female status x social treatment: estimate$_{receptive \times experienced}$: $0.26 \pm 0.08$, $t = 3.01$, $p = 0.002$; Figure 2.2a; Table 2.2).

Experienced males exhibited significantly more sneak attempts than naïve males (male social treatment: estimate$_{experienced}$: $0.49 \pm 0.22$, $t = 2.19$, $p = 0.028$; Figure 2.2b; Table 2.2), and this difference was due to greater frequency of sneak attempts by experienced males toward non-receptive females. I observed no difference in sneak frequency between experienced and naïve males in tests with receptive females ($\log_{\text{mean}} \pm SE$; non-receptive females: naïve males $1.16 \pm 0.18$, experienced males $1.65 \pm 0.14$, t-ratio$_{df=119} = -2.19$, $p = 0.030$; $\log_{\text{mean}} \pm SE$; receptive females: naïve males $1.14 \pm 0.16$, experienced males $1.16 \pm 0.15$, t-ratio$_{df=119} = -0.07$, $p = 0.94$; Fig. 2.2b; Table 2.2). Additionally, naïve and experienced males showed no significant difference in the time spent following females in the trials, or in overall time spent following receptive and non-receptive females (Figure 2.2c; Table 2.2).
Experienced males showed decreased latency to perform sexual behaviour compared to naïve males in tests with non-receptive and receptive females (male social treatment: estimate\textsubscript{experienced} - 1.51 ± 0.36, z = -4.17, p < 0.001; Figure 2.2d; Table 2.2) However, post-hoc tests indicate that this decrease was significant just in tests with non-receptive females (log\textsubscript{mean ± SE}; non-receptive females: naïve males 4.28 ± 0.26, experienced males 2.76 ± 0.25, t-ratio\textsubscript{df=115} = 4.17, p < 0.001; mean ± SE; receptive females: naïve males 3.75 ± 0.26, experienced males 3.23 ± 0.25, t-ratio\textsubscript{df=115} = 1.44, p = 0.15; Fig. 2.2d; Table 2.2).

**Figure 2.2. The effect of previous experience with female receptivity cues in male sexual behaviour.** Quantification of (a) the number of sigmoid displays, (b) number of sneak attempts, (c) total time spent following females, and (d) latency to first sexual behaviour performed towards non-receptive and receptive females in guppy males naïve (black circles) and experienced (gray squares) with female receptivity cues. Larger circles and squares indicate average values of each behaviours with 95% CI bars. For number of displays, experienced males significantly increased the number of displays towards receptive females compared to non-receptive females (female status x social treatment: p = 0.002; see Table 2.2). Stars indicate significance in post-hoc comparisons of the male response between female receptivity levels at pre-treatment and post-treatment time of testing (p < 0.001 ***, p < 0.05 *).
### Table 2.2
Statistical tests for models comparing potential differences in behaviour in no choice tests with non-receptive and receptive females performed to male guppies naïve and experienced with female receptivity cues. Parameters with significant differences in bold.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>Statistic</th>
<th>df</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Display</strong></td>
<td>Intercept</td>
<td>2.57</td>
<td>0.20</td>
<td>z : 12.70</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Female status (receptive)</td>
<td>-0.05</td>
<td>0.06</td>
<td>z : -0.85</td>
<td></td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Male social treatment (experienced)</td>
<td>-0.05</td>
<td>0.17</td>
<td>z : -0.28</td>
<td></td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Female status:male social treatment</td>
<td>0.26</td>
<td>0.08</td>
<td>z : 3.01</td>
<td></td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td><strong>Sneak</strong></td>
<td>Intercept</td>
<td>1.15</td>
<td>0.17</td>
<td>z : 6.48</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Female status (receptive)</td>
<td>-0.01</td>
<td>0.20</td>
<td>z : -0.07</td>
<td></td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>Male social treatment (experienced)</td>
<td>0.49</td>
<td>0.22</td>
<td>z : 2.19</td>
<td></td>
<td><strong>0.028</strong></td>
</tr>
<tr>
<td></td>
<td>Female status:male social treatment</td>
<td>-0.47</td>
<td>0.26</td>
<td>z : -1.79</td>
<td></td>
<td>0.07</td>
</tr>
<tr>
<td><strong>Zero Inflation:</strong> Intercept</td>
<td>0.49</td>
<td>0.10</td>
<td>z : -3.37</td>
<td></td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Latency</strong></td>
<td>Intercept</td>
<td>4.27</td>
<td>0.26</td>
<td>z : 16.42</td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Female status (receptive)</td>
<td>-0.52</td>
<td>0.36</td>
<td>z : -1.42</td>
<td></td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>Male social treatment (experienced)</td>
<td>-1.51</td>
<td>0.36</td>
<td>z : -4.17</td>
<td></td>
<td><strong>&lt; 0.001</strong></td>
</tr>
<tr>
<td></td>
<td>Female status:male social treatment</td>
<td>0.99</td>
<td>0.51</td>
<td>z : 1.93</td>
<td></td>
<td>0.053</td>
</tr>
<tr>
<td><strong>Zero Inflation:</strong> Intercept</td>
<td>0.00</td>
<td>0.00</td>
<td>z : -0.00</td>
<td></td>
<td>0.996</td>
<td></td>
</tr>
<tr>
<td><strong>Time spent following</strong></td>
<td>Intercept</td>
<td>351.27</td>
<td>44.13</td>
<td>t_{3.7} : 7.95</td>
<td></td>
<td><strong>0.002</strong></td>
</tr>
<tr>
<td></td>
<td>Female status (receptive)</td>
<td>24.51</td>
<td>42.85</td>
<td>t_{61.5} : 0.57</td>
<td></td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Male social treatment (experienced)</td>
<td>13.70</td>
<td>51.52</td>
<td>t_{105.5} : 0.26</td>
<td></td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td>Female status:male social treatment</td>
<td>-61.06</td>
<td>59.30</td>
<td>t_{61.8} : -1.03</td>
<td></td>
<td>0.31</td>
</tr>
</tbody>
</table>

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

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

Contrary to our prediction, males with social learning experience of female receptivity cues did not become choosier or increase their preference towards receptive females in dichotomous choice tests. It may be that my experimental treatment changed the perception of naïve and experienced
males in future reproductive opportunities, potentially biasing the investment of naïve males in sexual behaviours that I observed in the first sexual encounter of their pre-treatment test (Fischer et al., 2008, Aich et al., 2021). Furthermore, determining the costs of sexual behaviours are challenging in benign lab environments where food is not a limiting factor. Future work incorporating resource limitation will be helpful to determine ecologically-relevant effects of social learning in male mating preferences. Yet, it is important to note that our results are concordant with a recent meta-analysis showing no evidence that mating status is an important factor for preference. Specifically, across species, virgin females are as choosy as mated females across reproductive isolation, inbreeding avoidance, and sexually transmitted disease scenarios (Richardson & Zuk, 2022). Our study here presents a similar finding, as males with no previous mating experience (naïve males) presented similar preferences to males with mating experience (experienced males). Hence, our study focusing on male mate choice on preference for higher quality females is in broad agreement with previous observations on the role of mating status for female preference across species.

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

The changes I observe in male guppies in behavioural repertoire and latency towards females based on previous experience with female receptivity cues add to the evidence suggesting that social environment and learning from previous experience can affect male sexual behaviour. Overall, our results are consistent with the idea that male guppies use their social environment to efficiently
tune their mating tactics, soliciting copulation in higher rates to receptive females and performing higher coercive copulation attempts towards non-receptive females.
Chapter 3: Artificial selection for orange colouration increases overall mating effort of male guppies

3.1 Introduction

Sexually selected signals are traits that attract mates and increase fitness through reproduction and can include visual, acoustic, and olfactory traits (Andersson 1994; Johnstone 1995). Colour signals are often costly (e.g. carotenoid-based colouration, Olson & Owens, 1998). Some models of sexual selection predict that colour signals are displayed by the most fit individuals (Olson & Owens, 1998). Consistent with this, the presence of elaborate sexual ornaments is often associated with high fitness and quality in terms of disease resistance, foraging efficiency, resource defense, survival, and reproductive success (Andersson 1994; Møller & Alatalo 1999; Jennions et al., 2001; Maynard Smith & Harper 2003). However, the specific relationship between sexual ornaments and fitness varies across species and environments (Chaine & Lyon 2008).

Intra-sexual specific variation in mating tactics, reflecting different ways to obtain fertilizations, is common across taxa (Taborsky et al. 2008). Individuals that differ in colouration often display different reproductive behaviours (Arnason 1978, Ficken et al. 1978, Nuechterlein 1981, Roulin et al 2003; Hogan-Warburg 1966; Furness 1987, Johnston & Janiga 1995). Often, colour signals are indicative of social dominance, with individuals showing these traits tending to use courtship to attract females whereas submissive individuals (less colourful) rely on sneaker tactics (Taborsky, 2001). Yet, it is unclear how variation in colour signals may be associated with the use of courting and coercive reproductive strategies.

Guppies are good model species to study the association between a sexually selected signal and sexual behaviours. Male guppies have vivid colour spots which show a great deal of heritable variation among individuals (Houde, 1992; Morris et al., 2020; Sato & Kawata, 2020). The saturation and size of orange spots is determined by carotenoid uptake implying that these traits are reflective of a male’s ability to effectively forage. Female guppies prefer males with larger and more saturated orange spots, as it is a potential indicator of a male quality and fitness (Kawamoto et al., 2021). Additionally, larger and more saturated orange spots are correlated with lower parasite count and increased sperm competitiveness (Locatello et al., 2006; Evans et
al., 2004). These correlations suggest that orange spots may be an indicator of fitness and female preference for this sexual signal might operate under a good genes model (Hamilton & Zuk, 1982; Zahavi, 1975).

Male guppies employ two major mating tactics, courtship and coercion. Courtship displays occur when the male positions himself in front of the female with an S-shaped posture soliciting copulation. Coercive behaviours are unsolicited sneak attempts at inseminating a female from behind by thrusting his gonopodium at the female’s urogenital pore (Liley 1966). Most male guppies will use both of these tactics. However, the frequency of use may be different based on the different weights of costs and benefits. Some major factors that will affect the rate of use of different tactics include resource availability, energy reserves, predation, and condition of the participant (Farr, 1980a,b; Endler, 1987: Godin 1995). Courtships are thought to be more energetically costly than sneak attempts, but also more likely to result in fertilization (Magurran, 2005; Polverino, 2019).

The causal relationship between guppy courtship displays and male colouration is unclear. Some reports have found no association between orange area and courtship display (Herdegen-Radwan et al. 2021), whereas others have suggested that the orange area is associated with more displays (Jirotkul 2000). Here I build on this by testing for both the association between colour and display, as well as coercive mating tactics in male guppies artificially selected for high and low proportion of orange colouration. Based on theoretical expectations of fitness maximization and previous findings that orange was associated with more courtship displays (Jirotkul 2000), I predicted that high-orange males should perform more courtships than low-orange males. Courtship displays require a greater input of energy to elicit a female response, but are associated with higher reproductive fitness (Farr 1980b). I additionally predicted that low-orange males might bias their mating effort towards coercive behaviours, consistent with the theory that individuals express the mating tactic associated with the highest pay-off given their condition (Gross 1996).
3.2 Methods

3.2.1 Experimental evolution of orange colouration in guppies

I used artificial selection lines of guppies derived from our laboratory-adapted stock population, originally collected from the high predation region of the Quaré River, Trinidad. The artificial selection regime will be described elsewhere (van der Bijl et al in prep). Briefly, newborns were collected from our stock populations until they could be accurately sexed by the development of a gonopodium, a modified anal fin (Houde, 1997; Liley, 1966). Males were photographed once they reached sexual maturity, as evidenced by the development of male colouration, and the 30 males with the highest percentage of orange and the 30 males with the lowest percentage of orange (high orange, and low orange selection lines from hereon) were selected and paired with virgin females. The selection procedure was repeated on the offspring of these crosses for three generations. In total, I replicated this process three times to create six artificial selection lines (three high orange and three low orange lines). At generation F3, males from high orange and low orange selection lines presented a difference in total orange colouration in body and tail, with high orange males having a significantly more total orange colouration in body and tail (van der Bijl et al in prep).

Throughout the experiment, all aquaria contained gravel and water filters. Fish were raised at a water temperature of 25 °C with a 12:12 light:dark schedule, and fed a daily diet of flake food (Hikari Fancy Food) and live Artemia brine shrimp. Experiments were approved by institutional animal ethics protocols.

3.2.2 Sexual behaviour following experimental evolution for orange colouration

To evaluate sexual behaviour in male guppy selection lines, I used offspring from the F3 generation. Sample sizes from each selection line were $n_{\text{high1}}=22$, $n_{\text{low1}}=23$, $n_{\text{high2}}=22$, $n_{\text{low2}}=21$, $n_{\text{high3}}=19$, $n_{\text{low3}}=19$. I housed newborn F3 offspring from each selection line in groups and isolated males once they showed early onsets of sexual maturity with the development of a gonopodium. At approximately three months of age, all individuals had developed complete colour patterns associated to sexual maturity. At this age, I measured sexual behaviour in males from high orange and low orange selection lines by performing a no choice test with a
nonreceptive female. The use of nonreceptive females is common to evaluate male sexual behaviour in poecilids given that it allows standardization of female receptivity and behaviour towards different types of males (Guevara-Fiore et al 2010, Ptacek & Travis 1997). To generate nonreceptive females for behavioural trials, I placed 3-month-old virgin females with sexually mature males from our stock lab population in a 1:1 ratio 14 days prior to their use in behavioural trials with males of interest (Guevara-Fiore et al 2010).

I performed behavioural trials in a circular arena (diameter = 47 cm) which was sheltered to prevent disruption. From holding tanks, I netted fish, placed them in a glass bowl with water and transferred them to the testing apparatus to minimize handling stress prior to the trial. After a five-minute acclimatization period, I filmed fish behaviour for 15-minute periods using an OBSBOT webcam (1080P at 30 fps). For accurate identification of fish with tracking software, I placed focal individuals in the experimental arena in 20 second intervals, with females always placed first. I changed water between each test to avoid interference of olfactory cues from previous trials, and conducted all trials between 7am - 1 pm.

Using recorded videos in a randomized order, I scored male sexual behaviour to quantify: i) frequency and duration of sigmoid displays, characterized by an S-shaped posture soliciting copulation; ii) frequency of sneak attempts, unsolicited attempts of inseminating a female from behind by thrusting his gonopodium at the female’s urogenital pore iii) copulations following female consent (Liley 1966). From our behavioural scoring I obtained the number of displays shorter than one second (short displays), the number of displays longer than one second (long displays), the total duration of short and long displays (display duration), the number of sneak attempts (sneak attempts) and the time to the first sexual behaviour (latency) for each fish. I also obtained a ratio between courtship and coercive behaviours (display-sneak ratio) by subtracting normalized values of number of sneak attempts to normalized values of the number of total displays (short + long displays). Additionally, I used idTracker to track recorded videos and obtain positional data of both fish during each trial (Pérez-Escudero et al. 2014). From tracking data, I extracted the time that males spent following a female for each trial, i.e. the time spent at a distance lower than 4 cm, equivalent to two female body sizes (time following), and median speeds for male and female as an activity proxy (activity).
From our full dataset, I disregard trials in which females showed receptivity towards males (observation of consented copulation during scoring; n = 6). I additionally disregarded trials in which males or females presented signs of stress due to the experimental environment based on objective measurements from tracking data (activity < 0.5 or > 4; time following < 3.5% of the total time trial, n = 18). Our final dataset for statistical analyses included 95 individuals with balanced number between selection line treatments and replicates, $n_{\text{high1}}$ = 14, $n_{\text{low1}}$ = 16, $n_{\text{high2}}$ = 15, $n_{\text{low2}}$ = 16, $n_{\text{high3}}$ = 17, $n_{\text{low3}}$ = 17.

To compare short displays, long displays, display duration, and sneak attempts between high and low orange males, I fit independent generalized mixed models using a Poisson distribution and a logit link function for the conditional mean in the package glmer (Bates et al., 2007). I used each quantified behaviour as the dependent variable, selection line (high or low orange) as fixed effect, and female and male body size at the time of testing as covariates. I included the replicate line as a random factor in the model. For time spent following and latency to first sexual behaviour I fit a generalized mixed model with glmmTMB package (Brooks et al., 2017), the time spent using an analogous model structure, but adding a unique ID for each test to avoid overdispersion of the model. For display-sneak ratio I used a Linear Mixed Model with analogous structure using lmer package (Bates et al., 2007). I evaluated the adequacy of our fitted models using scaled-residuals quantile-quantile plots, residual versus predicted values plots in the DHARMa package (Hartig 2018). I processed the parameters of our statistical models using Wald tests obtained via the parameters package (Lüdecke 2020). All analyses were performed in R (v. 4.1.3; R Core Team 2022).

3.2.3 Quantification of colour patterns and morphology in artificially selected lines

After completion of each behavioural trial, I gently transferred males to a small glass aquarium and photographed them for colour and size quantification with a Canon EOS Rebel T7i camera. I photographed both sides of each fish and quantified the number of pixels with orange and black colouration separately. The artificial selection regime will be described elsewhere (van der Bijl et al in prep). I calculated the fish standard length (tip of the mouth to the end of the bottom, not including the tail), tail length and tail area using a scale and the quantified number of pixels in
I performed camera colour calibration with a Calibrite ColourChecker (X-Rite Inc.) daily.

I compared the percentage of orange colouration, the percentage of black colouration, tail standard area, body standard length and tail area between high orange males and low orange males. For this, I fit independent linear mixed models, with each morphological or colouration trait as the dependent variable. I included selection line as a fixed effect, and a random intercept for each replicate selection line and a random slope for colour selection line within each replicate. Analyses were carried out in the R statistical package, version 4.1.3 (R Core Team, 2022).

3.3 Results

3.3.1 Sexual behaviour following experimental evolution for orange colouration

High orange males performed short and long sexual displays significantly more frequently than low orange males (short displays: mean ± SE: high orange males 10.11 ± 1.30, low orange males 8.2 ± 0.91, z = 2.99, p = 0.0028; long displays: high orange 2.78 ± 0.52, low orange 2.21 ± 0.34, z = 2.12 p = 0.034). In addition, high orange males showed a longer display duration than low orange males (mean ± SE: high orange 10.89 ± 2.6, low orange 8.99 ± 1.61, z = 2.21, p = 0.027; Figure 3.1, Table 3.1). High orange males performed sneak attempts significantly more frequently (mean ± SE: high orange 1.78 ± 0.43, low orange 1.32 ± 0.25, z = 2.40, p = 0.016) and followed females significantly longer (mean ± SE: high orange 316.15 ± 29.59, low orange 261.96 ± 20.84, z = 2.40, p = 0.017) than low orange males. I found no differences between high orange and low orange males in the display-sneak ratio, although both groups showed higher rates of courtship than those of coercion in the test (mean ± SE: high orange 0.10 ± 0.36, low orange 0.10 ± 0.34, z = 0.03, p = 0.96; Figure 3.3). I observed no significant difference for the latency to first display between high orange and low orange males (mean ± SE: high orange 295.50 ± 31.35, low orange 281.15 ± 22.81, z = 0.31, p = 0.76; Table 3.1).
Figure 3.1. The effect of artificial selection for orange colouration on display sexual behaviour of male guppies. Comparison of a) frequency of short displays; b) frequency of long displays; and c) duration of display in male guppies following three generations of artificial selection for high (n = 49) and low (n = 46) proportion of orange in their body and tail. Low orange selected males in grey circles, high orange selected males with orange squares. Larger circles and squares indicate averages for each treatment and exposure time with 95% CI bars. All display sexual behaviours quantified showed significant differences between the two selection lines with high orange performing more. Stars indicate significance, with p< 0.01 **, p< 0.05 * (see Table 3.1).
Figure 3.2. The effect of artificial selection for orange colouration on coercive sexual behaviours of male guppies. Comparison of a) frequency of sneak attempts and b) time spent following in male guppies following three generations of artificial selection for high (n = 49) and low (n = 46) proportion of orange in their body and tail. Low orange selected males in grey circles, high orange selected males with orange squares. Larger circles and squares indicate averages for each treatment and exposure time with 95% CI bars. All sexual behaviours presented a significant difference between the two selection lines with high orange performing more. A star indicates significance p< 0.05 * (see Table 3.1).
Table 3.1. Results from statistical models comparing potential differences in sexual behaviour between high orange and low orange artificially selected male guppies. Parameters with significant differences in bold.

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Parameter</th>
<th>Estimate</th>
<th>SE</th>
<th>Z - value</th>
<th>P - value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short displays</td>
<td>Intercept</td>
<td>3.19</td>
<td>2.17</td>
<td>1.47</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Selection Line (high/low orange)</td>
<td>0.85</td>
<td>0.28</td>
<td>2.99</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Female body size (cm)</td>
<td>-0.50</td>
<td>0.44</td>
<td>-1.15</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Male body size (cm)</td>
<td>-0.76</td>
<td>1.37</td>
<td>-0.55</td>
<td>0.58</td>
</tr>
<tr>
<td>Long displays</td>
<td>Intercept</td>
<td>-1.90</td>
<td>2.68</td>
<td>-0.71</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>Selection Line (high/low orange)</td>
<td>0.73</td>
<td>0.35</td>
<td>2.12</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>Female body size (cm)</td>
<td>0.63</td>
<td>0.51</td>
<td>1.22</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>Male body size (cm)</td>
<td>0.094</td>
<td>1.68</td>
<td>0.056</td>
<td>0.96</td>
</tr>
<tr>
<td>Display duration</td>
<td>Intercept</td>
<td>-1.54</td>
<td>3.83</td>
<td>0.40</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>Selection Line (high/low orange)</td>
<td>1.11</td>
<td>0.50</td>
<td>2.21</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>Female body size (cm)</td>
<td>0.74</td>
<td>0.75</td>
<td>0.99</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Male body size (cm)</td>
<td>-0.080</td>
<td>2.38</td>
<td>-0.034</td>
<td>0.97</td>
</tr>
<tr>
<td>Sneak attempts</td>
<td>Intercept</td>
<td>-3.75</td>
<td>3.61</td>
<td>-1.04</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>Selection Line ((high/low orange)</td>
<td>1.14</td>
<td>0.47</td>
<td>2.40</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Female body size (cm)</td>
<td>-1.63</td>
<td>0.76</td>
<td>-2.14</td>
<td>0.033</td>
</tr>
<tr>
<td></td>
<td>Male body size (cm)</td>
<td>4.07</td>
<td>2.21</td>
<td>1.84</td>
<td>0.066</td>
</tr>
<tr>
<td>Time spent</td>
<td>Intercept</td>
<td>5.44</td>
<td>1.36</td>
<td>4.02</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>following (s)</td>
<td>Selection Line (high/low orange)</td>
<td>0.43</td>
<td>0.18</td>
<td>2.40</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>Female body size (cm)</td>
<td>Male body size (cm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------</td>
<td>---------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Display-sneak ratio</td>
<td>Intercep</td>
<td>0.76 0.43 1.75 0.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selection Line (high/low orange)</td>
<td>0.00 0.18 0.04 0.96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female body size (cm)</td>
<td>0.05 0.27 0.51 0.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male body size (cm)</td>
<td></td>
<td>-0.56 0.85 -2.24 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency to display (s)</td>
<td>Intercep</td>
<td>5.57 2.38 2.34 0.019</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Selection Line (high/low orange)</td>
<td>0.089 0.29 0.31 0.76</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female body size (cm)</td>
<td>0.29 0.50 0.59 0.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male body size (cm)</td>
<td>-0.45 1.45 -0.31 0.76</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.3. Comparison of mating tactics used by guppies artificially selected for total area of orange. Ratio of frequency of courtship display and coercive behaviours (sneak attempts) in male guppies following three generations of artificial selection for high (n = 49) and low (n = 46) proportion of orange in their body and tail. Low orange selected males in grey circles, high orange selected males with orange squares. Larger circles and squares indicate averages for each treatment and exposure time with 95% CI bars.
3.3.2 Quantification of colour patterns and morphology in artificially selected lines

Comparisons of male colouration between high orange and low orange males showed significant differences in the percentage of orange (mean ± SE: high orange 9.20 ± 2.69, low orange 6.72 ± 3.30, F = 52.98, df = 1, p = 0.002), but no significant difference in the percentage of black (mean ± SE: high orange 2.70 ± 0.722, low orange 2.79 ± 0.79, F = 1.12, t = -1.029, df = 1, p = 0.30; Figure 3.4, Table 3.2). I did not find significant differences between high orange and low orange males for any morphological trait measured: body length (mean ± SE: high orange 1.38 ± 0.10, low orange 1.40 ± 0.10, F = 2.33, df = 1, t = -1.51, p = 0.13); tail length (mean ± SE: high orange 0.50 ± 0.05, low orange 0.49 ± 0.06, F = 1.46, df=1, t = 1.18, p = 0.23) or tail area (mean ± SE: high 0.19 ± 0.04, low orange 0.19 ± 0.03, F = 0.22 df = 1, t = 0.50, p = 0.64; Figure 3.4, Table 3.2)
Figure 3.4. Colouration and morphological traits in guppies artificially selected for total area of orange. Comparison of a) % of orange colouration b) % of black colouration c) standard length d) tail length and e) tail area in male guppies following three generations of artificial selection for high (n = 49) and low (n = 46) proportion of orange in their body and tail. Orange colouration was significantly different between individuals from these selection lines used for behavioural experiments (see Table 3.2). Horizontal lines indicate medians, boxes indicate the interquartile range, and whiskers indicate all points within 1.5 times the interquartile range.
Table 3.2. Results for linear mixed model comparing morphological traits in high orange and low orange artificially selected male guppies. Significant effects (p < 0.05) in bold.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Df</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange Colouration</td>
<td>1</td>
<td>258</td>
<td>258</td>
<td>52.98</td>
<td>0.002</td>
</tr>
<tr>
<td>Black Colouration</td>
<td>1</td>
<td>0.72</td>
<td>0.72</td>
<td>1.12</td>
<td>0.29</td>
</tr>
<tr>
<td>Standard Length (cm)</td>
<td>1</td>
<td>0.024</td>
<td>0.024</td>
<td>2.33</td>
<td>0.13</td>
</tr>
<tr>
<td>Tail Length (cm)</td>
<td>1</td>
<td>0.0045</td>
<td>0.0045</td>
<td>1.46</td>
<td>0.23</td>
</tr>
<tr>
<td>Tail Area (cm²)</td>
<td>1</td>
<td>0.00025</td>
<td>0.00025</td>
<td>0.22</td>
<td>0.64</td>
</tr>
</tbody>
</table>

3.4 Discussion

Orange colour is particularly important in guppy sexual selection, as female guppies prefer males with larger and brighter orange colouration (Houde & Endler, 1990; Endler & Houde; 1995; Kawamoto et al., 2021; Karino et al 2010; Pitcher et al., 2007; Karino & Urano, 2008; Karino & Shinjo, 2004). The presence of more orange colouration in male guppies has been associated with higher fitness, such as lower parasite load, higher sperm competitiveness and better predator evasion (Evans et al., 2004; Godin & McDonough, 2003; Houde, 1997; Locatello et al., 2006; Prokop et al. 2012). However, the direct relationship between colour and mating tactics in guppies is unclear. Using male guppies artificially selected for high and low proportion of orange colouration, I performed a series of no-choice tests with nonreceptive females to determine the relationship between a key sexually selected colour signal in the species and sexual behaviours. I found that high orange males exhibit an increased overall mating effort by performing courting and coercive behaviours at higher rates than low orange males.

In line with our prediction, high orange males performed sigmoid displays more frequently and for longer time (Fig 3.1). These results are in agreement with a previous study in guppies that found a positive correlation between the proportion of orange colouration and the time that males spent courting females when swimming in groups. However, these results were not replicated in a recent study assessing courtship behaviour in males artificially selected for a high proportion of
orange (Herdegen-Radwan et al. 2021). It is however important to note that this study did not measure courtship duration and that the behavioural trials to assess the frequency of courtship display only run for five minutes. The direct association I found between orange colouration and courting behaviour following artificial selection for orange colouration suggests that these traits might co-evolve under a good genes model in this species, i.e. as indicators of male qualities that increase the fitness of offspring (Hamilton & Zuk, 1982; Zahavi, 1975). Indeed, given that all males were fed the same diet and that I observed no correlated changes in body size (condition) across high and low orange males, it is likely that co-variation in male orange area and courtship display rates in our experiment are driven by genetic heritability (Houde et al., 1992; Morris et al., 2020, Kawamoto et al., 2021).

Contrary to our prediction, high orange males also increase their mating effort in relation to coercive behaviours, i.e. they performed more sneak attempts and chased females for longer times in our experiments (Fig 3.2). These differences were not driven by changes in mating tactics of low orange males, as both high and low orange males on average performed more courting behaviours than coercive ones, with no observed differences in this ratio among groups (Fig 3.3.). To our knowledge, this is the first empirical test on the association between male colour and rate of coercive behaviours in the guppy. Our findings suggest that colouration is not associated with the use of alternative mating tactics in guppies, but with overall sexual vigour. It is plausible that high orange males through their overall increase in multiple mating tactics maximize their chances to inseminate females and increase their fitness this way. This might be related to previous results in guppies showing that males with lower parasite loads (associated with more vigour) are more aggressive and win more contests (Kennedy et al., 1987; Kolluru et al., 2008).

I observed no significant differences between high orange and low orange males in latency to perform sexual behaviours in the test (Table 3.1), suggesting that our results are not related to differences in stress response between them in the experimental setup. Furthermore, I observed no differences between high and low orange males in body size or tail size, traits that might be associated with a better condition in this species (Bischoff et al., 1985; Auld et al., 2017). As
such, I think it is unlikely that differences in overall sexual vigour were driven by a worse condition of males selected for lower orange colouration. However, it is worth noting that males in our laboratory tests were not subjected to energetic constraints, and I did not test potential effects of predation or social environment experienced, factors known to affect guppy mating tactics (Godin 1995; Plath et al., 2019; Kolluru et al., 2008; Guevara-Fiore & Endler, 2018; Řežucha & Reichard, 2014). Furthermore, male guppy colouration and courtship behaviour are regulated by androgens (Baatrup & Junge, 2001; Pandey, 1969; Borg, 1994), and increased orange area may be related to increased testosterone levels. It may be that selection to increase orange area has led to correlated changes in mating behaviour via testosterone. Future studies incorporating resource limitation, predation, and social environment factors, as well as the role of hormonal changes will be valuable in further assessing the link between mating tactics and colour in guppies.
Chapter 4: General Conclusion

4.1 Thesis Summary

The overall objective of this thesis was to investigate how male guppies differ in sexual behaviours depending on two different factors, social learning during early development, and overall colouration, measured by the proportion of orange on the body and tail. Specifically, I looked at how the mating effort in male guppies changed by measuring the use and potential difference in frequency of their mating tactics, coercion and courtships. I used mating tactics as a proxy of measurement because in many species it has been seen to be related to individual energy reserves and fitness. In our research there was no distinction between tactic and strategy. A distinction tends to be made in the evolutionary game theory models (Maynard Smith 1982) where strategy relates to a particular life-history pattern (Gross 1996) and tactic classifies the application of rules that are a part of the strategy (Shuster and Wade 2003). But in reality, the borders between the classification of strategy versus tactic are vague and flexible.

First, I investigated the effect of how the social environment affects male mate choice in the guppy (Poecilia reticulata). I evaluated whether male guppies with previous social experience of female receptivity cues learn to prefer and adapt their behavioural repertoire towards females with higher receptiveness levels, as this represents an optimal use of time and energy and is more likely to result in insemination. Sexual receptiveness towards males strongly correlates with the female guppy reproductive cycle. (Liley, 1966, Houde, 1997). Therefore, I predicted that male guppies would use learning from their social environment to shift their preference levels to favour receptive females. Similarly, I predicted that the social environment would affect how males adapt their behavioural repertoire depending on whether they encounter receptive or non-receptive females. I saw that males with previous access to female receptivity cues exhibited significantly greater frequency of coercive sexual behaviours and lower latency to first sexual behaviour to non-receptive females than naïve males. In addition, only experienced males significantly increased their number of displays towards receptive females compared to the number of displays performed with non-receptive females. However, previous experience with receptivity cues did not affect the strength of guppy male preference based on female mating status. Males in environments with social learning experience of female receptivity cues did not become choosier or increase their preference towards receptive females in dichotomous choice tests. Overall, our results are
consistent with the idea that male guppies use learning from ones social environment to efficiently tune their mating tactics, soliciting copulation in higher rates to receptive females and performing higher coercive copulation attempts towards non-receptive females.

Second, I tested for both the association between colour and display, as well as sneaky mating tactics in male guppies that were artificially selected for high and low proportion of orange colouration. Based on theoretical expectations of fitness maximization and previous findings that orange was associated with more courtship displays (Jirotkul 2000), I predicted that high orange males should perform more courtships than low orange males. Courtship displays require a greater input of energy to elicit a female response, but are associated with higher reproductive fitness (Farr 1980b). I additionally predicted that low orange males might bias their mating effort towards coercive behaviours, consistent with the theory that individuals express the mating tactic associated with the highest pay-off given their condition (Gross 1996). As our high orange colouration males sneaked and displayed more than our low orange colouration males I saw that colouration is not associated with the use of AMTs in guppies, but with overall sexual vigour.

4.2 Chapter 2 Results, Limitations and Potential Future Research

One of the most significant determinants of a male's mating success is his own mating effort (Edward and Chapman 2011). Our results add to research on mating effort and the ability to adjust one’s behavioural repertoires in guppies in a way to maximize fitness. First, I looked into the effect of the social environment on male mate choice in guppies. In previous research guppies have been shown to have a preference for receptive females (Guevara-Fiore & Endler, 2018; Guevara-Fiore et al., 2009). I chose to add to this research to see if males were able to learn about and have a preference for receptivity by setting up two different social environments. Experienced males were grown up in the presence of females and naïve males were grown up in the absence of females and no previous interactions were allowed. Our experiment did both a dichotomous choice test and a no choice test using receptive and nonreceptive females. Our dichotomous choice test did not show an increase in preference towards receptive females. This aligns with previous research showing that mating status may not be an important factor in preference (Richardson & Zuk, 2022). Our no choice test showed that males with previous access to female receptivity cues exhibited significantly greater frequency of coercive sexual
behaviours and lower latency to first sexual behaviour to non-receptive females than naïve males. In addition, only experienced males significantly increased their number of displays towards receptive females compared to the number of displays performed with non-receptive females. These results show a key role of learning from one’s social environment driving preferences for high quality females in this species.

It could be argued that mating preferences may be stronger in choice tests compared to no-choice design, as males can select the female that is more likely to result in insemination. However, one could also argue that there is an increased risk of being rejected by the only potential mate in a no-choice test (Dougherty & Shuker, 2015), and this could make males more careful in tuning their mate strategy to female receptivity cues. A very valuable component to our research was the use of both dichotomous and no-choice tests. Our different complementary experimental testing methods are in agreement in that there were no observed changes in overall preference for receptive females or differences in overall sexual behaviour levels based on female receptivity status. However, our observations of changes in rates of sexual display and coercive copulations depending on female mating status suggest that future mate choice studies should incorporate both methodologies. Including the results of our 2 experiments from the same study will help to reduce confounding factors such as effects associated with individual researchers, animal stocks, and so forth.

It is a potential that receptive females did not elicit a higher rate of courtship displays from experienced males in comparison to our naïve males because they were energy depleted, but in contrast I did see the number of sneak attempts remain high for nonreceptive females because they are less energetically expensive. Males may use the least expensive tactic when insemination probabilities are low. This is in agreement with previous studies which have shown that higher female encounter rate results in a reduction of courtship effort in guppies (Cattelan et al., 2016; Devigili et al., 2015; Jordan & Brooks, 2012). It was also seen that guppy males that experienced high mating success with receptive females decreased their courtship displays but increased the frequency of sneak attempts, whereas low mating success males previously rejected by nonreceptive females showed an increase in courtship and a decrease in sneak copulation attempts when mated to nonreceptive females (Guevara-Fiore & Endler, 2018). This could mean that experienced males may already have received high mating success with the
receptive females of their aquaria and present similar patterns that Guevara-Fiore & Endler (2018) found.

Our experimental design does not allow for us to see which specific mechanism leads to changes in behaviour between experienced and naïve males. Prior experience with females might lead to males better recognizing which mating tactics provide higher success. Therefore, it may be that our experimental treatment might have changed the perception of naïve and experienced males in future reproductive opportunities, potentially biasing the investment of naïve males in sexual behaviours that I observed in the first sexual encounter of their pre-treatment test (Fischer et al., 2008, Aich et al., 2021). An option for the future would be to add neurogenomic tests to see what types of cells/neurons are expressing genes related to male preference. Furthermore, determining the costs of sexual behaviours is challenging in benign lab environments where food is not a limiting factor. Future work incorporating resource limitation will be helpful to determine ecologically-relevant effects of learning from one’s social environment in male mating preferences.

4.3 Chapter 3 Results, Limitations and Potential Future Research

The presence of more orange colouration in male guppies has been associated with higher fitness, such as lower parasite load, higher sperm competitiveness and better predator evasion (Evans et al., 2004; Godin & McDonough, 2003; Houde, 1997; Locatello et al., 2006; Prokop et al. 2012). However, the direct relationship between colour and mating tactics in guppies is unclear. I found that high orange males exhibited an increased overall mating effort by performing courting and coercive behaviours at higher rates than low orange males. The direct association I found between orange colouration and courting behaviour following artificial selection for orange colouration suggests that these traits might co-evolve under a good genes model in this species, i.e. as indicators of male qualities that increase the fitness of offspring (Hamilton & Zuk, 1982; Zahavi, 1975). The colouration differences seen between our high and low orange males are likely due to genetic differences (versus difference in diet’s carotenoid level) because all guppies were fed the same diet. Genetic quality is defined based on the contribution a gene variant or genotype makes to an individual’s fitness; an individual is of higher genetic quality when it possesses an allele or genotype that increases its fitness relative to
that of an individual with a different allele or genotype. Fitness, in turn, can be defined by the individual’s lifetime reproductive success (LRS), which is composed of both survivorship and breeding success (Neff & Pitcher, 2005). The good genes model of sexual selection assumes that extreme ornaments indicate the genetic quality of the bearer (breeding value for fitness; Hamilton & Zuk, 1982; Zahavi, 1975).

A limitation of our study was similar to the other study in which our laboratory tests were not subjected to energetic constraints, and I did not test potential effects of predation or social environment experienced, factors known to affect guppy mating tactics (Godin, 1995; Plath et al., 2019; Kolluru et al., 2008; Guevara-Fiore & Endler, 2018; Řežucha & Reichard, 2014). For example, Godin (1995) found that focal male guppies displayed to females significantly less often on average, and conversely attempted sneak copulations more often, in the presence of the predator model than in its absence. It would be interesting to see if there was a difference in the number of sneak copulations from high or low orange males in the presence of a predator. Additionally running a fitness test on the high-orange and low-orange males would be beneficial as this would enable a direct evaluation of colouration’s effect on fitness. Previous literature has studied guppy fitness via swim performance (swimming is how fish escape predators, capture food, habitat shifts and reproduction; Gordon et al., 2015). This can be done by using flow chambers and changing the rate of flow of water (current) and calculate the guppies swim speed. Those who can swim faster in faster current water would be considered more fit (Nicoletto, 1991).

Another possibility for why high orange males performed more sexual behaviours is testosterone. Male guppy colouration, including orange, and courtship behaviour are regulated by testosterone (including androgen; Baatrup & Junge, 2001; Pandey, 1969; Borg, 1994). This suggests that the increased orange area in up-selected lines may be related to increased testosterone levels. Future research may want to expand the link that has been seen between colouration and sexual behaviour and link it to hormonal levels in animals. Currently, the understanding of the mechanisms that control hormonal pathways in male sexual characteristics of guppies are not fully understood. However, body colouration and courtship behaviours are known to be regulated by androgens (Baatrup & Junge, 2001; Pandey, 1969; Borg, 1994).
References


Kolluru GR, Grether GF, Dunlop E, South SH. 2008. Food availability and parasite infection influence mating tactics in guppies (*Poecilia reticulata*). *Behavioral Ecology. 20:*131–137


Appendix A

Table A1. Results of linear model comparing colouration and morphological traits in male guppies used for social experience experimental treatments

<table>
<thead>
<tr>
<th>Trait</th>
<th>Sum Sq</th>
<th>Mean Sq</th>
<th>F value (df = 1)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orange Colouration</td>
<td>1.1</td>
<td>1.09</td>
<td>0.12</td>
<td>0.72</td>
</tr>
<tr>
<td>Black Colouration</td>
<td>0.14</td>
<td>0.13</td>
<td>0.15</td>
<td>0.69</td>
</tr>
<tr>
<td>Standard Length (cm)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.93</td>
</tr>
<tr>
<td>Tail Length (cm)</td>
<td>0.012</td>
<td>0.01</td>
<td>3.08</td>
<td>0.08</td>
</tr>
</tbody>
</table>
Figure. A1. Body morphology and colouration in experienced and naïve males. No significant differences were found between randomly assigned males to naïve (n = 31) and experienced (n = 31) treatments for (a) tail length, (b) body length, (c) proportion of black, or (d) proportion of orange. For all boxplots, horizontal lines indicate medians, boxes indicate the interquartile range, and whiskers indicate all points within 1.5 times the interquartile range.