

**Influences of male and female phenotypes on male  
mate-choice copying in the Trinidadian guppy (*Poecilia  
reticulata*)**

**by**

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## ABSTRACT

Mate-choice copying in males is a form of social learning whereby an observer male modifies his inherent mating preference after observing a model male sexually interact with a female he did not initially prefer, and copies the mate preference of the model male. Little is known about such copying behaviour in males and how the phenotypes of males and(or) females interact to influence the likelihood of mate-choice copying and the strength of the copying response. Using the Trinidadian guppy (*Poecilia reticulata*), I investigated whether the relative sexual attractiveness of males influences the likelihood of mate-choice copying in males, and found that the highest rates of copying occurred when the model male was less sexually attractive than the observer male. Second, I tested whether the relative difference in the body size of paired females influenced the likelihood of male mate-choice copying, and did not find any evidence for such an effect.

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# **Chapter 1**

## **GENERAL INTRODUCTION**

## **Preamble**

Over the past 30 years, numerous studies have showed that individuals may use social information (*sensu* Danchin et al. 2004) to make mate-choice decisions under certain circumstance. A common form of socially-mediated mate choice is mate-choice copying, whereby an individual copies the observed mate choice of another same-sex conspecifics. Although female mate-choice copying has been extensively studied in several taxa, including insects, fishes, birds, and mammals, there is little information on male mate-choice copying (reviewed in Vakirtzis 2011, Witte et al. 2015). Furthermore, there are even fewer studies that investigate how male and female phenotypes might influence the frequency and strength of male mate-choice copying. Therefore, in this thesis, I investigate the potential influence of the phenotypes of sexually-interacting males and females on male mate-choice copying using the Trinidadian guppy (*Poecilia reticulata*) as a model system.

In this Chapter 1 (General Introduction), I summarize what is known about male mate-choice copying and under which circumstances it can occur. I then describe my study species, the Trinidadian guppy, and provide a rationale for using it as a model organism for investigating male mate-choice copying. I conclude the General Introduction with an overview of the organizational structure of my thesis.

## **An overview of mate-choice copying**

Animals continuously receive information from their internal and external environments via sensory inputs to the brain. Across species, some of this information may be biologically relevant while most information is irrelevant at any given time. The

ability of an individual to decipher relevant from irrelevant information can affect its fitness through its behavioural decisions in the contexts of foraging, predator avoidance and mating, for example (reviewed in Valone 2007).

Information from the external environment can be obtained personally (first-hand) or socially from others (Westneat et al. 2000; Danchin et al. 2004). Personal information is generally obtained via trial and error sampling of the environment, which could be more costly than beneficial (Danchin et al. 2004; Kendal et al. 2005). Social information, however, is usually less costly as the observing individual does not need to extensively sample the environment, which can be associated with costs such as energy expenditure, lost opportunities and risk of predation. Social information is useful when the focal individual does not have prior information on a particular environmental context or when acquiring personal information is very costly. For example, when a novel food item was presented to infant common marmosets (*Callithrix jacchus*) without parental guidance, the infants were more naïve and less likely to sample the novel food item (Voelkl et al. 2006). However, when adults were present, the infants were more likely to consume the novel food item, which suggests that adults can socially convey information regarding the safety of novel food items to their observing infants. Another example of neophobia and use of social information is in rat pups (*Rattus norvegicus*), which avoid new food items until the adults demonstrate that the novel item is non-toxic (Galef and Clark 1971). An animal can also avoid predation by observing another individual conduct risky behaviour towards a predator, which could result in the actor being killed (Valone 2007). As for mate choice, a sexually naïve individual may indirectly obtain information on prospective

mates by observing an experienced conspecific select a mate, and then subsequently use this socially-acquired information in making its own mating decisions.

The life-history phase, the internal state and phenotype of observers and models and certain ecological and demographic factors can potentially influence the likelihood and strength of mate-choice copying (reviewed in Vakirtzis 2011, Witte et al. 2015). For example, older, more experienced female guppies are less likely to copy the apparent mate choice of younger, less experienced females than *vice versa* (Dugatkin and Godin 1993). Thus, there is a perceived trade-off such that when an individual has had success selecting mates in the past, then using information from a younger individual may be less important than their own personal information (Kendal et al. 2005). Furthermore, when a young individual has not had experience choosing a mate, it might be in their best interest to attend to how more experienced individuals mate (Dugatkin and Godin 1993). However, it might be costly to use social information as it could conflict with the individual's personal information. This in turn could result in an unfavourable outcome when social information is used *in lieu* of personal information. In summary, using social information in a certain context can provide benefits to an observing individual, whereas the use of the same information in another context might be costly and disadvantageous.

In most species, mating is not random and individuals within populations vary widely in their mating and reproductive success (Andersson 1994). Mate-choice is a non-random process whereby individuals of one sex prefer to mate with certain individuals, and not others, of the opposite sex based on specific preferred traits, which can result in reproductive skew among individuals (Pruett-Jones 1992). In general, males select large and more fecund females, and females generally select large and(or) ornamented males

(Andersson 1994). Mate choice, however, does not come without costs. It may be costly to search for and assess mates as this detracts time from foraging or may lead to an increased predation risk (e.g., Patriquin-Meldrum and Godin 1998; Kendal et al. 2005; Danchin et al. 2004). Thus, certain tactics have evolved in some social species to mitigate the costs associated with mate choice. One such example would be mate-choice copying. Mate-choice copying occurs when an individual (i.e., the observer) eavesdrops on another same-sex individual's sexual interactions (including actual mating) with another individual (i.e., the model) of the opposite sex (Dugatkin and Godin 1992). In doing so, the eavesdropping individual may obtain social (public) information regarding the quality of a prospective mate at potentially little cost (Rieucou and Giraldeau 2011). Moreover, the use of mate-choice copying may override an individual's inherent preference for certain phenotypic traits in potential mates. More specifically, after obtaining social information on another male's choice of mate, an observer may subsequently reverse his own initial choice of a preferred female phenotype and copy the choice of the model male for a female that he would not have otherwise selected as a mate (e.g., Dugatkin and Godin 1992). Most interestingly, the probability of reversing one's own initial mate choice (i.e., mate-choice copying) may be influenced by the phenotypic traits of the model and(or) the relative difference in the phenotypes of the females being compared as potential mates (e.g., Auld et al. 2017). Mate-choice copying by either females or males can in theory affect the variance of a preferred trait in the opposite sex within the population, and thus influence both the direction and strength of sexual selection on that trait (Wade and Pruett-Jones, 1990; Pruett-Jones 1992; Kirkpatrick and Dugatkin 1994; Servedio and Kirkpatrick 1996).

To date, most studies of mate-choice copying have focused on female copying the observed choice of other females (Westneat et al. 2000; Vakirtzis 2011; Witte et al. 2015), and relatively little is known about male mate-choice copying despite increasing evidence that males can also be choosy under certain conditions (Edwards and Chapman 2011). Moreover, whether and how the phenotype of the model individual and the differences in the phenotype of prospective mates influence the tendency of an observer to copy the model's mate choice remains poorly understood (Westneat et al. 2000; Vakirtzis 2011, Witte et al. 2015).

### **Thesis objective, predictions and organization**

Therefore, the main objective of my thesis was to test experimentally the hypotheses that (i) differences in the phenotypes of a model and observer (focal) males and (ii) differences in the phenotype of paired stimulus females as prospective mates influence both the likelihood of an observer male copying the apparent mate-choice of a model male and the strength of any mate-choice copying response using the Trinidadian guppy as a model study system.

My study consisted of two distinct but related experiments, wherein focal (observer) males were presented with a dichotomous choice of two stimulus females as potential mates. In the first experiment, the stimulus females differed in body length (i.e., small vs. large females presented) and the average difference in their length was similar across treatments. I manipulated the relative sexual attractiveness and competitiveness of observer and model males by choosing males of same or different body length and body coloration, two sexually-selected traits in the guppy (Houde 1997; Auld et al. 2016), in

each of four treatments. I predicted that, focal observer males should be more likely to reverse their initial mate-choice (i.e., mate-choice copy) decision when social information is available, relative to when social information is not available. Given that larger and more colour ornamented male guppies are more sexually attractive (Houde 1997; Auld et al. 2016) and produce more and better quality sperm and are thus better sperm competitors (Pitcher and Evans 2001; Pilastro et al. 2002; Locatello et al. 2006) than smaller and less ornamented males, and that there is some degree of last male sperm-precedence (Evans and Magurran 2000), I further predicted that the likelihood and strength of mate-choice copying should be the greatest when the model male is relatively *less* sexually attractive and competitive than the observer male. In the second experiment, the body length and body colour ornamentation of observer and model males were matched as closely as possible in all trials, but paired stimulus females differed in body length by pre-determined amounts (i.e., <5%, ~15%, or ~30%) in each of three independent treatments, respectively. I predicted that, when paired stimulus females appeared to be similar in quality (i.e., similar body size; <5% size-difference treatment), focal observer males should be more uncertain of their choice (i.e., Kendal et al. 2005) and therefore should more likely to use social information and copy the apparent mate choice of the model male compared to when the females differed more greatly in body size (i.e., in the ~15% and ~30% size-difference treatments). Theory (Kendal et al. 2005; Witte and Ryan 1998) predicts that, in the latter two treatments, observer males should rely less on social information and more heavily on personal information in making mate-choice decisions and thus should be less likely to mate-choice copy.

Structurally, this thesis comprises five chapters, followed by a common Literature Cited section at the end. Chapter 1 consists of a literature review, a statement of the thesis' main objective and predictions, and a description of the study species. Chapter 2 describes the general materials and methods common to the two experiments forming the core of this thesis. Chapter 3 (the first data-based chapter) investigates the potential influence of male phenotype (body size and colour ornamentation) on male mate-choice copying, while controlling for female body size. Chapter 4 (the second data-based chapter) investigates the potential influence of female phenotype (body length) on male mate-choice copying, while controlling for male body size and colour ornamentation. Chapter 5 summarizes my salient results, compares them to existing knowledge, and discusses their importance to the field and their implications for future research.

## **Study species**

The Trinidadian guppy is a suitable model species for my proposed study for the following reasons. This species is sexually dimorphic and dichromatic, with adult males being smaller on average and more colourful than adult females, internally fertilizing, and exhibits a non-resource based, promiscuous mating system and mutual mate-choice (Houde 1997). Adult males vary widely in body coloration and body length within populations, with both phenotypic traits being normally distributed (Auge et al. 2016). Males guppies have determinate growth and do not grow further when they reach sexual maturity (Houde 1997; Magurran 2005). Therefore, the body size of sexually-matured, adult males in the guppy is not a reliable predictor of their chronological age. Females generally prefer larger and more colourful males as mates, and males prefer to mate with

larger, more fecund females (Endler and Houde 1995; Houde 1997; Herdman et al. 2004; Jeswiet et al. 2011; Godin and Auld 2013). Guppies are internal fertilizers and the young are born live and free swimming; there is no post-parturition parental care (Houde 1997). Males can achieve fertilizations through either of two alternative mating tactics (Houde 1997), namely, a courtship tactic (male courts a female using vigorous sigmoid displays to gain her acceptance and mate cooperatively), or a coercive (i.e., sneak) tactic (male circumvents female choice by rapidly approaching a female from behind and attempting to 'sneak' copulate with her). The courtship tactic is on average significantly more successful than the sneak tactic in transferring sperm into a female (Pilastro and Bisazza 1999).

Social (public) information also mediates mating preferences in the Trinidadian guppy. As previously noted above, mate-choice copying has been extensively studied in female guppies (e.g., Dugatkin and Godin 1992, 1998; Dugatkin et al. 2002, 2003; Briggs et al. 1996; Dugatkin 1996; Godin et al. 2005; Godin and Hair 2009), but only recently in male guppies (Auld and Godin 2015). Most adult females in natural populations are multiply mated (Kelly et al. 1999; Neff et al. 2008). Males thus experience very high levels of sexual and sperm competition, and are consequently sensitive to the presence and behaviour of nearby sexual rivals and adjust their mating behaviour and mating preferences to maximize their mating success and minimize their risk of sperm competition (Jeswiet et al. 2011, 2012; Auld and Godin 2015; Auld et al. 2015, 2017). Moreover, a male guppy can transfer up to 92% of his sperm in a single successful copulation, which may take 2-3 days to fully replenish (Pilastro and Bisazza 1999). Furthermore, the guppy mating system has some degree of last-male sperm precedence

(Evans and Magurran 2000). The use of social information in assessing sperm competition risk and female quality may therefore benefit an eavesdropping (observer) male in making mating decisions in such a competitive mating system.

## **Chapter 2**

### **GENERAL METHODS**

## **Experimental subjects and holding conditions in the laboratory**

The fish used for the current experiment were approximately 20<sup>th</sup> generation, lab-born descendants of a total of approximately 1000 wild adults collected from the Upper Aripo River in Trinidad, a low-predation population (Magurran 2005) between 2009-12. The fish were held in outbred mixed-sex stock aquaria, filled with aged, filtered tap water maintained at 24-26°C, and under a 13 h L: 11 h D illumination cycle. They were fed 2-3 times daily with commercial flake food (Nutrafin™) supplemented with live brine shrimp nauplii (*Artemia franciscana*).

Because guppies can become familiar with one another after 12 days of social association (Griffiths and Magurran 1997), I selected fish (both males and females) from different holding aquaria on different staging shelves for any given behavioural trial to ensure that they were unfamiliar with one another. Furthermore, the focal and model fish used in this study were not re-used to avoid pseudo-replication. For each behavioural trial, the focal, model, and pseudo-model males were isolated from each other and females into separate small holding aquaria (30 x 15 x 22 cm; L x W x H) the evening before the trial so as to minimize any differences in their sexual motivation prior to testing (Bierbach et al. 2011). Individual males were placed in one half of their respective holding aquarium, as the other half of the aquarium was occupied by a male to be used in another behavioural trial. The holding aquaria were equipped with an air stone and were divided using a transparent and perforated plastic divider which allowed males within the aquarium to view and smell each other. Each of the holding tanks held either a focal, model, or pseudo-model male. Holding aquaria were grouped in triplets (i.e., the focal, model and pseudo-model males to be used in a given trial) into a larger water bath, which

contained an immersion heater maintaining water temperature at 24-26°C. In their water bath, the holding aquaria were separated from each other using opaque Plexiglas screens to prevent visual contact and social association between the focal, model, and pseudo-model males to be used in a given trial. The fish were fed live brine shrimp *nauplii* the morning of the day they were used in a behavioural trial. Once the fish were removed from their respective holding aquaria, the aquaria were drained, rinsed thoroughly, and re-filled with aged tap water conditioned with approximately 1 mL of Stresscoat™.

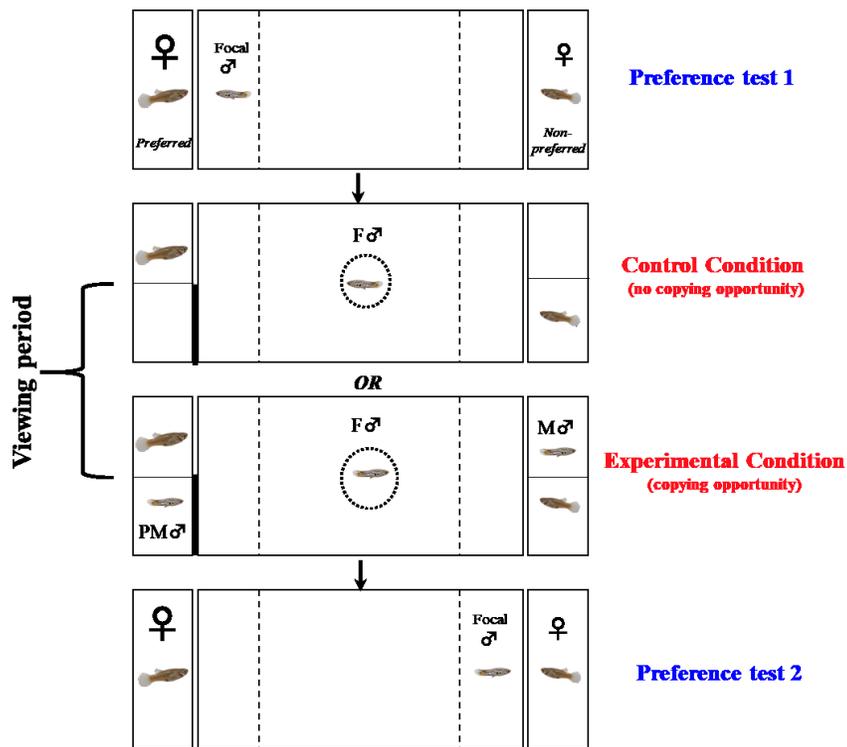
Following Dosen and Montgomerie (2004) and Auld and Godin (2015), gravid females with similar abdominal distention (assessed by eye), an indicator of stage of pregnancy (Houde 1997), were used as stimulus females as they are generally sexually unreceptive to males (Houde 1997) and to ensure that male mate choice would not be confounded by female sexual responses to male sexual activity and to minimize variation in male behaviour caused by any differences in female reproductive state. Although they prefer to mate with virgin over gravid females when given a choice (Guevara-Fiore et al. 2009), male guppies nonetheless sexually pursue, court and mate with gravid females in both the laboratory and field (e.g., Jeswiet et al. 2011; Godin and Auld 2013) and can successfully inseminate unreceptive gravid females through forced sneak copulations (Pilastro and Bisazza 1999) and sire offspring (Kelly et al. 1999; Herdman et al. 2004; Neff et al. 2008).

## **Experimental Apparatus**

I used the dichotomous choice paradigm and the reversal method (Dugatkin and Godin 1992) to test for mate-choice copying, and a similar experimental apparatus and protocol to that of Auld and Godin (2015). The apparatus (Figure 2-1) consisted of a central test aquarium (40 x 20 x 25 cm; L x W x H), flanked at either end with a smaller (15 x 20 x 22 cm; L x W x H) clear Plexiglas container (hereafter termed end compartment). The central aquarium held the focal (observer) male and served as the mate choice arena. Each end compartment housed a stimulus female. The bottoms of the end compartments and central aquarium were covered with aquarium gravel. The apparatus was filled to 15 cm depth with aged, aerated tap water (maintained at 24-26°C) conditioned with 1 mL of Stresscoat™, and illuminated overhead with full-spectrum fluorescent lights. Three sides of each end compartments and the front and back walls of the central aquarium were covered externally with tan paper to minimize any external disturbances. Using a Logitech C910 camera mounted overhead, I viewed the behaviour of the fish in real time from above on a PC monitor through YouCam Mobile software with the flip horizontal filter set to on (Cyberlink Corp., 2014). At the end of each trial, I emptied the central aquarium and end compartments and re-filled them with aerated, aged tap water conditioned with 1mL of Stresscoat™.

## **Experimental Protocol**

The experimental protocol used to test for mate-choice copying in this study is similar to that used by Auld and Godin (2015), which is based on the reversal method of Dugatkin and Godin (1992). A typical mate-choice trial consisted of three consecutive 20-min phases as follows (Figure 2-1).



**Figure 2-1.** Schematic top view of the experimental apparatus used in the current experiment and illustrating the set-up for the three 20-min phases of a typical mate-choice trial. The central test aquarium (40 x 20 x 25 cm; L x W x H) held the focal (F) male and served as the mate-choice arena and the end compartments (15 x 20 x 22 cm) held the stimulus females and, depending on the treatment, the model (M) and pseudo-model (PM) males. The circle represents a removable clear Plexiglas cylinder (7 cm diam.), in which the focal male could temporarily be placed. Dotted lines represent removable clear Plexiglas partitions that allowed end compartments to be either divided in half or left open. The thicker dark line represents a removable opaque screen and the dashed lines in the test aquarium demarcate 10-cm preference zones. Depending on the treatment, the body length and colour ornamentation of the focal and model males were either similar (matched) or dissimilar in a given trial.

*(i) Preference test 1*

In this initial mating preference test, the focal male's initial (baseline) mating preference for either of the paired stimulus females was ascertained. This mating preference test was 20 minutes in duration, and comprised two consecutive 10-min periods.

Prior to onset of a trial, I inserted opaque Plexiglas screens between the end compartments and the central test aquarium of the apparatus. A focal male was selected from one of the holding aquaria and gently placed in a clear Plexiglas cylinder (7 cm diam.) in the centre of the test aquarium, and one stimulus female was placed in each of the two end compartments, with the particular compartment determined at random. The fish were then allowed to acclimatize undisturbed for 10 min. Following this period, I removed the opaque screens and the focal male was allowed to view the stimulus females for 2 min, after which I gently raised the cylinder to allow the focal male to swim freely in the test aquarium and to choose between the two stimulus females. Once the focal male began to swim normally, I initiated a 10-min test during which I recorded the time that the male spent near (within 10 cm) and facing ( $0 \pm 90^\circ$ ) either female as a proxy for his mating preference. Such association time is a reliable predictor of mate choice in the guppy (Dugatkin and Godin 1992; Jeswiet and Godin 2011) and other species (e.g., Cummings and Mollaghan 2006; Witte 2006; Lehtonen and Lindström 2008; Witte and Ryan 1998).

At the end of this first test, the opaque screens were replaced and the focal male gently dip-netted and placed in the cylinder located in the centre of the tank. The positions of the female compartments were then switched to control for any potential side

bias of the focal male. A side bias was defined as the focal male spending more than 80% of his total association time on one side of the test aquarium, despite the switching of the positions of the stimulus females between the two halves of the 20-min preference test (Schlupp and Ryan 1997). Once the positions of the end compartments were switched, I removed the opaque screens, allowing the focal male to view the females for 2 min. Before being released from the cylinder and allowed to swim freely in the test aquarium. I then recorded the male's association time with the same pair of stimulus females during a second 10-min period. The proportion of the focal male's mating preference for a particular female in Preference test 1 was expressed as: (his association time near that female summed over both 10-min tests/sum of his total association time spent near both females). A preferred female is the one that the male spends more than 50% of his total association time summed over both females. Therefore, the initially non-preferred female is the female that the male spends less than 50% of his time associating with. Trials with a side bias (where the focal male spends 80% of his time on one side of the tank over the first or second preference tests) were discarded and excluded from further analysis (Schlupp and Ryan 1997).

(ii) Viewing period

At the end of Preference test 1, the opaque screens were replaced, the focal male returned to the central cylinder, and the end compartments were divided in half with a clear Plexiglas partition (Figure 2-1). Depending on the treatment (Figure 2-1), a model male could be placed in the empty half of the end compartment containing the initially non-preferred female so as to simulate an apparent mate-choice by the model male. The

model was used to demonstrate to the focal male that his initially non-preferred female may be sexually attractive to another male. Another male (the pseudo-model) would be similarly placed in the empty half of the other end compartment containing the initially preferred female to minimize potential differences in the activity level of the stimulus females. An opaque screen was placed in front of the half-compartment containing the pseudo-model male so that the focal male could not see him from his position in the central cylinder during the viewing period (Figure 2-1 Viewing period). For all trials, the focal and pseudo-model males were matched as closely as possible for body length and overall coloration.

Following a 2-min acclimatization period, I removed the opaque screens and allowed the focal male (whilst still in the cylinder) to view for 20 min either a model male near and visually interacting with his initially non-preferred female (thus simulating an apparent mate choice) or the same two stimulus females in the absence of any model male near either of them. At the end of this viewing period and in preparation for Preference test 2 that followed immediately, the opaque screens were replaced, and the model and pseudo-model males, along with the Plexiglas partitions in the end compartments, and the small opaque Plexiglas screen in front of the pseudo-model male's half-compartment were removed. The same protocol was applied for the Control Condition (model absent), whereby I gently dip-netted the end compartments which would normally hold the model and pseudo-model males. This was to control for the effect that handling the model males may have on the stimulus females' behaviour between the Control and Experimental Conditions.

### (iii) Preference test 2

In this second (repeated) 20-min mating preference test, the focal male's mating preference for either of the same two stimulus females was ascertained. The protocol for this second preference test is the same as that described above for Preference test 1.

If male guppies copy the mating preferences of other males when given the opportunity to do so, then a significant weakening or a complete reversal of a focal (observer) male's initial mating preference for a particular female (Preference tests 1 vs. 2; Figure 2-1) following his observing a model male near the other, initially non-preferred female (Viewing period, Figure 2-1), compared to his mating preferences in the absence of a model male is expected (cf. Dugatkin 1992).

## **Measuring fish body length and male colour ornamentation**

Prior to the start of each behavioural trial (described below), the stimulus females used in a trial were initially measured individually for their standard body length (distance from the tip of the nose to the caudal peduncle) using a metric measuring board without the use of anesthetic. However, the males were measured (using the metric unit board) and assessed for their relative colour ornamentation by eye (later confirmed using their digital photographs as described below) prior to isolating them overnight. At the end of each behavioural trial, all fish were lightly anaesthetized (with MS-222 at 1:10,000 dilution), placed on a piece of white Plexiglas, and their left side photographed next to a ruler using a digital camera under standardized lighting conditions. Following photography, the fish were placed in a container of aerated water to recuperate. From the

photographs, I measured the standard body length (to the nearest 0.1 mm) and area of the left side of the body (excluding the fins) for all fish, and the body colour ornamentation of all males using *Image J*® (<http://rsbweb.nih.gov/ij/>). I quantified the areas of black and orange pigmentation, which are two sexually-selected male traits in the guppy (Houde 1997; Auld et al. 2016), on the left side of each male's body, excluding the fins. A male's body colour score was expressed as the relative area of total body colour ([black area + orange area]/total body area) to control for variation in male body size. This proportion can be converted into a percent by multiplying the male's body colour score by 100.

For a given pair of stimulus females, the percent difference in their body length was calculated as [(standard length of the larger female – standard length of the small female)/standard length of the small female) x 100].

## **Statistical analyses**

All statistical analyses were carried out using the R-studio statistical framework (R Core Team 2014). The statistical tests used to analyse the data collected are described in the Methods for each of my two experiments (Chapters 3 and 4) separately.

## **Ethical note**

The two experiments (Chapters 3 and 4) described in this thesis received prior approval from the Carleton University Animal Care Committee (protocol #106386) and thus conform to the guidelines on the use and care of research animals of the Canadian Council on Animal Care and the laws of Canada.

## **Chapter 3**

# **THE INFLUENCE OF MALE PHENOTYPIC TRAITS ON MALE MATE-CHOICE COPYING**

## ABSTRACT

Mate-choice copying may mitigate the costs of searching for and assessing prospective mates. Mate-choice copying occurs when a bystanding individual observes another individual (of the same sex) sexually interact with a potential mate (of the opposite sex) and subsequently uses this socially acquired information to mate with the same (latter) individual. Male mate-choice copying has received less attention relative to female mate-choice copying, and much less is known about how the sexual attractiveness and competitiveness of males influences the likelihood of a given male copying another male's mate-choice decision. In the current study, I investigated whether the relative sexual attractiveness and(or) competitiveness of males (observers and models) influences the likelihood and strength of male mate-choice copying in the Trinidadian guppy. Focal observer males were presented with a large and a small female as potential mates in a dichotomous choice test, either in the presence or absence of a model male. I varied the relative differences in the body length and colour ornamentation (traits that determine sexual attractiveness and competitiveness in the guppy) of observer and model males in each of three treatments, wherein the males were either matched for attractiveness (similar body length and colour ornamentation), the model male was more attractive (larger and more colourful) than observer male, or the model male was less attractive (smaller and less colourful) than the observer male. Based on previous research, males were expected to initially prefer larger over smaller females on average. I predicted that focal observer males (i) would be more likely to reverse their initial mating preference (i.e., mate-choice copy) in the presence than in the absence of a model male placed near the initially non-preferred female, and (ii) would be more likely to reverse their initial

mating preference (i.e., more likely to mate-choice copy) when the model male was less sexually attractive than themselves compared to when the model male was similarly or more attractive than themselves. My results generally support these predictions.

## INTRODUCTION

In general, successful mating attempts are skewed towards a few individuals in the population who are the most sexually attractive and(or) sexually competitive (Andersson 1994). As with females, males can also be choosy of their mates under certain conditions (reviewed in Edward and Chapman 2011). Male mate choice is defined as the selection by individual males of particular females among those available based on certain phenotypic traits that are reliable indicators of their quality (Edward and Chapman 2011). A male may select a female mate either through direct (i.e., personal) sampling, whereby he assesses prospective female mates in the absence of social cues, or indirectly by using social information acquired from observing other males sexually interact with prospective female mates in subsequently making his own mating decision (Westneat et al. 2000; Danchin et al. 2004; Kendal et al. 2005). The use of social information via indirect sampling could mitigate the costs associated with searching for and assessing prospective mates and the risk of selecting an inferior mate (Andersson 1994), but at the potential cost of an increased risk of sperm competition (e.g., Wedell et al. 2002; Jeswiet et al. 2012).

When a model male is assessing two or more females in the perceived absence of other males, he will likely use personal information to pursue one female over the other (alternatively, he could simply prefer neither female). However, if there is an eavesdropping (observer) male nearby, then the eavesdropping male may use social information gleaned by observing the model male's mate choice to subsequently make his own mate choice decisions. In doing so, the eavesdropping male may select a different female (the one that he observed the model male sexually interact with) than he

might have otherwise chosen, and thereby potentially override his inherent preference for a particular female over others. Male mate-choice copying is deemed to have occurred if an observer male reverses his initial mating preference after he has viewed a model male sexually interact with a female that the observer male had not initially preferred as a mate (cf. Dugatkin and Godin 1992).

Female mate-choice copying has been extensively studied across several taxa (reviewed in Westneat et al. 2000; Vakirtzis 2011; Witte et al. 2015), including the guppy (see Chapter 1). For example, in the Japanese Quail (*Coturnix japonica*), a lekking species, females generally prefer males whom they have seen mating with another female (Galef and White 1998). Female sailfin mollies (*Poecilia latipinna*) reverse their initial mate choice decision (i.e., mate-choice copy) in the presence but not in the absence of a model female (Hill and Ryan 2006). In humans, mate-choice copying is based on the sexual experience of the female and the duration of the relationship she is seeking with a mate. When female humans are sexually experienced and are seeking a short-term relationship, they are less likely to copy another female's mate-choice decision. When the model female is more attractive and the focal observer female is less sexually experienced, then the latter female is more likely to copy the model's apparent choice (Waynforth 2007). Similarly, younger (and presumably less sexually experienced) female guppies are more likely to copy the mate choice of older (more experienced) females than vice versa (Dugatkin and Godin 1993). Sometimes it is costly to mate-choice copy. For example, in fruit flies (*Drosophila melanogaster*), females can distinguish between mated and unmated males and preferentially select to mate with virgin or unmated males (Loyau et al. 2012), presumably to avoid sperm competition.

Although females are generally regarded as the more choosy sex (Andersson 1994), males nonetheless can be choosy under certain circumstances (Edward and Chapman 2011), such as preferring larger (and more fecund) females over smaller ones as mates (e.g., Herdman et al. 2004). Compared with female mate-choice copying (Witte et al. 2015), male mate-choice copying using social information has received much less attention until recently. For example, male mate-choice copying has been experimentally demonstrated in threespined sticklebacks (Frommen et al. 2009), sailfin mollies (Schlupp and Ryan 1997), deep-snouted pipefish *Syngnathus typhyle* (Widemo 2006), Trinidadian guppy (Auld and Godin 2015), and in humans (Place et al. 2010). In the latter study (Place et al. 2010), the use of social information was most influential in sexually naïve males, and that the observer male was more likely to copy the mate choice when the model was more attractive than the focal male. Surprisingly, most male mate-choice copying studies to date have not manipulated phenotypic differences between observer and model males to investigate their importance in copying. Almost nothing is known about the roles that sexual attractiveness and competitiveness (i.e., relative phenotypes) play in determining the likelihood and strength of mate-choice copying in males.

Therefore, the current experiment was designed to address this knowledge gap. Here, I investigated the potential influence of differences in the phenotypes (i.e., body length and colour ornamentation) of focal observer and model males on the likelihood and strength of mate-choice copying behaviour by observer males in the Trinidadian guppy. To do this, focal observer males were presented with a dichotomous choice between a large and smaller stimulus females as potential mates and their mating preferences were assessed in the presence or absence of a model male simulating a

copying opportunity (see Chapter 2 for general methods). I experimentally varied the relative differences in body length and colour ornamentation (sexually-selected reliable proxy traits of male sexual attractiveness and competitiveness in this species; see Chapter 1) of observer and model males in each of three treatments. Depending on the treatment, males were either matched for sexual attractiveness/competitiveness (similar body length and colour ornamentation), the model male was more attractive/competitive (larger and more colourful) than observer male, or the model male was less attractive/competitive (smaller and less colourful) than the observer male. The guppy is a suitable study species to use for this experiment for reasons I outlined above in Chapter 1.

Based on previous research on the guppy (Dosen and Montgomerie 2004, Herdman et al. 2004; Jeswiet et al. 2011; Godin and Auld 2013; Auld et al. 2016), I expected that focal male guppies on average would initially prefer large over smaller females as mates in the absence of social information (i.e., no model males present). Based on previous research on mate-choice copying in the guppy using the reversal method (e.g., Dugatkin and Godin 1992; Auld and Godin 2015), I expected that focal males would weaken their initial preference for a particular female, and spend more time associating with the other (initially non-preferred) female, when a model male is placed near the latter female (thus simulating an apparent mate choice for this female). I predicted that focal observer males (i) would be more likely to mate-choice copy in the presence than in the absence of a model male, and (ii) would be more likely to reverse their initial mating preference (i.e., more likely to mate-choice copy) when the model male was less sexually attractive and competitive than themselves compared to when the model male was similarly or more attractive than themselves, because relatively larger

and more colourful (model) male rivals would be relatively more sexually attractive and better sperm competitors (Locatello et al. 2006) and focal males would likely incur a greater risk of sperm competition if they chose to mate with the same female previously mated by larger and more colourful model males.

## **METHODS**

### **Study subjects and maintenance conditions**

The source of the study subjects and the general fish husbandry conditions in the laboratory were described in Chapter 2.

### **Experimental apparatus**

The experimental apparatus used for this experiment (Figure 2-1) was illustrated and described in detail in Chapter 2.

### **Experimental protocol**

This experiment comprised four independent treatments (Table 3-1), one Control treatment (no copying opportunity) and three Experimental treatments (copying opportunity available).

The Control Condition (Treatment 1) tested for temporal consistency of mating preference by the focal male between two consecutive preference tests in the absence of any model male (Figure 2-1). Based on the previous findings of Godin and Auld (2013) and Auld and Godin (2015) for Upper Aripo River guppies, I expected males to be

consistent in their preference for a particular female between the repeated preference tests. The Experimental Condition (Treatment 2-4) ascertained whether a focal male's initial mating preference could be altered by an opportunity to view and copy the apparent mating preference of another male (the model) (Figure 2-1). These three Experimental treatments differed only in the body length and colour ornamentation of the focal (observer) male relative to the body length and colour ornamentation of the model male (Table 3-1). I manipulated the sexual attractiveness and competitiveness of the model male in any given trial by pre-selecting the model male to be either similar in body length and coloration to the focal male (Treatment 2), larger and more colourful than the focal male (Treatment 3), or smaller and less colourful than the focal male (Treatment 4) (Table 3-1). For all trials in the Experimental treatments, the body length and colour ornamentation score of focal and pseudo-model males were matched as closely as possible (Table 3-1). The above treatment combinations of body length and colour ornamentation for focal and model males reflect their occurrence in nature, as these two traits in free-ranging males in my study population are highly variable and normally distributed (Auge et al. 2016). By design, the body lengths of paired stimulus females in individual trials differed significantly within each treatment (Table 3-1) and were similar across treatments (one-way ANOVA,  $F_{3,156} = 0.89, p = 0.44$ ). The overall relative difference in the body length of paired stimulus females was approximately 29%.

All treatments followed the same general protocol testing for focal male mating preferences and mate-choice copying, as described in Chapter 2 and depicted in Figure 2-1. The treatments were carried out in random order, and each treatment was replicated 40 times with different fish that showed no side bias (cf. Chapter 2), for a total of 160 trials.

**Table 3-1.** Mean ( $\pm$  SE) standard body length and body colour ornamentation score for focal, model, and pseudo-model males, and standard body length of paired stimulus females, used in individual trials in each of four treatments (which are numbered in the first column of the table). The within-treatment comparisons are paired *t*-tests. The merged cells compare focal and model males, whereas the non-merged cells compare focal and pseudo-model male. The among-treatment comparison tests for similarities across the four treatments using the ANOVA.

	Male length (mm)			Male colour ornamentation score (%)			Stimulus female length (mm)	
	Focal	Model	Pseudo-model	Focal	Model	Pseudo-model	Large	Small
<b>1. Control</b>	15.8 $\pm$ 0.67	-	-	6.9 $\pm$ 0.38	-	-	21.6 $\pm$ 0.32	16.5 $\pm$ 0.19
<i>Within-treatment comparison</i>	-			-			<i>t</i> = 18.79 <i>p</i> < 0.001	
<b>2. Model matched</b>	15.6 $\pm$ 0.11	15.6 $\pm$ 0.11	15.4 $\pm$ 0.12	6.2 $\pm$ 0.38	5.8 $\pm$ 0.35	6.6 $\pm$ 0.33	21.8 $\pm$ 0.33	16.7 $\pm$ 0.31
<i>Within-treatment comparison</i>	<i>t</i> = 0.08, <i>p</i> = 0.93		<i>t</i> = 1.66 <i>p</i> = 0.10	<i>t</i> = 0.91, <i>p</i> = 0.37		<i>t</i> = -0.96 <i>p</i> = 0.35	<i>t</i> = 15.58 <i>p</i> < 0.001	
<b>3. Model more attractive</b>	14.6 $\pm$ 0.12	17.2 $\pm$ 0.16	14.9 $\pm$ 0.13	2.6 $\pm$ 0.30	9.6 $\pm$ 0.47	2.5 $\pm$ 0.36	21.6 $\pm$ 0.20	16.7 $\pm$ 0.21
<i>Within-treatment comparison</i>	<i>t</i> = -14.54, <i>p</i> < 0.001		<i>t</i> = 1.35 <i>p</i> = 0.18	<i>t</i> = -7.46, <i>p</i> < 0.001		<i>t</i> = 0.23 <i>p</i> = 0.82	<i>t</i> = 22.41 <i>p</i> < 0.001	
<b>4. Model less attractive</b>	17.0 $\pm$ 0.14	14.4 $\pm$ 0.12	16.5 $\pm$ 0.97	6.3 $\pm$ 0.44	2.2 $\pm$ 0.0027	6.2 $\pm$ 0.44	21.3 $\pm$ 0.27	16.8 $\pm$ 0.23
<i>Within-treatment comparison</i>	<i>t</i> = 14.31, <i>p</i> < 0.001		<i>t</i> = 2.81 <i>p</i> < 0.01	<i>t</i> = 7.50, <i>p</i> < 0.001		<i>t</i> = 0.14 <i>p</i> = 0.89	<i>t</i> = 17.10 <i>p</i> < 0.001	
<i>Among-treatment comparison</i>	<i>F</i> = 64.9 <i>p</i> < 0.001	<i>F</i> = 113.8 <i>p</i> < 0.001	<i>F</i> = 37.27 <i>p</i> < 0.001	<i>F</i> = 26.54 <i>p</i> < 0.001	<i>F</i> = 43.52 <i>p</i> < 0.001	<i>F</i> = 35.3 <i>p</i> < 0.001	<i>F</i> = 0.40 <i>p</i> = 0.76	<i>F</i> = 0.30 <i>p</i> = 0.82

If male guppies copy the mating preferences of other males when given the opportunity to do so, then I expected either a significant weakening or a reversal of a focal (observer) male's initial mating preference for a particular female following his observing a model male near the other, initially non-preferred female (in experimental Treatments 2-4) compared to his mating preferences in the absence of a model male (in the control Treatment 1) (cf. Dugatkin 1992; Dugatkin and Godin 1992).

### **Measuring fish body length and male colour ornamentation**

At the end of each behavioural trial, all fish used in the trial were lightly anaesthetized and their left side photographed with a digital camera under standardized lighting conditions. The methods used to photograph the fish and to measure their body length and to quantify the colour ornamentation of males from their individual photograph using *Image J*<sup>®</sup> software were described in Chapter 2.

### **Statistical analyses**

First, I tested whether focal males exhibited an overall preference for the larger of the two paired stimulus females in Preference test 1 in the absence of social information (data pooled across all four treatments) using the Binomial test (for number of males) and paired *t*-test (for association time).

Second, I used a general linear model to test for the main effects of the predictor variables, Preference test (i.e., Preference test 1 vs. 2) and Treatment (Treatments 1-4), and their potential interaction, on association time spent by focal males with their initially non-preferred female. The body length and colour ornamentation score of the focal male

and the difference in body length of the paired stimulus females in each trial were included as covariates in the model, as they are known to influence male sexual behaviour in the Trinidadian guppy (e.g., Dosen and Montgomerie 2004; Herdman et al. 2004; Jeswiet et al. 2012; Godin and Auld 2013; Auld et al. 2017). Because this response variable was not normally distributed (Shapiro-Wilk test), the data were normalized using square-root transformation prior to analysis.

Third, for each treatment separately, I compared the association time that individual focal males spent with the initially non-preferred female between Preference tests 1 and 2 using the paired *t*-test. A significant increase in this response variable between these paired preference tests in either of the three Experimental treatments (model male present), relative to the Control treatment (model male absent), is taken as evidence for mate-choice copying (cf. Dugatkin and Godin 1992).

Fourth, the number of focal test males reversing their mating preference for a particular female between Preference tests 1 and 2, as would be expected if mate-choice copying occurred (cf. Dugatkin and Godin 1992), was compared across treatments using the *G*-test with Williams correction.

Lastly, I compared the magnitude of the response variable among treatments using the ANOVA-based Tukey HSD test for post-hoc multiple comparisons of means.

## RESULTS

As expected theoretically and based on previous studies on the guppy, overall significantly more focal males (92 out of 160, or 57.5%) categorically preferred the larger over the small stimulus female in Preference test 1, in the absence of any social

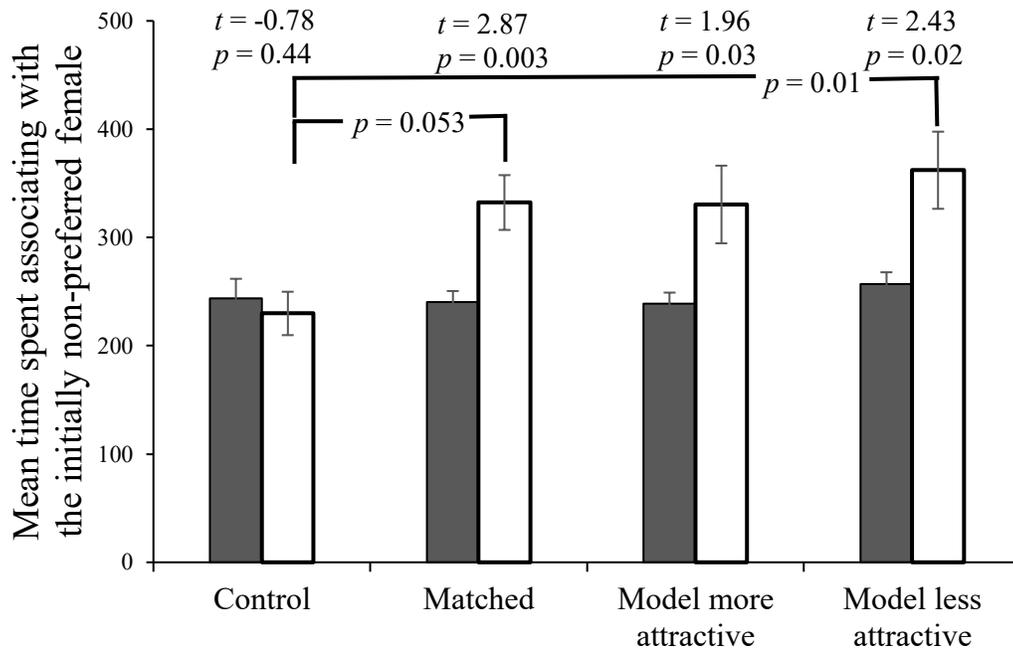
information about the mate choice of another (model) male, than expected by chance (Exact binomial test,  $p < 0.0001$ ). However, the strength of this preference was low because the time that focal males spent associating with the paired large (mean  $\pm$  SE =  $432 \pm 20.5$  s) and small ( $413.7 \pm 17.2$  s) females on average in Preference test 1 did not differ significantly (paired  $t$ -test,  $t_{159} = 0.499$ ,  $p = 0.61$ ). The association of time the focal male spent with the large (Shapiro-Wilk test,  $W = 0.99$ ,  $p = 0.37$ ) and small ( $W = 0.99$ ,  $p = 0.08$ ) female were normally distributed.

Overall, the association time that focal males spent with the initially non-preferred female was significantly influenced by the predictor variables, Preference test (linear model,  $F_{1,309} = 11.92$ ,  $p < 0.01$ ) and Treatment (linear model,  $F_{3,309} = 2.66$ ,  $p = 0.048$ ), when the difference in the body length of paired stimulus females and the body length and colour ornamentation score of the focal male were controlled for as covariates (Figure 3-1). There was a marginally non-significant interaction between Preference test and Treatment (linear model,  $F_{3,309} = 2.36$ ,  $p = 0.07$ ). None of the covariates were significant in the model. The model residuals approached normality (Shapiro-Wilk test,  $W = 0.99$ ,  $p = 0.025$ ). These results are as expected if the presence of a model male (i.e., social information) during the viewing period of a mate-choice trial, and the phenotype (body length and ornamentation colour) of the model male relative to that of the focal (observer) male, influence the initial mating preference of focal males and promote mate-choice copying behaviour.

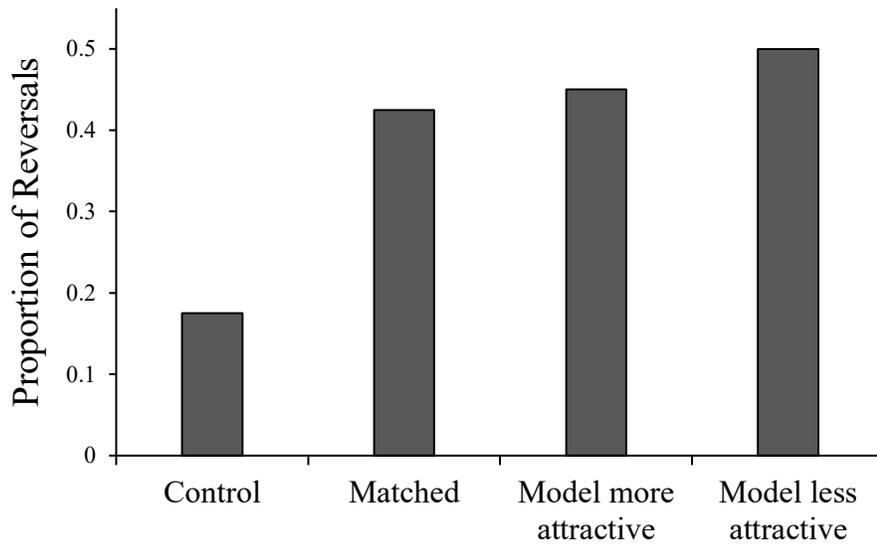
More specifically, in the Control treatment (Treatment 1), the time that focal males spent associating with the initially non-preferred female did not differ between Preference tests 1 and 2 (paired  $t$ -test,  $t = -0.70$ ,  $p = 0.44$ ; Figure 3-1) as expected,

suggesting that their mating preferences remained consistent over time in the absence of any social information about the mate choice of another (model) male nearby. The strength of the focal males' preference for a particular female in Preference test 1 did not differ across treatments (Tukey HSD test, all  $p > 0.99$ ; Figure 3-1). In contrast to the Control treatment and as expected, focal males significantly increased the time spent associating with their initially non-preferred female in Preference test 2 compared to Preference test 1, after having observed a model male near the latter female during the viewing period between these tests, in all three of the Experimental treatments (paired  $t$ -tests, all  $p < 0.05$ ; Figure 3-1). This increase in relative preference for the initially non-preferred female in Preference test 2 resulted in a larger proportion of males reversing their initial mating preference in each of the three Experimental treatments (model male present) compared to the Control treatment (model male absent) ( $G$ -test,  $G_{adj} = 7.94$ ,  $df = 3$ ,  $p < 0.05$ ; Figure 3-2).

Given that the response variable (Figure 3-1) varied significantly within and among treatments and that there was a moderate interaction between the predictor variables (see above linear model results), a post-hoc multiple comparisons of means Tukey HSD test revealed that focal males spent significantly more time with the initially non-preferred female in Preference test 2 (i.e., stronger copying response) when the model male was either less attractive than themselves ( $p = 0.011$ , Treatment 4) or when the model and focal males were phenotypically matched ( $p = 0.053$ , Treatment 2) compared to the Control Treatment 1, but not so for Treatment 3 ( $p = 0.19$ ) wherein the model male was more attractive than the focal male (Figure 3-1).



**Figure 3-1.** Mean ( $\pm$  SE) association time focal males spent near the initially non-preferred female in paired Preference test 1 (filled bars) and Preference test 2 (open bars) in each of the four treatments, namely, Control (Treatment 1, model male absent); Matched (Treatment 2, males have similar phenotypes), Model male more attractive than Focal male (Treatment 3), and Model males less attractive than Focal male (Treatment 4). Comparisons within treatments (Preference tests 1 vs. 2) was obtained using the paired  $t$ -test, and comparisons among treatments were based on the post-hoc Tukey HSD test. For the latter test, only significant  $p$ -values are shown.  $n = 40$  for each treatment.



**Figure 3-2.** The proportion of focal males that reversed their initial mating preference for a particular female between the paired Preference tests 1 and 2 in each of the four treatments, namely Control (Treatment 1, model male absent), Model and Focal males matched for body size and colour ornamentation (Treatment 2), Model male more attractive than Focal male (Treatment 3), and Model male less attractive than Focal male (Treatment 4). A reversal is defined as when the focal male reversed (i.e., switched) his initial mating preference for a particular female (in Preference test 1) towards the other stimulus female (i.e., spent >50% of his total association time near the latter female) in Preference test 2.  $n = 40$  for each treatment.

## DISCUSSION

Collectively, the results of the current study strongly suggest that male guppies, originating from the Upper Aripo River population, will copy the observed apparent mate choice of another (model) male when given the opportunity to do so, and importantly that the likelihood and magnitude of the copying response is dependent on the relative phenotypes of the focal (observer) and model males. In the absence of a copying opportunity (model male absent), focal males were consistent in their mating preferences over time. Consistent with my *a priori* predictions and with sperm competition theory (Wedell et al. 2002), focal males were more likely to mate-choice copy, and to exhibit a stronger copying response, when model and focal males were similar phenotypically and when model males were apparently less sexually attractive and presumably less sexually competitive than themselves. Conversely, focal males were less likely to mate-choice copy and to exhibit a weaker copying response when model males more sexually attractive and(or) competitive than themselves. Such a graded mate-choice response dependent on the phenotype of the model male relative to the focal male may minimize the risk of sperm competition of the focal male, a potential cost associated with copying the mate choice of another male (Vakirtzis 2011, Witte et al. 2015), and thus would appear adaptive.

In the current study, focal male guppies exhibited considerable inter-individual variation in their initial mating preference (in Preference test 1) for either of the paired stimulus females. Although significantly more males than expected by chance initially preferred the larger over the smaller stimulus females, there was no general consensus among males for a particular female phenotype based on body size. Regardless of their

individual initial preference for the larger or smaller female, individual focal males were highly consistent in their mating preference over time (Preference test 1 vs. 2) in the absence of a model male socially demonstrating an apparent opposite preference (Treatment 1, Control). These results corroborate the previous findings of Godin and Auld (2013) on wild-caught male guppies from the same study population as mine. They also reported wide variation in the mating preferences of males for females based on the latter's body size and showed that the mating preferences of individual males were consistent over time and highly repeatable in the absence of male rivals. Female mating preferences in the Trinidadian guppy are similarly consistent over time and repeatable (Godin and Dugatkin 1995). Notwithstanding such consistency and repeatability in individual mating preferences, it is now well established that the social environment, and in particular social information about the presence and sexual behaviour of other nearby conspecifics, can influence the mating decisions of individual animals, and thereby influence sexual selection, in a wide range of species (reviewed in Danchin et al. 2004; Vakirtzis 2011; Witte et al. 2015) including female (e.g., Dugatkin 1992; Dugatkin and Godin 1992, 1993; Godin et al. 2005; Godin and Hair 2009) and male (e.g., Jeswiet et al. 2011, 2012; Auld and Godin 2015; Auld et al. 2015, 2017; current study) Trinidadian guppies.

My current finding that male Trinidadian guppies, originating from the Upper Aripo River population, can copy the apparent mate choice of another (model) male when given the opportunity corroborates a similar result reported by Auld and Godin (2015) for male guppies of the same population. The observed increase in association time for the initially non-preferred female by focal males (in Preference 2 vs. 1) after they had

observed a model male near the latter female compared with the Control treatment is evidence for copying (cf. Dugatkin and Godin 1992). This result cannot be attributed simply to any differences in the sexual receptivity or differential activity of the paired stimulus females, as both females were exposed to a male (either model or pseudo-model male) during the viewing period of each experimental trial and both stimulus females were gravid and likely at similar stages of their reproductive cycle.

A particularly novel and important result of the current study is the modifying effect of the difference in the apparent sexual attractiveness and competitiveness (as determined by body length and colour ornamentation, Houde 1997; Auld et al. 2016; Locatello et al. 2006) of focal and model males on the likelihood and magnitude of mate-choice copying being exhibited by focal males when given the opportunity to do so. More specifically, focal males were less likely to copy the apparent mate choice (for the initially non-preferred female) of a model male, and more weakly so, when the model male was larger and more colour ornamented (and thus apparently more sexual attractive and competitive) than the focal male compared to when the model male was either smaller and drabber than, or of similar size and coloration to, the focal male. This result can plausibly be interpreted within the context of sperm competition theory (Wedell et al. 2002). Sexual selection should favour prudence in males that face sperm competition and the evolution of behavioural strategies and tactics that minimize their perceived risk of sperm competition when making mating decisions (Wedell et al. 2002). However, male humans have been shown to copy the mate choice of another male when the model is perceived to be more attractive than the observer male (Place et al. 2010). Male guppies are sensitive to the presence of nearby conspecific sexual competitors and, in response,

flexibly modify their mating tactics and mating preferences accordingly so as to seemingly minimize their risk of sperm competition (Jeswiet 2011, 2012; Auld and Godin 2015; Auld et al. 2015, 2017). Larger and more colour ornamented male Trinidadian guppies produce more and better-quality sperm than smaller and drabber males (Locatello et al. 2006). Therefore, it is possible that focal males in the current study perceived those model males who were larger and more colour ornamented than themselves (Treatment 3) as stronger sexual and sperm competitors and were thus less likely to copy their apparent choice of mate (i.e., the female that was not initially preferred by the focal) so as to minimize the risk that their sperm would be competing with those of the model male inside the reproductive tract of that female. Conversely, the risk of sperm competition may have been perceived by focal males as being lower when the model and focal males were matched phenotypically or when the model males were smaller and drabber than the focal males, which might explain the observed stronger mate-choice copying responses under these two scenarios (Treatments 2 and 4, respectively).

In conclusion, the current study has shown using the Trinidadian guppy as a model system that the mating preferences of individual males can be modified not only by the availability and use of socio-sexual information in their immediate social environment, but also by the nature of that information, more specifically and importantly by the relative phenotypes of focal (observer) and model males. Although putative fitness-related benefits and costs of mate-choice copying have been proposed (e.g., Vakirtzis 2011; Witte et al. 2015), empirical evidence for them has remained relatively elusive, and should therefore continue to be sought in the future. Moreover, knowledge of

the ecological circumstances or conditions favouring the use of social information over personal information, in mate choice (i.e., mate-choice copying) is also generally limited and should therefore be a promising area of future research.

Additionally, current knowledge of mate-choice copying in animals (e.g., reviewed in Westneat et al. 2000; Danchin et al. 2004; Vakirtzis 2001; Witte et al. 2015) might be practically applied to animal population management, whereby more or less attractive models could influence the mate-choice of prospective and sexually receptive males. If limiting population size is a management objective, then perhaps placing less attractive models near mates that have lower fitness outcomes (e.g., they have poor fertility) might be a possible management tool. If males sexually pursue less fit females based on their socio-sexual environment, then the population might be able to stabilize. Alternatively, placing less attractive models near attractive mates with a high fitness outcome might enhance genetic variability, abundance and overall fitness of populations of endangered species.

Lastly, future research should explore the potential importance of differences in female body size (and thus perceived quality), whilst controlling for the sexual attractiveness of the focal (observer) and model males, on the propensity of observer males to copy the apparent or actual mate choice of model males. I pursued this line of investigation in a second experiment using the Trinidadian guppy as a model system, which constitutes the next data chapter (Chapter 4).

## **Chapter 4**

# **THE INFLUENCE OF FEMALE PHENOTYPIC TRAITS ON MALE MATE-CHOICE COPYING**

## ABSTRACT

In theory, mate-choice copying may reduce the costs associated with finding and assessing prospective mates, but at the risk of increased sexual and sperm competition. This phenomenon occurs when a bystander individual observes another individual of the same sex (i.e., the model) sexually interacting or mating with an individual of the opposite sex and uses this socially-acquired information to subsequently mate with the same individual. Less is known about mate-choice copying in males than females, especially regarding how the differences in the phenotypes of available females as mates affect the likelihood and strength of a mate-choice copying in males. In the current study, I investigated whether the relative sexual attractiveness of stimulus females influences the likelihood and strength of male mate-choice copying in the Trinidadian guppy. Focal observer males were presented with two stimulus females in a dichotomous choice test, either in the presence or absence of an equally attractive model male. There were three treatments where females differed in body length to different extents (i.e., either <5%, ~15%, or ~30%). Based on previous research, males in general should initially prefer larger over smaller females on average. I predicted that focal males (i) would be more likely to reverse their initial mating preference (i.e., mate-choice copy) in the presence than in the absence of a model male placed near the initially non-preferred female, and (ii) would be increasingly more likely to reverse their initial mating preference (i.e., more likely to mate-choice copy) with decreasing difference in the body length of paired stimulus females. My results only partially support these predictions.

## INTRODUCTION

In general, the most sexually active and(or) competitive individuals in a population usually attain the highest proportion of successful mating attempts (Andersson 1994). Females are generally perceived as the choosier sex; however, males have been shown to be choosy as well in numerous taxa (Edward and Chapman 2011). Male mate choice occurs when a male selects a female based on her inheritable phenotypic traits (Edward and Chapman 2011). There are two ways by which males can assess females as prospective mates: (i) direct (i.e., personal) sampling in the absence of social cues regarding the quality of the females being assessed, and (ii) indirect (i.e., social) sampling, whereby males use social information, such as viewing other males interact with prospective female mates, to subsequently make their own mating decision (Danchin et al. 2004; Kendal et al. 2005). Social information may mitigate the costs associated with mate searching, assessing and selecting a mate (Andersson 1994; Danchin et al. 2004; Kendal et al. 2005), but at the potential risk of elevated sexual and sperm competition (e.g., Wedell et al. 2002; Jeswiet et al 2012).

The use of social information is important and is common in the contexts of predator avoidance, foraging, and mating (Danchin et al. 2004; Galef and Laland 2005; Kendal et al. 2005). The likelihood of utilizing social information may be influenced by the ‘personality’ of a given individual. Indeed, in the Trinidadian guppy, White et al. (2017) demonstrated that females that are more sociable were more likely to mate-choice copy than less sociable ones. However, there is usually a trade-off associated with the use of social and personal information in making behavioural decisions by individual animals

(Kendal et al. 2005). Generally, when females are closely matched for quality, males are expected to experience greater difficulty discriminating between them as mates (especially if the male is sexually naïve). Under this circumstance, it might be more beneficial for a male to use social information regarding the sexual attractiveness or quality of available females and to copy the observed mate choice of other nearby (model) males. Conversely, if there are large differences in the quality of prospective female mates, then males may have little difficulty in directly assessing females based on traits that indicate their quality (i.e., use personal information). Under this circumstance, the use of social information in making mating decisions (i.e., mate-choice copying) may be less beneficial.

When a model male is assessing two or more females in the perceived absence of other males, he will likely use personal information to pursue one female over the other (alternatively, he could simply prefer neither female). However, if there is an eavesdropping (observer) male nearby, then the eavesdropping male may use social information gleaned by observing the model male's mate choice to subsequently make his own mate choice decision. The eavesdropping male may select a phenotypically different female (the one he had previously observed the model male interact with) than he might have otherwise selected, in turn potentially overriding his inherent preference for a particular female over others. The likelihood and strength of such a reversal of a male's inherent mating preference owing to socially-acquired information could be influenced by differences in the phenotype of females being assessed as prospective mates. Male mate-choice copying is deemed to have occurred if an observer male reverses his initial mating preference after he has viewed a model male sexually interact

with a female that the observer male had not initially preferred as a mate (cf. Dugatkin and Godin 1992). When the apparent phenotypic difference between females is small, then the observing male may be more likely to use social information over personal information to select a mate. The converse may be true when the female phenotypes differ considerably (cf. Kendal et al. 2005).

There are many different variables that make a good quality mate, and these criteria vary based on the individual and over time. There are also many sensory modalities used in the assessment of mate quality (e.g., olfactory, Guevara-Fiore and Watt (2009); auditory, Kniel et al. (2015); visual, Witte and Ryan (1998)). Within visual cues, male guppies avoid females that they have previously viewed mating, but do not avoid a female if she is prevented (via a Plexiglas barrier) from mating (Dosen and Montgomerie 2004). Female Barbary macaque (*Macaca sylvanus*) have been known to broadcast mating vocalizations. Males are receptive to female vocalization such that the dominant male generally outcompeted a subordinate to the speaker which was playing the female vocalization recording (Semple 1998). This use of social information may be useful for nearby males to assess their relative sexual and sperm competitiveness so as to maximize their fitness. Similarly, female yellow baboon (*Papio cynocephalus*) broadcast mating vocalization, which males have been shown to discriminate and prefer certain female vocalizations over others (Semple 2001). The ability for a male to discriminate between individual females appears to be adaptive when selecting a mate. Under certain circumstances, social information may override the inherent mating preference of a given individual when the latter can view another individual interact with a prospective mate

that was not initially preferred, especially if the observer can outcompete the model male (Dugatkin 1996; Witte and Ryan 1998).

The number of potential sexual rivals (i.e., models) can influence mate-choice copying behaviour in males. For example, Witte and Noltemeier (2002) demonstrated that female sailfin mollies mate-choice copy under certain circumstances. When two model females were near the initially non-preferred female for five minutes the focal female was more likely to reverse her initial mating preference relative to when there was one model female for ten minutes. Therefore, a perceived increase in either the level of sexual competition or the sexual attractiveness of the initially non-preferred female may result in an increased likelihood of mate-choice copying by the observer individual.

Similarly, the relative phenotype of the stimulus males may also influence the likelihood and strength of female mate-choice copying. Dugatkin (1996) demonstrated that female guppies from the Paria River in Trinidad mate-choice copy. Focal females were presented with two stimulus males that differed in body coloration, once her initial preference was recorded, followed by a similar-sized female (i.e., a model) being placed near the initially non-preferred male. Females were more likely to mate-choice copy when a model female was placed near the initially non-preferred male and the paired stimulus males differed in coloration (area of body covered with orange pigmentation) by 12 or 24%. However, females were less likely to mate-choice copy when the model was present and stimulus males differed by 40% orange pigmentation. This context dependent case of mate-choice copying demonstrates that the females use social information to make mate-choice decisions when the phenotypic differences between stimulus males are

small, but do not copy the apparent mate choice of other females when there is a large difference in male body coloration.

Furthermore, the difference in the body size of stimulus males (commonly a sexually selected trait, Andersson 1994) could also influence female mate-choice copying. Witte and Ryan (1998) tested whether the difference in the body size of stimulus males influenced the strength of female mate-choice copying in the sailfin molly. In this species, females prefer large over smaller males. They observed that 10 out of 12 focal females copied the apparent preference of a model female when the stimulus males were matched for body length. However, when stimulus males differed in body length (i.e., one large and one small), only 3 out of 15 females switched to preferring the small male after viewing a model female interact with the small male. Therefore, female sailfin mollies mate choice copy when the apparent difference in quality of potential mates being assessed is low, whereas they are less likely to mate-choice copy when there is a large difference in quality between prospective mates.

To summarize, both males and females can be choosy when selecting a mate. Social information and mate-choice copying could be used to mitigate the cost of mate choice. Mate-choice copying, however, comes at a potential cost of increased sexual and sperm competition. The likelihood and strength of female mate-choice copying has been shown to be influenced by differences in sexually-selected traits of the stimulus males on which females base their mating decisions (e.g., body coloration, Dugatkin 1996; body length, Witte and Ryan 1998). Furthermore, when stimulus males are matched phenotypically, then females are more likely to copy the mate choice of a model female compared to when the stimulus males differ considerably in phenotype; in the latter

situation, observer females are less likely to use social information and more likely to retain their initial mating preference (Dugatkin 1996; Witte and Ryan 1998).

In the current experiment, I investigated whether the likelihood and strength of mate-choice copying in males of the Trinidadian guppy are influenced by the magnitude of the difference in the phenotypes of paired stimulus females presented as prospective mates, whilst controlling for any differences in the phenotypes (body length and coloration) of the focal observer and model males in each trial. I experimentally manipulated the difference in the body length (which is positively correlated with fecundity, Houde 1997) of paired stimulus females in each of three treatments, namely, 5%, ~15% or ~30% depending on the treatment. Each treatment consisted of a Control Condition (i.e., model absent) and an Experimental Condition (i.e., model present). In the first treatment, focal males were presented with two stimulus females which differed by less than 5% body length. In the second treatment, focal males were presented with two stimulus females which differed by 15% in body length. Lastly, in the third treatment, focal males were presented with two stimulus females which differ by 30% in body length.

Based on theory (Andersson 1994) and previous empirical work on male mate choice in the guppy (e.g., Dosen and Montgomerie 2004; Herdman et al. 2004; Godin and Auld 2013; Auld and Godin 2015), I predicted that the focal males' initial mating preference to be the strongest when females differed most widely in body length. Furthermore, within the Control Condition (i.e., model absent) for each of the treatments, I predicted that focal males would consistently prefer one stimulus female (most likely the large female) over the other stimulus female (i.e., most likely the small female) across

the two (paired) preference tests. The Control trials provide us the opportunity to tease apart inherent mating preference for a female and the effect of social information. However, within the Experimental Condition (i.e., model present) for each of the treatments, I predicted that the focal male should be more likely to reverse his initial mate preference in Preference test 2 compared to Preference test 1 (i.e., to mate-choice copy). When a model male is present and the perceived difference between mates is small (i.e., two potential mates appear more similar than dissimilar), then it is more likely that the individual selecting a mate would more likely rely on social cues or the perceived preference of the nearby male (Kendal et al. 2005). Therefore, I predicted that when the perceived difference in female body length is small (i.e., difference of < 5%), then focal males should be more likely to use the social information provided by the model male and more likely to reverse his initial mating preference (i.e., to mate-choice copy). In contrast, when the difference in female body length is moderate (i.e., ~15% difference) and/or large (i.e., ~30% difference), then focal males should be less likely to mate-choice copy and for any observed copying responses to be weaker relative to when the females are relatively similar in body length (i.e., Treatment 1). The predictions that males should switch their inherent mating preferences in the presence of a model and that the likelihood of occurrence, and that the strength of copying responses should be dependent on the magnitude of the difference in the phenotypes of females being assessed as prospective mates, are consistent with social information theory (Kendal et al. 2005) and mate-choice copying theory (Dugatkin and Godin 1992; Dugatkin 1996; Witte and Ryan 1998).

## **METHODS**

### **Study subjects and maintenance conditions**

The source of the study subjects and the general fish husbandry conditions in the laboratory were previously described in Chapter 2.

### **Experimental apparatus**

The experimental apparatus used for this experiment (Figure 2-1) was illustrated and described in detail in Chapter 2.

### **Experimental protocol**

The current experiment comprised of three independent Treatments (Table 4-1), each with two Conditions, one Control (no copying opportunity) and one Experimental (copying opportunity available).

The Control Condition tested for temporal consistency of the mating preference expressed by individual focal males between two consecutive preference tests in the absence of any model male (Figure 2-1). Based on the previous findings of Godin and Auld (2013), Auld and Godin (2015), and the results presented in Chapter 3 of this thesis for Upper Aripo River guppies, I expected males to be consistent in their mating preference for a particular female between the repeated preference tests. The experimental Treatments were designed to test whether a focal male's initial mating preference could be altered by an opportunity to view and copy the apparent mating preference of another male (the model; Figure 2-1), which was matched in body length

and coloration with the focal male in each trial (Table 4-1). The Control and Experimental Conditions were carried out in each of the three Treatments separately, wherein the difference in female length was either Small ( $< 5\%$ , Treatment 1), Moderate ( $\sim 15\%$ , Treatment 2), or Large ( $\sim 30\%$ , Treatment 3) (Table 4-1). I so manipulated the difference in the body length of paired stimulus females to test for its effect on the likelihood and strength on any mate-copying response exhibited by focal males in the presence of a model male in comparison with its absence. By design, the body length and colour score of the focal, model, and pseudo-model males were similar within and among treatments (Table 4-1).

All trials followed the same general protocol testing for focal male mating preferences and mate-choice copying, as described in Chapter 2 and depicted in Figure 2-1. The Treatments and Conditions were carried out in random order, and each was replicated 21 times with different fish that showed no side bias (cf. Chapter 2), for a total of 126 valid trials.

If male guppies copy the apparent mating preferences of other males, then I expected either a significant weakening or a reversal of a focal male's initial preference for a given female following his observing of a model male near the other, initially non-preferred female (Experimental Condition) compared to his behaviour in the absence of a model male (Control Treatment), especially when females are similar in body size (cf. Dugatkin 1992; Dugatkin and Godin 1992; Witte and Ryan 1998). Moreover, as predicted by social learning theory and the use of public information (Kendal et al. 2005) and previous work on another poeciliid fish species (Witte and Ryan 1998), I expected that the likelihood of copying and the strength of the copying response (i.e., response

difference score between the two preference tests; Figure 2-1) to be greatest when paired stimulus females were similar in body length (Treatment 1) and lowest when the difference in the body length of paired stimulus females was greatest (Treatment 3).

## **Measuring fish body length and male colour ornamentation**

At the end of each behavioural trial, all fish used in the trial were lightly anaesthetized and their left side photographed with a digital camera under standardized lighting conditions. The methods used to photograph the fish and to measure their body length and to quantify the colour ornamentation of males from their individual photograph using *Image J*<sup>®</sup> software were described in Chapter 2.

## **Statistical analyses**

First, I tested whether focal males exhibited an overall preference for the larger of the two paired stimulus females in Preference test 1 in the absence of social information (data pooled across all treatments and conditions) using the Binomial test (for frequencies) and paired *t*-test (for association time). I then tested whether focal males differed in their strength of initial preference (using association time) for the large female across treatments and condition using an ANOVA. I predicted that as the difference in the body length of paired stimulus females increases, so would the strength of the focal males' initial mating preference for the larger female on average.

Second, I used a general linear model to test for the main effects of the predictor variables, Preference test (i.e., Preference test 1 vs. 2), Condition (Control vs. Experimental), and Treatment (Small, Moderate, Large), and their potential interaction on

association time spent by focal males with their initially non-preferred female. The body length and colour ornamentation score of the focal males were included as covariates in the model, as the phenotype of male Trinidadian guppies is known to influence their sexual behaviour (Dosen and Montgomerie 2004; Herdman et al. 2004; Jeswiet et al. 2012; Godin and Auld 2013; Auld et al. 2017). Because this response variable was not normally distributed (Shapiro-Wilk test), these data were normalized using square-root transformation prior to analysis.

Third, I compared the magnitude of the response variable among treatments using the ANOVA-based Tukey HSD test for post-hoc multiple comparisons of means.

Fourth, for each Treatment and Condition separately, I compared the association time that individual focal males spent with the initially non-preferred female between Preference tests 1 and 2 using the paired *t*-test. A significant increase in this response variable between these paired Preference tests in either of the three Experimental treatments (model male present), relative to the three Control treatments (mode male absent), is taken as evidence for mate-choice copying (cf. Dugatkin and Godin 1992). Since time spent with the initially non-preferred female was not normally distributed (Shapiro-Wilk test), these data were normalized using square-root transformation prior to analysis.

Lastly, the number of focal test males reversing their mating preferences for a particular female between Preference tests 1 and 2, as would be expected if mate-choice copying occurred (cf. Dugatkin and Godin 1992), was compared across Treatments and Conditions using the *G*-test adjusted with Williams correction.

**Table 4-1.** Mean ( $\pm$  SE) standard body length and body colour ornamentation score for focal, model, and pseudo-model males, and standard body length of paired stimulus females, used in individual trials in each of the three Treatments (which are boldfaced numbered in the first column of the table), and between the two Conditions (i.e., Control and Experimental) within Treatments. The within-treatment comparisons were carried out using the paired *t*-test. The merged cells compare focal and model males, whereas the non-merged cells compare focal and pseudo-model males. The among-treatment comparison tests for similarities between Conditions and across the three Treatments using the ANOVA

Difference in stimulus female length	Male length (mm)			Male colour score (%)			Stimulus female length (mm)	
	Focal	Model	Pseudo-model	Focal	Model	Pseudo-model	Large	Small
<b>1. Small, Control</b>	15.6 $\pm$ 0.2	-		6.0 $\pm$ 0.7	-		19.1 $\pm$ 0.3	18.4 $\pm$ 0.3
Within-treatment	-						<i>t</i> = 7.18 <i>p</i> < 0.001	
<b>1. Small, Experimental</b>	16.2 $\pm$ 0.2	15.9 $\pm$ 0.2	15.8 $\pm$ 0.2	6.2 $\pm$ 0.48	5.3 $\pm$ 0.37	6.3 $\pm$ 0.44	18.4 $\pm$ 0.3	17.9 $\pm$ 0.3
Within-treatment	<i>t</i> = 1.69 <i>p</i> = 0.11		<i>t</i> = 1.67 <i>p</i> = 0.11	<i>t</i> = 1.4 <i>p</i> = 0.18		<i>t</i> = 0.13 <i>p</i> = 0.90	<i>t</i> = 7.13 <i>p</i> < 0.001	
<b>2. Moderate, Control</b>	15.6 $\pm$ 0.2	-		5.7 $\pm$ 0.44	-		20.0 $\pm$ 0.2	17.3 $\pm$ 0.2
Within-treatment	-						<i>t</i> = 39.6 <i>p</i> < 0.001	
<b>2. Moderate, Experimental</b>	15.7 $\pm$ 0.1	15.8 $\pm$ 0.1	15.6 $\pm$ 0.2	7.3 $\pm$ 0.48	6.6 $\pm$ 0.5	6.1 $\pm$ 0.48	20.3 $\pm$ 0.2	17.5 $\pm$ 0.2
Within-treatment	<i>t</i> = -1.24 <i>p</i> = 0.22		<i>t</i> = 0.2 <i>p</i> = 0.85	<i>t</i> = 1.57 <i>p</i> = 0.13		<i>t</i> = 2.11 <i>p</i> = 0.05	<i>t</i> = 32.72 <i>p</i> < 0.001	
<b>3. Large, Control</b>	15.7 $\pm$ 0.1	-		6.8 $\pm$ 0.55	-		22.1 $\pm$ 0.2	16.53 $\pm$ 0.2
Within-treatment	-						<i>t</i> = 30.2 <i>p</i> < 0.001	
<b>3. Large, Experimental</b>	15.87 $\pm$ 0.2	15.95 $\pm$ 0.2	15.78 $\pm$ 0.2	5.5 $\pm$ 0.57	5.2 $\pm$ 0.5	5.1 $\pm$ 0.42	21.5 $\pm$ 0.3	16.05 $\pm$ 0.2
Within-treatment	<i>t</i> = -0.46 <i>p</i> = 0.64		<i>t</i> = 0.45 <i>p</i> = 0.65	<i>t</i> = 0.71 <i>p</i> = 0.49		<i>t</i> = 0.62 <i>p</i> = 0.55	<i>t</i> = 30.7 <i>p</i> < 0.001	
Among-Conditions	<i>F</i> = 3.25 <i>p</i> = 0.07	-		<i>F</i> = 0.19 <i>p</i> = 0.66	-		<i>F</i> = 2.34 <i>p</i> = 0.13	<i>F</i> = 2.10 <i>p</i> = 0.15
Among-Treatments	<i>F</i> = 1.21 <i>p</i> = 0.30	<i>F</i> = 0.14 <i>p</i> = 0.87	<i>F</i> = 0.29 <i>p</i> = 0.75	<i>F</i> = 0.33 <i>p</i> = 0.71	<i>F</i> = 2.84 <i>p</i> = 0.07	<i>F</i> = 1.78 <i>p</i> = 0.18	<i>F</i> = 73.7 <i>p</i> < 0.001	<i>F</i> = 33.5 <i>p</i> < 0.001
Among Condition x Treatment	<i>F</i> = 0.86 <i>p</i> = 0.42	-		<i>F</i> = 3.63 <i>p</i> = 0.03	-		<i>F</i> = 2.23 <i>p</i> = 0.11	<i>F</i> = 1.87 <i>p</i> = 0.16

## RESULTS

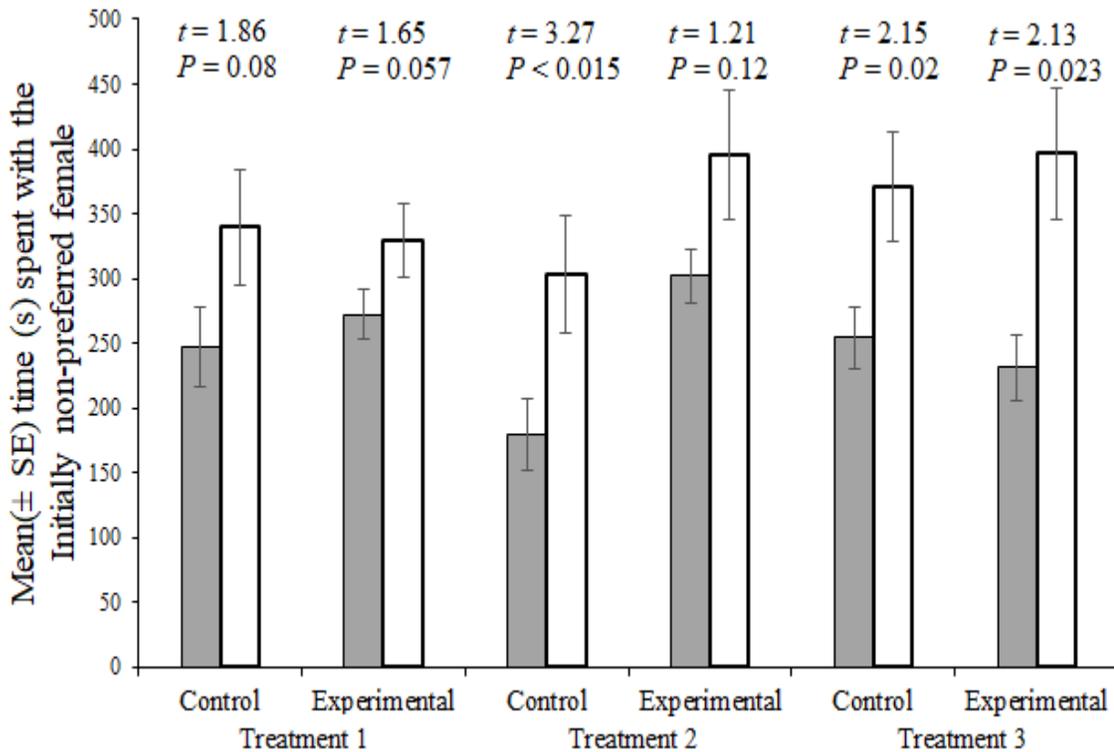
Although expected based on previous studies (Dosen and Montgomerie 2004; Herdman et al. 2004; Jeswiet et al. 2012; Godin and Auld 2013; Auld and Godin 2015), the number of focal male guppies in the current study that categorically preferred the larger over the smaller stimulus female in Preference test 1, in the absence of any social information about the mate-choice of another (model) male, was not significantly different from that expected by chance (72 out of 126, or 57.14%; Exact binomial test,  $p = 0.13$ ). Nonetheless, focal males spent significantly (paired  $t$ -test,  $t_{125} = 2.2$ ,  $p = 0.029$ ) more time overall associating with the larger stimulus female (mean  $\pm$  SE =  $502 \pm 24.4$  s) compared with the paired small female ( $405 \pm 22.8$  s) in Preference test 1. The square-root transformed association time the focal male spent with the large (Shapiro-Wilk test,  $W = 0.98$ ,  $p = 0.13$ ) and small ( $W = 0.99$ ,  $p = 0.78$ ) females were normally distributed. However, contrary to my *a priori* prediction, focal males did not differ overall in their association time with the larger female in Preference test 1 either among Treatments (Treatment 1,  $466 \pm 42$  s; Treatment 2,  $510 \pm 46$  s; Treatment 3:  $532 \pm 40$  s;  $F_{2,120} = 0.61$ ,  $p = 0.54$ ) or between Conditions (Control,  $553 \pm 40$  s; Experimental,  $453 \pm 27$  s,  $F_{1,120} = 2.21$ ,  $p = 0.14$ ), and there was no Treatment x Condition interaction ( $F_{2,120} = 0.36$ ,  $p = 0.696$ ). Therefore, male association time with the larger female was not influenced by the magnitude of the relative difference in the body length of the paired stimulus females.

Overall, the association time that focal males spent with the initially non-preferred female was significantly influenced by Preference test (Preference test 1,  $248 \pm 10.5$  s; Preference test 2,  $356 \pm 17.9$  s; linear model,  $F_{1,238} = 22.58$ ,  $p < 0.001$ ) and Condition

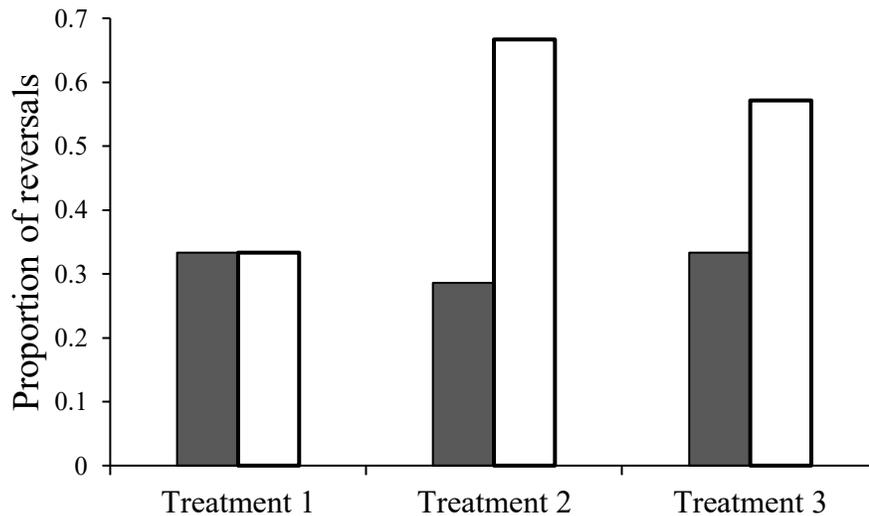
(Control,  $282 \pm 21.7$  s; Experimental,  $321 \pm 20.8$  s;  $F_{1, 238} = 5.01$ ,  $p = 0.03$ ), but not by Treatment (Small,  $297 \pm 22.5$  s; Moderate,  $295 \pm 27.8$  s; Large,  $313 \pm 27.5$  s;  $F_{2, 238} = 0.58$ ,  $p = 0.56$ ), when the body length and colour ornamentation score of focal males were controlled for as covariates (Figure 4-1). There was a marginally significant Condition x Treatment interaction ( $F_{2, 238} = 3.51$ ,  $p = 0.03$ ; see Figure 4-1). The residuals from this model are normally distributed (Shapiro-Wilk,  $W = 0.99$ ,  $p = 0.55$ ). Notwithstanding this overall effect of social information (i.e., presence of model male), focal males did not, however, alter their association time with the initially non-preferred female across Treatments; that is, the magnitude of the relative difference in the body length of the stimulus females had no effect on male mating preferences contrary to expectation.

In the Experimental Condition (i.e., model male present near the initially non-preferred female during the viewing period, Figure 2-1), focal males modified their mating preference for a particular female between the paired preference tests such that they significantly increased the time spent associating with their initially non-preferred female in Preference test 2 compared to Preference test 1 in Treatments 3 (Large difference in stimulus female body length) and tended to do so ( $p = 0.057$ ) in Treatment 1 (Small difference in body length of stimulus females), however, did not significantly ( $p = 0.12$ ) differ in Treatment 2 (Figure 4-1). Similarly, but unexpectedly, focal males in the Control Condition (i.e., model male absent) also increased their association time for the initially non-preferred female between the two consecutive preference tests, and significantly so in Treatments 2 and 3 (Figure 4-1), such that they were generally not consistent in their mating preferences in the absence of social information. In Treatment

1, focal males were only weakly consistent ( $p = 0.08$ ) in their initial mating preference between the consecutive tests in the Control Condition. Reflecting these findings based on association time, there were therefore no significant differences between the results of the Control and Experimental Conditions within Treatments (Figure 4-1) and no difference in the relative frequencies of mate-choice reversals by focal males between the two consecutive preference tests across Conditions and Treatments ( $G$ -test,  $G_{adj} = 1.11$ ,  $df = 7$ ,  $p > 0.05$ ; Figure 4-2).



**Figure 4-1.** Mean ( $\pm$  SE) association time (s) focal males spent near the initially non-preferred female in paired Preference test 1 (filled bars) and Preference test 2 (open bars) between the two Conditions (Control: model absent, and Experimental: model present) within each of the three Treatments, which varied in the relative difference of the body lengths of the paired stimulus females (Treatment 1: Small (<5%) difference; Treatment 2: Moderate (~15%) difference; Treatment 3: Large (~30%) difference).  $n = 21$  for each Condition within each Treatment. Comparison of means between the paired preference tests within each Condition were made using the paired  $t$ -tests and comparisons between Conditions and Treatments were made using the post-hoc Tukey HSD test. Only significant  $p$ -values for the latter test are shown.



**Figure 4-2.** The proportion of focal males that reversed their initial mating preference for a particular female between the paired Preference tests 1 and 2 in each of the three Treatments, when the relative difference in the body length of the paired stimulus females was either Small (< 5%, Treatment 1), Moderate (~15% Treatment 2), or Large (~30%, Treatment 3). Closed bars represent the Control Condition (no model present) and open bars represent the Experimental Condition (model present during the viewing period) within each Treatment. A reversal is defined as when the focal male reversed (i.e., switched) his initial mating preference for a particular female (in Preference test 1) towards the other stimulus female (i.e., spent >50% of his total association time near the latter female) in Preference test 2.  $n = 21$  for each Condition within each Treatment.

## DISCUSSION

In the current experiment, focal male guppies exhibited considerable inter-individual variation in their initial mating preference (Preference test 1) for either of the paired stimulus females, as they did in my first experiment (Chapter 3). Although the number of males preferring either the small or large stimulus female did not differ from that expected by chance ( $p = 0.50$ ), focal males nonetheless spent significantly more time (~25% more on average) associating with the larger female compared with the small one in Preference test 1 (i.e., no model male present). This result corroborates previous findings demonstrating a general preference for larger females as mates (in the absence of social information about the mating preferences of other nearby males) in males of the Trinidadian guppy originating from the same study population as mine (Godin and Auld 2013; Auld and Godin 2015; Auld et al. 2017) and other populations in Trinidad (Herdman et al. 2004; Jeswiet et al. 2012). By preferentially choosing larger females as mates, male guppies would mate with more fecund females (Houde 1997) and presumably gain a reproductive benefit in doing so.

Regardless of their individual initial mating preference for the larger or smaller female, individual focal males were inconsistent in their mating preference over time (Preference tests 1 vs. 2) when the model male was absent (i.e., the Control Condition) in Treatments 2 and 3 and only marginally consistent in Treatment 1 (Figure 4-1), such that focal males generally spent more of their association time with the initially non-preferred female in Preference test 2 than in Preference test 1. This result is unexpected and surprising given that male guppies originating from the Upper Aripo River were strongly

consistent in their initial mating preferences over time in the absence of a model male in my first experiment (Chapter 3) and in previous studies with both wild-caught and laboratory-born males from this same riverine population (Godin and Auld 2013; Auld and Godin 2015).

Following their observation of a model male interacting with their initially non-preferred female (Experimental Condition), focal males modified their initial mating preference and increased their associating time near the other female that they had not initially preferred (and significantly so in Treatments 3 and marginally so in Treatment 1, and not significantly so in Treatment 2). Although this socially-mediated increase in association time spent near the initially non-preferred female is as expected if males were copying the observed apparent mating preference of a nearby model male (cf. Dugatkin and Godin 1992), it cannot be attributed here to social learning and mate-choice copying because focal males in the Control Condition (i.e., model male absent), irrespective of Treatment, were generally not consistent in their mating preferences between the two consecutive preference tests but rather similarly increased their association time spent near their initially non-preferred female between Preference tests 1 and 2 (Figure 4-1). As a consequence of such similar temporal trends in response, there were no significant differences in the results between the Control (model male absent) and Experimental (model present) conditions within and between Treatments (Tukey HSD test). Overall, the results of the current experiment (Figures 4-1 and 4-2) are not as expected *a priori* and thus do not provide unequivocal evidence for mate-choice copying. More parsimoniously, the observed differences in mating preferences between Preference tests 1 and 2 (Figure 4-1) more likely reflect a temporal (sequence) effect on male mating

preferences in all Conditions and Treatments rather than mate-choice copying behaviour. As a consequence, none of my *a priori* predictions regarding the occurrence of mate-choice copying and the potential influence of the relative difference in the body length of paired stimulus females on mate-choice copying behaviour in males were supported by the results of the current experiment.

As mentioned above, the lack of evidence for male mate-choice copying in the current experiment using laboratory-born guppies originating from the Upper Aripo River in Trinidad is inconsistent with the results of Chapter 3 and previous results of Godin and Auld (2013) and Auld and Godin (2015) with male guppies from this same population; they reported highly consistent and repeatable male mating preferences in the absence of social information (model male absent) and mate-choice copying in the presence of social information (model male present). Moreover, my current results are also inconsistent with the results of my first experiment (Chapter 3), which showed that males were consistent in their initial mating preference over time in the Control treatment (model male absent) and exhibited mate-choice copying behaviour when given the opportunity to do so (Experimental treatment; model male present), especially so when the focal and model males were phenotypically matched.

Why laboratory-born male guppies originating from the Upper Aripo River expressed mate-choice copying behaviour in my first experiment (Chapter 3), but not in the current experiment, is perplexing and difficult to explain. One obvious difference between the two experiments is that there were almost twice as many trials per treatment in the first relative to the second experiment. Increasing the sample size in the current experiment to similar numbers from the first experiment may help lower the variance

within treatments and increase statistical power (Figure 4-1). This difference in experimental outcome cannot be readily attributable to any obvious differences in laboratory and husbandry conditions, experimental apparatus or experimental protocol because these were similar in the two experiments. Moreover, in both experiments and in particular treatments within the experiments, the focal and model males were matched phenotypically and focal males were exposed to paired stimulus females that differed considerably in body length as part of their respective experimental design. When comparing the timing of the two experiments, there was generally more human traffic and noise in the corridor outside our aquatics laboratory and the shift in ambient air temperature during the period (late Spring/Summer) that the current experiment was carried out compared to when the first experiment was carried out (Fall/Winter); this extra noise during the course of the current experiment may have disturbed and stressed the guppies in the latter experiment. Additionally, in my haste to complete my experimental work in a timely manner so as to submit my thesis by the university's deadline, I may have inadvertently and unconsciously stressed some of my experimental guppies by handling them more hastily than necessary. These latter two sources of potential external disturbance and stress may have adversely affected the behaviour of some of the experimental guppies used in the current experiment.

Although the results of current experiment were on the whole negative, the hypothesis that males should be more likely to use social information and to mate-choice copy when prospective mates are similar in phenotypic traits that indicate quality than when considerably different in phenotype is theoretically important (cf. Kendal et al.

2005) and thus warrants re-testing with the Trinidadian guppy as well as with other species in the future.

## **Chapter 5**

### **GENERAL DISCUSSION**

## Overview of salient results

I experimentally demonstrated that male guppies originating from the Upper Aripo River in Trinidad are able to copy the apparent mating preference of another nearby (model) male under certain conditions in one experiment (Chapter 3), but not in another (Chapter 4). Such socially-mediated mate choice behaviour corroborates the recent findings of Auld and Godin (2015), who reported that males from this same population mate-choice copy when given the opportunity to do so. Therefore, the presence of social information can influence the mate-choice decisions of male Trinidadian guppies under certain conditions, as it does in female guppies from various Trinidadian populations (e.g., Dugatkin 1992, 1996; Dugatkin and Godin 1992, 1993; Briggs et al. 1996; Godin et al. 2005; Godin and Hair 2009; but see Lafleur et al. 1997; Brooks 1999). In my first experiment (Chapter 3), focal males copied the apparent mating preference of a nearby model male conspecific. The magnitude of their mate-copying responses was greatest when the model male was smaller and less colour ornamented, and thus less sexually attractive and a poorer sperm competitor, than the observing male. Observer male guppies were apparently capable of assessing the relative sexual and potential sperm competitiveness of rival males in the current study, as previously established in other studies (Jeswiet et al. 2011, 2012; Auld et al. 2017), such that they were *less* likely to copy when model males were more attractive than the observer males, and were *more* likely to copy when model males were less attractive than the observer males. These results are novel and important, as they demonstrate that the use of social information by males in their mating decisions is contingent on the relative sexual attractiveness and sexual competitiveness of observer and model males, as indicated by honest phenotypic traits such as body length and colour ornamentation.

Unfortunately, the results of my second experiment (Chapter 4) did not provide any unequivocal evidence for male mate-choice copying in male Trinidadian guppies when the phenotype of observer and model males were matched and when difference in the body length of paired stimulus females was manipulated to varying extent depending on treatment. Such negative results were unexpected and are difficult to explain (see Discussion of Chapter 4) considering previous empirical evidence (Chapter 3; Auld and Godin 2015) for mate-choice copying in male Trinidadian guppies from my study population (Upper Aripo River). Nonetheless, the hypothesis under test in my second experiment (Chapter 4), that males should be more likely to use social information and to mate-choice copy when prospective mates are similar in phenotypic traits that indicate their quality than when considerably different in phenotype, is theoretically important (cf. Kendal et al. 2005) and thus warrants re-testing with the Trinidadian guppy as well as with other species in the future. A similar hypothesis has been tested for females in the context of mate-choice copying in at least two species. First, Dugatkin (1996) showed that female Trinidadian guppies were more likely to copy the apparent mating preference of a model female when paired stimulus males differed by 12 or 24% orange pigmentation, respectively, but they retained their initial mating preference (i.e., did not mate-choice copy) when stimulus males differed greatly (40%) in orange pigmentation. Second, female sailfin mollies were similarly more likely to mate-choice copy when paired stimulus males were similar in body length than when they differed considerably in body length (Witte and Ryan 1998).

Although female mate-choice copying has been extensively documented in the Trinidadian guppy (e.g., Dugatkin 1992, 1996; Dugatkin and Godin 1992, 1993; Briggs

et al. 1996; Godin et al. 2005; Godin and Hair 2009), studies of mate-choice copying in males, either using the guppy (current thesis; Auld and Godin 2015) or other species as model systems, have been very limited to date. Mate-choice copying in the Trinidadian guppy has been found in wild-caught male guppies from the Upper Aripo River (the same population used in this study). However, the strength and frequency of mate-choice copying using a laboratory-born and -reared Upper Aripo River guppies (current study) were lower than for wild-caught conspecifics from the same population (Auld and Godin 2015). Nonetheless, my current study is the first to demonstrate that the relative sexual attractiveness/competitiveness of the model male, relative to the focal observer male, influences the likelihood and strength of male mate-choice copying, such that observer males were more likely to copy the mate choice of less attractive models and less likely to copy the mate choice of more attractive models (Chapter 3). These results show that the nature of the social information received and used by observer males is important in their mating decision making and can determine their likelihood of either copying the observed mate choice of others or ignoring such social information and using their own personal information about the quality of prospective mates when choosing their mates.

### **Directions for future research**

Using the comparative approach, testing the hypotheses regarding male mate-choice copying elaborated in this thesis using other live-bearing fishes that differ in their mating systems (from entirely coercive to almost entirely courtship) should be a fruitful avenue for future research and would shed some insights into whether and how variation

in the risk and intensity of sexual and sperm competition across poeciliid taxa may have played a role in the evolution of mate-choice copying as a mating strategy.

To test for the external validity of male mate-choice copying, an experiment where the model male could fully interact with the female would test, under more natural conditions, how the observer male will respond once he views the model sexually pursuing and potentially mating with the initially non-preferred female (similar to Dosen and Montgomerie 2004). Would the observer male still reverse his preference and potentially risk increased sexual and sperm competition? One benefit is that the guppy has been shown to have some degree of last male sperm precedence (Evans and Magurran 2000); therefore, it might still be beneficial for the observer male to switch his mate preference (i.e., mate-choice copy), because if the observer male was observed mating with his initially-preferred female, then his sperm might be displaced by eavesdropping males.

My experimental design could be extended to characterize ‘personality’ differences (e.g., boldness, exploratory activity, etc.) among focal test fish before testing for their mate-choice copying tendencies under standardized conditions. Moreover, females generally prefer bolder males (Godin and Dugatkin 1996). This would combine what is known about social information theory and mate-choice copying. Recall that White et al. (2017) demonstrated that more sociable female guppies were more likely to mate-choice copy than less sociable ones. I would predict that more sociable males would similarly be more likely to mate-choice copy. The addition of personality data (perhaps from both observer and model males) might help explain some of the variance within and between my experiments (Chapters 3 and 4) in this current study.

Future studies could try to use results from mate-choice copying experiments in the context of population management. If a management goal is to increase population size, then it would be advisable to have unattractive model cues (visual, olfactory, and(or) acoustic) near more fecund females in order to increase male mating attempts with them. Conversely, if the management goal is to reduce population size (e.g., of an invasive species, Lindholm et al. 2005; Sievers et al. 2012), then the addition of unattractive model male cues, along with being paired with an attractive yet sterile female, may decrease the number of successful progeny produced

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