Ontogenetic environments and female mate choice in guppies, *Poecilia reticulata*

Submitted by Alessandro Macario to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Psychology

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I certify that all material in this thesis, which is not my own work, has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

Signature: ……

Abstract

Theoretical models of sexual selection assume that female mating preferences are fixed and variation found between individuals resulting solely from allelic variation at specific loci coding for sexual preferences. For the last decade, an increasing number of studies have demonstrated that individual phenotypic variation in preferences was common across a wide range of taxa and induced by the environmental context and the females' condition. Further, developmental stages of life are crucial in the formation of behaviours in general and have proven to be determinant to learn sexual preferences in some species that dispense care for their young. However, very little studies have analysed how the early social and physical environments shape female mate choice in species that lack parental care. In this thesis, I used guppies *(Poecilia reticulata)*, firstly, to investigate the influence of various aspects of the social environment provided by males during two ontogenetic phases. Secondly, I explored whether learned preferences in a foraging context during development could be transferred into a mating context.

Considering the early social environment, I explored three distinctive features potentially displayed by males and that females might experience while growing. Females were reared with different values of a sexual trait not genetically preferred in the population (orange colour) and different values of a trait for which they had innate predisposition (total colour area). In both cases, females were exposed to the different treatments for the whole developmental period or for its later phase. My results indicated that females changed their sexual behaviours in response to both type of traits experienced, reversing sometimes their genetic preferences. Moreover, the timing of exposure seemed to be a key factor in the acquisition of preferences as females exposed only to the later part of development with different values of total colour didn't rely anymore on colour patterns to discriminate among males. In a third body of experiment, I examined whether the overall phenotypic variance exhibited by males during whole development, independently of the values of a specific sexual cue, mediated female's behaviours. In a context of high variance, female became choosier relatively to those experiencing less variance. As a response, males switched mating tactics and attempted more forced copulations.

In its final part, my thesis searched for a link that might have arisen, owing to developmental conditions, between preferences using the same sensory modality in two behavioural contexts. Maturing females were given food that was associated to a certain colour and subsequently tested for both their coloured preference in a foraging and a sexual context. Although no foraging preference for the corresponding colour was detected, females that experienced a yellow stimulus preferred yellower males compared to females with other experiences.

Taken together these results suggest that developmental conditions and especially the social environment play a pivotal role in the process of mate choice. Under some circumstances, learned mate preferences override genetically-based preferences highlighting the importance of non-genetic mechanisms. Accordingly, it is urgent to integrate in the study of sexual selection and reproductive isolation this dimension. In guppies, for instance, the effect of early social life might contribute to the maintenance of colour pattern polymorphism found in males.

Table of Contents

List of Figures

List of Tables

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Chapter two:

I devised and designed the study, carried out the data collection and statistical analysis. John Endler provided guidance for planning the experiments and helpful comments on the manuscript. Sonia Chapman helped with the husbandry associated with the rearing treatments.

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1.Chapter I:
General introduction

1.1. Introduction

Sexual selection by female mate choice is widely recognised as a powerful driving force behind the origin and evolution of secondary sexual characters (Endler 1986; Andersson 1994; Hoekstra et al. 2001; Kokko et al. 2003; Shuster & Wade 2003; Andersson & Simmons 2006; Kokko, Jennions & Brooks 2006). In spite of a vast body of theoretical work and empirical studies emphasizing the importance of mate choice and its ubiquity across a wide range of taxa (Andersson 1994; Mead & Arnold 2004), a comprehensive understanding of the evolution of mate preference still needs to be achieved (Kokko et al. 2003). Traditional approaches accounting for the origin and evolution of female preferences are based on pure genetic models such as the Fisherian runaway process, the good-genes hypothesis or the pre-existing sensory bias model, ignoring non-genetic factors. However, during the last decade, interests in the effects that environmental and developmental changes could potentially have on the outcome of sexual behaviour has grown, highlighting how female mate preferences may vary within and between individuals in response to these changes *(Jennions & Petrie 1997; Wagner 1998; Widemo & Saether 1999; Brooks & Endler 2001b; Lehtonen & Lindstrom 2008)*. This phenomenon is also known as phenotypic plasticity (Pigliucci, Murren & Schlichting 2006; Fusco & Minelli 2010). This introduction aims to draw an inventory of the currently known source of phenotypic variation in female mate preferences and analyse the evolutionary implications of such variations.

Until a decade ago mating preferences were seen as species-specific and uniform (Jennions & Petrie 1997; Widemo & Saether 1999) even though geographical variation among populations was well documented in taxa like fish, amphibians, insects and birds (Endler & Houde 1995; Jennions & Petrie 1997). Behavioural flexibility in mate choice was thought to be the result of errors in mate assessment or limited availability of partners. However, there is now a large body of evidence showing that phenotypic variation in female mate preference within a population is biologically relevant and could have significant evolutionary consequences. Individual variation in preferences is common and can influence the

mode, direction and strength of sexual selection on male traits (Jennions & Petrie 1997; Brooks & Endler 2001b; Cornwallis & Uller 2010).

1.2. Variation in female mate preferences

1.2.1 Environmental contexts

Different environmental conditions can significantly modulate the choice than females exert on males (Table 1.1). In guppies and swordtails (Godin & Briggs 1996; Johnson & Basolo 2003), increased apparent risk of predation reduces the preference females have for elaborated ornaments. In guppies females diminish their sexual activity and preference for more colourful males whereas swordtail females prefer males without elongated swords. These results confirm theoretical models (Pomiankowski 1987) predicting that female preference should decrease with increasing costs of mate choice such as predation risk and thus relaxing sexual selection on males' sexual signals. The vast majority of studies follow that pattern but an exception has been found in fiddler crabs. In the species *Uca beebei*, females exposed to males with and without the preferred sexual trait (mud pillars at the entrance of their burrows) express stronger preference for the males' signal when the perceived risk of predation increases (Kim et al. 2009).

Some physical environmental elements such as lighting conditions and water turbidity could also alter female mate choice. Fuller & Noa (2010) reared bluefin killifish in different lighting environment (clear vs. tea-stained) and measured subsequent female preference under these two conditions. They observed that the environment experienced during development and the light conditions during mate selection interact with genetics to determine preferences. In guppies, temporal and spatial heterogeneity in ambient light spectrum affect female sexual responsiveness and males' attractiveness, influencing the mode and strength of sexual selection occurring in different environment (Gamble et al. 2003).

This review is non-exhaustive and these influences and others concern many other taxa (see table 1.1).

1.2.2 Female's condition and physiological state

In an elegant review, Cotton et al. (2006) put forward empirical evidence that female mate preferences are condition-dependent and potentially interact with the context in which the mating decision is made. Variation in female's condition (i.e. variation in female's viability) results from gene-environment interactions during development (West-Eberhard 2003) but also from short-term environmental variation such as resource availability, both of which lead to different physiological phenotypes. These differences mediate how resources are allocated to body maintenance and reproduction. Condition-dependent mate preferences depend on the ability an individual has to pay the costs involved in the process of mating. High-quality females are expected to show stronger preferences and/or increase sampling efforts compared to low-quality females resulting in having more attractive males in average.

This prediction is supported by Holveck & Riebel (2010) who experimentally manipulated the conditions of female zebra finches (*Taeniopygia guttata*) by varying the size of the broods producing low-quality females if raised in large broods and high-quality females if raised in small broods. They demonstrated that only high-quality females preferred high-quality males' displays while low-quality females preferred low-quality male's song. Alternatively low-quality females could end up mating with less attractive males and avoid losing breeding opportunities since high-quality males reject low-quality females or are monopolized by high-quality females. Mating costs are in these situations reduced per se.

In the swordtail fish (*Xiphophorus birchmanni*), experimentally food-deprived females show significantly stronger preference for chemicals cues indicating the nutritional conditions of males (Fisher & Rosenthal 2006). The authors emphasize that covariance between the strength of female preference and resource

availability might constitute a common cause of variation found in female mate preference within and between populations.

The different reproductive stages that females experience during a breeding cycle may also play a role in female mate choice flexibility. Females in Túngara frogs (*Physalaemus pustulosus*) base their choice almost entirely on acoustic signals produced by males. Lynch et al. (2005) examined whether different reproductive phases within a breeding cycle could mediate some aspects of female sexual behaviour. They found that when the time at which females need to release their eggs approaches the females lower their threshold criteria for accepting male's signals, thus becoming less choosy.

Age is another important source of variation in female mate preference. In satin bowerbirds (*Ptilonorhynchus violaceus*), females discriminate among males through a complex multistage process assessing various behavioural cues and decorated built-structures called bowers (Patricelli et al. 2002). Coleman et al. (2004) showed that there is plasticity in female mate preference due to age-specific and stage-specific differences for the different males 'displays; younger females relying more on the quality of the bowers whereas older females prefer intense behavioural display. Such variation could account for the evolution of multiple ornaments widely found throughout the animal kingdom.

1.2.3 Social environment

The social environment has also proved to be an important factor accounting for variation found in female mate preference.

Manipulation of the operational sex ratio (defined in this case as the number of sexually active males divided by the total number of sexually active adults of both sexes) in guppies (*Poecilia reticulata*) alters females mate choice (Jirotkul 1999); females showing stronger preference for orange colour (a visual cue on which females base their choice) when the OSR is more male biased. Cratsley & Lewis (2005) analyzed the effect of seasonal variation in OSR (defined here as the

ratio of fertilizable females to sexually active males) on mate choice in fireflies, *Photinus ignitus*. As the season progresses, sex ratio is less and less male biased which leads to a behavioral shift in female responsiveness to male flashes since they become more responsive as the number of courting males decrease.

Another factor is the behaviour of other females engaged in sexual activities that could influence the decisions made by an observing individual. This phenomenon known as “mate choice copying” is found in an increasing number of taxa – fishes (Dugatkin 1992; Dugatkin & Godin 1993), birds (Hoglund et al. 1995; Swaddle et al. 2005), invertebrates (Mery et al. 2009) and mammals (Galef, Lim & Gilbert 2008) including humans (Little et al. 2008). The propensity to favor public information (the choice of others) over personal information (individual underlying preferences) in a mating context can in some cases outweigh genetically determined preferences (Dugatkin 1992, 1996) and thus alter the evolution of male traits. Such behaviour increases the reproductive success of inexperienced individuals conforming to the choice of more experienced females (often older females) (Dugatkin & Godin 1993). Independently of the age of the observer and of the “model(s)”, mate choice copying can also be adaptive when costs associated with mate choice are high or when the observer is less efficient in searching potential mates than eavesdropped females.

Animals living in groups or in close geographical proximity may breed with genetically related individuals which may lead to inbreeding depression issues such as reduced quality and number in offspring. To avoid such deleterious effects, some species have implemented some strategies like recognizing and avoiding mating with kin or multiply mating opportunities. In the polyandrous field cricket (*Gryllus bimaculatus*), females who mate with sibling and non-sibling individuals increased hatching success compared with females that mate only with siblings (Tregenza & Wedell 2002).

1.2.4 Social experience with males

Another component of the social environment that accounts for variation in female mate preference is the experience that females gain through being exposed to males throughout their life. We can divide that experience into experience with males before and after maturity. Experience acquired after maturity is reviewed in this section and pre-maturity experience is developed in the next section.

Learning (defined here as a change in behaviour through individual experience) during adulthood in a context of mate choice through experience with conspecifics has been reported in vertebrates such as the japanese quail (*Coturnix coturnix japonica*, (Domjan 1992)), guppies (*Poecilia reticulata*, (Magurran & Ramnarine 2004) and zebra finches (*Taeniopygia guttata*, (Collins 1995)) and in invertebrates such as fruit flies (*Drosophila melanogaster*, (Dukas 2005). Each of these studies establishes that learning influences the outcomes of mate choice but do not always distinguish between the different forms of experiences that underpin the change in decisions made by females. That is, experience is a term that covers different aspects of the interactions that females can have with males.

A female's experience with males consists of (also see table 1.1):

- Males' sexual behaviour (including alternative reproductive tactics)
- Previously seen males
- Distribution of males' phenotype within a population
- Familiarity of the females with potential mates
- Mating history

Bakker & Milinski (1991) demonstrated that in a sequential mate choice experiment (females cannot see more than one male simultaneously but instead are presented with males one after the other) choices made by female sticklebacks are affected by the attractiveness of the male seen previously. A given male is rated higher when preceded by a lower quality male or rated lower if the previous-seen male is of better quality. This phenomenon is known as the "previous male effect". Fawcett & Bleay (2009) expanded the understanding of that effect by analyzing how individuals adjust the perception of their own attractiveness given

the outcome of previous encounter. They developed a model of mutual mate choice that shows that individuals are sensitive to previous encounters and tune their mate preference according to the response they got from the opposite sex. Experience of acceptance tends to increase their choosiness whereas rejection provokes the opposite.

Familiarity is defined as repeated encounters of one or more individuals (or some of their phenotypic traits like odours). An individual is thus defined as unfamiliar if it has never been encountered or encountered less often than a familiar individual. Female mate choice has been shown to vary according to male familiarity, but interestingly in some species this favours familiar males, while in others unfamiliar males are preferred. Preference for familiar males has been demonstrated in mammals such as rodents (Patris & Baudoin 1998; Ricankova, Sumbera & Sedlacek 2007) and primates (pygmy loris; (Fisher, Swaisgood & Fitch-Snyder 2003)). Assessing scent marks gives monogamous females an idea of the quality of the males who become, as a consequence, familiar males. These familiar males are preferred over males that haven't deposited any olfactive cues that could have signaled their territory and/ or social status. On the contrary, unfamiliar males are preferred in other social context or other mating systems. Fitness benefits associated to multiple mating (Jennions & Petrie 2000) explain preference for non-familiar males. In guppies, females familiarized (but not mated) with males of a particular colour morph are significantly more likely to mate subsequently with a male bearing a novel colour pattern than with a familiar colour-type male (Hughes et al. 1999).

Previous mates, male's phenotypes commonly found in the local environment and kin could also be considered as familiar males, thus particular attention should be paid to avoid misinterpretation between these confounding effects. Further studies on female mate preference in guppies disentangled the relative importance of familiarity, relatedness, mating history and phenotypes rarity. Female guppies, which use visual signals to choose mates, tend to prefer novel or rare males (Zajitschek, Evans & Brooks 2006; Zajitschek & Brooks 2008) and

discriminate against previous mates and those that look like previous mates (Eakley & Houde 2004; Hampton, Hughes & Houde 2009).

1.2.5 Ontogeny and phenotypic plasticity in female mate preference

Morphology, life-history tactics and behaviour can be developmentally plastic and change in response to environmental variation experienced during ontogeny (West-Eberhard 2003). If initially dismissed in the elaboration of the modern evolutionary synthesis, development and adaptive phenotypic flexibility became a major concern during the latter part of the twentieth century and triggered a lot of interest in an attempt to reunify development and evolution (West-Eberhard 2003). Many features of the environment such as resource availability, temperature, photoperiod, predation level, and social environment experienced during ontogeny has long-term effects on the developing offspring (Monaghan 2008; Prudic et al. 2011). Only the influence of the early social environment is developed here.

Plasticity in sexual preferences as a function of an individual's social rearing environment originally stem from research on the well-known and extensively investigated sensory learning mechanism called sexual imprinting (Lorenz 1935). Sexual imprinting could be defined as a process in which individuals, early in their development, learn during a relatively short sensitive period, about the appearance (visual, auditory or olfactive) of parents and siblings and use that information accordingly when choosing a mate. This phenomenon is widespread among vertebrae taxa and has been experimentally demonstrated in birds (Slagsvold et al. 2002; ten Cate, Verzijden & Etman 2006), fish (Verzijden & Ten Cate 2007) and mammals (Kendrick et al. 1998) in species that present a high level of parental care (Grant & Grant 1997; Shettleworth 1998). Interspecific cross-fostering experiments have been particularly useful to show the parental influence on subsequent sexual preferences since offspring would preferentially choose to mate with the closely related species experienced as parents rather than with the genetic species (Kendrick et al. 1998; Slagsvold et al. 2002). Sexual imprinting is affecting

both sexes in their subsequent sexual behaviour (male: (Kendrick et al. 1998; Slagsvold et al. 2002), female: (Slagsvold et al. 2002; Verzijden & Ten Cate 2007)) and shape mate preferences such as offspring will prefer, once mature, to mate with individuals that are phenotypically similar (ten Cate et al. 2006) or dissimilar (Kruczek 2007) to their parents and/or siblings.

As mentioned above, sexual imprinting is most likely to occur in systems where offspring are raised by their parents. By contrast, we can wonder how female preferences could be modified or shaped by the social environment in the countless species in which there are neither parental care nor learning from parents. Surprisingly however, how development and the early social environment contribute to variation in female mating preferences remain poorly understood and have rarely been addressed experimentally outside the context of sexual imprinting.

In vertebrates, the few examples of early social experience modifying subsequent mate choice (outside the context of sexual imprinting) come from fish in the Poeciliidae family. Breden et al. (1995) found a significant effect of prior experience in guppies (*Poecilia reticulata*), with females preferring to associate with male phenotypes experienced during development. In another significative study carried out by Rosenqvist & Houde (1997), females guppies were reared in three different conditions among which two treatments were displaying low variance of the male sexual signals and one displaying high variance of the male sexual trait. Only females raised in the mixed treatment (high variance) showed a significant preference for the higher value of the sexual character. More recently, in another Poeciliid species, the green swordtail (*Xiphophorus helleri*) in which some female preferences are based on pre-existing sensory biases (Basolo 1990a; Basolo 1995), Walling et al. (2008) showed that heritable preferences can be altered by manipulating early social experience, reversing the innate predisposition. Learned mate preference have also been identified in another species of swordtails, *Xiphophorus birchmanni*, where both olfactory and visual preferences were formed through exposure during development (Verzijden &

Rosenthal 2011). The authors have also pointed out the role of the timing of exposure where visual cues were longer to acquire than olfactive cues.

Some insight into the potential influence of subadult social experience on female mate preference comes from studies on invertebrates. In two studies carried out on wolf spiders (Hebets 2003; Hebets & Vink 2007), females choose differentially between male phenotypes based on their prior experience during development. In the first study (Hebets 2003), females reared with a specific morph preferred the phenotypes experienced. In contrast to exposed females, unexposed individuals were not influenced by male phenotype during the test, showing that there is no innate preference for any one particular phenotype. In the second study (Hebets & Vink 2007), experienced females mate significantly more with brush-legged males (brush legs being the sexual signal assessed), regardless of the form of the males they have been exposed to (brush-legged vs. non-ornamented), whereas inexperienced females mate equally with both morphs. To expand on these earlier works, Rutledge et al. (2010) analysed the influence of subadult exposure to multiple sensory modalities (chemical vs. visual) on female mate preferences in a closely related species (*Schizocosa rovneri*). Although they confirmed that juvenile experience played an essential role in the acquisition of sexual preferences, their results contrasted with earlier studies as females preferred unfamiliar visual phenotypes and unfamiliar chemical phenotypes. Finally, in a recent study, Bailey & Zuk (2008) showed that female field crickets (*Teleogryllus oceanicus*) adjust their responsiveness to signalling males depending on the acoustic environment they have experienced during the rearing period.

Early social experience is then also an important determinant of plasticity in female mate preference. To conclude this review, I summarize in table 1.1 the different sources of variation mentioned previously, which allows grasping in a glimpse their large diversity and the numerous taxonomic groups involved. The evolutionary consequences of such variation are then analysed.

Table 1.1: Sources of non-heritable variation in female mate preferences

Sources of variation	Description	Model system	Studies
Environmental conditions	- Predation (threat level and assemblage)	- Guppy, swordtail, fiddler crab	- Godin & Briggs (1996), Johnson & Basolo (2003), Kim et al. (2009), Bierbach et al. (2011)
	- Parasite load	- Guppy, sand goby	- Kennedy (1987), Barber (2005)
	- Lighting conditions	- Bluefin killifish	- Fuller & Noa (2010)
	- Water turbidity	- Cichlids	- Maan et al. (2010)
	- Visual backgrounds and background noise	- Guppies	- Endler (1980)
Female's condition and physiological state	- Age	- Satin bowerbird	- Coleman (2004)
	- Quality	- Zebra finch	- Holveck & Riebel (2010)
	- Hunger	- Swordtail	- Fisher & Rosenthal (2006)
	- Diet regime	- Wolf spider	- Hebets et al. (2008)
	- Hormones	- Túngara frog	- Lynch et al. (2005)
Experience with males after maturity	- Perception of their own attractiveness	- Mathematical model	- Fawcett & Bleay (2009)
	- Phenotypes of current and previously seen males	- Sticklebacks	- Bakker & Milinski (1991)
	- Rarity or novelty of male's phenotype	- Guppy	- Zajitschek & Brooks (2008)
	- Familiarity with potential mates/ males' phenotype	- Guppy	- Mariette et al. (2010)
	- Mating history (polyandry)	- Different taxa	- Jennions & Petrie (2000)
Experience with males before maturity	- Sexual imprinting	- Goose, Tits, Ungulates, Sticklebacks	- Lorenz (1935), Slagsvold et al. (2002), Kendrick et al. (1998), Kozak & Boughman (2009)
	- Oblique imprinting (early social exposure in species without brood care)	- Butterfly, swordtails, wolfspiders, Cricket	- Westerman et al. (2012), Walling et al. (2008), Rutledge et al.(2010), Bailey & Zuk (2008)
Other aspects of the social environment	- Population density and population composition (OSR)	- Guppy, Firefly	- Jirotkul (1999), Cratsley & Lewis (2005)
	- Behaviour of other females = mate copying	- Black grouse, zebra finches, guppy, Drosophila	- Hoglund et al. (1995), Swaddle et al. (2005), Dugatkin &Godin (1993), Méry et al.(2009)
	- Availability of potential mates	- Human	- Bateson & Healy (2005)
	- Female's relatedness to potential mates	- Field cricket	- Tregenza and Wedell (2002)

1.3 Evolutionary implications of behavioural plasticity in female mate choice

Behavioural plasticity has an adaptive value when it allows an individual to cope with a new or changing environment (Price, Qvarnstrom & Irwin 2003; West-Eberhard 2003). In the context of mate choice, selection is expected to favour individuals that can adjust their preferences to variation in the social and/or ecological environment through behavioural flexibility or learning. Plasticity in preferences allow a female to maximize her own fitness via direct benefits and/or her offspring fitness thanks to indirect benefits (Qvarnström 2001).

Reducing preferences for conspicuous males in response to increased predation risks allow females to decrease the time spent with males that are more likely to be preyed upon and thus increase females' viability. Prior experiences (before and after sexual maturity) with male phenotypes provide information about the expected quality of males in the population. For instance, if little or no information is furnished about males (small variance in phenotypic cues), benefits of choice (indirect and direct) are low. Thus, females may reduce their level of choosiness, avoiding some of the costs associated with mate choice and pair with available males rather than missing breeding opportunities. Changing mate-sampling tactics in response to different males' phenotypes distributions can present an adaptive interest if females reduce the time and energy devoted to gather information about prospective mates. Plasticity in female preferences may also increase female fitness based on the genetic quality of her offspring when sexual signals indicating males' quality vary across breeding events (Chaine & Lyon 2008).

Plasticity in female choice could also favour mating between more genetically compatible individuals (i.e. how well the alleles of the parents function together in their offspring) than possible in a rigid system. In sexually reproducing organisms, offspring fitness will largely depend on the quality of the match between females and males genotypes (Neff & Pitcher 2005). It is important to note, however, that genetic compatibility does not equate to genetic dissimilarity. Genetic

dissimilarity being a continuum, in the low dissimilarity end of the continuum, reproduction between closely related individuals yield low fitness (i.e. inbreeding depression) as do reproduction between related species or even between different conspecific populations at the other end of the continuum (i.e. outbreeding depression). Maximal fitness is achieved at intermediate levels of genetic dissimilarity (Bateson 1983). Inbreeding depression which can be considered a special case of genetic incompatibility and mechanisms of inbreeding avoidance are well documented and found in a large range of taxonomic group (Pusey & Wolf 1996; Stow & Sunnucks 2004) (even if inbreeding avoidance through mate choice is not observed in all species (Hansson et al. 2007)). Overall individual heterozygosity or heterozygosity at specific loci are special cases of genetic compatibility providing fitness benefits (Brown 1997; Hansson & Westerberg 2002; Kempenaers 2007; Fromhage, Kokko & Reid 2009) even though studies show contrasting results regarding the generality and the magnitude of the effect of heterozygosity on fitness (Kempenaers 2007; Mays et al. 2008; Chapman et al. 2009). Best evidence for female choice based on genetic compatibility comes from optimal MHC-based preferences (Tregenza & Wedell 2000; Penn 2002; Milinski 2006; Lenz et al. 2009) and from studies showing patterns of extra-pair paternity; extra-pair young being more dissimilar than within-pair young (Foerster et al. 2003). Tarvin et al. (2005) found that levels of extra-pair paternity in broods of the splendid fairy wren increased with genetic similarity between social mates.

Behavioural compatibility within a pair can also present an adaptive value achieved through variation in female mate choice - especially in species with bi-parental care. For instance, behaving similarly is advantageous if it reduces conflict between sexual partners (van Oers et al. 2005). On the opposite, disassortative mating pairs might have an advantage as they might have a larger behavioural repertoire that ensure good foraging success in changing environment (Both et al. 2005) or allow parents to cover wider ecological niche.

The extent to which female preferences direct the evolution of male traits may ultimately depend on the level of plasticity in female mate preferences.

Plasticity could alter the strength and outcome of sexual selection on male ornamentation (Chaine & Lyon 2008). Depending on how the preferences are shaped, sexual selection may be stabilizing, directional or disruptive and its strength may be enhanced or weakened. Sexual selection might also be annihilated if female choices vary across years.

In the lark bunting, *Calamospiza melanocorys*, different male traits serve as fitness indicators in different years. Flexible female choice allows tracking temporal variation in the trait that predicts enhanced fitness. Hence, if in a given year there is a strong selection for or against some male traits, overall, across years there is very weak selection for most traits (Chaine & Lyon 2008). This example highlights the importance of choosing the appropriate time scale to detect any patterns of selection.

Let's consider a case where preferences are mediated by early social experience: if a female did not encounter mature males during development, she'll rely essentially on her genetic predisposition to choose a mate. In that situation, the combined evolution of female preferences and male secondary sexual trait(s), within a population, will depend on the frequency of the different alleles coding for sexual preferences (considering that any other non-heritable sources of variation are kept constant). In contrast, a male bearing a non-genetically preferred sexual trait (because it is not signalling a high quality male, for instance) could increase his mating success through female mate preference acquired during ontogeny and thus weaken directional sexual selection on the signalling trait. This could occur in Guppies as it has been shown that novel or rare male colour patterns are favoured over common ones (Eakley & Houde 2004; Zajitschek et al. 2006). However, contrary to the predictions made, no influence of pattern rarity on the strength and form of sexual selection on ornamental traits has been found to operate (Zajitschek & Brooks 2008). Alternatively, preference plasticity through previous experience of highly variable pool of potential mates leads female guppies to become choosier as they trade-up on male quality (Rosenqvist & Houde 1997; Pitcher et al. 2003), thereby imposing stronger sexual selection on male ornamentation. It also

encourages non-genetic phenotypic differences in preferences, which will further select for male diversity.

Flexibility in females' preferences may also provide a mechanism for the preservation of genetic polymorphism found in sexually selected traits. In the context of mate choice, strong directional preference is expected to erode additive genetic variation by fixing favourable alleles; this is called the lek paradox (Kirkpatrick & Ryan 1991). Thus, the maintenance and evolution of a high level of genetic polymorphism in sexual signals (like in Guppies, stalk-eyed flies or flycatchers) has long been a topic of discussion between evolutionary biologists. Theoretically, different factors such as frequency-dependent selection, environmental heterogeneity (spatial and temporal), mutation-selection balance, heterozygote advantage and antagonist pleiotropy are thought to account for maintaining genetic variance in morphological traits (Maynard Smith 1998). Frequency-dependent selection and fluctuating selection owing to environmental heterogeneity are directly underpinned by the process of mate choice and help additive genetic variance in sexual traits to be maintained. Frequency-dependent mating success in male is supported by studies demonstrating that female guppies actively discriminate against common phenotypes within population (Farr 1977; Hughes et al. 1999; Eakley & Houde 2004; Zajitschek & Brooks 2008), enabling for the maintenance of high level of colour pattern polymorphism within a population. Very recently, Hampton et al. (2009) brought new evidence supporting possible negative frequency-dependent mating success in males' guppies as females were more sexually responsive to novel males over redundant males' morphs. Fluctuating selection arguments depend on the idea that the optimal phenotype varies either in space (Jia, Greenfield & Collins 2000) or in time (Chaine & Lyon 2008) which means that there is not one genotype that should always be favoured by females over the others. Flexibility in female mate choice imposes fluctuating selection on male traits and allows genetic variants to be maintained within populations.

Finally, variation in female mate preferences is invoked to explain for the evolution and maintenance of ubiquitous multiple cues used in mate choice

(Candolin 2003; Bro-J¯rgensen 2010). Variation in preferences impose dynamic selection regimes whereby different multiple signals can coexist. In fluctuating environments where ecological and social conditions can change drastically the content of a sexual trait, a single signal is not reliable enough to convey an honest message (or alternatively do not carry enough information) about the direct and/or indirect benefits a male could provide to a female. In such situations, there are strong selection pressures on males to evolve another signal that would be a better indicator of quality in the new environment (Bro-J¯rgensen 2010). Females can, thus, choose the best male or the one that best complement their needs in a specific place at a specific point in time.

Recently, few studies have suggested that variation in female mate preferences across time and space is also a mechanism that contributes to maintain alternative reproductive strategies in swordtails (courter versus sneaker males) (Rios-Cardenas, Tudor & Morris 2007; Morris, Rios-Cardenas & Brewer 2010).

Reproductive isolation is based upon a suite of mechanisms that prevent two or more populations from exchanging genes. The separation of the gene pools of populations, under some conditions, can lead to the genesis of distinct species (Mayr 1963). Reproductive isolation can occur either by preventing fertilization, or by the creation of sterile hybrids. Obstacles in fertilization could arise from pre-mating isolation as a consequence of a geographical barrier, which can lead to geographical variation in morphological, behavioural and life history traits or as a consequence of (dis)assortative mating within the same population. The presence of phenotypically similar closely related species may increase error rate in mate recognition and might favor reinforced mate preferences towards conspecifics. One of the mechanisms that can promote reinforcement (i.e. selection against deleterious hybrids), and thus species divergence, is variability in female mate preference through learning and developmental plasticity (West-Eberhard 2003) which can enhance species recognition (Price 2008; Servedio, Saether & Saetre 2009). Evidence of learned mate preference are accumulating and come from

different taxa such as birds, fish and arthropods (Magurran & Ramnarine 2004; Dukas 2005; ten Cate et al. 2006; Hebets & Vink 2007; Verzijden & Ten Cate 2007; Kozak & Boughman 2009). Svensson et al. (2010) presented data on female mate preferences of the banded demoiselle (*Calopteryx splendens*) showing a strong role for learning in population divergence and species recognition. Very recently, Westerman et al. (2012) demonstrated that in the butterfly species *Bicyclus anynana*, females shift their preference in response to social encounter. The authors also emphasized the existence of a learning bias to enhanced sexual signals over reduced signals – females learning more readily an enhanced-ornamentation phenotype than a reduced-ornamentation phenotype. These preference shifts and the bias towards enhancement learning suggest that premating experience may play a role in population divergence and thus reproductive isolation.

1.4 Questions addressed and aim of the thesis

There is a very large diversity of biotic and abiotic sources of variation giving a much more complex picture in the attempt to understand female mate preference and female mate choice than thought a decade ago. Phenotypic variation in female mate preference is important in microevolutionary processes within populations because it affects the strength and direction of pre-copulatory sexual selection and thus can have a major impact on the evolution of male sexual signals and potentially the rate of population divergence and ultimately speciation.

To broaden the comprehension we have on the various factors inducing variation in female mate choice, I have decided to examine the role played by the ontogenetic environment on developing individuals. My decision has been motivated by the importance of the early stage of life in an individual's life and the paucity of studies investigating the influence of early social and physical environment in species lacking parental care. Moreover, these studies present contradictory results and do not take into account factors such as the duration of the environmental experience or the complexity of systems in which females base their choice on multicomponent signals.

In the first part of my thesis (chapters two, three and four), I investigated how females shape their sexual preferences according to the male phenotypic distributions experienced during development. To do so, I used guppies (*Poecilia reticulata*) as a model system (see next section).

In chapter two, I explored the effects of exposing young guppy females to different amount of orange coloration (a commonly preferred sexual trait in guppies throughout populations) on their subsequent mate choice. Once adult, I tested their preferences for orange but also for other colour patterns. Besides I analysed the effect of the length of exposure to orange.

Question 1: How is the female preference for orange, a popular sexual signal in guppies, mediated through the experience of different level of orange while maturing?

Question 2: Are female mate preferences for other sexual signals (other colour pattern) affected by experiencing different value of orange while maturing?

Question 3: Does the duration of exposure to different amount of orange play a role in the acquisition of preferences for orange and/or for other colours borne by males?

Chapter three tested the same hypothesis as chapter two but used a different sexual signal. Here the social experience is made of varying values of the total colour covering the body. Once adult, I tested female preferences for the different colour patterns displayed by males and analyzed the effects of the rearing treatments. Once more, females are exposed to varying distribution of total colour traits for different timing of exposure.

Question 4: How different distributions of total colour experienced during early life could influence female mate choice subsequently?

Question 5: Is the acquisition of preferences dependent on the duration of exposure?

In chapter four, I aimed to explore a different aspect of early social environment on learned mate preferences. In the previous chapters, I manipulated quantitatively one (chapter 2) or all (chapter 3) sexual signals and analyzed the effects of such variation on mate choice. Here, I searched for the effect of different level of overall phenotypic variance in males independently on whether the traits experienced predicted mating success. The focus was centered on the variance of male phenotypes as a whole and not anymore on variation in male sexual signals.

Question 6: Does phenotypic variation influence mate choice whether or not the sexual traits experienced during ontogeny are good predictors of mating success?

The second part of my thesis (chapter five) was devoted to analyze how the sensory environment, in which individual females and their cognitive systems matured, could influence subsequent detection and processing of sexual signals and accordingly attuned mate preferences. The sensory drive model predicts that male traits and female preferences for these traits evolve in a given environment to enhance the communication between the sender (usually males in a mating context) and the receiver (usually females in a mating context). To my knowledge, however, the influence of the physical environment experienced during development on the acquisition of mate preference remains poorly understood. In chapter 5, I tested the hypothesis that the colours of the environment in which maturing females fed, could induce some changes in subsequent mate preferences for coloured visual cues.

Question 7: How does the coloured environment in which developing females feed, tune their subsequent mate choice for coloured male signals?

Chapter six takes the form of a general discussion of the results and proposes avenues for future research.

The aim of the thesis was to emphasize the importance of the social and physical environment experienced during ontogeny in the formation of female mate preferences.

1.5 The model system

Guppies (*Poecilia reticulata*) have been widely used as a model system in biology. Used in various fields such as ecotoxicology, genetics, biomedical research and since recently functional genomics, the guppy system has proved to be particularly fruitful in behavioural ecology and evolutionary biology (reviewed in (Endler 1995; Houde 1997; Magurran 2005)) over the last five decades. Although I have tried to cite studies dealing with various animal taxa, there is an overrepresentation of work using guppies (see table 1 and references throughout this thesis chapter), underlining the importance of that species in behavioural and evolutionary studies.

Pioneering research in sexual selection (Haskins & Haskins 1949), reproductive isolating mechanisms (Liley 1966) and sperm competition (Schmidt 1920) conducted on guppies, go back to the early twentieth century. Due to a particular geographical arrangement of several rivers in different drainage basins, the scientific attention has been put on guppy populations from the island of Trinidad. The natural barriers within (like waterfalls) and between rivers have allowed guppies to evolve differentially under different selection pressures (essentially different level of predation and resource availability due to differences in canopy cover) making this spot a “natural laboratory” as stated by Cary Haskins, a pioneering guppy ecologist.

1.5.1 A quick overview of the ecology and behaviour of guppies

Guppies are small livebearer fish native to coastal streams and rivers of northeastern South America and adjacent islands (essentially Trinidad). They have a continuous, non-resource based and non-territorial mating system (Liley & Seghers 1975). They are sexually dimorphic in body size and body pigmentation (Haskins et al. 1961), females being bigger and drab while males display highly complex, conspicuous, polymorphic and heritable colour patterns. Males fertilize females internally thanks to an intromittent organ, called a gonopodium, which is a modified anal fin. The observed sexual dimorphism is the result of different selection forces acting within population; that is sexual selection, life-history

strategies and predation pressure (Reznick, Bryga & Endler 1990; Houde 1997; Magurran 2005).

The colour patterns represent a balance between selection for crypsis through natural selection and selection for conspicuousness through sexual selection (Endler 1978, 1980). Sexual selection occurs in the form of female mate choice based on these colour patterns (Houde 1987; Houde & Endler 1990b; Endler & Houde 1995; Houde 1997; Evans, Bisazza & Pilastro 2004a) but also on male size (Reynolds & Gross 1992; Endler & Houde 1995) and courting intensity (Stoner & Breden 1988; Nicoletto 1993).

Figure 1.1: Example of guppies, male and female

1.5.2 A good model system for my research?

Firstly, guppies present the general characteristics found in other extensively used model system in biology (e.g. mouse or fruit fly). They are easy to keep in large numbers, their generation time is short and their behaviours are easily observed and can be reliably measured. Most importantly, for the study of mate choice and sexual selection, these livebearing fish have internal fertilization, which allow females to have a relatively good control over the mating process resulting from the female mate preferences.

Secondly, guppies present more specific features that fit the questions raised. Individual phenotypic variation in female mate preference in guppies has been found to occur (Brooks & Endler 2001b; Brooks 2002) and, as reviewed in previous paragraphs, experience with males during adulthood and during development might contribute to that plasticity. Moreover, social interactions between males and immature individuals are reinforced since they share the same local microhabitat. In wild guppy populations, some studies have found that the pattern of sexual segregation is associated with the level of predation (Croft, Botham & Krause 2004; Croft et al. 2006) and the level of male harassment (Darden & Croft 2008). Males, being more vulnerable to predation (Magurran 2005), tend to live in shallow waters where the predation pressure is lower (Mattingly & Butler 1994) than in deeper water where the sex ratio is female-biased. Fry and juveniles tend to occupy also the shallowest area of the river where the predation and the current rates are lower (Magurran 2005). Young guppies are, thus, constantly exposed to males. The guppy system offers a unique opportunity to investigate the potential effect of early social experience with different male phenotypes for two other reasons: male phenotypes differ dramatically between and within populations (Endler 1978, 1980) and female mate preference criteria vary among populations according to the variation in male patterns (Endler & Houde 1995; Brooks & Endler 2001b). As a consequence there is no universally attractive male phenotype.

2. Chapter II

Early social experience of a sexual trait not primarily involved in mate choice affects sexual behaviours in female guppies

2.1. Abstract

Individual phenotypic variation in female mate preference is common and can influence the mode, strength and direction of sexual selection on males' traits but also the evolution of reproductive isolation. This variation is shaped, among other factors, by the social environment but surprisingly little research has been conducted on the early stages of social experience. I used guppies, *Poecilia reticulata*, to investigate the possibility that females alter their sexual preferences in response to the male phenotypic distribution encountered during development. To manipulate their juvenile experience, I exposed maturing females, either during half or during the whole developmental period, to groups of males differing in the values of a sexual trait not genetically favoured in the population under scrutiny (i.e. high, low, and mixed value of orange colouration). Both choosiness and preference functions changed following the rearing treatments. Short-exposed females became less choosy when raised in contact with males displaying either high- or low-value of orange. Moreover, it appears that females discriminated against males based on the level of orange experienced while growing. Finally, being exposed to different values of orange during ontogeny influenced female preferences for other sexual traits. This study highlights the importance of the early rearing environment for the acquisition of sexual behaviours even when exposed to a trait not genetically preferred.

2.2. Introduction

Phenotypic variation in female mate preferences within population has important consequences for both the evolution of male sexual traits and female mate choice (Jennions & Petrie 1997; Widemo & Saether 1999; Brooks & Endler 2001b; Cornwallis & Uller 2010) but also for the evolution of reproductive isolation leading ultimately to speciation (Verzijden, Lachlan & Servedio 2005; Servedio et al. 2009; Svensson et al. 2010). Alongside with additive and non-additive genetic differences among individuals, a number of non-genetic factors accounts for the variation found in mate preferences across taxa. These include perceived risk of predation (Johnson & Basolo 2003; Greig & Pruett-Jones 2010; Bierbach et al. 2011), parasite load (Barber 2005), lighting conditions (Fuller & Noa 2010) or female condition (Cotton et al. 2006; Holveck & Riebel 2010). The social environment is also a major source of flexibility in mating preferences as familiarity (Mariette et al. 2010), male phenotype rarity (Zajitschek & Brooks 2008) operational sex ratio (Jirotkul 1999) or mate copying (Witte & Ryan 2002; Mery et al. 2009) alter the outcome of female mate choice.

Of increasing interest is the influence that the social environment experienced during early ontogeny has on the formation of mate preferences. Early rearing environment can affect the development and expression, throughout lifetime, of fitness-related traits such as anti-predator defences (Chapman et al. 2008a), social behaviours (Laviola & Terranova 1998; Chapman, Ward & Krause 2008b; Arnold & Taborsky 2010) or learning abilities (Liu et al. 2000; Levy et al. 2003). Moreover, a growing body of evidence emphasizes the importance of early social experience on the acquisition of mate preferences (e.g. learned mate preferences) and thus on the process of sexual selection. This has been demonstrated in vertebrates (Breden et al. 1995; Rosenqvist & Houde 1997; Walling et al. 2008; Verzijden & Rosenthal 2011) and invertebrates (Hebets 2003; Hebets & Vink 2007; Rutledge et al. 2010; Westerman et al. 2012) however, studies remain very scarce. At this point, it is important to note that different type of learning can be involved in mate choice depending on the ecology and the biology of species. Sexual imprinting, a learning process by which young individuals

acquire sexual preferences based on the observation of one their parents has been extensively studied in the context of species recognition, cost avoidance related to heterospecific matings and speciation (Irwin & Price 1999; Slagsvold et al. 2002; Verzijden & Ten Cate 2007). Outside the framework of sexual imprinting, the paucity of studies dealing with species without parental care is striking and the aim of my work is to extend the understanding of the role that developmental social experience has on mate preference plasticity and analyse its evolutionary consequences.

To do so, I capitalize on the Trinidadian guppy (*Poecilia reticulata*) as a model system. Previous work proved that early social experience was influential in mate choice learning. For instance, Breden (1995) demonstrated that females prefer to associate with males exhibiting similar (but not equal) phenotypes to the phenotypes experienced during early life. Subsequently, Rosenqvist & Houde (1997) showed that only some conditions experienced during development were efficient at shaping female mate choice. Females reared with a group of males displaying a large variability in the value of the sexual cue (orange hue) were preferentially associating with males bearing higher value of that sexual trait, contrary to females reared in low variance treatments (groups of males displaying either high value or low value of the sexual trait) that lost any significant preference. Recently, the Poeciliid family has provided more insight into the role of the social environment experienced during early ontogeny. Walling et al. (2008) proved that heritable preferences could be reversed after having manipulated the rearing environment of the green swordtail (*Xiphophorus helleri*), a species known for having evolved mate preferences through pre-existing sensory bias (Basolo 1990b). Learned mate preference have also been identified in another species of swordtails, *Xiphophorus birchmanni*, where both olfactory and visual preferences were formed through exposure during development (Verzijden & Rosenthal 2011).

In my study, I investigate how females adjust their sexual behaviours when reared in visual contact with males varying in the phenotypic distribution of orange body colouration. My experiment follows up with Rosenqvist and Houde's work widening the scope of the analysis in the following way. Firstly, even though

orange is attractive to females in many populations, there is no universally attractive male phenotypes (Endler & Houde 1995). Thus, testing for the innate predispositions of the population under scrutiny provide valuable and accurate information on which sexual traits are liked or disliked and help with the interpretation of the effects of the rearing treatments. Secondly, I examine the effect of the length of exposure to adult males as females were exposed, in the different treatment, either for the whole duration of the developmental period or for the second half of it. The temporal dimension might emerge as a substantial factor for preference learning because of biological and ecological reasons. It is well established that learned preferences in the context of sexual imprinting or learned traits in some species (e.g. bird songs) are restricted to a limited period during development called the “sensitive period” (Knudsen 2004). Even though, early experience gained from conspecifics might involve a different neural circuitry than the one underlying filial imprinting, a sensitive period is plausible in the formation of mate preference based on non-parental stimuli. On ecological grounds, it also makes sense to investigate the influence of different timing of exposure. Croft et al. (2003) demonstrated that movement in guppies are sex-biased, males emigrating significantly more than females. Consequently, the pattern of male phenotypes distribution within female’s habitat is changing over time prompting females to vary learning strategy as a function of the timing of exposure. Thirdly, guppies, being a multiple sexual signalling and thus multiple mate preferences species (Brooks & Couldridge 1999), it is worth investigating how variance in one sexually selected trait could influence not only subsequent preference for that specific trait (here orange colouration) but also preferences for other sexual traits.

2.3. Methods

2.3.1 Study organisms

Guppies used for the experiment were second and third generation descendants of individuals collected in the lower part of the Aripo river (high predation zone) in Trinidad in March 2008 (N 10°. 39.031; W 61°13.404; 37m altitude). All fish housed in the laboratory are maintained on a 12h light:dark cycle at 24°C. They were fed twice daily: in the morning with commercial flakes and in the afternoon with brine shrimp (*Artemia*). All the housing tanks had a gravel substrate and were aerated through an undergravel filtering system. Plastic plants were placed into the tanks to physically enrich the environment of the fish and to let them have some room to hide.

Parental females' fish are kept individually in 4L plastic tank. Female poeciliids can store sperm (Constanz 1989) that can fertilize eggs for up to eight month (Winge 1937). Recently inseminated sperm will, however, secure most fertilizations (Constanz 1984) and within a given brood cycle the last male to mate is likely to father most offspring (Evans & Magurran 2001). Thus, to reduce the probability of producing half-siblings for the rearing treatments, females were kept individually until they gave birth to two consecutive broods that were replaced in stock tanks. Then, a single male sired them. Such procedure ensures that one male fathered broods. Each brood was kept for five days in 4L plastic tanks, visually isolated from other fish, before being divided in three equal experimental groups and placed in rearing tanks (see Fig. 2.1 and Fig. 2.2). Only broods of eighteen or more individuals were used to have a sufficient number of female per family and decrease the variance in the size of brood from which fry came (Mean ± standard deviation = 22.2 ± 4.6).

2.3.2 Rearing treatments

Prior to rearing females in different social contexts, I analysed their genetic preferences. To do so, young females were brought up in the same laboratory

conditions as the females used for the experiments but in the absence of any stimuli males. Once mature, they were tested following the procedure described below.

The experiment consisted of rearing groups of virgin females experiencing three different treatment conditions for either the whole period of development (84 days post birth) or only during the second-half of the developmental period (from day 42 post-birth until day 84 post-birth). Reznick et al. (1997) determined that females from high predation site in the Aripo river were mature at 55.6 +/- 2.2 days however personal observations showed that females were not engaged in sexual behaviours before day 80 post-birth. The three different treatments correspond to exposure of the experimental fish to three different sets of male trait values: high, medium and low. Females were reared in visual contact with 4 males expressing high-level of orange, 4 males expressing low-level of orange and a third group in which there are 2 males with low- and 2 males with high- level of orange. Fry within the high-level of orange treatment experienced males displaying all more than 8% of orange (Mean (%) $\pm$ standard deviation = 11.1 ± 2.4) when the fry experiencing low-level of orange were presented males that displayed less than 4% (Mean (%) $\pm$ standard deviation = 2.6 $\pm$ 1.1) of orange on their body. In the mixed treatment, they experienced males displaying a mixture of the two phenotypes (High orange: mean (%) $\pm$ standard deviation = 12.4 ± 3.3; Low orange: mean (%) $\pm$ standard deviation = 3.1 $\pm$ 0.5).

Since not all females from one rearing tank or one replicate could be tested on the same day (day 84 + 2), stimuli males were removed from the tanks at the end of day 84 post-birth to control for the duration of exposure that each female had experienced. The females that were not tested on day 86 stayed in their rearing tank (without stimuli male) until the mate preference trials. The maximal time range that females spent without seeing males before being tested was six days. The experimental design is summarised in figure 2.1.

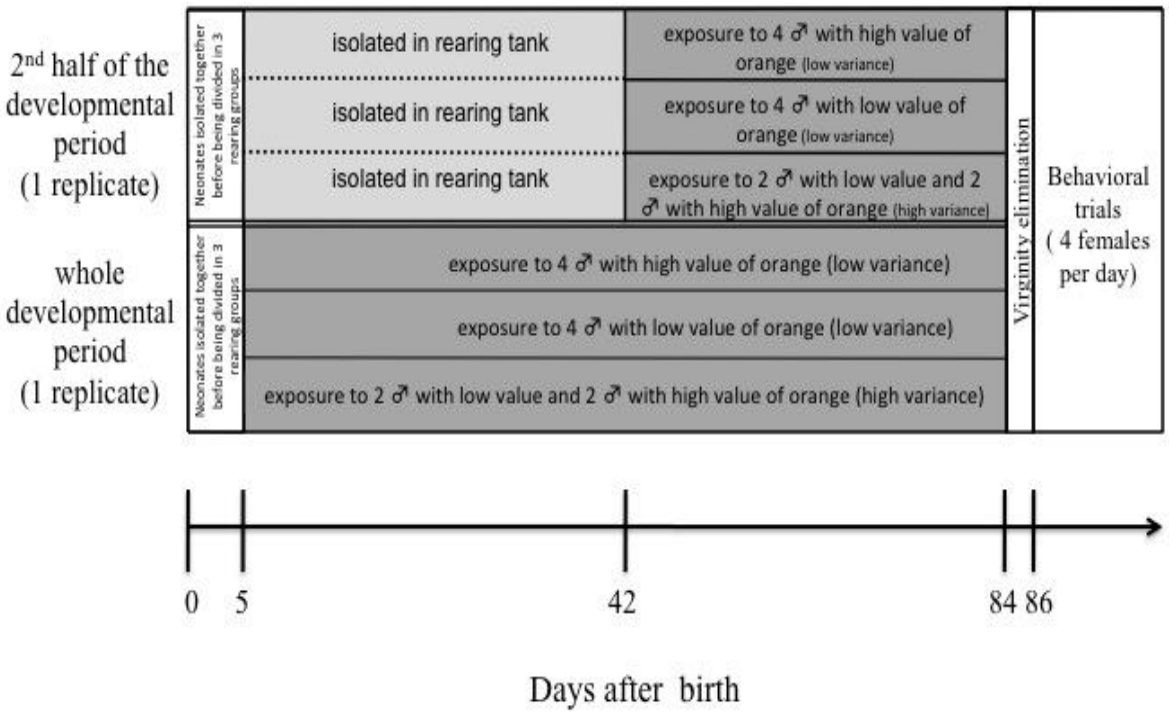

Figure 2.1: Timeline of the experimental design. Is represented one replicate either for half of the developmental period or for the whole development.

The rearing tanks (fig. 2.2) contained 25 litres of water filtered by an undergravel filter.

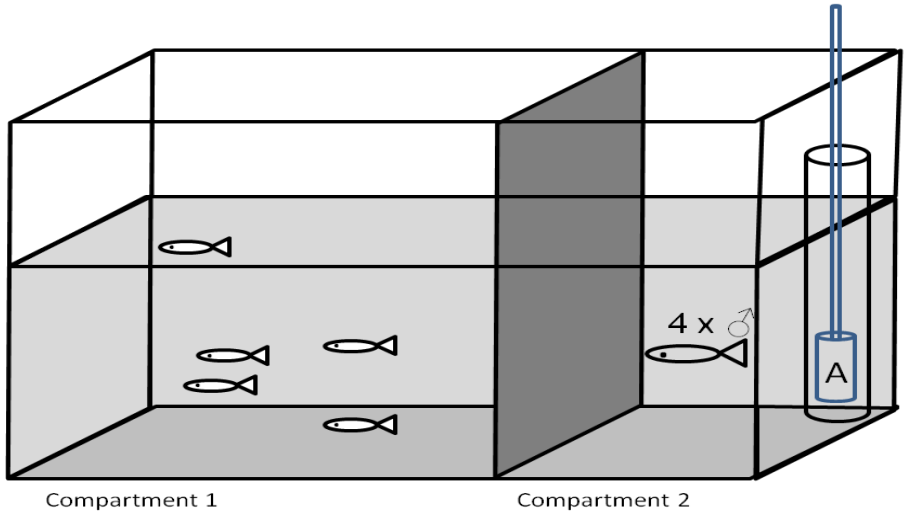

Figure 2.2: Rearing tank for the different rearing treatments

Fry are reared in compartment 1 (Fig. 2.2, 30cm X 30cm X 18cm) separated by transparent Perspex partition from compartment 2 (Fig. 2.2, 15cm X 30cm X 18cm) into which four “stimuli” males are placed. The partition is not sealed which allows olfactive cues to pass from one compartment to the other. This design enabled developing females to grow in situations close to natural conditions. Within the groups of fry, males are removed before reaching sexual maturity that is before the gonopodial hood extends beyond the tip of the fin (Reznick 1990). This prevented growing females from experiencing sexual signals of their siblings but also from sexual harassment and potential fertilization (that could occur at the end of the female development) as males were mature around 50 days (personal observation and Reznick et al. (1997)).

2.3.3 Mate Choice trials

The choice tank (four in total) contained eight enclosures, six containing one male each and two controls with one female each (fig. 2.4). Control females were not virgin and present in two different body sizes (one similar to and one larger than the tested female). The six males displayed a range of values that the tested females experienced during the rearing treatment and were chosen in different housing tanks in which they grew up in the presence of females. None of these eight individuals could see each other but could be seen by the focal female through clear glass (fig. 2.3 and fig. 2.4). The presence of the control females was to test whether the tested females expressed a sexual behavior or simply a tendency to associate with conspecifics. Four choice tanks are used on any given day. Measurements of light intensity within tanks and among tanks were carried out to evaluate any potential differences in light conditions. The measures were made above each preference zone (see fig. 2.4) and above the center of the tank for each tank twice a day during 3 days. Two-levels nested-ANOVA with position in tanks (9 groups: 8 preferences zone + center of the tank) nested within tanks revealed some differences between positions within tanks ($F(32,180)=10.5$, $p<0.001$) in light intensity but not between choice tanks ($F(3,32)=2.14$, $p=0.11$). Because the chambers are sealed, visual but not olfactory communication is

possible between the focal females and the fish in the enclosures (Brooks 2000; Brooks & Endler 2001b).

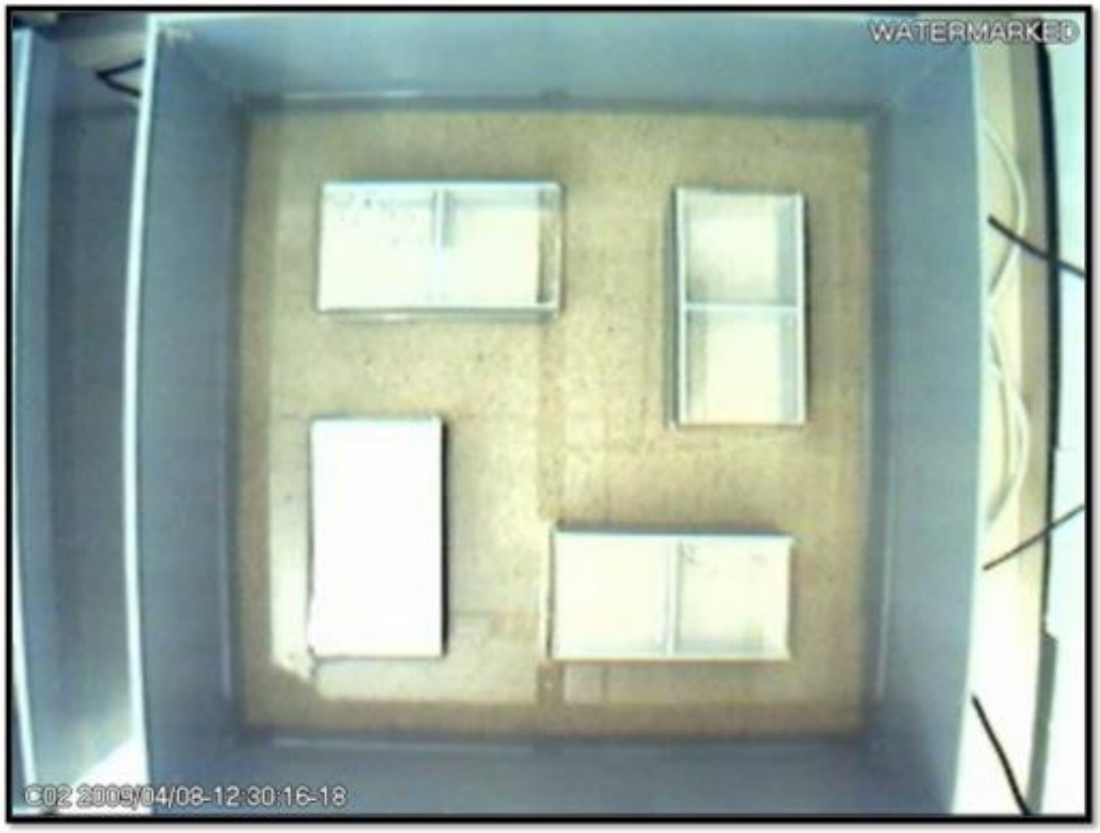

Figure 2.3: Picture of the choice tank taken from above with the video camera and representing what the experimenter saw during data collation. There is no experimental female in the tank.

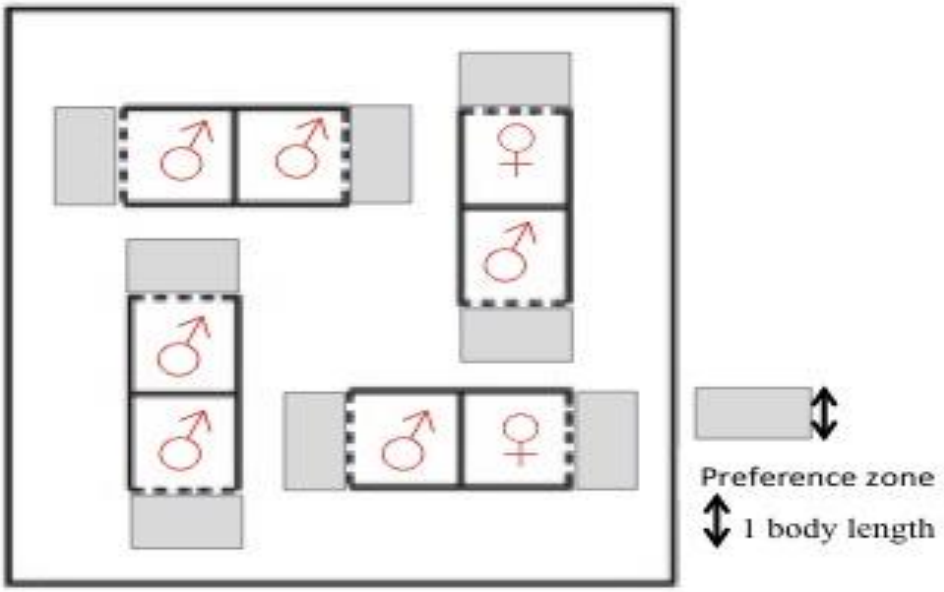

Figure 2.4: Diagram of the choice arena used in measuring female preferences. The tank floor is covered by light brown sand. Sides of chambers indicated by dotted line are transparent. The outer tank is a square 45 cm on a side.

Because virgin female guppies may show little mate discrimination in their first mating (Endler & Houde 1995; Houde 1997; Hughes et al. 1999; Brooks & Endler 2001b), females were allowed to copulate freely with a stock male during the afternoon prior to the testing day (virginity elimination, fig. 2.1). This male didn't resemble either the males experienced during development or the males found in the choice arena. At the end of the afternoon before the trial, the focal females were moved to the choice tank and allowed to acclimatize to their new environment until the next morning. Trials were video-recorded from above and the trial lasted for one hour. Each observation involved scoring the number of occurrence and the total duration that the focal female spent within one body-length of the front of each chamber (so-called preference zone, fig. 2.4). Data were collated with a laptop using JWatcher, an event-recording program written in Java (http://www.jwatcher.ucla.edu/jwfaq.html). To be included in the analyzed data set, a female had to visit the eight chambers at least once during the recording session. Male body size was controlled within each tank and these males were reassigned randomly each day in the enclosures to avoid any pre-existing (or biased) preference for a particular position within the choice arena. I used a set of six

males and two females with seven to twelve different focal females on consecutive days (see table 2.1). None of the fish in the enclosure were related to the focal females.

Table 2.1: Detail of the fish used for the mate choice trials

Rearing treatments	Number of focal females	Number of sets of males
HO+1[1]	35	5
MO+1[2]	34	4
LO+1[3]	34	4
HO[4]	33	4
MO[5]	31	4
LO[6]	33	3

[1] females reared with high value of orange during the 2nd half of the developmental period
[2] females reared with high and low value of orange during the 2nd half of the developmental period
[3] females reared with low value of orange during the 2nd half of the developmental period
[4] females reared with high value of orange during the whole developmental period
[5] females reared with high and low value of orange during the whole developmental period
[6] females reared with low value of orange during the whole developmental period

2.3.4 Male traits analysis

Male colour patterns were photographed with a digital camera (Nikon coolpix 8800) in a narrow plastic box filled with a small volume of water where fish were free to swim. All the pictures were taken under the same light conditions, when fish were parallel to the front of the box. Both sides of the each guppy were photographed and the images analysed using the UTHSCSA ImageTool program (developed at the University of Texas Health Science Center at San Antonio, Texas, http://compdent.uthscsa.edu/dig/). Colour patches were grouped into the following colour classes: black, orange (including red), yellow, silver (including white), blue and violet, and finally bronze-green. The colour classes were measured as relative total area (relatively to the body + caudal fin) since it usually explains most of the variance in male attractiveness (see appendix for more information on the use of the word “attractiveness” in this thesis). The data for each male consisted of the mean of the right and left side of the body for the relative area. A measure of the diversity of the colour pattern was also calculated for each male. This male trait, which is rarely examined in the study of female preference, was computed thanks to a variant of the Simpson Diversity Index (i.e. Simpson’s Reciprocal Index, see

appendix). The values span from 1 to X with X being the number of category being used (for example if there are five colour classes, the higher value is X=5). The lower the value the lesser diversity and vice et versa.

2.3.5 Female preference analysis:

2.3.5.1 *Innate preference – Innate predisposition*

To increase the understanding of the effects of the rearing treatments on female mate choice, genetically determined female preferences in the Lower Aripo population were analysed for different sexual traits known to be good predictors of mating success in various guppy population (Endler & Houde 1995; Houde 1997). I investigated the relationship between female preference, measured as the mean proportion of time that females spent with different males, and, male trait values using a multiple regression. Partial regression coefficient for a particular trait gave a measure of the degree of female preference for that trait. I was interested in female innate preferences for colour pattern as a whole so male traits under investigation were: orange-red, yellow, black, silver-white, blue-violet, bronze-green, total coloured area and Simpson index of diversity. Body sizes were not analysed but were controlled within choice tanks.

Model 1

A forward (i.e. positive) stepwise multiple-regression procedure was carried out using the default 0.15 inclusion criteria. The criteria of inclusion/rejection (α level) is rather liberal because the significance value associated to a regression coefficient cannot be understood as a threshold that determines what traits are used or not by females. The *p*-value associated with a colour class must be seen as an indication of the relative importance of a colour in the process of mate choice. Normality of the residuals was checked using Shapiro-Wilks test. The Shapiro-Wilks test compares the scores in a sample (here the residuals of the regression model selected) to a normally distributed set of scores with the same mean and standard deviation. Thus, a non-significant test tells that the distribution

of the sample is normally distributed. Residuals in this model were normally distributed as W=0.984, p=0.524. The Durbin-Watson test checked for the independence of the residuals. For any two observations, residuals must not be correlated (i.e. lack of autocorrelation). The Durbin-Watson test can vary between 0 and 4 with a value of 2 meaning that the residuals are uncorrelated. With a value of 1.833, there were no reason for concern (Durbin & Watson 1951). Multicollinearity between colour classes was checked through various methods. A correlation matrix between all predictor variables did not reveal any correlation coefficients above 0.83 (coefficients higher than 0.9 are a sign of multicollinearity). The tolerance statistics used for collinearity diagnostic and given for each predictors kept in the model were not lower than 0.229 when values below 0.2 are worthy of concern (Menard 1997).

Model 2

Orange was not included in the previous rather liberal regression model emphasizing its little, if any, contribution to male attractiveness (see appendix for more information on the use of the word “attractiveness” in this thesis). However it would be interesting to know how the colour, used to make up the different rearing treatments, influenced the predictive power of the model. In other words, it allowed me to see how much more variance in the data was explained by the model (increase in “R^2” and coefficient of the semi-partial correlation between orange and the outcome) when orange was included and how orange affected the regression coefficients of significant predictors. To do so, I entered in model 2 the previously significant predictors and orange. Residuals were normally distributed (W=0.984, *p*=0.503) and there was no autocorrelation between residuals (Durbin-Watson value is 1.836). There was a little concern with multicollinearity as “total area” explanatory variable had a tolerance statistic of 0.186, however, this value remained close to the 0.2 threshold. Moreover, this model had only a “consultative” purpose and was only used to look at the orange contribution. Model 1 was the model whereby female preferences were evaluated.

Principal component analysis and model 3

Finally, a principal component analysis (PCA) was conducted to uncover potential underlying clusters of colours as it might do because single genes control several colours at a time (see appendix for more information on the genetic basis of male traits in guppies). A PCA is a coarse method to detect colours that are potentially under the control of the same gene(s). It is particularly interesting to know whether some colours are genetically correlated as it could explain the evolution of some colour patterns through indirect selection.

The PCA was performed on orange, yellow, black, silver/white, blue/violet and bronze/green relative area. The data reduction model met the different assumptions required: the determinant of the R-matrix (0.278) did not show any signs of multicollinearity; the KMO measure of sampling adequacy provided a value of 0.59, which was greater than the 0.5 value of acceptation and the Bartlett's test of sphericity was significant (meaning that there are some relationships between the variables I included in the analysis). It was therefore appropriate to conduct a principal component analysis on these variables. Components extraction was based on eigenvalues greater than 1. I chose an oblique rotation method since principal components could be correlated (one gene or a set of genes could contribute strongly to a colour loaded onto one component and to a smaller extent to a colour loaded onto another one; see appendix).

Once the principal components had been extracted and rotated, I used the component scores (computed with the regression method) to carry out a new multiple regression (model 3) to see whether female preference could be predicted, at least partly, by the principal components. I entered in the multiple regression model the principal components (PC1 and PC2) and the variables that were not included in the PCA that was total colour area and index of diversity. Residuals were normally distributed (W=0.992, p=0.949) and there was no autocorrelation between residuals (Durbin-Watson value is 1.79). There was a little concern with multicollinearity as the PC1 had a tolerance statistic of 0.178, however, this value remained far away from the 0.1 tolerance value that indicates a serious collinearity problem.

2.3.5.2 *Preference of experimental females*

There are different ways to measure a female sexual response. Following a terminology used in two seminal reviews (Jennions & Petrie 1997; Widemo & Saether 1999) extended by Brooks and Endler (2001b), we can divide individual female sexual behavior into three measurable components (see fig. 2.5):

- Choosiness that is the investment into mating itself sub-divided in:
    - Receptivity (or mean responsiveness) defined as female willingness to respond positively to male solicitations and measured as the mean response to the displays of all males in a trial.
    - Discrimination (selectivity) describes the degree to which females distinguish variation in male traits.
- Preference function that is the ranking order of male sexual signals; measured as the relationship between females' response and the male trait(s) they are evaluating (see fig. 2.5).

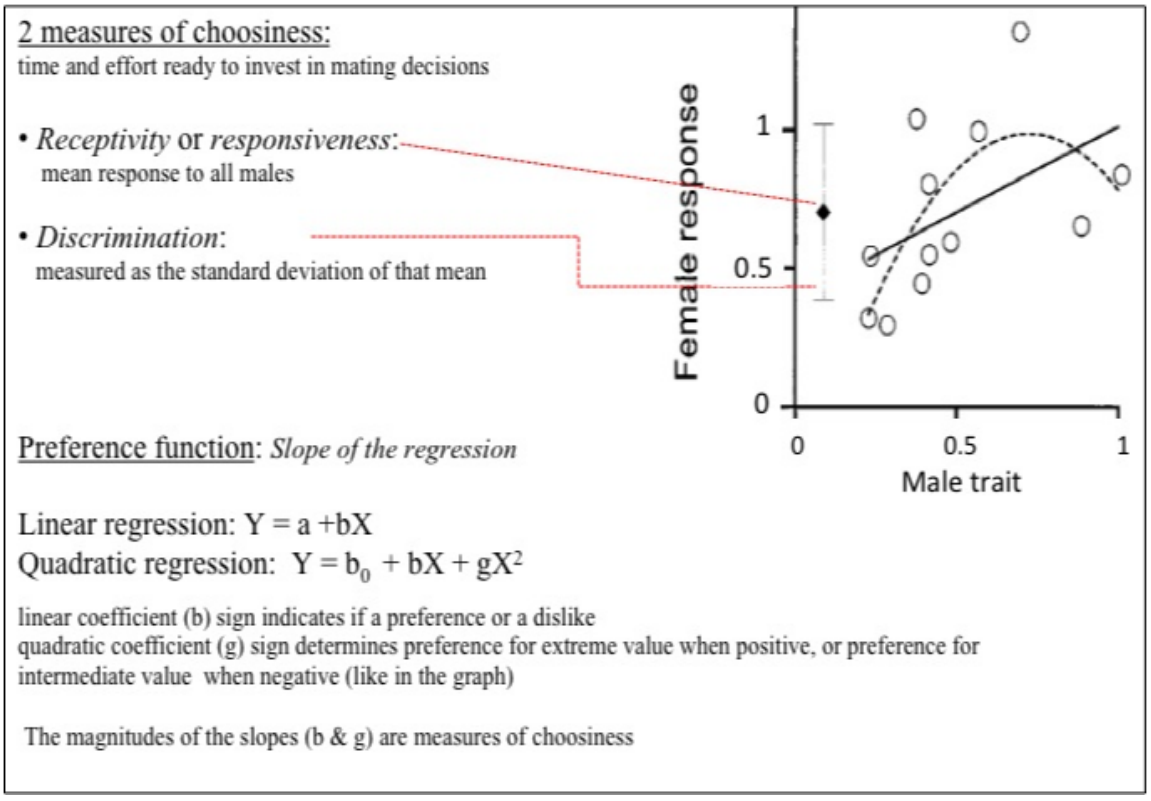

Figure 2.5: The different components of female choice's behaviour. Data are fictional and represent the response of a single female (angular transformed) to 12 males displaying a gradient of a sexual trait

The female response to a given male was measured as the proportion of time spent in the preference zone of that male and angular transformed (arc sine square root transformation) before analysis. As shown in figure 2.5, individual female preference functions were described by the linear and quadratic regression coefficients. Males traits (for measurement methodology, see above) taken into account to examine female preference functions were orange, yellow, black, blue, silver, green, total colour, colour diversity index and a variable that I call "preference for attractive males". This variable estimates the extent to which each female resembled the group (or population) norm in her mating preferences. Each male's mean attractiveness (see appendix for more information on the use of the word "attractiveness" in this thesis) was computed and adjusted by subtracting the contribution of the female of interest to this mean. I then estimated the regression coefficient of male attractiveness to the female of interest on adjusted mean attractiveness. A positive slope indicates that the female's responses were very similar to those of the other females having seen those males whereas a negative slope indicates that she differed from the responses of other females.

Female responsiveness may influence variation in preference functions (Bailey 2008) so I calculated Pearson's correlation coefficients between the quality of the linear regression ("R^2") representing the individual preference function for a trait and female's mean responsiveness. A significant positive correlation suggests that, as mean responsiveness increased, females were expressing stronger preferences (negative or positive) for that trait whereas a negative correlation shows that when responsiveness increased, females were less likely to express a preference for the trait under scrutiny (responsiveness masks preference). No correlation means that female's responsiveness hadn't any influence on female's preference function.

When females were found to have significant individual preference function for a trait in one or more rearing treatment, I performed a linear mixed model to test for phenotypic variation in preference for that trait between rearing treatments and duration of exposure. Slope of preference functions were generally normally distributed without transformation. When they were not normally distributed, slopes

were transformed appropriately before subsequent analysis. Rearing treatments and duration of exposure were considered as fixed factors with three and two levels respectively. In each case, I included the family variable (family was made up of female siblings who were split among the rearing treatments) as a random factor to examine how it contributed to the variability found in preference function (using the SUBJECT option within SPSS MIXED procedure). Moreover, the male trait (family centered) corresponding to the colour of the preference function under scrutiny was also added as a fixed and random covariate. Adding a random component to the covariate allowed quantifying the variation in the influence of the trait on preference function across family (random slope model). All main- and interaction-effects that reduced the Bayesian information criterion (BIC) were kept in the final model.

Analyses were done in various versions of SPSS (SPSS Inc., Chicago, IL, USA).

2.4. Results

2.4.1. Innate preference

Table 2.2 presents the results of the female preference in the Lower Aripo population for a set of male sexual traits known to be used in other populations. I interpret these results as the genetic preference found in the population because all fish tested were born and reared in the laboratory under controlled conditions ruling out most of the environmental effects (see chapter 1) that could shape females preferences in guppies. The three models considered are, overall, a good fit of the data (see table 2.2, model 1,2,3: $p<0.001$) and male traits selected explain a large amount of variation found in female preference. The significance levels of the regression coefficients are indicators of how important a colour class is in the process of mate choice and not a threshold delimiting the use of the trait. The model 1 (R^2_{adj} = 69.1%) suggests that females base their choice mainly on three colour classes. Females have strong preferences for yellow and black colouration and do like also males with more colour overall. Even if not significant

Table 2.2: Partial regression coefficients (and standard errors) from the stepwise multiple regression of male sexual traits on female innate preferences. The one-sample *t*-tests indicate whether coefficients differ from zero and contribute significantly to the model. The semi-partial correlation represents how each explanatory variable contributes to female preference while controlling for the effects that the other variable have on female preference (or male attractiveness).

Innate preference	Unstandardized regression coefficients	Standard error	*t*-test	*p*-value	Semi-partial correlation coefficients
Model 1 (N=69)					
Intercept	0.234	0.055	4.245	0.001	-
Yellow	1.2	0.351	3.423	0.001	0.231
Black	1.011	0.39	2.593	0.012	0.175
Total area	0.32	0.159	2.012	0.048	0.136
Diversity index	-0.016	0.011	-1.485	0.142	-0.1
Model 2 (N=69)					
Intercept	0.233	0.058	4	0.001	-
Orange	-0.026	0.305	-0.085	0.933	-0.006
Yellow	1.195	0.358	3.34	0.001	0.227
Black	1.007	0.395	2.55	0.013	0.173
Total area	0.326	0.178	1.84	0.071	0.125
Diversity Index	-0.016	0.012	-1.39	0.169	-0.094
Model 3 (N=69)					
Intercept	0.214	0.08	2.68	0.009	-
PC1 (see table 2.3)	0.037	0.017	2.24	0.028	0.163
PC2 (see table 2.3)	-0.001	0.008	-0.15	0.885	-0.011
Total area	0.571	0.179	3.19	0.002	0.232
Diversity Index	-0.008	0.013	-0.59	0.555	-0.043

Model 1: R^2adj = 69.1%; F(4,64)=38.97, p<0.001
Model 2: R^2adj = 68.6%; F(5,63)=30.69, p<0.001
Model 3: R^2adj = 64%; F(4,64)=31.22, p<0.001

at the standard α-level of 0.05, it seems that females dislike males with increased colour diversity (table 2.2). Model 1 is the reference model for female innate preferences in Lower Aripo guppies. Model 2 (R^2_{adj} = 68.6%) was only carried out to have an estimate of the female preference for orange in lower Aripo population. The regression revealed that females are not using orange to discriminate among males. The signs and magnitudes of the regression coefficients of the previously selected colour class do not change in the new model even though orange inclusion changes slightly their *p*-values (table 2.2).

The PCA has extracted two principal components (see table 2.3). PC1 is primarily composed of roughly equivalent loadings of orange, yellow and black. PC2 is composed of roughly equivalent absolute loadings for the iridescent colour patches (silver, blue and green). This would mean that to a certain degree a set of genes would code for the expression of carotenoid pigments (orange + yellow) and black coloration and another set of genes would control the expression of iridescent colours. Given the complexity of colour pattern genetics in guppies (see appendix), such conclusion should be used with care but confirms findings from Winge (1927).

Table 2.3: Principal component loadings for each variable (after oblique rotation) onto the 2 components extracted (Pattern matrix)

Loading	PC1	PC2
Orange	0.851	0.067
Yellow	0.845	0.127
Black	0.804	-0.176
Silver/White	-0.144	-0.740
Blue/Violet	-0.075	0.698
Bronze/Green	-0.029	0.613
Variance explained (%)*	36	23.7

*Variance after extraction but before rotation

Model 3 (see table 2.2, R^2_{adj} = 64%) is assessing the predictive power of the regression model when colour classes are grouped into principal components. Total area and PC1 are the main predictors of male attractiveness. It is not surprising that PC1 contributes significantly to female preference since females

have strong preferences for yellow and black. A higher semi-partial correlation in total area than in PC1 (and thus more variance in female preference explained) is explained by the presence of orange in PC1, a colour to which females are indifferent in the population used.

2.4.2. Female preferences following the rearing treatments

Females from the three different treatments in the two temporal conditions spent more time on average with each of the six males than with each of the two control females (see table 2.4) suggesting that females were associating with males for sexual reasons rather than as a tendency to shoal with conspecifics.

Table 2.4: Paired-sample *t*-test testing female' receptivity in each experimental treatments

Rearing treatments	*df*	T-test	*p*-value
HO+1[1]	34	5.12	<0.001
MO+1[2]	33	4.86	<0.001
LO+1[3]	33	8.44	<0.001
HO[4]	32	4.53	<0.001
MO[5]	30	5.01	<0.001
LO[6]	32	3.65	0.001

[1] females reared with high value of orange during the 2nd half of the developmental period
[2] females reared with high and low value of orange during the 2nd half of the developmental period
[3] females reared with low value of orange during the 2nd half of the developmental period
[4] females reared with high value of orange during the whole developmental period
[5] females reared with high and low value of orange during the whole developmental period
[6] females reared with low value of orange during the whole developmental period

2.4.2.1 Individual female preference functions after exposure to stimuli males for half of the developmental period

Females having been exposed to high value of orange during the second half of their developmental period (HO+1 treatment) tended to prefer males that other females of the same rearing treatment found attractive and that displayed large area of yellow and black. By contrast, they were attracted by males with low value of orange. This was indicated by mean preference functions that differ

significantly, after sequential Bonferroni correction, from zero in one-sample *t*-tests (table 2.5). Females having been exposed to high and low value of orange during the second half of their developmental period (MO+1 treatment) preferred males that other females of the same rearing treatment found attractive, that had large area of yellow, black and total colour (significant after sequential Bonferroni correction). They also had a significant preference for males displaying more diversity in their colour pattern (Table 2.5). They tended to reject males with large value of iridescent green although this result was not significant after correction for multiple *t*-tests. Females having been exposed to low value of orange during the second half of their developmental period (LO+1 treatment) did not display any significant preference for any male traits (table 2.5).

A quadratic term strongly improved the fit of the statistical models for all male parameters and in all treatments (see table 2.6) although very few linear and quadratic components were significant after sequential Bonferroni adjustment. In the “HO+1” treatment, only attractive males were significantly preferred by females who had also a non-significant preference for intermediate value of yellow (mean g significantly negative before adjustment, table 2.6). In the “MO+1” treatment, once again, females orientated their choices towards attractive males but not only, since orange was used to discriminate among males. They preferred very high value of orange as indicated by the significant positive quadratic coefficient and almost significant negative linear component of the regression. The quadratic regressions confirm the absence of preferences found previously for females having been reared with low value of orange (“LO+1” treatment). Due to the lack of any consistency in the quadratic regressions to account for individual choices, I consider only linear regression coefficients for these treatments in further analysis.

Pearson correlations didn’t reveal much relationship between individual mean responsiveness and the linear preference functions for various traits (table 2.7). The only significant correlation coefficient (after sequential Bonferroni adjustment) was found in the “LO+1” treatment. Quality of the linear regression for blue preference was negatively correlated with female mean responsiveness. Such negative relationship could mask preference for blue in the “LO+1” treatment

however, in most cases, the absence or presence of preferences for male traits had not been influenced by female responsiveness.

Table 2.7: Correlation coefficients between individual female mean responsiveness and quality of the linear regression (representing individual preference functions) for different male traits.

Treatments		N	Pearson correlation coefficient	*p*-value
HO+1[1]	Orange area	35	**0.354**	**0.037**
	Yellow area	35	0.089	0.611
	Black area	35	0.019	0.914
	White	35	0.065	0.709
	Blue area	35	0.251	0.146
	Green area	35	-0.069	0.695
	Total colour area	35	-0.188	0.28
	Simpson Index	35	-0.237	0.17
MO+1[2]	Orange area	34	-0.104	0.56
	Yellow area	34	-0.089	0.617
	Black area	34	-0.078	0.662
	White	34	-0.119	0.503
	Blue area	34	**-0.38**	**0.026**
	Green area	34	0.131	0.461
	Total colour area	32	-0.052	0.779
	Simpson Index	34	-0.133	0.453
LO+1[3]	Orange area	34	0.214	0.225
	Yellow area	33	-0.112	0.529
	Black area	33	-0.163	0.356
	White	34	0.014	0.935
	Blue area	34	**-0.492**	**0.003***
	Green area	34	0.118	0.507
	Total colour area	34	-0.048	0.787
	Simpson Index	34	0.098	0.583

* Significant after sequential Bonferroni correction for number of tests in column

[1] females reared with high value of orange during the 2nd half of the developmental period

[2] females reared with high and low value of orange during the 2nd half of the developmental period

[3] females reared with low value of orange during the 2nd half of the developmental period

Table 2.5: Means and standard errors of the slopes (b) of the linear regression representing the individual female preference functions. One-sample Student's t-tests were used to test whether coefficients tend to differ from zero. Means qualities of the linear regressions are also represented ("R^2").

			Linear regression				
Treatments		N	mean R^2 (%)	mean b	SE	*t*	*p*
HO+1[1]	Orange area	35	49.4	**-2.52**	0.87	-2.90	**0.006***
	Yellow area	35	28.6	**1.01**	0.33	3.10	**0.004***
	Black area	35	19.5	**1.31**	0.39	3.35	**0.002***
	White area	35	15.8	0.17	1.07	0.16	0.875
	Blue area	35	16.6	-0.19	0.67	-0.29	0.776
	Green area	35	15.4	-0.08	0.54	-0.15	0.884
	Total colour area	35	25.2	0.58	0.30	1.91	0.065
	Simpson Index	35	17.3	0.05	0.03	1.75	0.089
	Attractive males	31	55.9	**0.60**	0.15	3.97	**<0.001***
MO+1[2]	Orange area	34	26.0	-0.08	1.22	-0.06	0.951
	Yellow area	34	25.4	**2.52**	0.52	4.75	**<0.001***
	Black area	34	15.5	**2.29**	0.61	3.72	**0.001***
	White area	34	15.0	-0.43	1.11	-0.37	0.711
	Blue area	34	29.9	1.08	1.04	1.03	0.312
	Green area	34	18.3	**-1.40**	0.53	-2.62	**0.013**
	Total colour area	32	23.7	**1.01**	0.25	4.09	**<0.001***
	Simpson Index	34	19.8	**0.09**	0.02	5.07	**<0.001***
	Attractive males	31	64.8	**0.58**	0.17	3.31	**0.002***
LO+1[3]	Orange area	34	20.1	1.09	0.61	1.79	0.083
	Yellow area	33	18.4	0.32	0.71	0.45	0.655
	Black area	33	17.1	-0.64	0.69	-0.93	0.357
	White area	34	17.5	1.29	1.03	1.25	0.222
	Blue area	34	21.3	0.67	1.23	0.54	0.590
	Green area	34	16.6	-0.33	0.46	-0.71	0.485
	Total colour area	34	23.1	0.46	0.31	1.46	0.153
	Simpson Index	34	16.1	0.01	0.02	0.24	0.815
	Attractive males	32	46.4	0.28	0.18	1.56	0.13

* Significant after sequential Bonferroni correction for number of tests in column

[1] females reared with high value of orange during the 2nd half of the developmental period

[2] females reared with high and low value of orange during the 2nd half of the developmental period

[3] females reared with low value of orange during the 2nd half of the developmental period

Table 2.6: Means and standard errors of the linear (b) and quadratic (g) components representing the individual non-linear preference functions. One-sample Student's t-tests were used to test whether coefficients tend to differ from zero. Means qualities of the linear regressions are also represented ("R^2").

				Quadratic regression							
			mean R^2 (%)	**Linear component**				**Quadratic component**			
Treatments		*N*		mean b	SE	*t*	*p*	mean g	SE	*t*	*p*
HO+1[1]	Orange area	35	58.0	-9.94	5.25	-1.89	0.067	65.13	43.81	1.49	0.146
	Yellow area	35	48.8	**5.09**	2.01	2.53	**0.016**	**-61.70**	26.23	-2.35	**0.025**
	Black area	35	44.5	29.13	23.95	1.22	0.232	-173.95	144.32	-1.21	0.236
	White area	35	35.5	10.72	11.00	0.98	0.336	-773.11	818.12	-0.94	0.351
	Blue area	35	40.5	0.61	7.97	0.08	0.940	4.47	79.47	0.06	0.955
	Green area	35	35.9	5.08	9.33	0.54	0.590	-22.23	42.96	-0.52	0.608
	Total colour area	35	46.9	-4.44	5.92	-0.75	0.458	6.85	7.82	0.88	0.387
	Simpson Index	35	36.7	1.05	0.68	1.55	0.130	-0.12	0.08	-1.45	0.157
	Attractive males	31	63.8	**0.67**	0.22	3.08	**0.004***	-8.59	9.82	-0.87	0.388
MO+1[2]	Orange area	34	48.0	**-17.62**	6.32	-2.79	**0.009**	**156.21**	51.93	3.01	**0.005***
	Yellow area	34	43.1	-1.34	2.72	-0.49	0.629	45.48	32.69	1.37	0.180
	Black area	34	40.5	8.62	6.87	1.24	0.225	-74.71	71.68	-1.03	0.312
	White area	34	34.9	6.73	5.22	1.25	0.219	-314.33	200.00	-1.52	0.137
	Blue area	34	46.2	6.77	7.13	0.94	0.356	-97.74	119.34	-0.81	0.425
	Green area	34	33.8	-16.55	8.19	-1.99	0.055	57.76	30.92	1.84	0.075
	Total colour area	32	37.0	8.31	8.69	0.96	0.346	-9.72	12.77	-0.76	0.452
	Simpson Index	34	34.6	0.35	0.48	0.72	0.479	-0.02	0.07	-0.35	0.728
	Attractive males	31	71.3	**0.56**	0.17	3.23	**0.003***	1.13	0.85	1.33	0.194
LO+1[3]	Orange area	34	37.2	8.23	4.6	1.80	0.080	-65.11	38.94	-1.67	0.104
	Yellow area	33	36.9	-5.31	7.3	-0.73	0.472	-38.20	102.77	-0.37	0.717
	Black area	33	37.3	1.18	4.8	0.25	0.806	-20.74	52.39	-0.40	0.695
	White area	34	41.3	20.05	10.6	1.90	0.067	-1088.71	845.00	-1.29	0.207
	Blue area	34	44.0	7.75	6.2	1.25	0.220	-68.10	67.97	-1.00	0.324
	Green area	34	37.0	10.72	10.0	1.07	0.294	-56.61	51.59	-1.10	0.280
	Total colour area	34	50.8	0.88	5.1	0.17	0.864	-1.00	8.11	-0.12	0.903
	Simpson Index	34	33.6	-1.46	1.7	-0.87	0.392	0.16	0.18	0.85	0.399
	Attractive males	32	69.7	0.47	0.26	1.82	0.078	2.02	1.19	1.69	0.102

* Significant after sequential Bonferroni correction for number of tests in column; [1, 2, 3] see previous table for abbreviations

2.4.2.2 Individual female preference functions after exposure to stimuli males for the whole developmental period

After having experienced males with high value of orange for the whole duration of the developmental period (HO treatment), females developed preferences for males that are generally attractive to other females in the same treatment, that had large value of yellow and total colour although only yellow and "attractive male" preferences were significant after sequential bonferroni correction (Table 2.8). Pearson correlation didn't reveal any significant relationship between quality of the linear regression (representing preference functions) and female mean responsiveness except for total colour area (Table 2.10). A significant (but not after sequential Bonferroni adjustment) negative correlation suggests that responsiveness might have masked, to some extent, the females' preference function for total colour area. In the absence of such relationship, the preference might have been stronger and could have been significant after correction.

After exposure to high and low value of orange during the whole developmental period (MO treatment), females acquired significant preferences (after adjustment) for males that other females found attractive, that have large value of yellow, black and total colour area (Table 2.8). A preference for males with increased diversity in their colour pattern was only significant before correction and female responsiveness didn't seem to affect the strength of this preference, as the Pearson correlation didn't reveal anything (Table 2.10). Only preference for white colouration could have been influenced by responsiveness (correlation significant before correction, Table 2.10) but it is unlikely given the value of the white preference slope (Table 2.8) that does not differ from zero.

Experiencing males with low value of orange during development (LO treatment), affected female preferences for black, blue and for males that other females of the same treatment found attractive. Females tended to favour males with low value of black and blue but these preferences were not significant after correction (Table 2.8). A significant negative correlation between responsiveness

and the quality of the linear regression concerning blue (Table 2.10) indicates that females' responsiveness could have reduced their preference for that colour.

Adding a quadratic term to the regression defining preference functions improved the fit of the statistical models for all male parameters and in all treatments (see table 2.9). However, just as for females having experienced the different treatments during half of their development, very few linear and quadratic components were significant before or after sequential Bonferroni adjustment. Males found to be attractive in average were also preferred by individual females in all three treatments (Table 2.9). The other significant contribution to female preferences was made by black coloration in the MO treatment. Females tended to prefer males with high value of black (marginally significant negative *b* and positive *g*, table 2.9). This result contradicts the preference for black found with the linear regression. Just like with the other treatments and for the sake of simplicity, the outcomes of the quadratic regression won't be considered in further analysis.

Table 2.10: Correlation coefficients between individual female mean responsiveness and quality of the linear regression representing individual preference functions for different male traits

Treatments		N	Pearson correlation coefficients	*p*-values
HO[1]	Orange area	33	-0.151	0.4
	Yellow area	33	-0.293	0.098
	Black area	33	-0.049	0.785
	White area	33	-0.164	0.362
	Blue area	33	-0.105	0.56
	Green area	33	0.16	0.375
	Total colour area	33	**-0.38**	**0.029**
	Simpson Index	33	-0.333	0.058
MO[2]	Orange area	31	-0.218	0.239
	Yellow area	30	-0.273	0.138
	Black area	31	-0.287	0.118
	White area	31	**0.418**	**0.019**
	Blue area	31	0.012	0.948
	Green area	31	0.163	0.381
	Total colour area	31	-0.021	0.911
	Simpson Index	31	0.199	0.282
LO[3]	Orange area	33	0.042	0.818
	Yellow area	33	-0.094	0.608
	Black area	33	-0.111	0.537
	White area	33	0.22	0.218
	Blue area	33	**-0.521**	**0.002***
	Green area	33	-0.199	0.268
	Total colour area	33	0.087	0.631
	Simpson Index	33	0.194	0.28

* Significant after sequential Bonferroni correction for number of tests in column

[1] females reared with high value of orange during the whole developmental period

[2] females reared with high and low value of orange during the whole developmental period

[3] females reared with low value of orange during the whole developmental period

Table 2.8: Means and standard errors of the slopes (b) of the linear regression representing the individual female preference functions. One-sample Student's t-tests were used to test whether coefficients tend to differ from zero. Means qualities of the linear regressions are also represented ("R^2").

			Linear regression				
Treatments		N	mean R^2 (%)	mean b	SE	*t*	*p*
HO[1]	Orange area	33	18.5	0.75	0.59	1.26	0.215
	Yellow area	33	29.5	**3.38**	0.79	4.26	**<0.001***
	Black area	33	21.9	0.25	1.24	0.21	0.839
	White area	33	18.7	0.49	2.07	0.24	0.815
	Blue area	33	24.7	-0.04	1.05	-0.03	0.974
	Green area	33	25.9	-0.28	0.93	-0.30	0.764
	Total colour area	33	25.6	**1.06**	0.40	2.66	**0.012**
	Simpson Index	33	25.8	0.07	0.04	1.89	0.067
	Attractive males	30	70.3	**1.01**	0.07	14.32	**<0.001***
MO[2]	Orange area	31	23.7	-0.86	0.93	-0.92	0.367
	Yellow area	30	19.7	**2.08**	0.35	5.89	**<0.001***
	Black area	31	23.4	**4.97**	1.09	4.55	**<0.001***
	White area	31	23.4	-0.22	1.52	-0.14	0.886
	Blue area	31	24.6	-0.26	0.97	-0.27	0.786
	Green area	31	25.6	1.05	0.88	1.19	0.243
	Total colour area	31	20.8	**0.76**	0.21	3.61	**<0.001***
	Simpson Index	31	16.5	**0.06**	0.02	2.51	**0.018**
	Attractive males	29	55.2	**0.93**	0.11	8.09	**<0.001***
LO[3]	Orange area	33	22.5	-0.21	0.70	-0.30	0.764
	Yellow area	33	24.1	-1.48	1.09	-1.34	0.190
	Black area	33	20.1	**-2.18**	0.97	-2.24	**0.032**
	White area	33	18.3	-0.62	1.53	-0.76	0.451
	Blue area	33	24.9	**-2.53**	1.11	-2.28	**0.029**
	Green area	33	21.4	1.23	1.06	1.16	0.255
	Total colour area	33	33.2	0.14	0.38	0.37	0.715
	Simpson Index	33	18.4	-0.01	0.02	-0.32	0.748
	Attractive males	30	65.9	**1.08**	0.09	11.34	**<0.001***

* Significant after sequential Bonferroni correction for number of tests in column

[1] females reared with high value of orange during the whole developmental period

[2] females reared with high and low value of orange during the whole developmental period

[3] females reared with low value of orange during the whole developmental period

Table 2.9: Means and standard errors of the linear (b) and quadratic (g) components representing the individual non-linear preference functions. One-sample Student's t-tests were used to test whether coefficients tend to differ from zero. Means qualities of the linear regressions are also represented ("R^2").

			Quadratic regression							
Treatments		**Mean R^2 (%)**	**Linear component**				**Quadratic component**			
			mean b	SE	*t*	*p*	mean g	SE	*t*	*p*
HO[1]	Orange area	39.8	-4.80	6.38	-0.75	0.457	43.18	53.05	0.81	0.422
	Yellow area	51.8	1.28	4.77	0.28	0.779	22.21	92.32	0.24	0.812
	Black area	41.9	20.09	17.53	1.15	0.260	-261.48	209.85	-1.25	0.222
	White area	31.1	2.41	5.80	0.41	0.681	-18.64	245.92	-0.08	0.940
	Blue area	46.5	5.51	9.32	0.58	0.564	-51.53	81.45	-0.62	0.538
	Green area	50.8	49.25	57.73	0.85	0.400	-187.24	223.16	-0.84	0.408
	Total colour area	47.9	-2.45	8.41	-0.29	0.773	22.77	12.16	1.87	0.070
	Simpson Index	49.7	-0.94	0.59	-1.61	0.118	0.13	0.08	1.66	0.107
	Attractive males	88.4	**1.16**	0.24	4.76	**<0.001***	1.76	1.44	1.22	0.234
MO[2]	Orange area	44.7	-2.52	6.29	-0.40	0.692	16.42	48.91	0.34	0.739
	Yellow area	47.7	1.20	2.31	0.51	0.613	17.27	38.93	0.43	0.671
	Black area	46.4	**-18.57**	7.74	-2.40	**0.023**	**271.53**	96.89	2.80	**0.009**
	White area	44.5	9.01	13.12	0.69	0.497	114.06	621.42	0.18	0.856
	Blue area	51.2	-16.16	8.18	-1.98	0.057	124.23	66.76	1.86	0.073
	Green area	45.2	4.31	12.83	0.34	0.739	-43.34	71.39	-0.61	0.548
	Total colour area	44.5	0.52	5.26	0.10	0.922	0.31	8.39	0.04	0.971
	Simpson Index	33.9	1.44	0.87	1.65	0.109	-0.15	0.09	-1.66	0.107
	Attractive males	65.7	**0.87**	0.14	6.25	**<0.001***	0.98	0.76	1.30	0.204
LO[3]	Orange area	41.4	-2.40	5.27	-0.46	0.651	39.19	49.05	0.80	0.430
	Yellow area	54.3	-7.33	7.01	-0.94	0.353	64.76	148.62	0.41	0.686
	Black area	46.3	-13.26	22.68	-0.58	0.563	190.93	284.82	0.67	0.507
	White area	43.8	0.57	10.39	0.06	0.956	233.34	419.51	0.56	0.582
	Blue area	47.2	-19.22	10.28	-1.87	0.071	131.91	80.52	1.64	0.111
	Green area	44.3	-72.62	50.11	-1.45	0.157	309.22	193.62	1.60	0.120
	Total colour area	52.0	11.53	13.86	0.83	0.412	-15.52	20.33	-0.76	0.451
	Simpson Index	49.2	1.69	0.84	2.02	0.052	-0.21	0.10	-1.99	0.055
	Attractive males	79.5	**1.04**	0.11	8.81	**<0.001***	5.14	2.60	1.97	0.057

* Significant after sequential Bonferroni correction for number of tests in column; [1, 2, 3] see previous table for abbreviations

2.4.2.3 Variation in preference functions between rearing treatments

i. Variation in orange preference between treatments

Long- and short- exposed females changed in their preference for orange after the exposure treatments. I found a significant interaction between treatment and time of exposure (Table 2.11). No other main factors or interactions were significant. There was more within-family variation (σ^2=23.9, Table 2.11) than among-family variation (σ^2=0.38) as the Wald Z statistic suggests (Table 2.11). This might reflect that a large part of the variance in orange preference was environmental. An ANOVA with one fixed factor "exposure group" and six levels (exposed to high value of orange, half and whole development, exposed to high and low value, half and whole development and exposed to low value of orange, half and whole development) was performed to disentangle the effects of the interactions. The omnibus test was significant ($F_{(5,194)}$=2.27, p=0.049) and post-hoc analysis (using Bonferroni test to correct for multiple comparisons) showed that females from "HO+1" treatment preferred significantly more males with low value of orange than females reared in "LO+1" who preferred males with more orange (p=0.043, Fig. 2.6). In spite of the significant interaction between treatment and time of exposure found in the main model, no more differences in preference between exposure groups were significant after correction although significant before adjustment (Fisher LSD post-hoc: "HO" versus "HO+1", p=0.009, "LO" versus "HO+1", p=0.057 and "HO+1" versus "MO+1", p=0.042; see fig. 2.6).

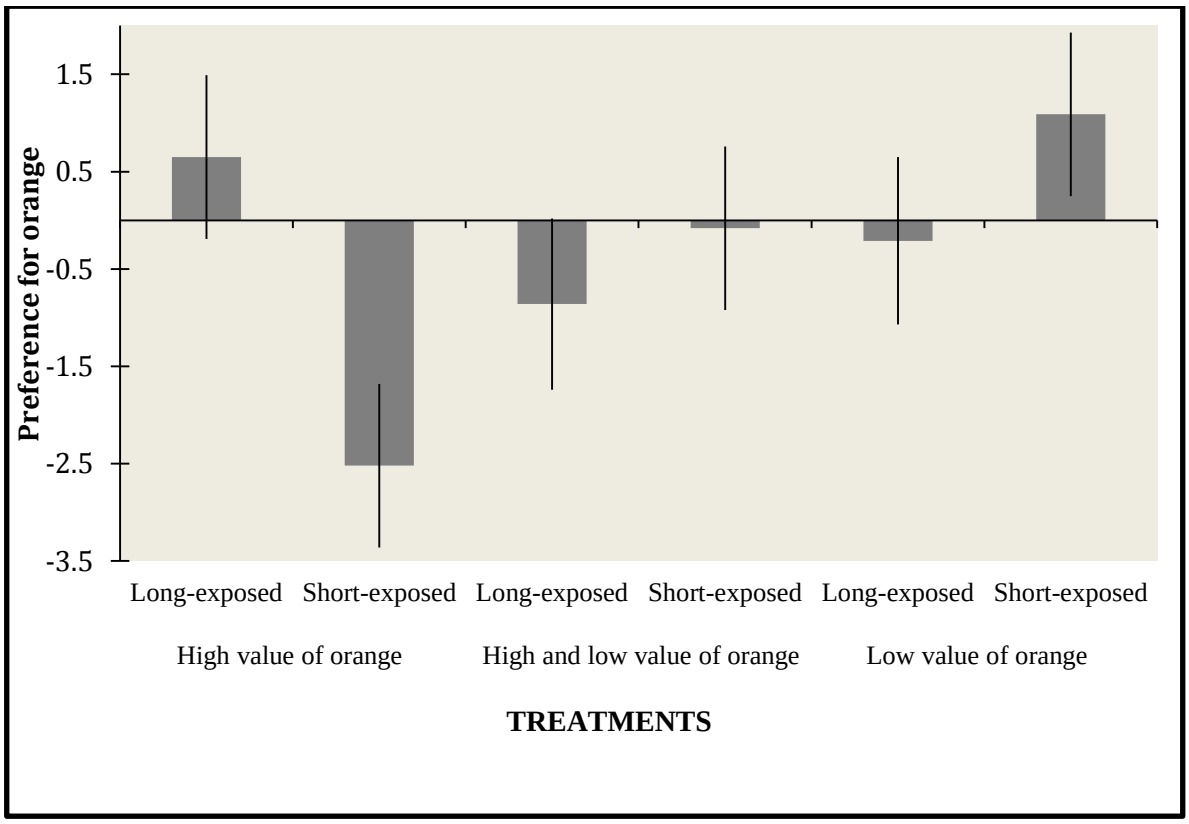

Figure 2.6: Females' orange preference after exposure to high, mixed, and low value of orange for half or whole development. Bars represent the estimated marginal means of the regression slopes representing the preference functions +/- SE.

Table 2.11: Linear mixed model of fixed and random effects influencing differences in orange preference across treatments

	Factor	*df*	F	*p*
Test of fixed effects	Intercept	1, 8.4	0.37	0.56
	Treatments	2, 185.7	1.36	0.26
	Time of exposure	1, 8.4	0.026	0.88
	Orange covariate	1, 3.33	3.5	0.15
	Treatment*Time of exposure	2, 185.7	**5.2**	**0.006**
	Time of exposure*Orange covariate	1, 3.33	0.023	0.89
	Treatment* Orange covariate	2, 85.6	0.84	0.43
	Treatment*Time of exposure*Orange covariate	2, 85.6	0.07	0.93
	Parameter	**Estimate**	**SE**	**Wald Z**	**Significance**
Estimates of covariance parameters	Residuals	23.9	2.54	9.39	<0.001
	Intercept	0.38	0.914	0.42	0.68
	orange covariate	2103	11527	0.18	0.86

ii. Variation in yellow preference between treatments

Experiencing different level of orange during development affected females' preference for yellow ($F_{(2, 180.3)}$=4.12, p=0.017, Table 2.12). Independently from the time of exposure, females having been reared with high value of orange (HO and HO+1 treatments) and with high and low value of orange (MO and MO+1 treatments) preferred significantly yellower males than females reared with low value of orange (LO and LO+1 treatments) (p=0.054 and p=0.028 respectively, corrected with Bonferroni test for multiple comparisons, Fig. 2.8) although there were no differences in preferences for yellow between females after HO/HO+1 and MO/MO+1 treatments. The main factor duration of exposure has no significant effect on preference for yellow (table 2.12) however short- and long-exposed females differed after the exposure treatments as shown by the significant interaction between treatments and time of exposure ($F_{(2, 176.2)}$=3.79, p=0.024, Table 2.12). An ANOVA with one fixed factor "exposure group" and six levels (exposed to high value of orange, half and whole development, exposed to high and low value, half and whole development and exposed to low value of orange, half and whole development) was performed to disentangle the effects of the interactions. The omnibus test was significant ($F_{(5,191)}$=6.38, p<0.001) and multiple comparison (adjusted with Bonferroni correction) showed that females reared with low value of orange during whole development preferred significantly less yellower males than HO females (p<0.001, see fig. 2.7), than MO females (p=0.007, see fig. 2.7) and, than MO+1 females (p=0.001, see fig. 2.7). HO females liked also higher value of yellow than LO+1 females (p=0.027, see fig. 2.7).

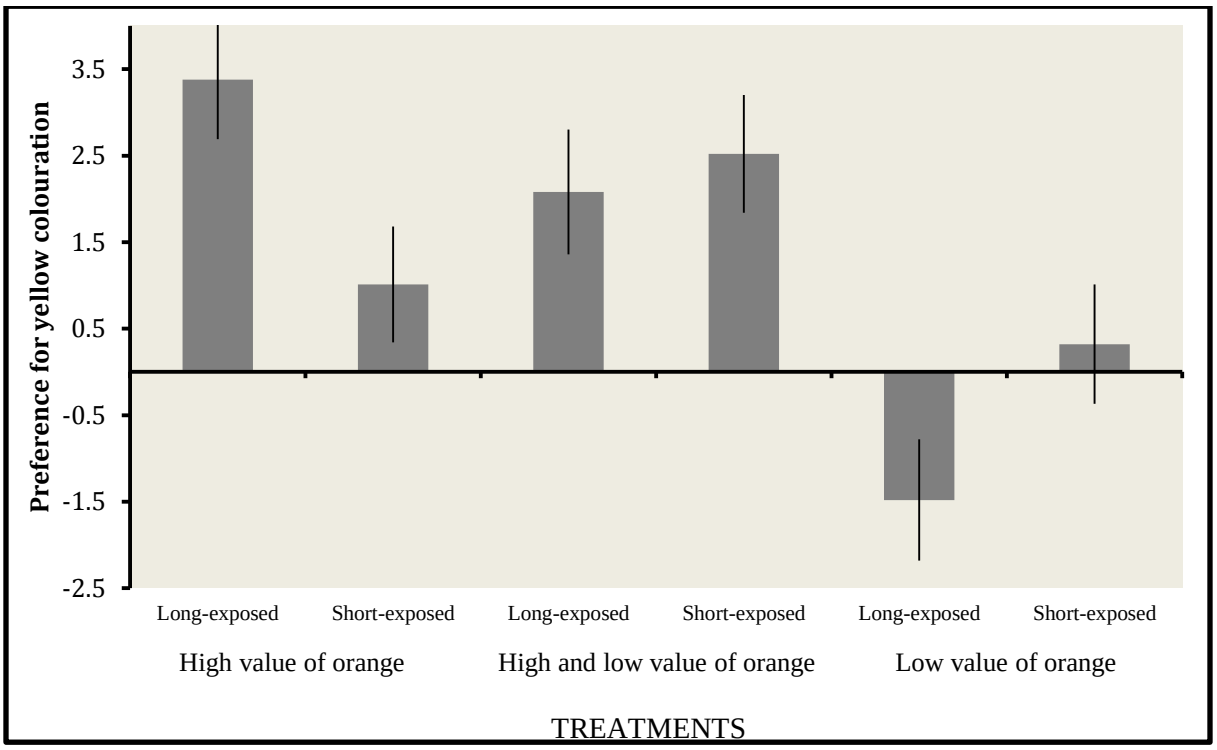

Figure 2.7: Females' yellow preference after exposure to high, mixed, and low value of orange for half or whole development. Bars represent the estimated marginal means of the regression slopes representing the preference functions +/- SE.

Table 2.12: Linear mixed model of factors (fixed and random effects) influencing differences in yellow preferences across treatments

	Factor	*df*	F	*p*
Test of fixed effects	Intercept	1, 10.7	20.1	**0.001**
	Treatments	2, 176.2	4.15	**0.017**
	Time of exposure	1, 10.7	0.046	0.833
	Yellow covariate	1, 7.2	5.29	0.054
	Treatment*Time of exposure	2, 176.2	3.79	**0.024**
	Time of exposure*Yellow covariate	1, 7.2	1.1	0.33
	Treatment* Yellow covariate	2, 181.3	8.09	**<0.001**
	Treatment*Time of exposure*Yellow covariate	2, 181.3	1.73	0.18

	Parameter	Estimate	SE	df	*t*	Wald Z	*p*
Estimates of fixed effects	Intercept	-0.34	0.71	49.5	-0.47	-	0.64
	HO treatment[1]	3.25	0.91	179	3.6	-	**<0.001**
	MO treatment[1]	1.97	0.93	168.8	2.11	-	**0.036**
	Time of exposure[2]	1.41	1.04	55.8	1.36	-	0.178
	Yellow covariate	289	73	21.4	3.98	-	**0.001**
	HO*Yellow[3]	-286	81	176.5	-3.5	-	**0.001**
	MO*Yellow[3]	-193	83	177.2	-2.3	-	**0.022**
Estimates of covariance parameters	Residuals	11.9	1.33	-	-	8.98	**<0.001**
	Intercept	0.45	0.54	-	-	0.83	0.41
	yellow covariate	12288	9391	-	-	1.31	0.19

Not all the estimates included in the model are represented
[1] LO treatment is the reference category
[2] Whole developmental period used as the reference category
[3] LO*Yellow is the reference category

iii. Variation in black preferences between treatments

Experiencing different level of orange during development affected females' preference for black spots ($F_{(2,\ 177.5)}$=15.4, p<0.001, Table 2.13). Females having been reared with high and low value of orange (MO/MO+1 treatment) significantly preferred, after Bonferroni adjustment, blacker male than HO/HO+1 females (p=0.018, see fig. 2.8) and, than LO/LO+1 females (p<0.001, see fig. 2.8). Moreover, females having been reared with high value of orange preferred males with higher value of black than LO/LO+1 females (p=0.012, see fig. 2.8) who disliked black, confirming the mean individual preferences (Table 2.5, table 2.8).The linear mixed model didn't present any other significant factors that could account for the variation found in black preferences across the rearing treatments (Table 2.13).

Variance estimates showed that they were no significant differences in black preference across family (σ^2=2.84, p=0.19, Table 2.13).

Table 2.13: Linear mixed model of factors (fixed and random effects) influencing differences in black preferences across treatments

	Factor	*df*	F	*p*
Test of fixed effects	Intercept	1, 10	1.99	0.188
	Treatments	2, 177.5	15.4	**<0.001**
	Time of exposure	1, 10	0.132	0.724
	Black covariate	1, 107.8	0.922	0.339
	Treatment*Time of exposure	2, 177.5	2.1	0.125
	Time of exposure*Black covariate	1, 107.8	3.68	0.058
	Treatment* Black covariate	2, 181	0.986	0.375
	Treatment*Time of exposure*Black covariate	2, 181	1.46	0.24

	Parameter	Estimate	SE	df	*t*	Wald Z	*p*
Estimates of fixed effects	Intercept	-1.83	1.1	21.2	-1.67	-	0.11
	HO treatment[1]	2.22	1.19	174.1	1.87	-	0.064
	MO treatment[1]	6.68	1.23	175.1	5.44	-	**<0.001**
	Time of exposure[2]	-0.34	1.88	43.4	-0.18	-	0.86
	Black covariate	305	201	183.3	1.51	-	0.132
Estimates of covariance parameters	Residuals	22.9	2.48	-	-	9.22	**<0.001**
	Intercept	2.84	2.18	-	-	1.3	0.19
	Black covariate	482	3668	-	-	0.13	0.90

Not all the estimates included in the model are represented
[1] LO treatment is the reference category
[2] Whole developmental period used as the reference category

iv. Variation in total colour preferences between treatments

Experiencing different level of orange during half or the whole developmental period didn't influence females' preference for total colour area among females (Table 2.14, fig. 2.8). However the average value of total colour borne by males in the experimental tanks (e.g. how colourful they are) plays a role on female preference for that male trait. As indicated by the significant positive coefficient of the covariate, females increased their interest for total colour as the sexual cue augment in magnitude (Table 2.14). I also found a significant interaction between duration of exposure to males and total colour covariate showing that females having been exposed to males for half of the developmental period were less sensitive to total colour than females exposed to males for their whole development although the coefficient was not significantly different from zero (Table 2.14). Variance estimates showed that they were no significant differences in total colour preference across family (σ^2=0.03, p=0.81, Table 2.14).

Table 2.14: Linear mixed model of factors (fixed and random effects) influencing differences in total colour preference across treatments

	Factor	***df***		**F**			***p***
Test of fixed effects	Intercept	1, 10.2		19.9			**0.001**
	Treatments	2, 184.8		1.35			0.26
	Time of exposure	1, 10.2		0.188			0.67
	Total colour covariate	1, 182.6		7.6			**0.006**
	Treatment*Time of exposure	2, 184.8		0.55			0.58
	Time of exposure*Total colour covariate	1, 182.6		4.32			**0.039**
	Treatment* Total colour covariate	2, 181.4		0.21			0.81
	Treatment*Time of exposure*Total colour covariate	2, 181.4		0.65			0.52
	Parameter	**Estimate**	**SE**	**df**	*t*	**Wald Z**	***p***
Estimates of fixed effects	Intercept	0.13	0.32	43.2	0.39	-	0.7
	HO treatment[1]	0.81	0.45	177.9	1.82	-	**0.07**
	MO treatment[1]	0.67	0.45	181.2	1.49	-	0.14
	Time of exposure[2]	0.43	0.58	80.5	0.75	-	0.46
	Total colour covariate	36.2	17.3	185.8	2.1	-	**0.04**
	Time of exposure*Total colour covariate[3]	-33	20.9	186	-1.58		0.12
Estimates of covariance parameters	Residuals	3.23	0.35	-	-	9.34	<**0.001**
	Intercept + Total colour covariate	0.03	0.12	-	-	0.24	0.81

Not all the estimates included in the model are represented
[1] LO treatment is the reference category
[2] Whole developmental period used as the reference category
[3] Whole developmental period*total colour covariate is the reference category

v. Variation in female preference for colour pattern diversity across treatments

The amount of orange experienced during development is the only factor that influenced female preferences for colour pattern diversity in guppies ($F_{(2, 190.7)}$=4.2, p=0.017, Table 2.15). Female having been reared with high and low value of orange preferred males with more colour diversity than LO/LO+1 females (p=0.024, Bonferroni adjusted, see fig. 2.8) just as HO/HO+1 females who had a marginal preference for increased diversity relatively to LO/LO+1 females (p=0.077, Bonferroni adjusted, see fig. 2.8).

Variance estimates showed that they were no significant differences in total colour preference across family (σ^2=4.10^{-5}, p=0.96, table 2.15).

Table 2.15: Linear mixed model of factors (fixed and random effects) influencing differences in colour diversity preferences between treatments

	Factor	***df***		**F**			***p***
Test of fixed effects	Intercept	1, 6		17.6			**0.006**
	Treatments	2, 190.7		4.2			**0.017**
	Time of exposure	1, 6		0.14			0.72
	Colour pattern diversity covariate	1, 178		3.23			0.074
	Treatment*Time of exposure	2, 190.7		1.13			0.33
	Time of exposure*Colour diversity covariate	1, 178		1.11			0.30
	Parameter	**Estimate**	**SE**	**df**	*t*	**Wald Z**	***p***
Estimates of fixed effects	Intercept	-0.002	0.03	46.4	-0.08	-	0.93
	HO treatment[1]	0.08	0.04	182.9	2.14	-	**0.03**
	MO treatment[1]	0.05	0.04	184.5	1.34	-	0.18
	Time of exposure[2]	0.01	0.04	43.6	0.24	-	0.81
	Colour pattern diversity covariate	0.1	0.06	183.6	1.84	-	0.068
	Time of exposure*Colour diversity covariate[3]	-0.07	0.07	178	-1.05		0.30
Estimates of covariance parameters	Residuals	0.02	0.002	-	-	9.38	<**0.001**
	Intercept + Colour diversity covariate	4.10^{-5}	7.10^{-4}	-	-	0.05	0.96

Not all the estimates included in the model are represented
[1] LO treatment is the reference category
[2] Whole developmental period used as the reference category
[3] Whole developmental period*colour diversity covariate is the reference category

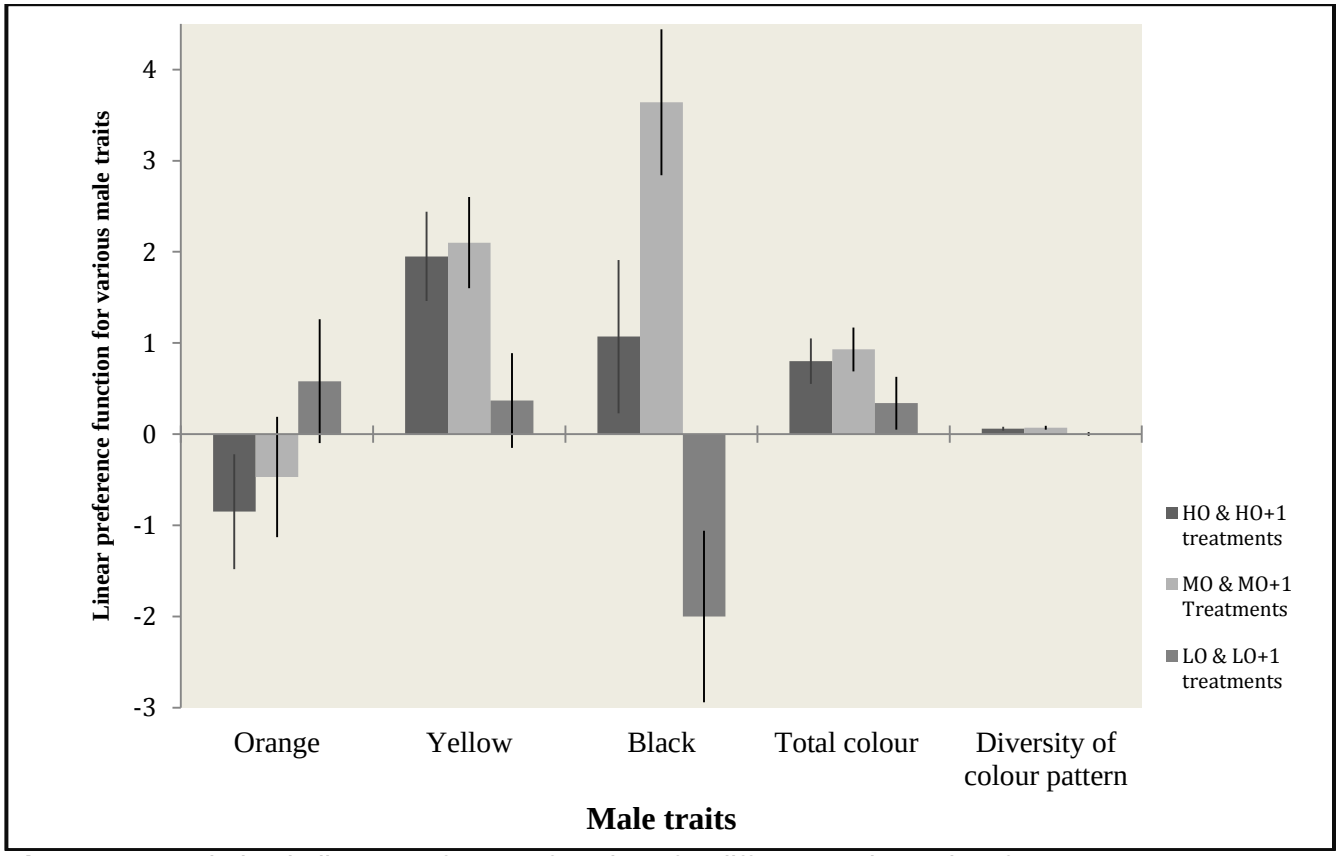

Figure 2.8: Variation in linear preference functions for different males traits after exposure to different values of orange colouration during ontogeny. Bars represent the estimated marginal means of the regression slopes representing the preference functions +/- SE.

2.4.2.4 Variation in mean responsiveness and choosiness between rearing treatments.

i. Variation in mean responsiveness between treatments

Females mean responsiveness didn't seem to be dependent on the amount of orange experienced or the duration of the exposure to males but the interaction of these two factors was influential ($F_{(2,\ 184.8)}$=4.1, p=0.018, see table 2.16 and fig. 2.9). An ANOVA with one fixed factor "exposure group" and six levels (exposed to HO, HO+1, MO, MO+1, LO, LO+1) was performed and the omnibus test was significant ($F_{(5,194)}$=2.66, p=0.024). After multiple comparisons among groups, fisher's LSD tests showed that HO females were less responsive than HO+1 females (p=0.008, see fig. 2.9) and, than LO+1 females (p=0.011, see fig. 2.9).

The same pattern affected MO+1 females (HO+1: p=0.016 and LO+1: p=0.021; see fig. 2.9) although there were no differences with MO females. LO+1 females were marginally more responsive than LO females (p=0.086). By contrast, Bonferroni adjustments, which are more conservative, did not reveal any differences between groups. Family didn't differ in mean responsiveness variability ($\sigma^2=7.10^{-5}$, p=0.2, see table 2.16).

Table 2.16: Linear mixed model of factors (fixed and random effects) influencing differences in mean responsiveness across treatments

	Factor	***df***		**F**			***p***
Test of fixed effects	Intercept	1, 6.2		193.2			**<0.001**
	Treatments	2, 185		0.58			0.559
	Time of exposure	1, 9.9		1.05			0.33
	Orange covariate	1, 6.1		1.14			0.72
	Treatment*Time of exposure	2, 184.8		4.1			**0.018**
	Parameter	**Estimate**	**SE**	***df***	*t*	**Wald Z**	***p***
Estimates of fixed effects	Intercept	0.395	0.03	6.6	13.1	-	**<0.001**
	HO treatment[1]	-0.004	0.01	183.2	-0.62	-	0.53
	MO treatment[1]	0.006	0.01	180.8	0.84	-	0.41
	Time of exposure[2]	0.01	0.01	27	1.48	-	0.15
	Orange covariate	-0.18	0.46	6.1	-0.38	-	0.72
Estimates of covariance parameters	Residuals	7.10^{-4}	8.10^{-5}	-	-	9.4	**<0.001**
	Intercept	7.10^{-5}	6.10^{-5}	-	-	1.28	0.2
	Orange covariate	0.98	1			0.97	0.33

Not all the estimates included in the model are represented
[1] LO treatment is the reference category
[2] Whole developmental period used as the reference category

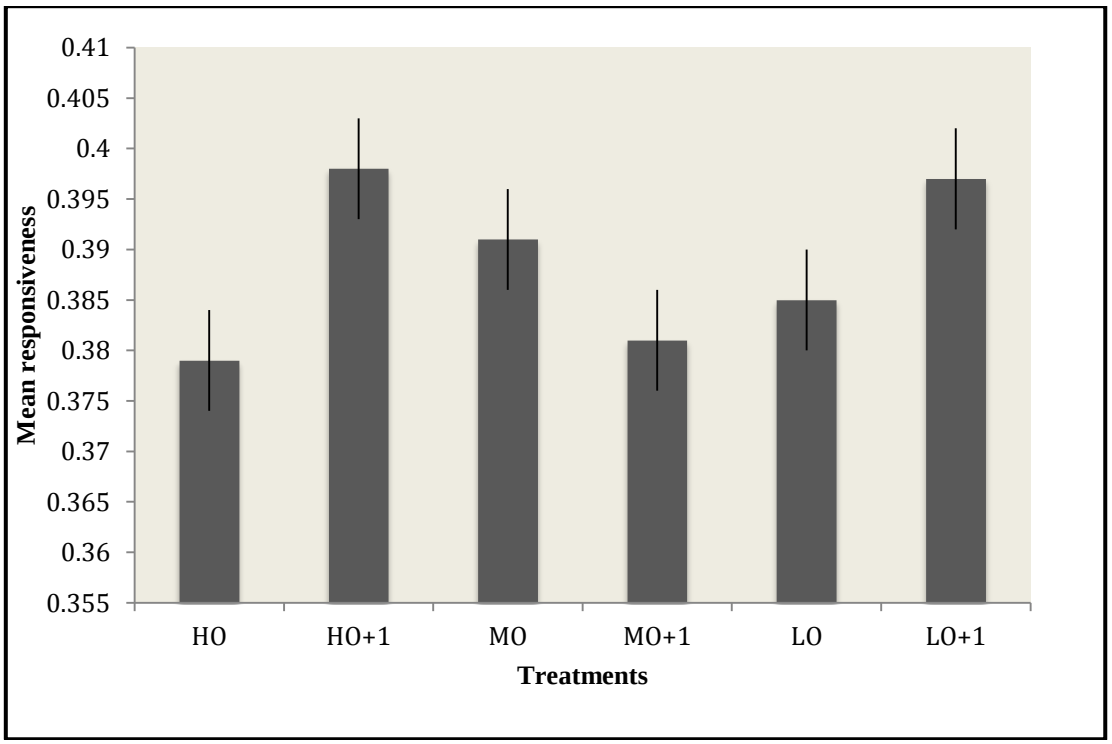

Figure 2.9: Mean responsiveness of females exposed to high, mixed, and low value of orange for half or whole development. The error bars represent one SE.

ii. Variation in discrimination between treatments

Long- and short- exposed females differed in their discrimination after the exposure treatments. I found a significant interaction between treatments and duration of exposure ($F_{(2, 188)}$=4.1, p=0.018, see table 2.17) although no other main or interactions terms were influential. To investigate this effect further, an ANOVA with one fixed factor "exposure group" and six levels (exposed to HO, HO+1, MO, MO+1, LO, LO+1) was carried out and found to be significant ($F_{(5,194)}$=2.92, p=0.015). After multiple comparisons among groups, fisher's LSD tests showed that HO females were more discriminating than HO+1 (p=0.005, see fig. 2.10) and LO+1 (p=0.01, see fig. 2.10) females. By contrast, HO+1 females were less discriminating than MO+1 (p=0.009, fig. 2.10) and, than LO females (p=0.051, fig 2.10). Eventually, MO+1 females were more discriminating than LO+1 females (p=0.018, fig. 2.10). Discrimination didn't differ in variability across family (σ^2=0.008, p=0.21, see table 2.17).

Table 2.17: Linear mixed model of factors (fixed and random effects) influencing differences in discrimination across treatments

	Factor	*df*		F			*p*
Test of fixed effects	Intercept	1, 159.4		38.9			**<0.001**
	Treatments	2, 188		0.68			0.51
	Time of exposure	1, 9		1.58			0.24
	Orange covariate	1, 188.2		0.41			0.53
	Treatment*Time of exposure	2, 188		4.1			**0.018**
	Parameter	**Estimate**	**SE**	**df**	*t*	**Wald Z**	*p*
Estimates of fixed effects	Intercept	0.62	0.11	127.7	5.6	-	**<0.001**
	HO treatment[1]	0.05	0.07	184.7	0.66	-	0.51
	MO treatment[1]	-0.05	0.07	185.4	-0.71	-	0.48
	Time of exposure[2]	-0.14	0.09	27.5	-1.55	-	0.13
	Orange covariate	-0.89	1.39	188.2	-0.64	-	0.53
Estimates of covariance parameters	Residuals	0.09	0.009	-	-	9.5	**<0.001**
	Intercept	0.008	0.006	-	-	1.27	0.21

Not all the estimates included in the model are represented
[1] LO treatment is the reference category
[2] Whole developmental period used as the reference category

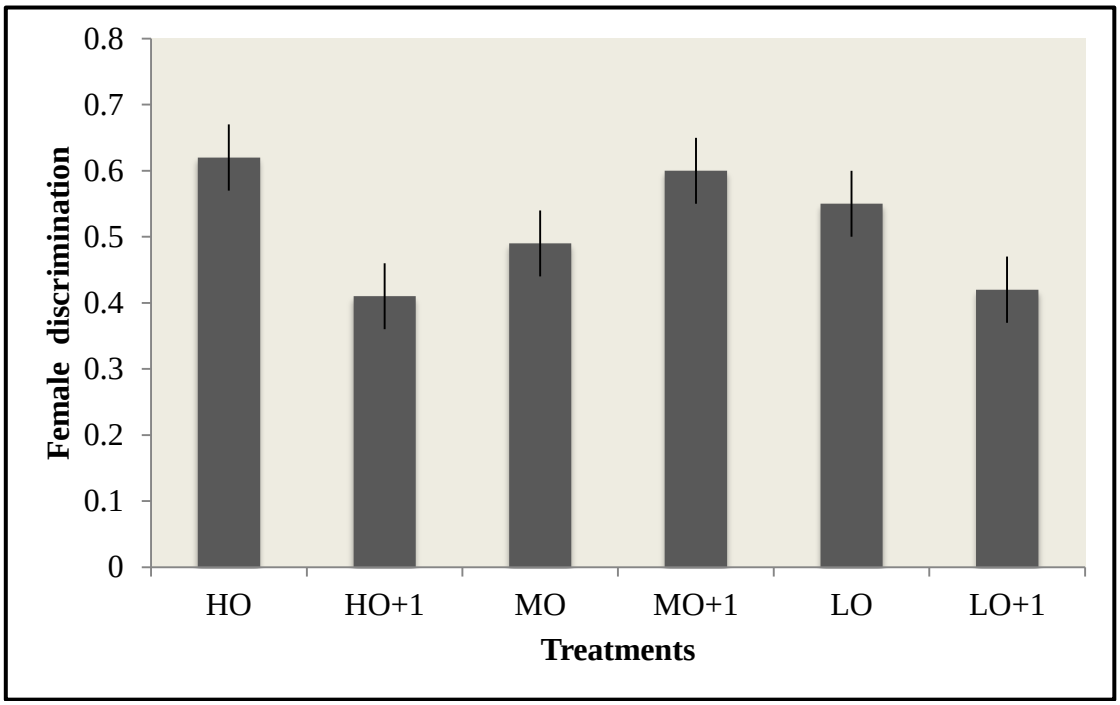

Figure 2.10: Sexual discrimination of females exposed to high, mixed, and low value of orange for half or whole development. The error bars represent one SE.

2.5. Discussion

The genetic predispositions of Lower Aripo females have been established in laboratory conditions. Females had a strong preference for males expressing high values of yellow and black. They also favoured males displaying more colour overall and to a lesser extent discriminated against males with increased colour pattern diversity. Although guppy females from various populations typically exhibit strong sexual preferences for males with high levels of orange pigmentation (Houde & Endler 1990a; Endler & Houde 1995; Houde 1997; Evans, Bisazza & Pilastro 2004b), these females seemed to be indifferent to that particular trait. This result is consistent with a previous study showing that Aripo females don't use orange as a sexual cue (Houde 1988b). Unlike many populations in which females reject particular colour class (Endler & Houde 1995), Lower Aripo females didn't seem to have any dislike. At first glance, it looks contradictory that females significantly preferred males with more colours overall but less diverse colour pattern however these two traits refer to different features. Total colour quantifies the body surface area covered with colours independently of the colours themselves when the Simpson Index of Diversity gives an indication of the abundance as well as the evenness of colours found on a male. A body largely covered with one or two colours (when four or five colour classes are present) has a high value of "total colour" but a relatively small value of "colour pattern diversity". Hence, females can express some preferences for colourful males of whom colours are not equally distributed but biased towards a few classes. In a context of high predation (as it is for Lower Aripo females), it is advantageous to diminish the time spent assessing males. An increased diversity in colour patterns might augment the time the cognitive system needs to process the information displayed by males and then act upon it, namely make a choice. Hence, favouring less colour diversity reduces the cost associated with mate choice. This analysis must be taken with caution, as the regression coefficient of the Simpson Index in the multiple regressions was not significant at the standard α-level suggesting that colour diversity was not a key element in mate choice. The presence of orange, a gaudy colour, is puzzling in a population experiencing high level of predation if not selected for by female choice. A principal component analysis extracted a

component composed of orange, yellow and black emphasizing the possibility that the same gene or clusters of genes are controlling the expression of these colours. Such pleiotropy between male traits could account for the persistence of orange in Aripo males. Directional female mate choice for yellow and black pigments results indirectly in the evolution of orange spots.

Exposing females during ontogeny to different values of a non-genetically preferred trait question the importance of environmental effects and how genetic and non-genetic factors interact. I provided good evidence that females shaped their sexual behaviours from exposure as juveniles. All three components of female mate choice were modified following the rearing treatments they experienced. In the process of producing a final mating decision, choosiness, that is, responsiveness and discrimination and preference function can interact (Brooks & Endler 2001b; Ritchie, Saarikettu & Hoikkala 2005; Bailey 2008) and obscure the full expression of female preferences. Absence of significant correlation between responsiveness and the quality of regressions representing individual linear preference functions for all colours in all exposure groups but one, suggested that there were no such interplays.

Regarding responsiveness, that is the likelihood a female will react to male's solicitation, females reared with high or low value of orange (low variance) presented the same pattern. Females were more responsive after exposure to males during half of the developmental period. The opposite pattern was observed when females were exposed to the high-variance treatment even though not significant. Greater responsiveness of females having experienced males for a shorter period of time might indicated the urge to mate rapidly since they learned that males were not present all the time in their habitat. Moreover, the relatively little amount of information provided by males (only high value or low value of orange) prompted females to reduce their level of choosiness (i.e. more responsive) which in turn reduced the costs associated with mate choice. This result was backuped by females' discrimination across exposure groups. Discrimination is the degree to which females distinguish variation in male traits. More discriminating females (i.e. choosier females) invest more in responding to

preferred mates instead of non-preferred mates. In this study, discrimination was the mirror image of responsiveness; greater mean responsiveness was associated with lesser discrimination and vice et versa. Unlike, Reinhold et al. (2002) who found that responsiveness and discrimination were uncorrelated in the grasshopper *Chorthippus biguttulus,* my findings suggested that these two components operated together in guppies adjusting for the level of choosiness. On the other hand, my work was partly supported by a recent study from Bailey (2008) that showed behavioural linkage between discrimination and responsiveness in crickets, *Teleogryllus oceanicus*, although in this case the effects were pleiotropically linked. Such analysis should be considered cautiously as the differences in responsiveness and discrimination across exposure groups were non-significant after corrections. The high level of heritability in discrimination and, more particularly, in responsiveness found in guppies (Brooks & Endler 2001b) might explain that phenotypic variance owing to environmental factors was not more marked.

I also investigated whether manipulating early social experience can induce variation in female preference functions for various male traits. The manipulation involved varying the value of a trait to which females were genetically indifferent. To my knowledge, it is the first time that female preferences are shown to fluctuate in response to different phenotypic distributions of a trait not primarily involved in mate choice. Interestingly, females from the same exposure group, generally, found the same males attractive except for females reared with low value of orange during half development (LO+1 group). Following the rearing treatments, preference functions for orange, yellow, black, total colour and for colour diversity were considered. In every instance, an effect was detected. The estimation of individual preference functions highlighted two main trends across rearing treatments. Firstly, females reared with low value of orange during development (both half and full developmental period) did not display any more significant preferences for the different colorations borne by males. This absence of preferences could mean that females would either mate at random or base their choice on other sexual cues. Few studies have reported that body size (Reynolds & Gross 1992; Endler & Houde 1995; Magellan, Pettersson & Magurran 2005;

Karino & Urano 2008) or tail area (Bischoff, J.L & D.I 1985; Karino & Kobayashi 2005) could be used as mate choice criteria. Rosenqvist & Houde (1997) already demonstrated that females lost their preference for orange following ontogenetic exposure to low-orange males however these females came from a population in which levels of orange accounted for variation in male mating success. Secondly, females reared with low and high value of orange during ontogeny (MO/MO+1 groups) acquired preferences for males with increased colour diversity, altering their innate predispositions. More information about male quality in the social environment drove females to favour more diversity in individual patterns, namely, more information within a single male. There are two plausible scenarios here: either females developed a preference for the trait itself or increased their preference for multiple components independently, which would, as a side effect, results in higher preference for colour diversity. When looking in details at the preferences for different male traits, females showed stronger black preference relatively to low variance treatments. Such dissimilarity could account for the difference detected but do not allow to choose between the two scenarios. Experimental designs that would permit to control for the value of "colour diversity", such as those incorporating digitally modified videos techniques (Sato & Karino 2006) would allow disentangling the females' behaviour.

When looking more in details at phenotypic variation in preference functions across exposure groups, the effect of duration stood out showing that only preferences for orange and yellow were modified by the time of exposure. In both case, this effect was dependent on the effect of the male stimuli. Experiencing high value of orange during half of the developmental period induced a strong dislike for males with more orange and emphasized the preference for rare and/or novel male phenotypes. Because guppies occurring in the same habitat are more likely to be related to each other, and because dispersal can be limited by climatic conditions, avoiding common colour patterns may prevent from inbreeding depression. Inbreeding in guppies can lead to potential costs such as reduction in male courtship (Mariette et al. 2006), in sexually selected traits (van Oosterhout et al. 2003; Zajitschek & Brooks 2010), in life history traits (Nakadate, Shikano & Taniguchi 2003) and in fertilization success (Zajitschek et al. 2009) prompting

females to evolve mechanisms to diminish detrimental costs. Females having been reared with low value of orange for half of the developmental period tended to prefer males with more orange and were significantly different from HO+1 females. This confirms the “rare male” effect aforementioned and lay the ground for a mechanism to avoid mating with relatives. In the hypothesis that it is evolutionary important to limit inbreeding (but see Kokko & Ots (2006)), the tactic of mating with rare male is reinforced by the absence, in guppies, of kin recognition mechanism (Viken, Fleming & Rosenqvist 2006; Guevara-Fiore, Rosenqvist & Watt 2010). My finding supports previous studies, which highlighted the importance of rare phenotypes, in mate choice, based on prior adult experience (Farr 1977; Eakley & Houde 2004; Zajitschek & Brooks 2008). However it is not clear why such pattern of preferences was not found in females having been reared with low variance males during their entire development (LO and HO exposure group). It would suggest that these females relied on their genetic preferences.

Time of exposure across treatments also mediated female preferences for yellow. Long- and short- exposed females in the low variance treatments (HO/HO+1 and LO/LO+1) differed but in opposite manner. HO+1 significantly decreased their preference for yellow relatively to HO females when LO+1 females increased their preference compared to LO females (significant before but not after Bonferroni adjustment). Strong yellow preferences for HO females did not depart form innate preference and seemed to indicate the absence of early social environment influence. The drop found for HO+1 females could be pleiotropically linked to the fall observed for orange preference in the same group. Even though a marginal difference was observed between LO and LO+1 females, the estimation of their individual yellow preference was not significantly different from zero and recall the absence of preferences in LO/LO+1 treatments discussed previously. High variance treatments (MO/MO+1) didn’t differ and were not changed after ontogenetic experience. Eventually, females didn’t tune their total colour preference according to the social environment experienced.

Taken together these findings give rise to the role that non-genetic transmission of mate preference (in this case via early social experience) has in

guppies even when the socially induced mate choice contrasts with the genetically based preference. Surprisingly, variability in a trait that is not genetically preferred can mediate learned preferences for other sexual cues and partly explain the considerable variation found in female preference within population. Furthermore, it contributes to the evolution of multiple signals and to some extent to the maintenance of polymorphism in male colour patterns. Different evolutionary processes are brought into play here. First, the preference for rare patterns is a source of negative frequency-dependent selection, which is the best-established mechanism acting to maintain additive genetic variation (Turelli & Barton 2004; Zajitschek & Brooks 2008). Negative frequency-dependent selection has already been put forward in guppies either through mate preferences (Hughes et al. 1999; Zajitschek & Brooks 2008; Hampton et al. 2009) or through frequency-dependent survival (Olendorf et al. 2006). Secondly, the lost of clear preferences in a social context where males displayed low value of a sexual trait relaxes the selection exerted by mate choice on male characters and allows non-genetically preferred phenotypes to spread, augmenting the within population polymorphism. Such dynamic fluctuation in selection pressures due to variation in preference functions can work alongside with plasticity in choosiness reinforcing or hampering the effects of the preferences. Mating at random in association with a drop in choosiness will drastically decrease the variance in mating success in a population where hypothetically every male will contribute to the gene pool. In species, like guppies, where sexual behaviours in males occupy a large part of the time activity budget, polymorphism will evolve relatively rapidly reaching a threshold where the intensity of sexual selection will rise again and reverse the phenomenon. Finally, female preference for increased colour diversity when reared in high variance treatments is also, potentially, a mechanism accounting for male traits polymorphism since patterns of diversity are made of a large number of combinations between sexual signals. It is, however, essential to investigate whether such preference is an artefact of preferences for multiple traits or a preference for the trait “colour diversity” itself.

In the next chapter, the same paradigm is used to examine variation in female mate choice within population. Developing females had been exposed, this

time, to different values of a sexual cue that is genetically favoured. In parallel with the findings of this chapter, the next chapter is offering more insights into the understanding of the complex influence of the early social environment on female preference plasticity.

3. Chapter III

Different male trait distribution experienced as juveniles determined female sexual behaviours in Guppies

3.1. Abstract

Models of sexual selection generally assume that female mate preferences are fixed but over the last decade a growing body of evidence has shown that individual variation in mate choice is frequent across taxa. Understanding how such variation affects the strength and direction of selection within population is a central aim in the study of sexual selection. The social environment is a source of flexibility in mate preferences but studies examining its role before individuals reach maturity in species without parental care are lacking. I analysed in guppies, *Poecilia reticulata*, how variation in the phenotypic distribution of a male sexual trait affected the acquisition of mate preferences. Growing females were exposed to low, high and mixed values of a trait called “total colour” in the guppy literature, representing the entire area of male body colouration and known to be a good predictor of mating success in this population. Additionally, I explored the timing of exposure (short- versus long-exposed) to these different rearing treatments on learned mate preferences. My results showed that the temporal factor was essential as short-exposed females preferred to rely on other sexual cues than colour patterns to choose a mate. When long-exposed, females from the high and low total colour treatments exhibited disassortative mating preferences regarding the phenotypes experienced as juveniles. Finally, I found that females reared with low amount of total colour for their whole development were more likely to respond sexually to males than females from the other treatments. My findings showed that genetically-based preferences can be overridden by socially-based preferences under specific circumstances. Moreover, the disassortative mating pattern detected could generate negative frequency-dependent selection that will in turn contribute to the maintenance of colour polymorphism found in guppies.

3.2. Introduction

Research on female mating preferences has historically been conducted on mean preferences across populations and species, investigating the role of average preferences on the evolution of elaborated male traits and species recognition (Andersson 1994; Andersson & Simmons 2006). Recently, however, more attention has been placed on variation in female mate preference found both among and within individuals (Jennions & Petrie 1997; Widemo & Saether 1999; Brooks & Endler 2001b; Lehtonen & Lindstrom 2008). Genetic differences, developmental histories and environmental conditions are all factors that account for the variability found between individuals (Jennions & Petrie 1997; Schielzeth, Bolund & Forstmeier 2010; Guevara-Fiore 2012; Guevara-Fiore, Svensson & Endler 2012). Quantifying, disentangling the genetic and environmental components of phenotypic variance found between individuals within population remains particularly challenging (Jennions & Petrie 1997; Widemo & Saether 1999; Cornwallis & Uller 2010; Schielzeth et al. 2010). Moreover, it is not always possible to identify the outcome of a mate choice process, namely the chosen male(s), through direct observations of copulation or genetic analysis. Thus, to simplify the characterization of variation in females' sexual behaviours, mate choice can be dissected into *preference functions*, that is, "the order in which an individual ranks prospective mates" and *choosiness*, that is, "the time and/or energy that an individual is prepared to invest in assessing mates", following Jennions & Petrie (1997) and Brooks & Endler (2001b). The analysis of heritability (i.e. the degree to which variation in a trait is under genetic control) of female mate choice within population has produced variable results. Within populations, there is good evidence for heritable variation in choosiness (Collins & Cardé 1990; Bakker 1993; Brooks & Endler 2001b; Rodríguez & Greenfield 2003) but the situation is more confusing for preference functions. Some studies have found heritable variation for preference functions (Collins & Cardé 1989; Houde 1994; Iyengar, Reeve & Eisner 2002) whereas others fail to detect it (Breden & Hornaday 1994; Brooks & Endler 2001b; Hall, Lindholm & Brooks 2004; Simmons 2004; Ritchie et al. 2005) suggesting that a large part of the variation in mate choice is due to environmental factors experienced throughout life.

In my study, I focus on the role that the social environment experienced during early development can have on the expression of the different components of female sexual behaviours. To do so, I capitalize on the guppy, *Poecilia reticulata*, a species that lacks parental care. The classical learning mechanism of sexual imprinting whereby growing individuals acquire sexual preferences based on the phenotypes of their genetic or social parents (Immelmann 1972; Bateson 1978; Slagsvold et al. 2002) has been extensively studied in birds. In contrast, mate choice imprinting in species with no parental care but living in groups has rarely been examined despite its strong potential for mate choice learning. Imprinting on non-parental adults is called "oblique imprinting" and refers to a learning mechanism by which developing individuals acquire long-term sexual preferences through social interactions with the opposite sex. Although uncommon, evidence suggests that prior social experience is central in the acquisition of preferences and widespread in the animal kingdom since invertebrates (Hebets 2003; Hebets & Vink 2007; Bailey & Zuk 2008; Rutledge et al. 2010) and vertebrates (Breden et al. 1995; Rosenqvist & Houde 1997; Walling et al. 2008; Verzijden & Rosenthal 2011) rely on oblique imprinting. In order to understand the ontogeny of female mate preferences in guppies, I experimentally manipulated the distribution of males varying in the values of a sexual signal known to be attractive and exposed them to developing females. The sexual signal consisted of the total area covered by the different colour patterns ("total colour") found on the body of guppy males and has proven to be a good predictor of mating success in that population (chapter two). Once adults, females are tested for the effects of the rearing treatments on their *preference functions* and their *choosiness.* Given that guppies are a multiple signalling species with multiple mate preferences (Brooks & Couldridge 1999; Brooks & Endler 2001a; Brooks 2002), it is not clear whether females perceive "total colour" as a single trait or as a composite signal made of the multiple traits fro which they have multiple preferences (or dislikes). Such a difference has consequences on the evolution of female preferences and male sexual traits and will be discussed in the light of the results. More generally, whether plasticity in mate choice is adaptive and how it affects the process of sexual selection in males and females is analysed in the discussion.

3.3. Methods

3.3.1. Study organisms

Guppies used for the experiment came from the same population (Lower Aripo) as the one used for the previous experiment (chapter two). In this case, they were fourth and fifth generation descendants. All fish housed in the laboratory are maintained on a 12h light:dark cycle at 24°C. They were fed twice daily: in the morning with commercial flakes and in the afternoon with brine shrimp (*Artemia*). All the housing tanks had a gravel substrate and were aerated through an undergravel filtering system. Plastic plants were placed into the tanks to physically enrich the environment of the fish and to let them have some room to hide.

Some large females were taken in the stock tanks and kept individually in 4L plastic tank to be used as parental females. Female poeciliids can store sperm (Constanz 1989) that can fertilize eggs for up to eight month (Winge 1937). Recently inseminated sperm will, however, secure most fertilizations (Constanz 1984) and within a given brood cycle the last male to mate is likely to father most offspring (Evans & Magurran 2001). Thus, to reduce the probability of producing half-siblings for the rearing treatments, females were kept individually until they gave birth to two consecutive broods. Then the females were placed in a separate chamber to mate with a single male. This increased the likelihood that one male fathered the broods. Each brood was kept for five days in 4L plastic tanks, visually isolated from other fish, before being divided in three equal experimental groups and placed in rearing tanks (see Fig. 3.1 and Fig. 2.2 in chapter two). Only broods of eighteen or more individuals were used to have a sufficient number of female per family and decrease the variance in the size of brood from which fry came (Mean $\pm$ standard deviation = 21.6 ± 3.9).

3.3.2. Rearing treatment

The experiment consisted of rearing groups of virgin females experiencing three different treatment conditions for either the whole period of development (84 days post birth) or only during the second-half of the developmental period (from day 42 post-birth until day 84 post-birth). Although Reznick et al. (1997) established that females from high predation sites in the Aripo river were mature at 55.6 +/- 2.2 days, I chose to expose females until day 84 since personal observations showed that very few females were receptive to male courtship before that. The second half of the developmental period was chosen instead of the first one because, from a functional point of view, it is more advantageous for females to learn about males present in their environment when they approach the time of first mating, making the second half of development more likely to incorporate learning than the first one. The three different treatments correspond to exposure of the experimental fish to three different sets of male trait values: high, medium and low. Females were reared in visual contact with 4 males expressing high-level of “total colour”, 4 males expressing low-level of “total colour” and a third group in which there are 2 males with low- and 2 males with high- level of “total colour”. Investigation of the innate preferences (see previous chapter) in Lower Aripo females demonstrated that preference for “total colour” was genetically based. Fry within the high-level treatment experienced males with more than 40% of their body covered with colours (Mean (%) $\pm$ standard deviation = 43.4 ± 3.1). Fry experiencing low-level of body colours experienced males with less than 25% (Mean (%) $\pm$ standard deviation = 22.6 $\pm$ 2.5) of total colour. In the mixed treatment, they experienced males displaying a mixture of the two phenotypes (High total colour: mean (%) $\pm$ standard deviation = 42.2 ± 2.7; Low total colour: mean (%) $\pm$ standard deviation = 24.1 $\pm$ 1.8).

To control for the duration of exposure that each female had experienced, stimuli males were removed from the tanks at the end of day 84 post-birth. The females that were not tested on day 86 stayed in their rearing tank (without stimuli male) until the mate preference trials. The maximal time range that females spent

without seeing males before being tested was six days. The experimental design is summarised in figure 3.1 (for more details, see methods section in chapter two).

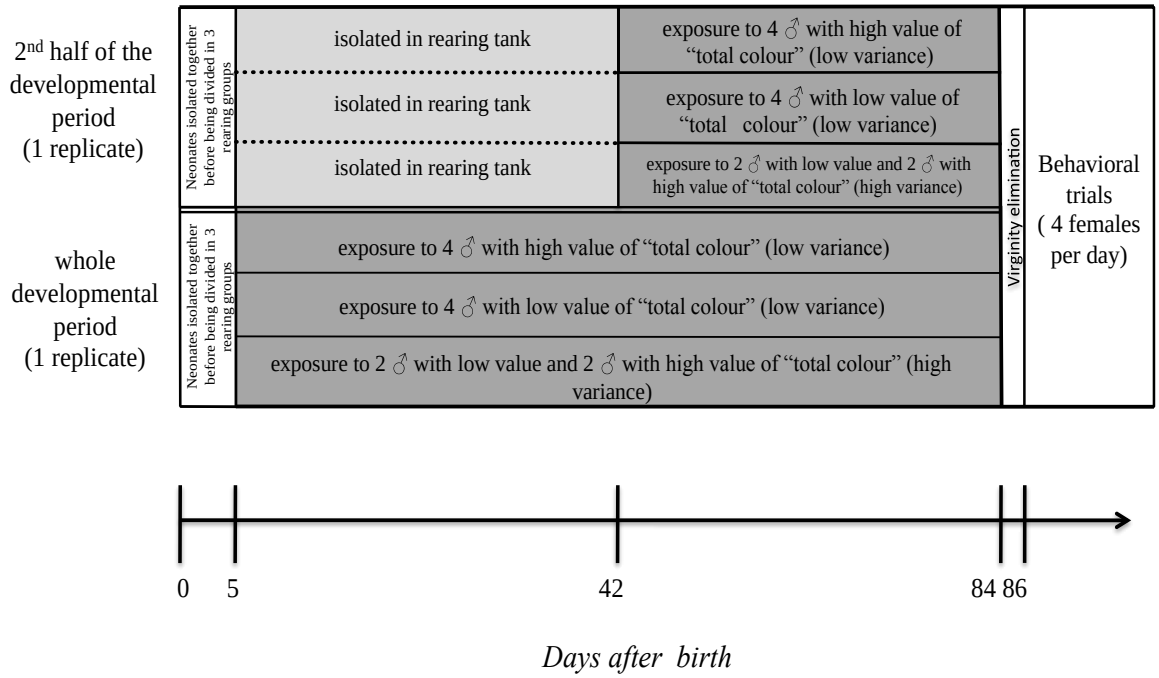

Figure 3.1: Timeline of the experimental design representing one replicate either for half of the developmental period or for the whole development.

3.3.3. Mate Choice trials

The choice tank (four in total) contained eight enclosures, six containing one male each and two controls with one female each (fig. 2.4, chapter two). Control females were not virgin and present in two different body sizes (one similar to and one larger than the tested female). The six males displayed a range of values that the tested females experienced during the rearing treatment and were chosen in different housing tanks in which they grew up in the presence of females. None of these eight individuals could see each other but could be seen by the focal female through clear glass (fig. 2.3 and fig. 2.4, chapter two). The presence of the control females was to test whether the tested females expressed a sexual behavior or simply a tendency to shoal with conspecifics. Four choice tanks are used on any given day. Because the chambers are sealed, visual but not olfactory

communication is possible between the focal females and the fish in the enclosures (Brooks 2000; Brooks & Endler 2001b).

Because virgin female guppies may show little mate discrimination in their first mating (Endler & Houde 1995; Houde 1997; Hughes et al. 1999; Brooks & Endler 2001b), females were allowed to copulate freely with a stock male during the afternoon prior to the testing day (day 85 on the timeline). This male didn't resemble either the males experienced during development or the males found in the choice arena. At the end of the afternoon before the trial, the focal females were moved to the choice tank and allowed to acclimatize to their new environment until the next morning. Trials were video-recorded from above and the trial lasted for one hour. Each observation involved scoring the total duration and the number of occurrence the focal female spent within one body-length of the front of each chamber (so-called preference zone). Data were collated with "JWatcher", a software specifically designed to quantify behavioural measurements. To be included in the data statistically analyzed, a female had to pass in front of all eight chambers at least once during the recording session. Male body size was controlled within each tank and these males were reassigned randomly each day in the enclosures to avoid any pre-existing (or biased) preference for a particular position within the choice arena and to control for the potential bias introduced by any small differences in light intensity between preference zones (see section 2.2.3, chapter two). I used a set of six males and two females with seven to eleven different focal females on consecutive days (see table 3.1). None of the fish in the enclosure were related to the focal females.

Table 3.1: Detail of the fish used for the mate choice trials

Rearing treatments	Number of focal females	Number of sets of males
HNS+1[1]	24	3
MNS+1[2]	27	3
LNS+1[3]	28	4
HNS[4]	22	2
MNS[5]	20	2
LNS[6]	24	3

[1] females reared with high value of total colour during the 2nd half of the developmental period
[2] females reared with high and low value of total colour during the 2nd half of the developmental period
[3] females reared with low value of total colour during the 2nd half of the developmental period
[4] females reared with high value of total colour during the whole developmental period
[5] females reared with high and low value of total colour during the whole developmental period
[6] females reared with low value of total colour during the whole developmental period

3.3.4. Male trait analysis

Male colour patterns were photographed with a digital camera (Nikon coolpix 8800) in a narrow plastic box filled with a small volume of water where fish were free to swim. All the pictures were taken under the same light conditions, when fish were parallel to the front of the box. Both sides of the each guppy were photographed and the images analysed using the UTHSCSA ImageTool program (developed at the University of Texas Health Science Center at San Antonio, Texas, http://compdent.uthscsa.edu/dig/). Colour patches were grouped into the following colour classes: black, orange (including red), yellow, silver (including white), blue and violet, and finally bronze-green. The colour classes were measured as relative total area (relatively to the body + caudal fin) since it usually explains most of the variance in male attractiveness. The data for each male consisted of the mean of the right and left side of the body for the relative area. A measure of the diversity of the colour pattern was also calculated for each male (see appendix).

3.3.5. Female preference analysis

There are different ways to measure a female sexual response. Following a terminology used in two seminal reviews (Jennions & Petrie 1997; Widemo &

Saether 1999) extended by Brooks and Endler (2001b), we can divide individual female sexual behavior into three measurable components (see fig. 3.2):

- Choosiness that is the investment into mating itself sub-divided in:
    - Receptivity (or mean responsiveness) defined as female willingness to respond positively to male solicitations and measured as the mean response to the displays of all males in a trial.
    - Discrimination (selectivity) describes the degree to which females distinguish variation in male traits.

- Preference function that is the ranking order of male sexual signals; measured as the relationship between females' response and the male trait(s) they are evaluating (see fig. 3.2).

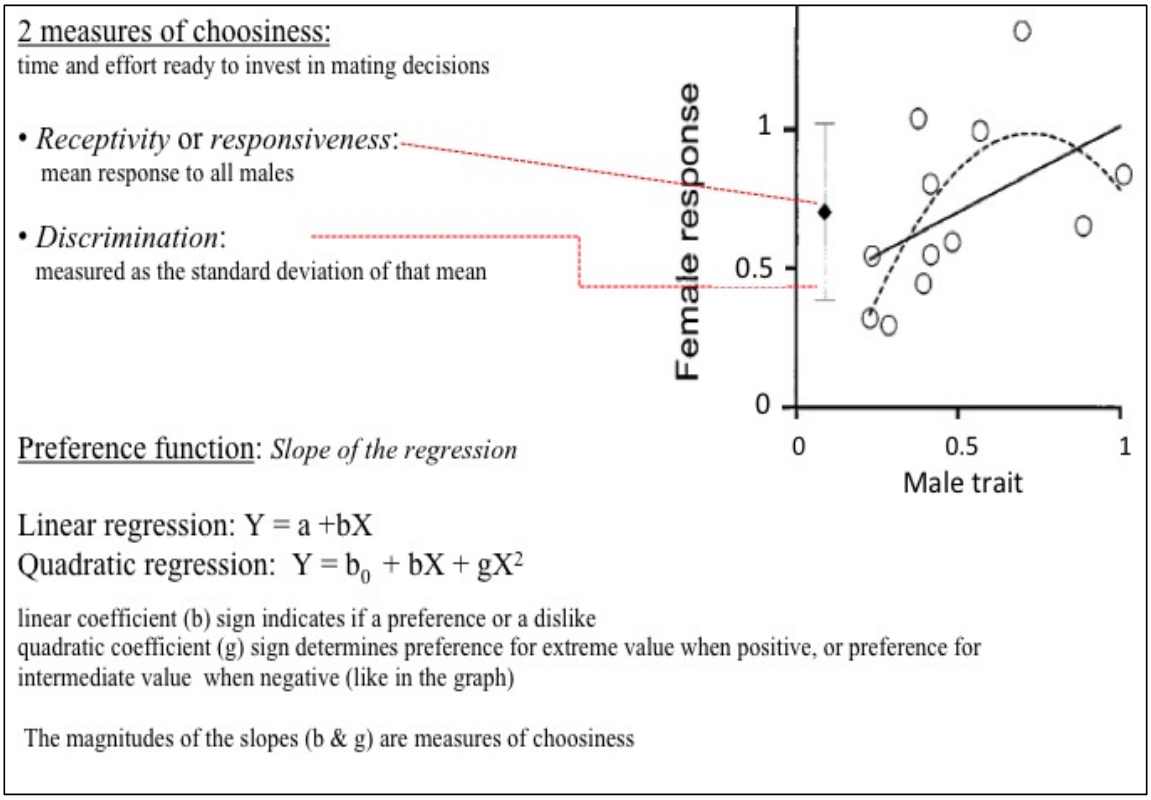

Figure 3.2: The different components of female choice's behaviour. Data are fictional and represent the response of a single female (angular transformed) to 12 males displaying a gradient of a sexual trait

The female response to a given male was measured as the proportion of time spent in the preference zone of that male and angular transformed (arc sine square

root transformed) before analysis. Individual female preference functions are described by the linear and quadratic regression parameters (based on six males and not twelve as shown on figure 3.2). A linear regression slope gives the direction and strength of an individual female preference for the particular trait on which the regression has been calculated. A quadratic regression allows describing non-linear preference functions in terms of a linear component and a quadratic component (figure 3.2). Simple linear and quadratic regressions were calculated using orange, yellow, black, blue, silver, green, total colour, colour pattern diversity and a variable called “preference for attractive males”. Preference for attractive males estimates the extent to which each female resembled the preference norms of the group she belonged to (see section 2.2.5.2, chapter 2). One-sample Student’s *t*-tests were carried out to test whether the mean regression coefficients for each male trait tended to differ from zero.

Linkage between preference functions and choosiness (e.g. responsiveness and discrimination) can obscure the interpretation of the final mate choice and its evolutionary implications. In an attempt to estimate the effects of interactions among the different behavioural elements, I calculated Pearson’s correlation coefficients between the individual linear regression slope and individual mean responsiveness. A significant positive correlation would mean that as responsiveness increased females would be more willing to express a preference or a dislike for a sexual character. A negative correlation would indicate that responsiveness masks the full expression of female preference. An absence of correlation would show that these behavioural elements are independent.

When females displayed significant individual preferences (e.g. regression slopes differed from zero) for a particular trait, I used a linear mixed model to test for the effect of “total colour” values and time of exposure on phenotypic variation in female mate choice. Slopes of preferences were generally normally distributed among treatments groups without transformation. Rearing treatments and time of exposure were considered as fixed factors with three and two levels respectively. In the model, a random intercept was included for the family where the females came from (using the SUBJECT option within SPSS MIXED procedure). This

allowed controlling for the non-independence of full- or half- sibling females behaviour (and potentially quantifying variation in female preference between and within families). Moreover, the average value, in a mate choice trial, of the trait of which I compare the preference function was added as a covariate to control for differences between trials. This covariate was also family centered and included as a random slope to quantify its potential influence across families. To estimate variance components, I used restricted maximum likelihood method (REML method). All main- and interaction- effects that reduced AIC (Akaike Information Criterion) were kept in the final model. Based on the "smaller is better" interpretation, the random intercept was needed in the models for orange, yellow and blue preferences but not total colour preferences. The random slope was only needed in the model describing yellow preferences.

Differences in mean responsiveness and discrimination were also tested. Discrimination, defined as the standard deviation of the mean representing responsiveness (figure 3.2) was scaled by the magnitude of that mean (i.e. the discrimination is now defined as the coefficient of variation of the mean which amounts to discrimination divided by responsiveness) to control for variation in female receptivity. Because responsiveness and discrimination were not normally distributed (even after various transformations), generalized linear mixed models (GLMM) were performed to determine whether social experience during ontogeny shaped females choosiness. A gamma-GLMM with log link function was used for responsiveness and a gamma-GLMM with identity link function for discrimination. Fixed and random terms are the same as here above.

Analyses were done in SPSS version 16.0 and subsequent release (SPSS Inc. an IBM company, Chicago, IL, USA).

3.4. Results

In all but one exposure group, females spent more time on average with each of the six males than with each of the two control females (see table 3.2) indicating that females are engaged in sexual behaviours when associating with males.

Table 3.2: Paired-sample *t*-tests testing females' receptivity in each experimental treatments

Rearing treatments	*df*	T-test	*p*-value
HNS+1[1]	23	2.54	0.018
MNS+1[2]	26	0.36	0.722
LNS+1[3]	27	2.24	0.033
HNS[4]	21	2.65	0.015
MNS[5]	19	4.91	<0.001
LNS[6]	23	5.00	<0.001

[1] females reared with high value of total colour during the 2nd half of the developmental period
[2] females reared with high and low value of total colour during the 2nd half of the developmental period
[3] females reared with low value of total colour during the 2nd half of the developmental period
[4] females reared with high value of total colour during the whole developmental period
[5] females reared with high and low value of total colour during the whole developmental period
[6] females reared with low value of total colour during the whole developmental period

3.4.1. Individual preference functions after exposure to stimuli males for half development

The slopes of the linear regression were not significantly different from zero, after sequential Bonferroni corrections, in the different rearing treatments except for the trait "attractive males". Within treatments, females tended to find the same males generally attractive although the sexual traits measured didn't seem to be used as a criterion of mate choice. The lack of response in female preference for the various colour patterns might be accounted for by low statistical power owing to the relatively small sample size and/or a small magnitude in the preferences although the sample size was big enough to detect some rearing effects for long-exposed females (see below). Alternatively, variation in responsiveness could mask variation in preference functions, as it is the case with virgin guppy females

(Houde 1997) but I didn't detect any significant correlations between responsiveness and linear preferences (see table 3.5) that would indicate a possible behavioural linkage between these two components. There was, however, an exception for the yellow trait in the MNS+1 treatment where preference and responsiveness were positively correlated. In parallel, the only marginally significant individual preference in the MNS+1 treatment was for yellow (see table 3.3). Taken together, these results suggest that responsiveness had a pleiotropic effect on preference function at least for one colour pattern but in any case could explain the absence of preferences.

Table 3.5: Correlation coefficients between individual female mean responsiveness and quality of the linear regression (representing individual preference functions) for different male traits.

Treatments	Male traits	N	Pearson correlation coefficients	*p*-values
LNS+1 [1]	Orange area	28	-0.33	0.09
	Yellow area	28	0.19	0.33
	Black area	28	-0.28	0.16
	White area	28	-0.07	0.74
	Blue area	28	0.19	0.33
	Green area	28	0.08	0.69
	Total colour area	28	0.18	0.36
	Simpson Index	28	0.21	0.28
MNS+1 [2]	Orange area	27	-0.09	0.65
	Yellow area	27	0.53	**0.005***
	Black area	27	-0.09	0.67
	White area	27	0.01	0.95
	Blue area	27	0.26	0.19
	Green area	27	0.05	0.81
	Total colour area	27	0.2	0.33
	Simpson Index	27	0.17	0.4
HNS+1 [3]	Orange area	24	0.22	0.31
	Yellow area	24	-0.39	0.06
	Black area	24	0.11	0.62
	White area	24	0.16	0.45
	Blue area	24	-0.01	0.97
	Green area	24	0.4	0.06
	Total colour area	24	0.04	0.85
	Simpson Index	24	-0.24	0.27

* Significant after sequential Bonferroni correction for number of tests in column
[1] females reared with low value of total colour during the 2nd half of the developmental period
[2] females reared with high and low value of total colour during the 2nd half of the developmental period
[3] females reared with high value of total colour during the 2nd half of the developmental period

Fitting a quadratic regression significantly improved the description of the outcome for all preference functions in all three treatments (table 3.4) although very few linear and quadratic coefficients were significant after Bonferroni adjustment. Again, within treatments, females agreed in the extent to which they preferred the same males that other females found attractive (table 3.4) but no other preferences were clearly expressed. Females reared in visual contact with males bearing large amount of colours (HNS+1 treatment) are the only one who expressed a consistent (even though not significant after correction) quadratic preference (table 3.4). Females tended to like more colourful males as indicated by marginally significant b and g coefficients ($b<0$ and $g>0$). I didn't find any significant correlation between the qualities of the individual quadratic regressions and female's mean responsiveness (table not represented in the thesis). Due to a lack of significant *g* coefficients, only the outcomes of the linear regression are considered in further analysis.

Table 3.3: Means and standard errors of the slopes (b) of the linear regressions representing the individual female preference functions. One-sample Student's t-tests were used to test whether coefficients tend to differ from zero. Means qualities of the linear regressions are also represented ("R^2").

		Linear regression					
		N	mean R^2 (%)	mean b	SE	*t*	*p*
HNS+1 [1]	Orange area	24	24.8	-0.25	0.9	-0.28	0.78
	Yellow area	24	34.3	1.19	1.5	0.80	0.43
	Black area	24	21.1	-1.04	1.5	-0.71	0.48
	White area	24	26.6	-1.37	2.1	-0.66	0.52
	Blue area	24	17.5	-0.48	1	-0.48	0.64
	Green area	24	17.0	0.29	0.6	0.51	0.62
	Total colour area	24	25.3	-0.12	0.4	-0.34	0.74
	Simpson Index	24	16.0	0.06	0.1	1.12	0.28
	Attractive males	24	63.7	1.00	0.1	8.65	<**0.001***
MNS+1 [2]	Orange area	27	16.7	-0.28	1.	-0.27	0.792
	Yellow area	27	13.8	1.98	0.9	2.14	**0.042**
	Black area	27	22.1	0.32	1.5	0.21	0.838
	White area	27	26.1	-2.88	1.7	-1.65	0.111
	Blue area	27	29.3	0.64	1.4	0.47	0.642
	Green area	27	28.8	3.83	0.6	0.35	0.730
	Total colour area	27	14.9	-0.24	0.3	-0.83	0.412
	Simpson Index	27	22.3	-0.05	0.03	-1.44	0.161
	Attractive males	27	71.6	0.99	0.1	14.3	<**0.001***
LNS+1 [3]	Orange area	28	19.9	0.88	0.6	1.46	0.155
	Yellow area	28	31.6	1.61	1.3	1.22	0.234
	Black area	28	24.1	-1.73	1.1	-1.64	0.113
	White	28	20.3	0.54	2.1	0.26	0.800
	Blue area	28	20.7	-0.35	0.8	-0.46	0.651
	Green area	28	23.6	0.56	0.7	0.85	0.401
	Total colour area	28	14.3	0.00	0.3	0.02	0.987
	Simpson Index	28	33.8	-0.01	0.1	-0.19	0.848
	Attractive males	27	72.7	1.01	0.5	10	<**0.001***

*Significant after sequential Bonferroni correction for number of tests in column

[1] females reared with high value of total colour during the 2nd half of the developmental period

[2] females reared with high and low value of total colour during the 2nd half of the developmental period

[3] females reared with low value of total colour during the 2nd half of the developmental period

Table 3.4: Means and standard errors of the linear (b) and quadratic (g) components representing the individual non-linear preference functions. One-sample Student's t-tests were used to test whether coefficients tend to differ from zero. Means qualities of the linear regressions are also represented ("R^2").

Treatments		*QUADRATIC REGRESSION*								
				Linear component				Quadratic component			
		N	mean R^2 (%)	mean b	SE	*t*	*p*	mean g	SE	*t*	*p*
HNS+1 [1]	Orange area	24	56.4	5.78	14.8	0.39	0.70	-74.2	129.7	-0.57	0.57
	Yellow area	24	46.7	-3.75	2.5	-1.52	0.14	50.6	66.4	0.76	0.45
	Black area	24	39.4	-7.72	7.7	-1.01	0.32	60.8	90.1	0.67	0.51
	White area	24	39.8	-13.4	9.3	-1.44	0.16	215.9	238.1	0.91	0.37
	Blue area	24	42.7	-2.03	7.4	-0.27	0.79	-4.3	82.2	-0.05	0.96
	Green area	24	38.5	10.7	6.1	1.75	0.09	-40.8	24.8	-1.69	0.11
	Total colour area	24	50.1	-13	5.5	-2.38	**0.026**	20.6	8.00	2.57	**0.017**
	Simpson Index	24	37.6	-0.38	0.9	-0.41	0.69	0.1	0.12	0.42	0.68
	Attractive males	24	76.1	0.76	0.1	5.6	**<0.001***	3.5	0.75	4.62	**<0.001***
MNS+1 [2]	Orange area	27	47.3	-2.24	6.5	-0.35	0.73	30.7	57.7	0.53	0.599
	Yellow area	27	32.7	-1.9	7.3	-0.26	0.8	31.8	97	0.33	0.747
	Black area	27	43.4	-15	11.7	-1.28	0.21	107.5	99.1	1.08	0.288
	White area	27	40.9	-9.22	7.4	-1.25	0.22	48.2	151	0.32	0.751
	Blue area	27	45.9	-13.6	9.5	-1.43	0.16	108.3	90.9	1.19	0.245
	Green area	27	43.2	15.3	2	0.05	0.96	-5.9	18.2	-0.32	0.753
	Total colour area	27	31.1	-1.47	4.3	-0.34	0.74	1.5	6.7	0.23	0.823
	Simpson Index	27	42.7	-0.22	0.7	-0.30	0.77	0.1	0.1	0.47	0.645
	Attractive males	27	78.3	0.91	0.1	10.4	**<0.001***	0.4	0.7	0.57	0.576
LNS+1 [3]	Orange area	28	46.6	6.82	8.2	0.83	0.41	-54.9	70.1	-0.78	0.440
	Yellow area	28	44.3	-3.07	4.4	-0.66	0.52	124.2	88.8	1.38	0.179
	Black area	28	43.1	-8.02	7.1	-1.13	0.27	92.2	111	0.83	0.414
	White area	28	38.4	-1.50	9	-0.17	0.87	102.4	263	0.39	0.700
	Blue area	28	47.5	-0.34	12.1	-0.03	0.98	0.2	92.6	0.00	0.999
	Green area	28	45.9	-18.1	15.8	-1.15	0.26	75	61.9	1.21	0.236
	Total colour area	28	43.0	12.8	8.9	1.43	0.16	-17.7	13	-1.37	0.183
	Simpson Index	28	54.7	-1.14	1	-1.13	0.27	0.1	0.1	1.00	0.328
	Attractive males	27	80.0	0.95	0.7	7.09	**<0.001***	1.3	3.3	2.08	**0.048**

*Significant after sequential Bonferroni correction for number of tests in column; [1, 2, 3] see previous table for abbreviations

3.4.2. Individual preference functions after exposure to stimuli males for the whole development

In contrast to females who were exposed to males half of their developmental period, clear-cut preferences were observed with females reared with males during their whole development. When brought up with males bearing high values of total colour (HNS treatment), they chose to associate significantly more with males displaying high value of yellow (table 3.6). By contrast, they had a significant dislike for males with high value of orange and a marginal preference for males with less blue and less colour overall (table 3.6). Females reared in the presence of males with high and low value of “total colour” (mixed or high variance, MNS, treatment) preferred males with more yellow and more colour overall although the coefficients of the preference slopes didn’t differ significantly from zero after Bonferroni corrections. Exposed to males with few colours overall, females expressed strong interest for males with more blue, more colourful and surprisingly with little yellow (table 3.6). Within the three treatments, females agreed on the preference for males that are generally found to be attractive. The absence of correlations between mean responsiveness and quality of the linear regressions (table 3.8) showed behavioural independency between sexual components.

Table 3.6: Means and standard errors of the slopes (b) of the linear regression representing the individual female preference functions. One-sample Student's t-tests were used to test whether coefficients tend to differ from zero. Means qualities of the linear regressions are also represented ("R^2").

Treatments		***Linear regression***					
		N	mean R^2 (%)	mean b	SE	*t*	*p*
HNS [1]	Orange area	22	22.9	-1.47	0.4	-3.31	**0.003***
	Yellow area	22	43.0	2.70	0.8	3.41	**0.003***
	Black area	22	17.9	-1.25	0.8	-1.59	0.128
	White area	22	16.6	-1.50	1.5	-1.01	0.325
	Blue area	22	25.5	-2.22	1	-2.25	**0.035**
	Green area	22	28.7	-0.68	0.5	-1.46	0.161
	Total colour area	22	29.1	-0.94	0.4	-2.21	**0.04**
	Simpson Index	22	15.1	0.05	0.03	1.70	0.104
	Attractive males	22	50.8	1.04	0.1	12.3	<**0.001***
MNS [2]	Orange area	20	34.2	0.42	1.2	0.4	0.717
	Yellow area	20	35.2	2.76	1.1	2.5	**0.022**
	Black area	20	39.2	-2.83	1.5	-1.8	0.081
	White area	20	22.3	2.15	1.3	1.6	0.132
	Blue area	20	30.1	-0.61	1.3	-0.5	0.643
	Green area	20	23.6	0.58	0.4	1.4	0.169
	Total colour area	20	33.4	1.03	0.4	2.4	**0.029**
	Simpson Index	20	26.0	0.05	0.1	1.0	0.342
	Attractive males	20	87.6	1.09	0.1	16.7	<**0.001***
LNS [3]	Orange area	24	29.5	0.87	0.8	1.06	0.300
	Yellow area	24	22.9	-2.21	0.8	-2.83	**0.009**
	Black area	24	12.4	-0.01	0.5	-0.03	0.976
	White area	24	18.5	-0.79	1.4	-0.55	0.587
	Blue area	24	20.4	2.36	0.7	3.39	**0.003***
	Green area	24	28.6	0.45	0.5	0.89	0.382
	Total colour area	24	32.1	1.85	0.5	3.89	**0.001***
	Simpson Index	24	23.6	-0.04	0.03	-1.27	0.217
	Attractive males	24	76.6	0.93	0.1	17.6	<**0.001***

* Significant after sequential Bonferroni correction for number of tests in column

[1] females reared with high value of total colour during the whole developmental period

[2] females reared with high and low value of total colour during the whole developmental period

[3] females reared with low value of total colour during the whole developmental period

Table 3.7: Means and standard errors of the linear (b) and quadratic (g) components representing the individual non-linear preference functions. One-sample Student's t-tests were used to test whether coefficients tend to differ from zero. Mean qualities of the linear regressions are also represented ("R^2").

QUADRATIC REGRESSION

Treatments				Linear component				Quadratic component			
		N	Mean R^2 (%)	mean b	SE	*t*	*p*	mean g	SE	*t*	*p*
HNS [1]	Orange area	22	45.8	0.04	4.7	0.01	0.99	-7.8	33.3	-0.24	0.82
	Yellow area	22	58.6	-2.54	3.9	-0.65	0.52	63.8	38.6	1.65	0.11
	Black area	22	39.8	17.1	31.7	0.54	0.6	-143	250	-0.57	0.57
	White area	22	33.6	-2.53	7.9	-0.31	0.76	-20.2	154	-0.13	0.9
	Blue area	22	48.1	-0.76	6.9	-0.11	0.91	-31.9	61.5	-0.52	0.61
	Green area	22	45.3	-6.88	5	-1.41	0.18	22	16.1	1.39	0.18
	Total colour area	22	29.1	-3.75	12.1	-0.31	0.76	8.9	18.6	0.48	0.64
	Simpson Index	22	41.9	0.32	0.7	0.54	0.6	-0.05	0.1	-0.54	0.6
	Attractive males	22	60.9	1.01	0.1	8.60	**<0.001***	5.68	1.7	3.34	**0.003***
MNS [2]	Orange area	20	57.9	-0.89	3.7	-0.24	0.81	18.9	34.8	0.54	0.59
	Yellow area	20	63.0	5.35	17.9	0.30	0.77	27.2	148.7	0.17	0.86
	Black area	20	56.2	14.2	13	1.09	0.29	-162.7	103	-1.58	0.13
	White area	20	40.3	5.32	7.9	0.66	0.52	-133	155	-0.83	0.42
	Blue area	20	54.1	22.4	12.7	1.76	0.09	-207.8	102	-2.05	**0.05**
	Green area	20	39.2	0.70	2.2	0.32	0.75	-5.5	9.4	-0.59	0.56
	Total colour area	20	51.3	-5.10	3.9	-1.30	0.21	8.4	5.9	1.42	0.17
	Simpson Index	20	39.6	-1.88	0.9	-2.20	**0.04**	0.3	0.11	2.30	**0.03**
	Attractive males	20	90.0	1.04	0.1	16	**<0.001***	0.5	0.54	0.99	0.33
LNS [3]	Orange area	24	46.2	8.6	6.1	1.43	0.17	-66.6	47.9	-1.39	0.18
	Yellow area	24	40.7	-7.7	2.8	-2.80	**0.01**	39.2	42.5	0.92	0.37
	Black area	24	38.0	-9.4	10.9	-0.86	0.40	28.7	85.2	0.34	0.74
	White area	24	38.7	7.2	7.8	0.93	0.36	-7.4	272	-0.03	0.98
	Blue area	24	43.4	-13.2	13.9	-0.95	0.35	132.3	118	1.13	0.27
	Green area	24	48.0	-0.5	3.3	-0.16	0.87	3.0	10.9	0.27	0.79
	Total colour area	24	53.2	28.3	12.9	2.19	**0.04**	-29.2	17.1	-1.71	0.10
	Simpson Index	24	48.7	0.5	0.5	0.84	0.41	-0.1	0.1	-0.93	0.36
	Attractive males	24	83.7	1.4	0.3	5.11	**<0.001***	-0.4	0.7	-0.59	0.56

*Significant after sequential Bonferroni correction for number of tests in column; [1, 2, 3] see previous table for abbreviations.

Table 3.8: Correlation coefficients between individual female mean responsiveness and quality of the linear regression (representing individual preference functions) for different male traits.

Treatments	Male traits	N	Pearson correlation coefficients	*p*-value
LNS [1]	Orange area	24	0.08	0.72
	Yellow area	24	0.3	0.16
	Black area	24	0.05	0.83
	White area	24	0.12	0.59
	Blue area	24	0.14	0.51
	Green area	24	-0.2	0.34
	Total colour area	24	0.26	0.22
	Simpson Index	24	-0.16	0.46
MNS [2]	Orange area	20	0.19	0.43
	Yellow area	20	0.16	0.51
	Black area	20	0.38	0.1
	White area	20	0.03	0.92
	Blue area	20	0.25	0.29
	Green area	20	0.28	0.24
	Total colour area	20	-0.16	0.5
	Simpson Index	20	0.05	0.82
HNS [3]	Orange area	22	-0.21	0.35
	Yellow area	22	0.23	0.3
	Black area	22	0.07	0.75
	White area	22	-0.15	0.52
	Blue area	22	0.08	0.74
	Green area	22	-0.2	0.34
	Total colour area	22	-0.08	0.74
	Simpson Index	22	0.36	0.12

[1] females reared with low value of total colour during the whole developmental period
[2] females reared with high and low value of total colour during the whole developmental period
[3] females reared with high value of total colour during the whole developmental period

Adding a quadratic component improved the models' efficiency but the coefficients *g* and *b* were not significant except for the trait "attractive males" which demonstrates, as it is the case with linear regression that females tended to find the same males, generally, attractive (table 3.7). In the LNS treatment, the quadratic regressions confirmed the linear preferences and in the MNS treatment, females seemed to prefer males with increased colour diversity ($b<0$, $g>0$ marginally significant). Due to a lack of significant *g* coefficients, only the outcomes of the linear regression are considered in further analysis.

3.4.3. Phenotypic variation in preference functions between rearing treatments

3.4.3.1 Variation in orange preference between rearing treatments

The linear model didn't reveal phenotypic variation in orange preferences across rearing treatment for long- and short- exposed females although the main effect "treatment" was marginally significant ($F_{(2,\ 121.1)}$=2.57, p=0.08, table 3.9). Pairwise comparisons between the three levels, adjusted with Bonferroni method, demonstrated that females reared with males bearing low value of total colour (during half and whole developmental period) marginally preferred males with more orange than females reared with high value of total colour (p=0.076, see fig. 3.3).

Moreover, the small non-significant variance estimate for the random intercept (0.43, p=0.32, Table 3.9) indicates that heterogeneity among families could be ignored.

Table 3.9: Linear mixed model of fixed and random effects influencing differences in orange preference across treatments

	Factor	***df***	**F**	***p***
Test of fixed effects	Intercept	1, 83.6	2.25	0.14
	Treatments	2, 121.1	2.57	0.08
	Time of exposure	1, 4.4	0.002	0.96
	Orange covariate	1, 104.4	2.25	0.14
	Treatment*Time of exposure	2, 120.4	0.46	0.63

	Parameter	**Estimate**	**SE**	**df**	*t*	**Wald Z**	***p***
Estimates of fixed effects	Intercept	3.81	2.1	79	1.78	-	0.08
	HNS treatment[1]	-2.47	1.2	120	-2	-	**0.048**
	MNS treatment[1]	-0.74	1.3	120.7	-0.58	-	0.56
	Time of exposure[2]	-0.05	1.3	28.8	-0.04	-	0.97
	Orange covariate	-43.6	29	104.4	-1.5	-	0.14
Estimates of covariance parameters	Residuals	17.3	2.3	-	-	7.5	**<0.001**
	Family	0.43	1.3	-	-	0.32	0.75

Not all the estimates included in the model are represented
[1]LNS treatment is the reference category
[2]Whole developmental period used as the reference category

3.4.3.2. Variation in yellow preference between rearing treatments

Experiencing different levels of total colour during development shaped females' preference for yellow pigmentation ($F_{(2, 117.4)}$=6.33, p=0.002, Table 3.10). Independently of the time of exposure, females reared with low value of total colour (LNS/LNS+1 treatments) preferred significantly less yellow males than females reared with mixed value of total colour (MNS/MNS+1 treatments) and females reared with high value of total colour (HNS/HNS+1 treatments) (p=0.003 and p=0.038 respectively, corrected with Bonferroni test for multiple comparisons, Figure 3.3). The time of exposure was not influential in itself but a marginal interaction between treatment and time of exposure was observed ($F_{(2, 117.4)}$=2.47, p=0.09, Table 3.10). To analyse this effect further, an ANOVA with one fixed factor made of six levels (HNS, HNS+1, MNS, MNS+1, LNS, LNS+1) was carried out. The model was significant ($F_{(5, 133)}$=2.88, p=0.017) and comparisons between levels showed that females reared with low value of total colour during whole development were significantly less attracted by yellow males than HNS females (p=0.029, see fig. 3.4), than MNS females (p=0.033, see fig.3.4) and than MNS+1 females (p=0.079, see fig. 3.4). When an LSD post-hoc test was used (no correction applied), the two remaining groups also appeared to prefer yellower males (p=0.027 for HNS+1 and p=0.059 for LNS+1, see fig. 3.4).

Variance components suggested that there were no variability in yellow preference at the between-family level (0.85, p=0.63, see table 3.10) and no variation in the influence of the yellow covariate on yellow preference owing to variation across families (table 3.10).

Table 3.10: Linear mixed model of factors (fixed and random effects) influencing differences in yellow preferences across treatments

	Factor	*df*	F	*p*
Test of fixed effects	Intercept	1, 9.6	0.78	0.4
	Treatments	2, 117.4	6.33	**0.002**
	Time of exposure	1, 9.6	1.42	0.26
	Yellow covariate	1, 13.2	0.14	0.72
	Treatment*Time of exposure	2, 117.4	2.47	0.09
	Time of exposure*Yellow covariate	1, 13.2	1.48	0.24

	Parameter	Estimate	SE	df	*t*	Wald Z	*p*
Estimates of fixed effects	Intercept	-3.9	2.2	10	-1.75	-	0.1
	HNS treatment[1]	4.9	1.5	115.3	3.24	-	**0.002**
	MNS treatment[1]	5.5	1.6	114.2	3.52	-	**0.001**
	Time of exposure[2]	5.9	2.9	14.2	2	-	0.065
	Yellow covariate	37.5	45.9	7.2	0.82	-	0.44
	HNS*Half development[3]	-4.4	2.1	112.2	-2.1	-	**0.04**
	MNS*Half development[3]	-3.6	2.2	118.1	-1.67	-	0.1
Estimates of covariance parameters	Residuals	24.8	3.4	-	-	7.2	**<0.001**
	Family	0.85	1.75	-	-	0.49	0.63
	Yellow covariate	29748	28232	-	-	1.1	0.29

Not all the estimates included in the model are represented
[1] LNS treatment is the reference category
[2] Whole developmental period used as the reference category
[3] LNS and Whole development are the reference categories

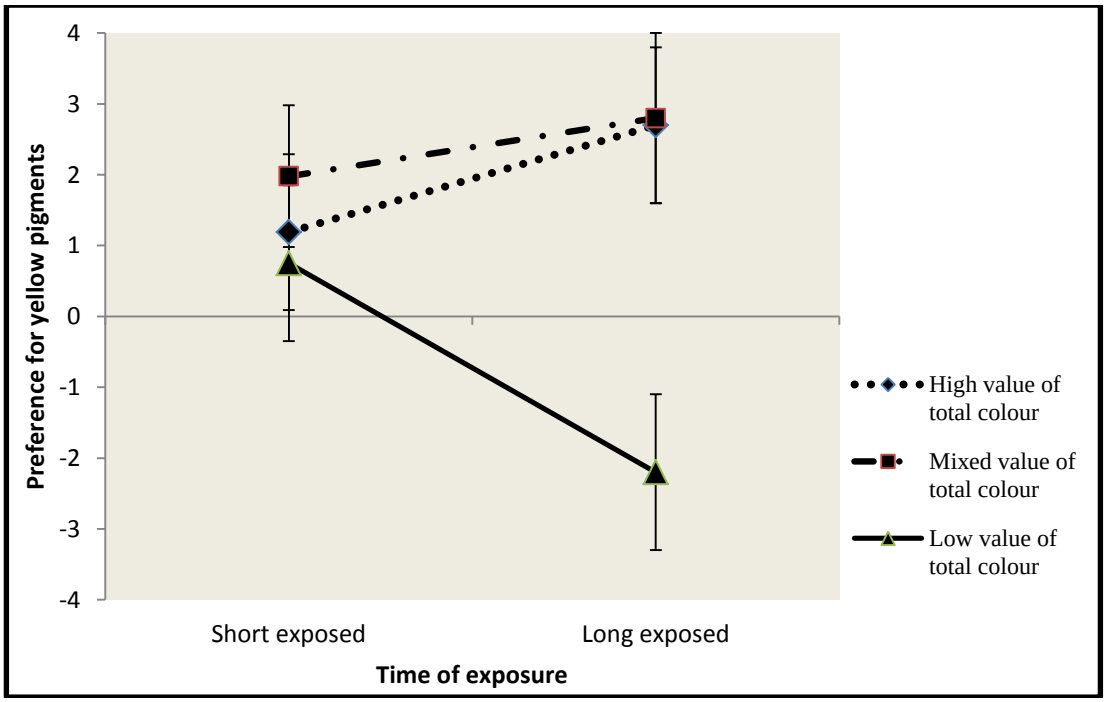

Figure 3.4: Females' yellow preference after exposure to different values of total colour during half (short-exposed) or whole development (long-exposed). Preferences are represented by the least square means (± SE) of regression slopes for high value (···♦···), mixed value (-·-·-·-□-·-·-·-) and low value (——□——) of total colour treatments.

3.4.3.3. *Variation in blue preference between the rearing treatments*

Long- and short- exposed females varied in their preference for blue coloration following exposure to different values of total colour. I found a significant interaction between treatment and time of exposure (Table 3.11). The effects of the two main factors and of the covariate were not significant in the main model (Table 3.11). However, an ANOVA with "treatment" as a single fixed factor showed the influence of the amount of coloration experienced during ontogeny on blue preference ($F_{(2,\ 128.7)}$= 3.1, p=0.049). Females having been reared in the presence of males with high level of colours (HNS/HNS+1) preferred significantly less blue males than females having been reared with males with low value of total colour (Bonferroni adjusted, p=0.05, figure 3.3). To investigate the effects of the interaction between "treatment" and "time of exposure", I performed an ANOVA with one fixed factor "exposure group" and six levels (HNS, HNS+1, MNS, MNS+1, LNS, LNS+1). After Bonferroni correction for multiple comparisons, I found that LNS females preferred significantly bluer males than HNS females (p=0.044, Fig. 3.5). When LSD post hoc tests were performed, more differences were found. LNS females preferred bluer males than HNS+1 females (p=0.057, see fig.3.5), than MNS females (p=0.058, see fig.5) and than LNS+1 females (p=0.059, see fig.3.5). Moreover, MNS+1 females preferred bluer males than HNS females (p=0.054, see fig. 3.5).

Variance estimate suggested there were no significant heterogeneity among families in blue preference (2.6, p=0.3, table 3.11).

Table 3.11: Linear mixed model of factors (fixed and random effects) influencing differences in blue preference across treatments

	Factor	*df*	F	*p*
Test of fixed effects	Intercept	1, 102.1	1.24	0.27
	Treatments	2, 133.5	1.35	0.26
	Time of exposure	1, 8.5	0.01	0.93
	Blue covariate	1, 131.1	1.24	0.27
	Treatment*Time of exposure	2, 129.1	3.33	**0.039**
	Treatment* Blue covariate	2, 133.9	0.77	0.47

	Parameter	Estimate	SE	df	*t*	Wald Z	*p*
Estimates of fixed effects	Intercept	3.37	3.6	124.8	0.93	-	0.36
	HNS treatment[1]	-10.2	5.1	129.7	-1.98	-	**0.049**
	MNS treatment[1]	-7.4	5.2	135	-1.4	-	0.16
	Time of exposure[2]	-2.9	1.7	25.2	-1.8	-	0.089
	Blue covariate	-16.6	56.6	135.9	-0.29	-	0.77
	HNS*Half development[3]	4.5	2.1	131.3	2.2	-	**0.029**
	MNS*Half development[3]	4.6	2.1	131.3	2.2	-	**0.029**
Estimates of covariance parameters	Residuals	24.5	3.1	-	-	7.9	**<0.001**
	Family	2.6	2.4	-	-	1.1	0.28

Not all the estimates included in the model are represented
[1] LNS treatment is the reference category
[2] Whole developmental period used as the reference category
[3] LNS and Whole dev. are the reference categories

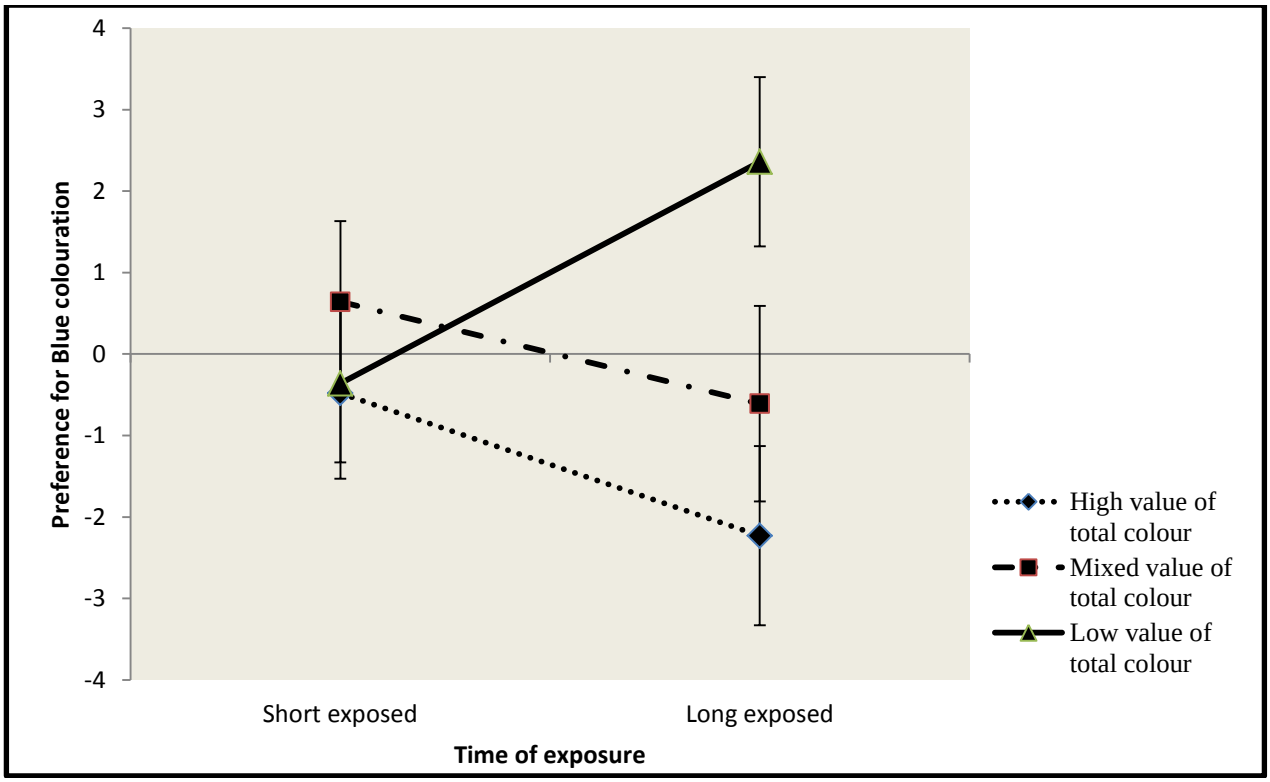

Figure 3.5: Females' blue preference after exposure to different values of total colour during half (short-exposed) or whole development (long-exposed). Preferences are represented by the least square means (± SE) of regression slopes for high value (···♦···), mixed value (- - - - - -□- - - - - -) and low value (——□——) of total colour treatments.

3.4.3.4. Variation in total colour preference between the rearing treatments

A GLM procedure showed that the amount of total colour experienced during ontogeny independently of the duration of exposure mediated female mate preference for total colour ($F_{(2, 137)}$= 7.69, p=0.001, table 3.12, figure 3.3). Females having matured in visual contact with males displaying low value of total colour (LNS/LNS+1 treatment) had a significant preference for colourful males relatively to females reared with high value of total colour (p<0.001, see fig. 3.3). Females reared with mixed value of total colour also preferred males with more colours than HNS/HNS+1 females (p=0.05, fig. 3.3) who seemed to favour little amount of total colour as indicated by the negative linear coefficients (table 3.6, fig.3.3). The time of exposure influenced how the amount of colour experienced was shaping female preference as indicated by the significant interaction ($F_{(2,137)}$=7.54, p=0.001, table 3.12, fig. 3.6). The difference between the six exposure groups was revealed by an ANOVA ($F_{(5, 139)}$= 7.01, p<0.001). After application of Bonferroni adjustment to the multiple comparisons, I found that LNS and HNS females diverged significantly in their preference (p<0.001, fig. 3.6) - HNS females discriminating against colourful males unlike LNS females. LNS females preferred also, significantly more, colourful males than HNS+1 females (p=0.004), MNS+1 females (p=0.001) and LNS+1 females (p=0.005). Further, HNS females chose drabber females than MNS females (p=0.009, fig. 3.6).

Table 3.12: General linear model of factors influencing differences in total colour preferences across treatments

	Factor	*df*	F	*p*
Test of fixed effects	Intercept	1, 137	3.53	0.06
	Treatments	2, 137	7.69	**0.001**
	Time of exposure	1, 137	3.07	0.08
	Total colour covariate	1, 137	4.1	**0.045**
	Treatment*Time of exposure	2, 137	7.54	**0.001**
	Time of exposure*Total colour covariate	1, 137	2.63	0.11

	Parameter	Estimate	SE	df	*t*	Wald Z	*p*
Estimates of fixed effects	Intercept	0.94	1.7	137	0.55	-	0.58
	HNS treatment[1]	-2.8	0.5	137	-5.23	-	**<0.001**
	MNS treatment[1]	-0.8	0.6	137	-1.42	-	**0.16**
	Time of exposure[2]	-8.1	4.1	137	-2	-	**0.05**
	Total colour covariate	2.43	4.4	137	0.55	-	0.58
	HNS*Half development[3]	2.7	0.7	137	3.7	-	**<0.001**
	MNS*Half development[3]	0.54	0.7	137	0.73	-	0.46

Not all the estimates included in the model are represented
[1] LNS treatment is the reference category
[2] Whole developmental period used as the reference category
[3] LNS and Whole development are the reference categories

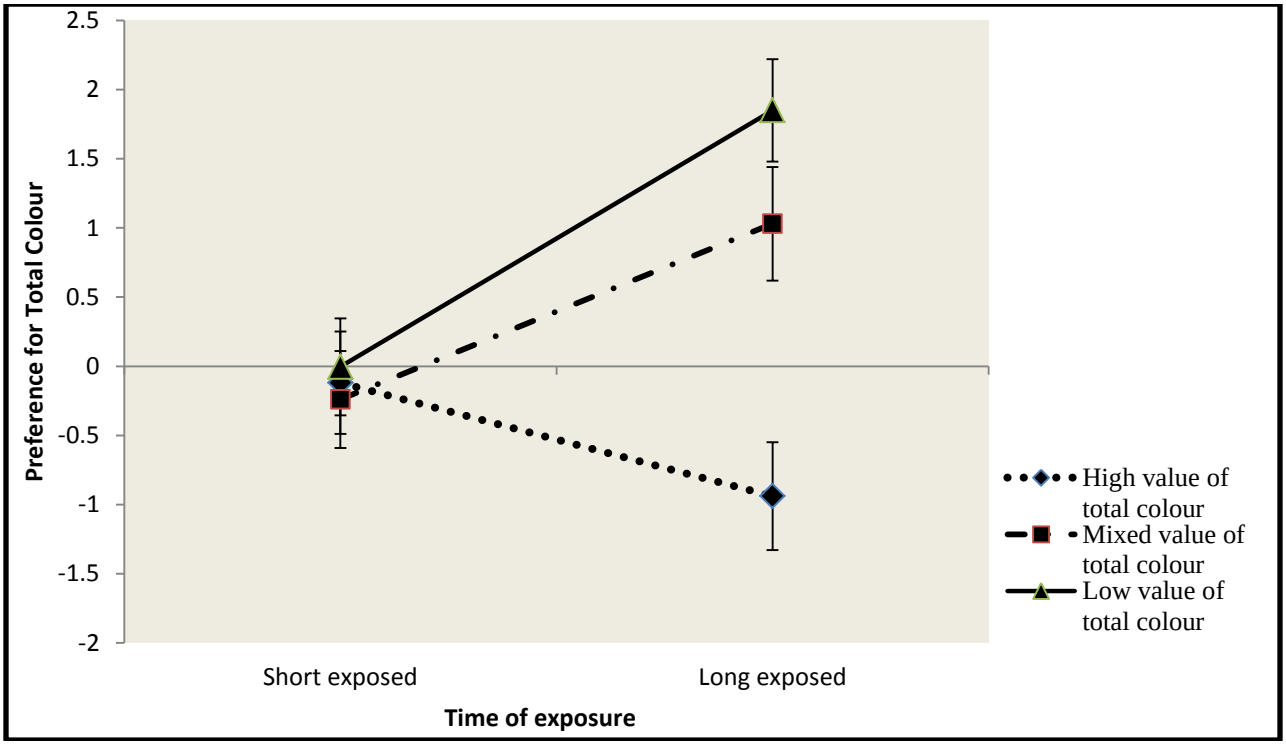

Figure 3.6: Females' total colour preference after exposure to different values of total colour during half (short-exposed) or whole development (long-exposed). Preferences are represented by the least square means (±SE) of regression slopes for high value (···♦···), mixed value (- - - - - □ - - - - -) and low value (——□——) of total colour treatments.

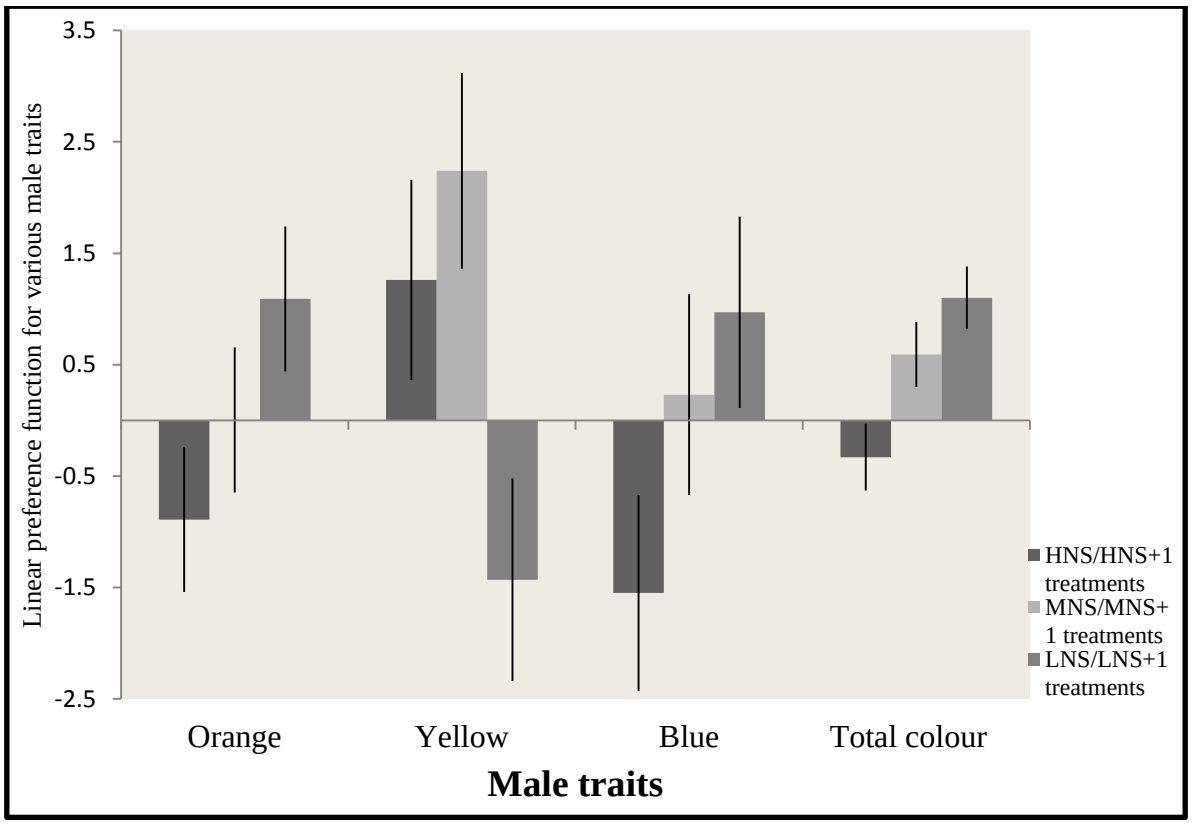

Figure 3.3: Variation in linear preference functions for various male traits after exposure to different values of total colour during ontogeny. Bars represent the estimated marginal means of the regression slopes (+/- SE).

3.4.4. Phenotypic variation in mean responsiveness between rearing treatments

Female responsiveness depended on the amount of colour experienced during ontogeny ($F_{(2, 24)}$= 5.32, p=0.012, table 3.13). Females exposed to low value of total colour are significantly more receptive to males than HNS/HNS+1 females (p=0.028 after sequential Bonferroni adjustment, fig. 3.7) and, than MNS/MNS+1 females (p=0.022 after sequential Bonferroni adjustment, fig. 3.7). Further, the length of exposure influenced the effects of the of colour on learned preferences as indicated by the significant interaction ($F_{(2, 24)}$=3.9, p=0.034, table 3.13). An ANOVA with one fixed factors “exposure group” ($F_{(5, 139)}$=8.63, p<0.001) made of six levels – HNS, HNS+1, MNS, MNS+1, LNS, LNS+1 – confirmed the interaction in the main model and showed that females reared with low value of total colour during whole

development (LNS treatment) were more responsive than females in any other groups ($p<0.001$ after sequential Bonferroni adjustment for LNS against the five other groups, fig 3.8).

A shown by the variance estimates, relatedness didn't contribute much to the variance found in females' responsiveness (estimate ±SE = 0.002 ± 0.002, table 3.13). Similarly, the variation in treatment effects owing to variability across family didn't explain much variation (table 3.13).

Table 3.13: Generalized linear mixed model of factors (fixed and random effects) influencing differences in mean responsiveness across treatments

	Factor	***Df***	**F**	***p***
	Treatments	2, 24	5.32	**0.012**
	Time of exposure	1, 13	2.36	0.15
	Total colour covariate	1, 85	2.72	0.10
	Treatment*Time of exposure	2, 24	3.9	**0.034**

	Parameter	**Estimate**	**SE**	*t*	***p***	95% Confidence Interval	
						Lower	Upper
Estimates of fixed effects	Intercept	-1.01	0.14	-7.25	**<0.001**	-1.29	-0.73
	HNS treatment[1]	-0.16	0.05	-3.39	**0.001**	-0.26	-0.06
	MNS treatment[1]	-0.17	0.05	-3.55	**0.001**	-0.28	-0.07
	Time of exposure[2]	-0.17	0.06	-3.07	**0.003**	-0.28	-0.06
	Total colour covariate	0.59	0.36	1.65	0.1	-0.12	1.3
	HNS*Half development[3]	0.15	0.07	2.37	**0.02**	0.02	0.29
	MNS*Half development[3]	0.16	0.07	2.43	**0.02**	0.023	0.29
Estimates of covariance paramaters	Residuals	0.013	0.002	-	-	0.01	0.016
	Family	0.002	0.002	-	-	0.0001	0.013
	Treatment	0.003	0.002	-	-	0.001	0.01

Not all the estimates included in the model are represented
[1] LNS treatment is the reference category
[2] Whole developmental period used as the reference category
[3] LNS and Whole dev. are the baseline categories

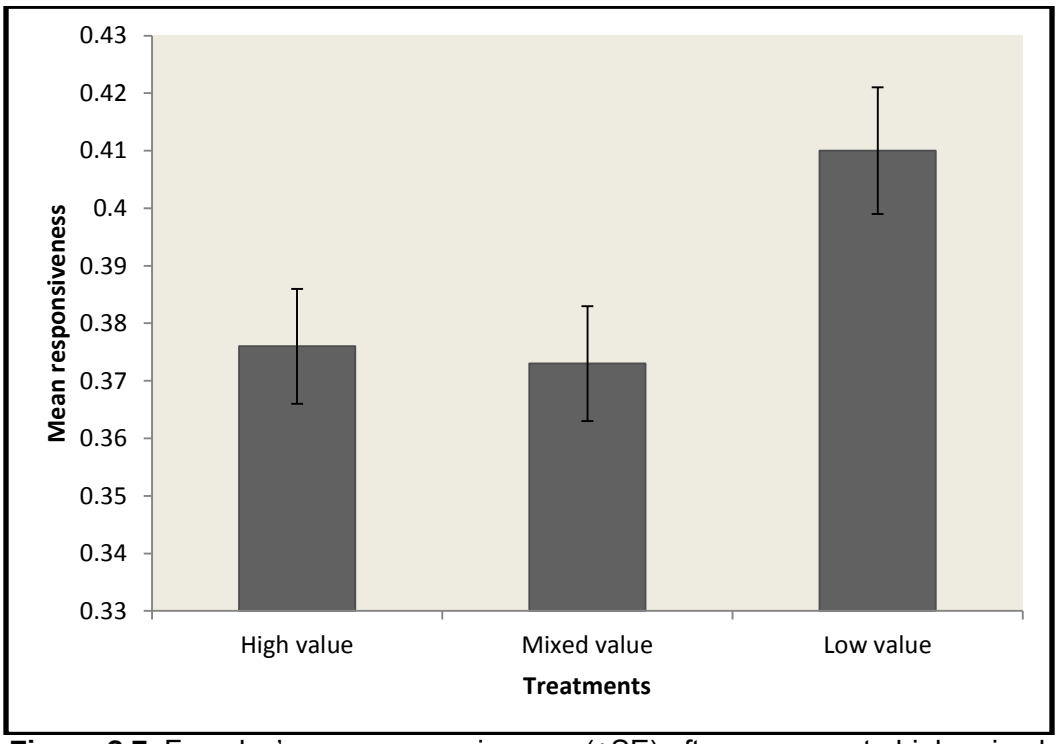

Figure 3.7: Females' mean responsiveness (±SE) after exposure to high, mixed and low value of total colour during development (data are merged for half and full development).

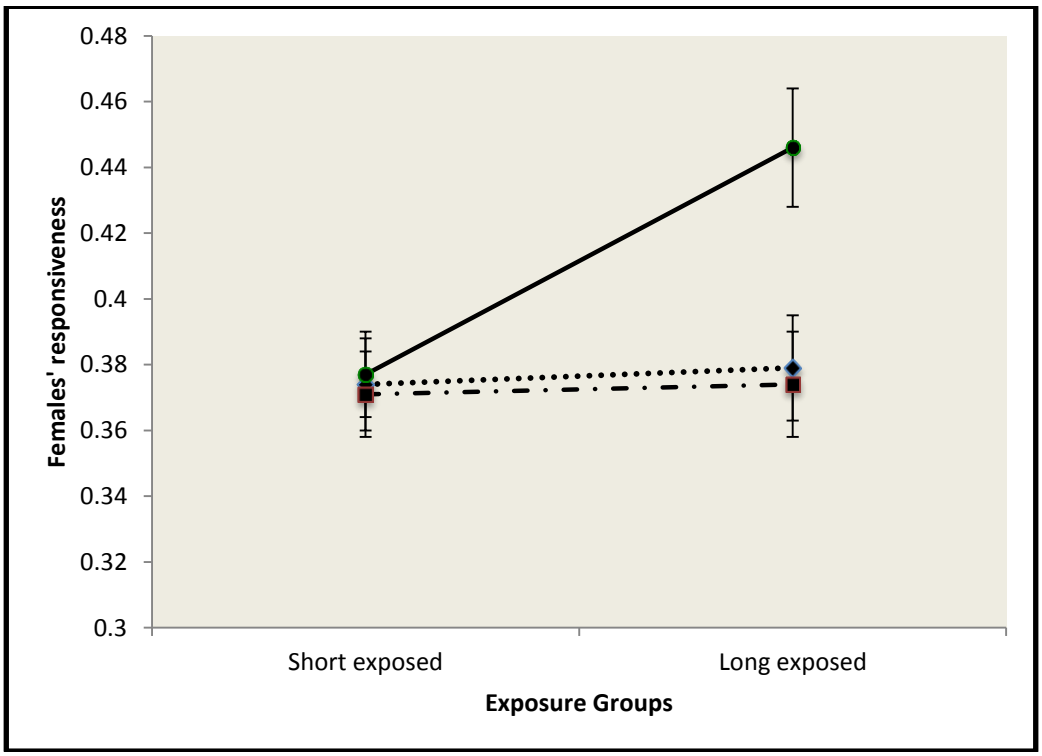

Figure 3.8: Females' responsiveness (±SE) after exposure to different values of total colour during half (short-exposed) or whole development (long-exposed). Responsiveness is represented by the least square means (±SE) for high value (···♦···), mixed value (----□----) and low value (—□—) of total colour treatments.

3.4.5. Phenotypic variation in discrimination between rearing treatments

Unlike responsiveness, which varied in response to the different treatments, discrimination didn't seem to change after exposure to different social environment (table 3.14). Neither the amount of total colour nor the duration of visual contact with males influenced the degree to which females distinguished male trait variation.

Table 3.14: Generalized linear mixed model of factors (fixed and random effects) influencing differences in discrimination across treatments

	Factor	***df***	**F**	***p***			
	Treatments	2, 16	1.4	0.28			
	Time of exposure	1, 11	0.65	0.44			
	Total colour covariate	1, 113	2	0.16			
	Treatment*Time of exposure	2, 16	0.08	0.92			
	Parameter	**Estimate**	**SE**	*t*	***p***	95% Confidence Interval	
						Lower	Upper
Estimates of fixed effects	Intercept	0.87	0.27	3.2	**0.002**	0.33	1.41
	HNS treatment[1]	0.03	0.08	0.3	0.77	-0.16	0.21
	MNS treatment[1]	0.09	0.09	0.97	0.33	-0.11	0.28
	Time of exposure[2]	0.05	0.12	0.46	0.65	-0.19	0.3
	Total colour covariate	-0.95	0.67	-1.41	0.16	-2.3	0.39
	HNS*Half development[3]	0.05	0.12	0.39	0.7	-0.21	0.31
	MNS*Half development[3]	0.03	0.13	0.27	0.79	-0.23	0.29
Estimates of covariance paramaters	Residuals	0.16	0.02	-	-	0.13	0.21
	Family	0.02	0.01	-	-	0.005	0.07
	Treatment	0.008	0.008	-	-	0.001	0.05

[1] LNS treatment is the reference category
[2] Whole developmental period used as the reference category
[3] LNS and Whole dev. are the baseline categories

3.5. Discussion

This study demonstrated the importance of the early social environment in mate preference acquisitions in guppies. Both preference functions and choosiness were divergent after different ontogenetic paths. In a species where females base their choice on multiple criteria, the exposure to various values of a genetically preferred trait (i.e. total colour) influenced not only the preference for that trait but also preferences for other sexual cues. Moreover, the length of exposure to stimuli males seemed to play a crucial role on female subsequent choices.

Unlike other studies in which females were reared with different values of orange, discrimination was not dependent on prior experience. Conversely, responsiveness (i.e. the other aspect of choosiness) was altered after females were brought up during their whole development with males bearing low value of total colour (LNS treatment), becoming more receptive to males' solicitations. This result contrasts with my previous finding (chapter two) which showed females being more willing to respond to males after short exposure to low variance orange treatments. The difference might stem from the nature of the trait to which females have been exposed; orange not being a colour primarily involved in mate choice in this population, unlike "total colour" which is a key element to discriminate among males. On the whole, variability in choosiness was stronger after experiencing variation in a non-preferred trait than in a trait directly concerned in the mate choice process. This suggests the possibility of epistatic (up regulation) and/or hypostatic (down regulation) rearing effects on different components of sexual behaviours. For instance, the early experience of a trait like "total colour" might constrains future choosiness whereas exposure to orange eases it. Independently of the direction of the rearing treatment effects, these results showed that environmental factors can tune female's responsiveness even when a large part of its phenotypic variance is explained by additive genetic variation (Brooks & Endler 2001b; Brooks 2002; Rodriguez & Greenfield 2003). Incidentally, I found a positive correlation between responsiveness and yellow preference in the MNS+1 treatment. In this case, responsiveness promoted the expression of a genetically based preference

in a context where it seemed that environmental factors outweighed genetical determinism (see below).

Early social experience of total colour also modified the direction and the strength of preference functions. Three main outcomes are noteworthy. First, the timing of exposure seemed to play a decisive role as short-exposed females didn't display any preferences based on colours borne by males (except yellow preference after MNS+1 treatment as discussed here above) yet not associating at random, since within treatments, females preferred the same males that other females found attractive (coefficient of "attractive male" trait significantly differed form zero, table 3.3 and table 3.6). From a mechanistic point of view, it is possible that imprinting on a nonparental adult (i.e. oblique imprinting) is restricted to a sensitive period (just as traditional sexual imprinting), which was not included in the second half of development. However, this hypothesis is not very plausible in this case because females reared in the absence of males expressed preferences (see chapter two, section on innate preference). Furthermore, to maximize their fitness, guppy females ought to evolve plasticity in preference acquisition during a period close to first mating rather early in ontogeny. A low variance level in the sexual cue experienced during maturation could also account for the absence of preference, as demonstrated by Rosenqvist and Houde (1997). Such hypothesis would be supported if females reared in the mixed treatments (MNS+1 treatment) displayed some preferences but they didn't show any interest for black or total colour (innate preference) and yellow preference seemed to be linked to responsiveness. Consequently, the most likely reason for the lack of choice based on colour is that females used other sexual cues. Besides being attracted by colour spots, females also discriminate among males based on courtship display (KodricBrown & Nicoletto 1996; Houde 1997), male body size (Reynolds & Gross 1992; Magellan et al. 2005; Karino & Urano 2008) and tail area (Bischoff et al. 1985; Karino & Kobayashi 2005). Since male body size was controlled in the experimental arena, males might have varied in display rate and/or tail size, which offered to females the opportunity to make a choice. The experimental setting didn't allow me to measure these variables and the possibility that females prioritize display rate

and/or tail size over colour spots after short exposure to males during ontogeny offers interesting avenue for future research.

The second noticeable outcome of this study indicated that ontogenetical experience altered and sometimes reversed preferences genetically determined. I discussed above the possible reasons of an absence of colour preference for short-exposed females. Conversely, long-exposed females to low variance treatments (HNS and LNS treatment) acquired preferences that diverged from their innate predispositions. Only females exposed to mixed value of total colour (high variance: MNS treatment) resembled the preference norm found in the population. Between females exposed to low value of total colour and those exposed to high value of total colour, an opposite pattern took shape. HNS females tended to choose males displaying low value of orange, blue and total colour and favoured males with high value of yellow, which significantly differed from LNS females who made the exact inverse choice (Fig. 3.3, 3.4, 3.5, 3.6). This pattern contrasts with Rosenqvist and Houde (1997) who showed that only females reared in a social context with high variance level of orange subsequently discriminated among orange males. Alternatively, in my study, even low variance treatments (HNS and LNS groups) provided a good ground for females to make subsequent choice once adult because, if, overall the variance is low, there is variation in the colours making up the “total colour” trait. On the whole, females from low variance treatments exhibited disassortative mating regarding the phenotypes experienced as juveniles. This pattern appears to emphasize preferences for novel and/or rare male phenotypes recalling previous findings on learned mate preferences in adults (Farr 1977; Eakley & Houde 2004; Zajitschek & Brooks 2008) and in immature individuals (previous work seen in chapter 2).

Finally, the results provided some insight into the attribute of the trait “total colour”. The absence of interest in black pigmentation (a colour on which genetical preference are based) displayed by long-exposed females seems to indicate that “total colour” is seen as a single trait. If the preference was an artefact of multiple preferences for diverse colour patterns, it’s likely that females would have responded to variation in black colouration found in the experimental trials. The

clear-cut preference or dislike for total colour in response to the rearing treatments also suggests that it is a trait in itself. No matter how “total colour” was made up, females were choosing males according to the extent of body surface covered, confirming that “total colour” is a trait in itself on which a specific preference operates.

3.5.1. Individual variation in female mate choice and evolution of male sexual traits

Individual variation in the different aspects of mate choice has profound influences on the strength, the direction and the mode of sexual selection on male traits (Jennions & Petrie 1997; Brooks & Endler 2001b; Cornwallis & Uller 2010). The results of this study provide insights into how early life experience alters the strength and direction of genetically based preference in the guppy mating system. An increase in responsiveness, as it is the case for females from the LNS treatment, might relax the pressure on preferred traits because female tend to accept more males. In the absence of other aspects of mate choice or other extraneous factors (e.g. male-male competition or male mate choice), non-preferred traits might expand in the population. However, when females express directional preferences (determined genetically or environmentally like in my study), evolutionary change might be annihilated because of contrasting selection pressures exerted independently by the two behavioural components. Alternatively, an increase in discrimination (see chapter two) associated with strong directional preference might stimulate female to spend more time and energy searching and evaluating suitable males. In this situation, the interaction of choosiness and preferences work together to impose a selection regime that follows the shape of the preference function. Consequently, to understand the overall strength and direction of selection on males, it is crucial to take into account how these components interact to produce a final mating decision (Bailey 2008). Although focused on the effect of early social life, it is important to remember at this point that many other biotic and abiotic factors need to be contemplated to grasp how preference function and choosiness are shaped. The female condition, for example, is particularly relevant to the expression of choosiness (Jennions & Petrie

1997; Widemo & Saether 1999). Eventually, as sampling tactics adopted by females might also interfere with preference function and choosiness, it's worth considering this parameter when analysing potential interactions between components of mate choice.

These results also contribute to a long debated discussion on the maintenance of additive genetic variance for traits under sexual selection (Kirkpatrick & Ryan 1991; Tomkins et al. 2004; Kotiaho et al. 2008). In guppies, early social experience could lead females to promote rare or novel phenotypes relatively to common ones (phenotypes experienced during ontogeny are the common ones) and thus creating a rare-male effect whereby rare males have a higher mating success. Moreover, Olendorf et al. (2006) demonstrated that males with rare colour patterns survived better, perhaps as a result of predator search images for common males. These two factors operating together can maintain the extreme polymorphism found in male colour pattern because of the negative frequency-dependent selection they exert.

These results highlight another evolutionary mechanism that could maintain genetic variation in sexual traits that influence male fitness. Spatial or temporal heterogeneity in environmental signalling conditions may induce fluctuating sexual selection on male ornaments. For instance, Gamble et al. (2003) showed that variation in incident light spectrum affected female responsiveness and male mating tactics, both of which influencing the mode and strength of sexual selection on male traits in different environment leading potentially to the maintenance of polymorphism. Here, I demonstrated that the timing of juvenile exposure to varying value of the trait "total colour" determined whether females used colour patterns or other sexual cues to discriminate among males. After having been in contact with males during their whole development, females varied in their choice accordingly to the distribution of male phenotypes experienced. If the phenotypical environment varies from one generation to another due to differential gene flow, various predation regime or any other environmental factors, learned mate preferences impose fluctuating selection pressures that in turn may maintain genetic variation in male ornaments. Alternatively, if females rely on other sexual cues to make a

choice, as it is the case when short-exposed to males, colour patterns are not directly selected for or against anymore and their evolutionary trajectory become either stochastic or dependent on the genetic architecture of sexual signals as a whole. Gametic phase disequilibrium, pleitropy and/or epistatis between alleles coding for sexual traits lay the ground for indirect selection on characters that are not the primary target of female choice. In guppies, several studies have shown Y linkage in traits related to mating success (Lindholm & Breden 2002; Postma et al. 2011) such as courtship (Farr 1983), colour patterns (Winge 1927; Brooks & Endler 2001a; Tripathi et al. 2009b), tail area (Brooks & Endler 2001a) or body size (Hughes, Rodd & Reznick 2005) and covariation among male sexual traits (Brooks & Endler 2001a; Postma et al. 2011). Depending on the nature, the sign and the strength of genetic correlations, indirect selection might favour the maintenance of a high level of polymorphism in colour patterns or on the contrary lead to reduced additive genetic variance.

Despite that not being in contact with males during a significant part of the development is rather unlikely for growing females in natural conditions, my results gave rise to the existence of a temporal factor in the acquisition of mate preference. The large difference between short- and long-exposed females in learned mate preferences suggests that a narrower range of temporal variation in duration of exposure would also generate some differences in mate choice. Refining the scale of variation in length of exposure offers interesting avenues for future research and will allow approaching realistic conditions found in the wild.

3.5.2. Individual variation in female mate choice and evolution of female preferences

Socially induced flexibility in female preference has several consequences for the evolution of female mate preference itself. The evolution of mate choice occurs in response to direct selection on genetic variation in choice and to indirect selection that operates on genetically correlated traits. Direct selection can operate on choice essentially in three ways: direct benefits (and direct costs), sensory bias

and sexual conflict. In a promiscuous mating system as it is the case in guppies, direct benefits are rare or non-existent since males don't provide any other resources than their genes. There is, however, an exception if males vary in their insemination abilities (Pilastro et al. 2002) or provide fecundity benefits (Pilastro et al. 2008). In parallel, reproduction in guppies can incur costs owing to increased exposure to predation (Godin & Briggs 1996), sexual harassment (Magurran & Seghers 1994) or parasitic infection (Cable et al. 2002; Johnson et al. 2011) that in turn oppose the evolution of female mate preference. Adjusting preference functions or choosiness in response to the social environment experienced as a juvenile allow diminishing associated costs and thus increase females fitness. Despite the critical role of costs in the expression of mate choice revealed by theoretical work, very little studies have quantified these costs (Head, Wong & Brooks 2010). Measuring costs, how they could be reduced (through early life experience for example) and how they influence the outcome of mate choice evolution are key questions in contemporary study of mate choice. Variation in mate preference due to sensory bias and its consequences on mate choice evolution is the topic of chapter five, thus not addressed in this discussion.

Although measures of heritability of mate choice behaviour haven't been carried out, the results indicated that the phenotypic variance observed in female mate preference is mainly environmentally induced. If the social environment account for most of the variation found, the evolution of preference depends on the heritability of genes coding for learning abilities and not anymore on preference genes per se. However, we cannot rule out the existence of "preference for rarity" alleles appearing either by mutation or through genetic assimilation (West-Eberhard 2003; Crispo 2007). Such phenomena would increase the genetic component of variation for that preference and allow selection to operate. Kokko et al. (2007) provided significant insight modelling the evolution of preferences for rarity. When the preference is rare, it spreads in the population following a Fisherian evolutionary process until the sons produced are more and more common and lose their mating advantage. At that point, over-represented females carrying the preference allele(s) for rarity decline in frequency as they select against the phenotype previously rare but presently common. Ultimately, the

mating preference is maintained by negative indirect frequency-dependent selection and reach an equilibrium value that depends mainly on the type of preference (absolute or relative) and the number of different male phenotypes present in the population (Kokko et al. 2007).

Finally, individual variation in female mate preference opens a field of investigation that remains largely unexplored despite its potential to extend the understanding we have of mate choice evolution. Whether preference is the result of alleles coding for the preference itself or alleles coding for learning capabilities, in both case it could be subjected to genotype-by-environment interaction (GxE). The possibility that the relative performance of different genotypes are dependent on the environments in which they are expressed has consequences on direct and indirect benefits that female can get from non-random mating, on the maintenance of variation in male sexually selected trait (Kokko & Heubel 2008) and on the strength of the genetic correlation between preference and attractiveness (Rodriguez & Greenfield 2003; Narraway et al. 2010). Although more and more empirical studies, across various taxa, demonstrated that variation in the social environment induce variation in mate preference, very few tested explicitly for GxEs (Rodriguez & Greenfield 2003; Ingleby, Hunt & Hosken 2010; Narraway et al. 2010). In such species, the first step would be to identify the existence, the occurrence and the strength of GxEs in both male traits and female preference. The next step is to test whether these GxEs have fitness consequences and finally analyse their effect on the joint evolution of preference and signals.

In the next chapter, I turn my attention to the effect of another parameter of the social environment provided by males. In the last two chapters, growing females were exposed to varying values of sexual traits to which they displayed genetically based preference or, on the contrary, non-genetically based preference. In chapter four the focus is out on the level of phenotypical variance independently of the value or the message conveyed by a sexual signal in itself.

4. Chapter IV

Female mate preferences mediated through prior exposure to male overall phenotypic variance

4.1. Abstract

Female mating preferences are often flexible and reflect variation in the social and ecological environment. Several studies analysed the role played by the early social environment and showed its importance in mate choice of various species. In these studies, different social environments were represented by different sexual trait values of the males with whom the females interacted. Although essential, this approach does not capture all the distinctive features of male phenotypes. Hence, other phenotypic aspects could be important in the formation of mate preferences if, for example, they provide additional information about the males available. I explored how the level of overall phenotypic variance (independently of the sexual traits' values) experienced during ontogeny, mediated female mate preference in guppies, *Poecilia reticulata*. In order to do so, maturing females were reared during their whole development with males either all different (high variance) or all similar (low variance) or with adult females (no variance). I found that females reared in the high variance treatment strongly increased the strength of their preferences for some colours compared to the other groups. Moreover, females reared in the presence of females were more sexually responsive than females reared in the presence of males. For their part, males switched mating tactics in response to choosier females, increasing the rate of coerced copulation attempts, possibly to balance the loss in mating opportunities. Taken together, these results demonstrate the adaptive plasticity of female mate preferences and the dynamic selection they might impose on male traits.

4.2. Introduction

Sexual selection by female mate choice is widely accepted as a powerful source of selection (Andersson 1994; Andersson & Simmons 2006) and thus a strong agent of evolution. Variation in mating preferences arises through a balance between genetic determination and environmental determination (Brooks 2002; Pfennig et al. 2010; Schielzeth et al. 2010). Examining how these determinants affect variation in preferences is thus essential to understand the evolution of both sexual traits and mate choice (Widemo & Saether 1999) or the evolution of reproductive isolation (Svensson et al. 2010). Although important, the effects of development and the early rearing environment remains poorly understood (Jennions & Petrie 1997). Developmental plasticity enables juveniles to evaluate environmental conditions and adaptively shape behavioural and morphological traits to maximize fitness later in their adult environment (West-Eberhard 2003). Acquiring information about potential mates prior mating may help females to make an optimal decision and decrease the costs associated.

Different sensory learning modes can be involved in the development of individual mate preferences following a period of exposure to sexually mature adults. Sexual imprinting via visual (Kendrick et al. 1998), acoustic (Riebel 2003, 2009) and olfactory (Penn & Potts 1998) cues has been extensively studied. It is usually observed in species with parental care, where the young individuals use the parent of the opposite sex as a model upon which they will base their subsequent mate preferences. Parental imprinting (e.g. sexual imprinting using either the male or the female parent as a model) function as a mechanism to secure an accurate recognition of conspecifics as prospective partners and thus avoiding the cost of heterospecific matings.

However mate preferences can also be formed during the developmental period in species that lack parental care. This other type of imprinting is referred to as “oblique imprinting” and hasn’t been explored sufficiently. Little is known about how the phenotype distribution of sexually signalling individuals in species with no

brood care influences the development of mating preferences and contribute to the variation in mate choice found within population.

To date, ten or so studies have addressed the possibility that early exposure to mature non-genetically related males could shape female mating preferences. The Poeciliid fish family (Breden et al. 1995; Rosenqvist & Houde 1997; Walling et al. 2008; Verzijden & Rosenthal 2011) and the *Schizocosa* genus in wolf spiders (Hebets 2003; Hebets & Vink 2007; Rutledge et al. 2010) are the model systems that have provided conclusive evidence on the role of early social experience shaping subsequent mate preferences. Two other studies have expanded our current knowledge in early acquisition of mate-preference across taxa: female field cricket – *Teleogryllus oceanicus* – adjust their responsiveness to signalling males depending on the acoustic environment they experienced during ontogeny (Bailey & Zuk 2008) and females butterflies *Bicyclus anynana* alter their mating preferences after exposure to males with enhanced wing ornamentation (Westerman et al. 2012).

The aim of my study was to broaden the understanding we have about the type of social environment that mediates the acquisition of mate preferences. In the previous studies, even though the effects of the ontogenetic conditions were not identical among species, the experimenters had always manipulated qualitatively or quantitatively sexual signals known to be good predictors of male mating success in the population and the species under scrutiny. For instance, Rosenqvist & Houde (1997) or Breden et al. (1995) reared young female guppies with males varying in the value of traits (orange colouration) that have proven to be selected for by females of the same population. Walling et al. (2008) varied the size of the sword – an important criterion of mate choice in the genus swordtails - to which growing females were exposed to. Similarly, studies carried out on wolf spiders addressed the effect of exposure to artificial phenotypes by modifying qualitatively the sexual signal either in the visual modality (brushed legs) (Hebets 2003; Hebets & Vink 2007) or in both chemical and visual modalities (Rutledge et al. 2010). In the present study, I consider another aspect of the social environment that is experienced by growing females during ontogeny, the distribution of male

phenotypes. The focus is not solely on variation in traits that influence directly male attractiveness but now on the phenotypic distribution as a whole. Rather than varying the value of a trait under sexual selection, I examine the effects of experience with varying levels of among male variance (independently of the value of the sexual signal itself) on female preference acquisition. The extreme polymorphism in colour patterns exhibited by guppy males and the strong component of social environmental variation found in female mate preferences are features that conduced me to use guppies as a model system. Conceptually, preferences can be divided into preference functions and choosiness (Jennions & Petrie 1997; Widemo & Saether 1999; Brooks & Endler 2001b). Preference functions come from the rank order of stimuli, and choosiness is the relative investment into mating with the preferred stimulus. Both components contribute to a final mate choice and are thus relevant for sexual selection. I therefore included both of them in the analysis. I also investigated the possibility that males shift their reproductive tactics – courtship display versus sneak matings (e.g. forced copulation attempts) – in response to potential changes in female mate preferences.

4.3. Method

4.3.1. Study organisms

Guppies are small livebearer freshwater fish native to the coastal streams of northeastern part of South America. Those used in this study are third and fourth generation descendants of individuals collected in the lower part of the Aripo river in Trinidad in March 2008 (N 10°. 39.031; W 61°13.404; 37m altitude). Neonates are picked up from housing tanks at day 5 post-birth and placed into treatment groups. All fish housed in the laboratory are maintained on a 12h light:dark cycle at 24°C. They are fed twice daily: in the morning with commercial flakes and in the afternoon with brine shrimp (*Artemia*). All the rearing tanks are covered by a gravel substrate and aerated through an undergravel filtering system. Plastic plants are placed into the tanks to physically enrich the environment of the fish and to let them have some room to hide.

4.3.2. Experimental treatments and experimental settings

Neonates were separated and reared during the whole duration of their development with one of three groups of males representing three levels of phenotypic variance (high, low and no variance, see fig. 4.1). The “high variance” treatment is composed of males, which differ greatly in their phenotypes, the “low variance” treatment is achieved by assembling males having nearly identical phenotypes, and the “no variance” treatment is composed of mature females with a mating history (no body colouration). Once mature, focal females are tested for their mating preferences. Due to the Y-linked inheritance of a large part of the colour pattern alleles (Winge 1927; Haskins & Haskins 1951; Yamamoto 1975; Houde 1992), the “low variance” condition is represented by collections of half or full brothers. Guppies are highly polymorphic so the “high variance” condition consists of mostly unrelated individuals. To ensure heterogeneity between males’ phenotype in the “high variance” condition, I used a variant of the Simpson’s index of Diversity named the Simpson’s Reciprocal Index. The inverse Simpson diversity gives the number of equivalent equally common colour classes (see appendix). The experiment consists of four replicates of each treatment.

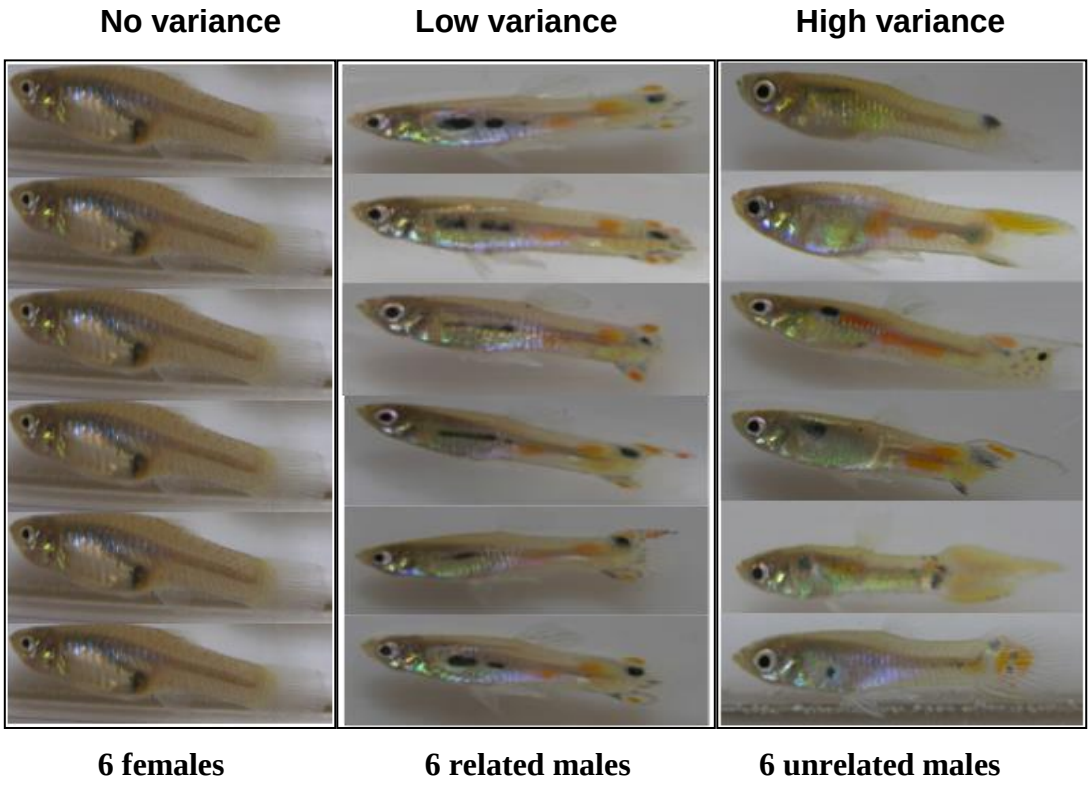

Figure 4.1: Example of one replicate made of the three different level of phenotypic variance to which fry are exposed.

Males composing the “low variance” treatment are similar as nearly all of the elements (patches) coincide in both colour classes and topographic position but might slightly vary in their shape (see fig. 4.1).

Fry were reared in compartment 1 (30cm X 30cm X 18cm) separated by transparent Perspex partition from compartment 2 (15cm X 30cm X 18cm) into which six “stimuli” males or 6 females are placed. The partition is not sealed which allows olfactive cues to pass from one compartment to the other. This design enabled developing females to grow in situations close to natural conditions. Within the groups of fry, to prevent any fertilization of the experimental females, males are removed before reaching sexual maturity, that is before the gonopodial hood extends beyond the tip of the fin (Reznick 1990). The transparent partitions are not sealed to the edge of the tank, which allows the developing fry and the stimuli fish to communicate through visual and olfactory cues.

4.3.3. Behavioural trials

Following Houde (Houde 1987; 1988c; Houde 1997) and Grether (2000; 2005), mate choice is measured by observing females' response to males' courtship in a 40 litre open aquarium (see fig. 4.2). Female preferences were analysed in terms of variation in male attractiveness (see appendix), that is, by calculating the effect of particular male traits on females' responsiveness. It is a standard method used in the study of mate choice in guppies and is biologically relevant as it allows the expression of the full repertoire of sexual behaviours between males and females (Houde 1997).

Each experimental group consisted of the same numbers of individuals of both sex (in general 6 males and 6 females). Within a group, males were randomly drawn from different stock tanks where they developed and grew in physical contact with mature females. They display different colour patterns and different sizes. Females in a given test were from the same rearing treatment and had not been exposed during development to the phenotypes of the experimental males. The day before the trial, virginity of the tested females is eliminated to ensure the full expression of their preference, as naive females are known to show little mate discrimination in their first matings (Endler & Houde 1995; Houde 1997; Hughes et al. 1999; Brooks & Endler 2001b). To do so, they are placed individually in 4-l plastic tanks, containing gravels and a plastic plant, for approximately 5 hours with one male that hasn't been experienced during development and that won't be seen during the experiments. On the day of the trial, females were released in the testing tank two hours before the observation started to let them acclimatize to the new environment.

Observation sessions involved 5 min focal observation of each male in turn, in random order. Three sessions in the morning and three sessions in the afternoon were carried out. At the end of the last afternoon session, I continued to observe males for 20 min (focal of 30 sec/male) to ensure that all females have been visited. After the daily experiment, females were released in housing tanks and males were kept in the observational aquarium to be reused until all the females from the same replicate had been tested. Male colour patterns were sketched to

ease the distinction between males but it was in general easy to tell them apart. After all the females of one replicate (females from the three different treatments) were tested, experimental males were changed.

During each focal observation, I recorded each of the male's sigmoid displays (Houde 1997) and the female's response to these courtship displays. The relative attractiveness of a given male to females in an experimental group is estimated as the proportion of his displays that elicit at least a "glide" response (the "fraction response" D; see table 4.1 for details of the female sexual response). Individual females are not distinguishable so D represents an aggregate measure of the preference of all females in the experimental group for that particular male. D is a reliable predictor of male mating success (Houde 1987, 1988a). Furthermore, D controls for variation in display rate among males, which can affect female preferences (Farr 1980). The degree of preference for a sexual trait is calculated as the regression of D on that trait for all males used in that session. The slope of the regression is a measure of the overall degree of female preferences for that sexual trait in a given observational trial. Males that perform less than five displays throughout the observation sessions are excluded from the analysis. Male displays were recorded only if they were directed toward a particular female, if other males do not interrupt them and if they started after the male becomes the focal male. In addition, the number of sneak attempts was recorded for each focal male during the 6 sessions of observation but not during the last twenty minutes.

Being more biologically relevant than other experimental design like dichotomous choice settings, the open aquarium design has also its shortcomings. The main one, here, is that the outcome of mate choice could be influenced by male-male competition. Studies have shown that mate competition in guppies increases with male-biased operational sex-ratio (Jirotkul 1999) or when males have been reared together (Houde 1997). Accordingly, experiments were always conducted at an even sex ratio and males were drawn from different stock tanks.

The tests were conducted in a windowless room presenting the same lightning characteristics as the room in which the fry were reared. The observation aquarium was covered with natural gravel on the bottom and opaque paper on

three sides; the observations were made from the fourth side (Fig. 4.2, 4.3). The aquarium was illuminated with daylight spectrum fluorescent tube and one incandescent bulb placed above the tank (40 W) yielding a light intensity at the water surface of roughly 900 lux.

Figure 4.2: Aquarium used for the observational trials

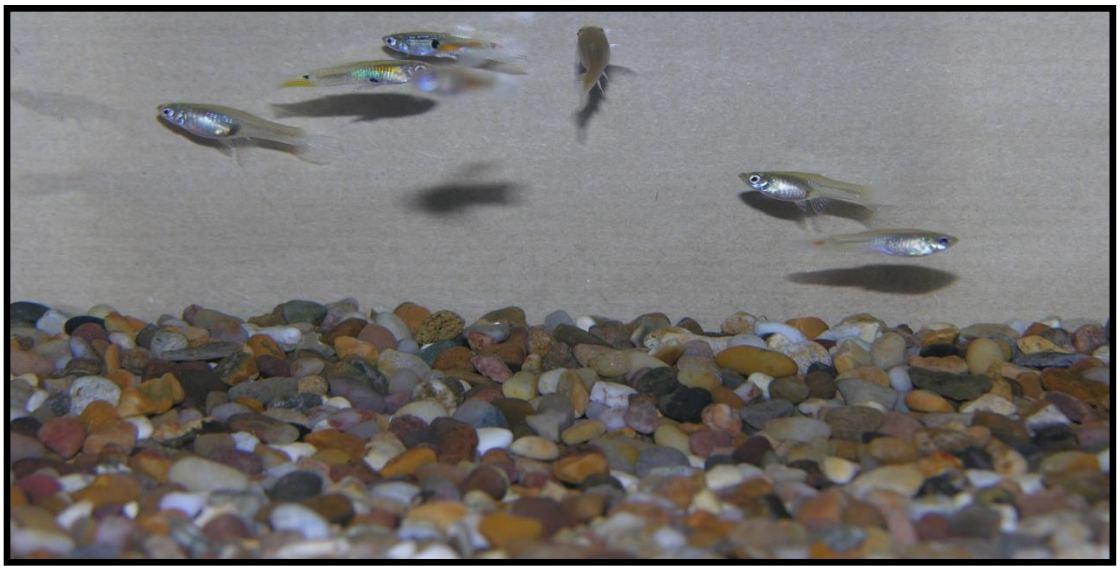

Figure 4.3: Zoom of the observer's viewpoint during experimental trials

Table 4.1: Measure of preference = D^1 =∑ (score ≥ 2) / ∑ (all male display)

Score	Female and Male behaviour
0	No response; Female ignores male
1	Female orients toward male but does not move closer
2	Female glides toward the male
3	Male and female circle around each other
4	Copulation attempt; mate thrust and makes gonopodial contact
5	Copulation; gonopodial contact followed by male jerk

[1]aggregate measure of preference of all females in the group
[1]relative attractiveness of a given male to all females in the group

4.3.4. Male trait analysis

Male colour patterns were photographed with a digital camera (Nikon coolpix 8800) in a hand-made box filled with a small volume of water where fish are free to swim. All the pictures were taken under the same light conditions when the fish was parallel to the glass of the box. Both sides of the each guppy were photographed and the images analysed with the UTHSCSA ImageTool program (developed at the University of Texas Health Science Center at San Antonio, Texas and available from the Internet at http:// compdent.uthscsa.edu/dig/itdesc.html). Colour patches were grouped into the following colour classes: black, orange (including red), yellow, iridescent (including silver/white, blue, violet, and bronze-green) and total colour area. The colour classes were measured as relative total area (relative to the body + caudal fin) and number of spots per colour class. The data for each male consists of the mean of the right and left sides of the body for both relative area and of the right side for the number of spots. The total body length (from the tip of the snout to the tip of the longer lobe of the caudal fin) of each male was recorded using a digital caliper.

A measure of the diversity of the colour pattern was calculated for each male. This male trait, which is rarely examined in the study of female preference, was computed with Simpson's Reciprocal Diversity Index. The values span from 1 to X with X being the number of categories being used (for example if there are five colour classes, the highest possible value is X=5 when each of the 5 colours have equal areas on the guppy). The lower the value the less diversity and vice versa (see appendix).

4.3.5. Female preference analysis

There are different ways to measure a female sexual response. Following a terminology used in two seminal reviews (Jennions & Petrie 1997; Widemo & Saether 1999) extended by Brooks and Endler (2001b), we can divide individual female sexual behavior into three measurable components (see fig. 4.4):

- Choosiness that is the investment into mating itself sub-divided in:
    - Receptivity (or mean responsiveness) defined as female willingness to respond positively to male solicitations and measured as the mean response to the displays of all males in a trial.
    - Discrimination (selectivity) describes the degree to which females distinguish variation in male traits.
- Preference function that is the ranking order of male sexual signals; measured as the relationship between females' response and the male trait(s) they are evaluating (see fig. 4.4).

Data in the example of figure 4.4 are fictional and represent the response of females in an observational group to 12 males displaying a gradient of a sexual trait

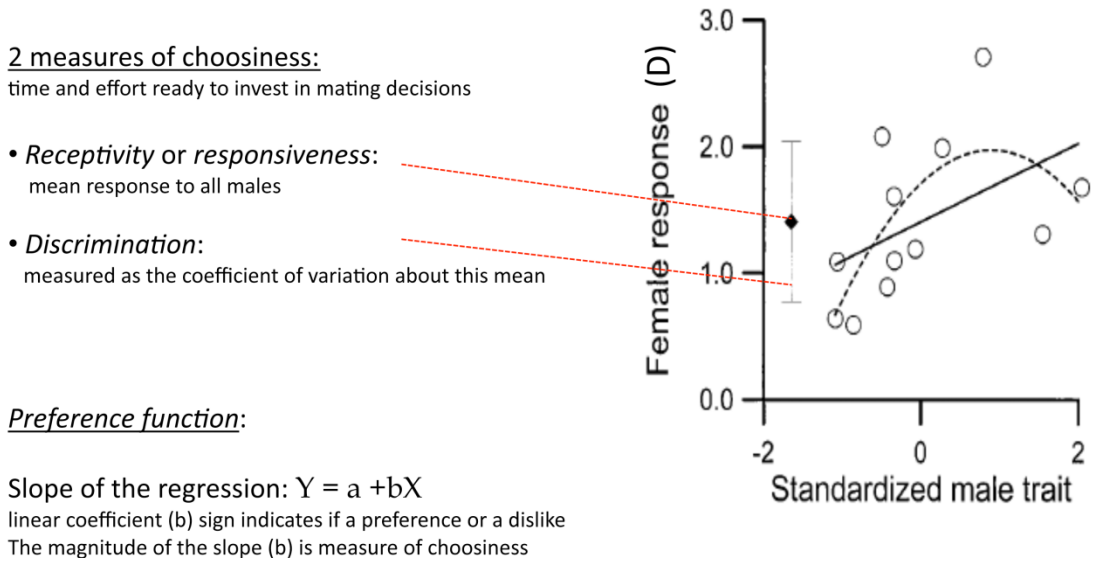

Figure 4.4: Diagram showing how female sexual behaviour is divided into choosiness and preference function.

The variables that were measured as proportions (response rate, relative colour areas) were angular transformed to meet the assumptions of normality and homoscedasticity. For the count variables (displays and sneaky attempt), I used a square-root transformation after which parametric assumptions were met.

I tested for treatment differences in females' responsiveness using a linear mixed model with female response (D) as the dependent variable and treatments and male characters as fixed effects. The male traits included as continuous covariates were relative area of orange, yellow, black, iridescent, total colour and total body length. Main effect and interaction factors that were not significant were removed from the final model. Because of the possible non-independence of the behaviours of females (and males) within a single group (cluster data), I used a mixed-model version of repeated-measures ANOVA, which I implemented with the "subject" and "repeated" options within MIXED procedure in SPPS (IBM SPSS statistics release 19.0.0). Males within a single group were treated as repeated measures on the dependent variable. For the repeated measures I employ a

heterogeneous covariance structure in which behaviours between treatments are allowed to have independent covariance structure.

I used the same kind of statistical model to test for differences in display rates and sneak copulations across the different rearing conditions. Again covariates and interactions that were not significant were not included in the final model.

For each male character, the overall degree of female preference was analyzed among treatments. To do so, preference slopes of these characters were compared using a Kruskal-Wallis procedure, followed by multiple comparisons if the main analysis was found to be significant. Finally, I tested for a correlation between female preference slopes and the level of variance experienced as juveniles using a spearman rank correlation.

4.4. Results

4.4.1. Responsiveness

The general linear model analysis controlling for the effects of the covariates indicate that females from the no variance group (female stimuli) are more responsive than females from higher variance (male stimuli) groups ($F_{(2;\ 24.8)} = 8.8$; p=0.001, see table 4.2 and fig. 4.5).

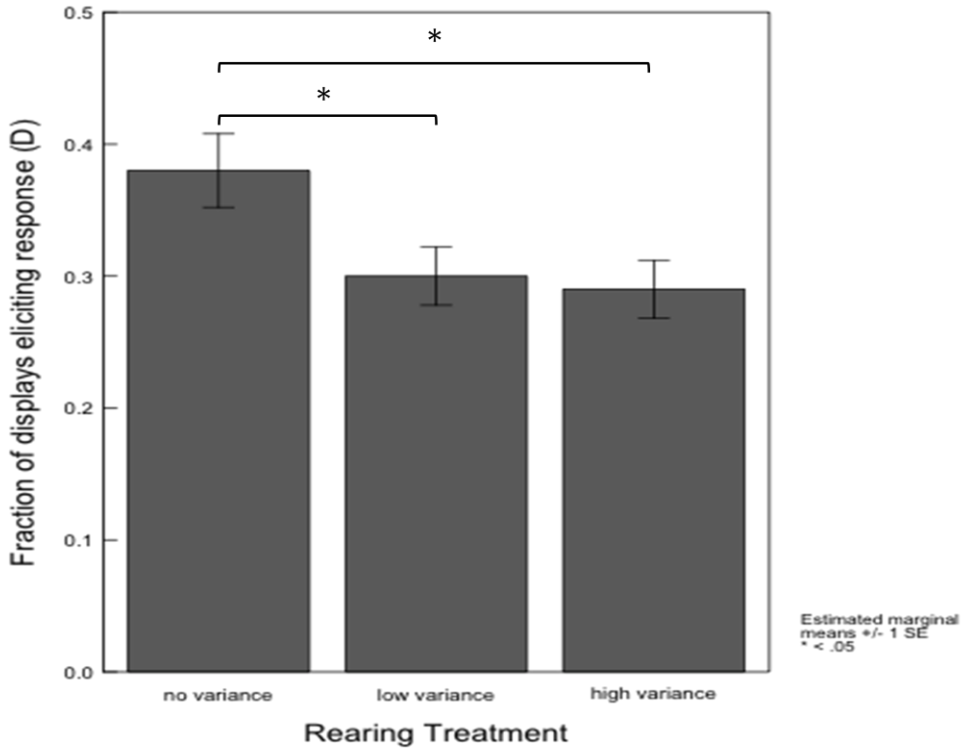

Figure 4.5: Fraction of female responses in the 3 different treatments. Values are reported in table 4.2.

Females could vary in responsiveness due to confounding factors (other than their ontogenetic experience) such as males' behaviour (i.e. male display rate). The model shows that there is no significant differences ($F_{(2;\ 19.4)} = 1.8$; p=0.18, see fig. 4.6 and table 4.2) in male courtship displays directed towards females reared in the different conditions.

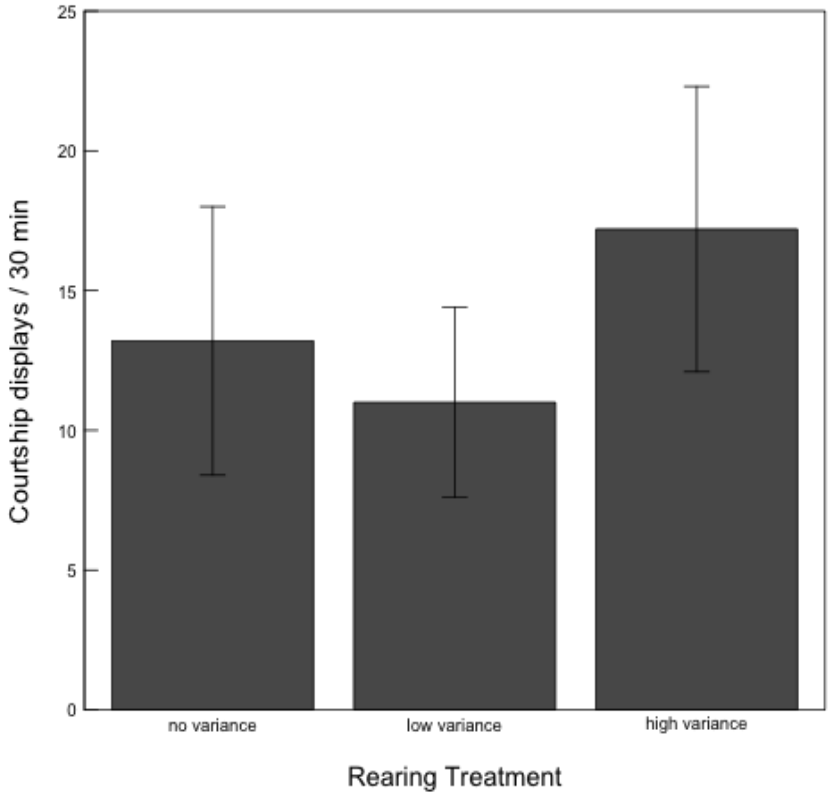

Figure 4.6: Total male displays directed towards females reared in the 3 different treatments. Values are estimated marginal means +/- SE and are reported in table 4.2.

Table 4.2: Estimated marginal means (SE) for female responses and male displays

	No variance	Low variance	High variance	F-value	*df*	*p-value*
Female response	0.38 (0.03)	0.3 (0.02)	0.29 (0.02)	3.98	2; 11.8	0.048
Male display	13.2 (4.8)	11 (3.4)	17.2 (5.1)	0.53	2; 8.8	0.606

4.4.2. Linear Preference function

Female preference functions for male traits were estimated as the linear regression slope coefficients of female response on the male trait being evaluated. I estimated the female preference function for each male trait (orange, yellow, black, iridescent, total colour area, total body length and diversity of colour pattern) separately within each observational tank. For each treatment, I carried out four observational sessions yielding four slopes per treatments per male traits. In chapters three and four, I demonstrated the importance of the phenotypic distribution of specific sexual traits experienced during ontogeny in the subsequent expression of mate choice. Accordingly, we need to ensure that observed differences between treatments resulted from variation in the overall phenotypic variance per se and not variation in a certain colour. To do so, I compared the average values of sexual traits experienced by females in high- and low-variance treatments and for which females displayed a preference during the tests. Comparisons were not made with the no-variance treatment as a matter of course.

4.4.2.1. Preference for relative yellow area

Overall there was a difference in yellow preference between treatments. Females that had experienced different phenotypes during development showed a stronger preference for yellow than females having experienced similar males. When treatments are ordered from “no variance” to “high variance”, there is a significant positive correlation between the strength of yellow preference and the amount of phenotypic variance experienced as juveniles (see table 4.3, figure 4.7). Females from high- and low- variance conditions didn’t experience different values of yellow during ontogeny ($t_{(46)}$= -0.66, p=0.52) confirming the true effect of the treatments.

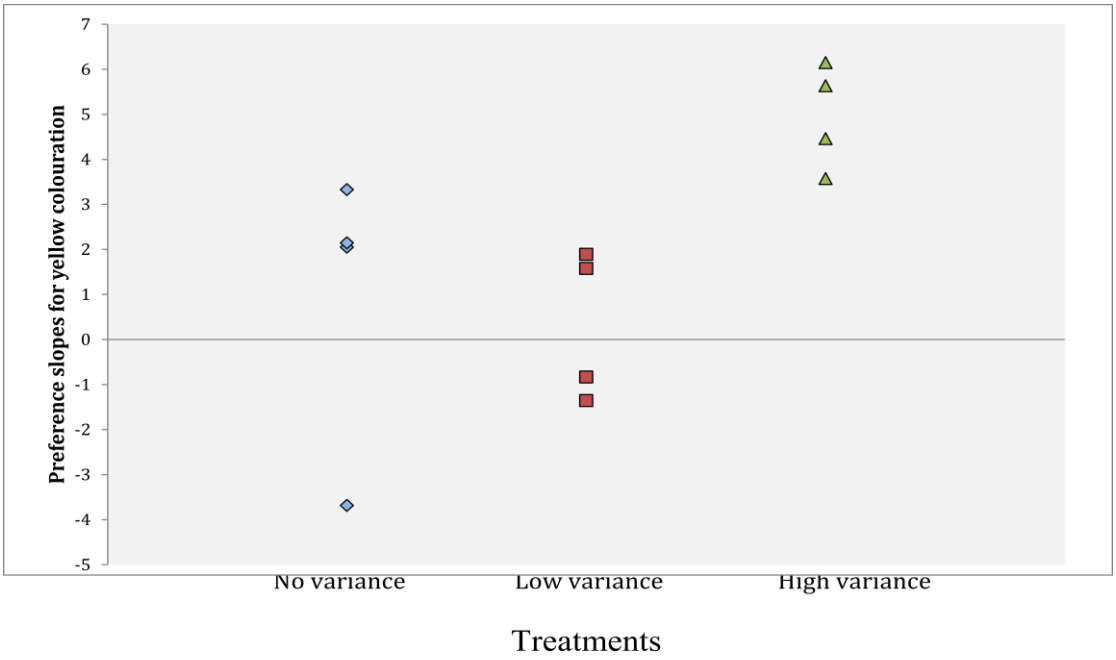

Figure 4.7: Degree of female preference for relative yellow area across rearing treatments. Each point gives the degree of preference in one observation session.

4.4.2.2. Preference for relative black area

Overall, there was a difference in black preference between treatments. Females that had experienced different phenotypes during development showed a stronger preference for black than females having experienced similar males (see table 4.3, and fig. 4.8). No correlation was found between treatments and preference for black colour. Females from high- and low- variance conditions didn't experience different values of black during ontogeny ($t(34)= -1.54$, $p=0.13$) ruling out the possibility that females differed because of variation in black spots.

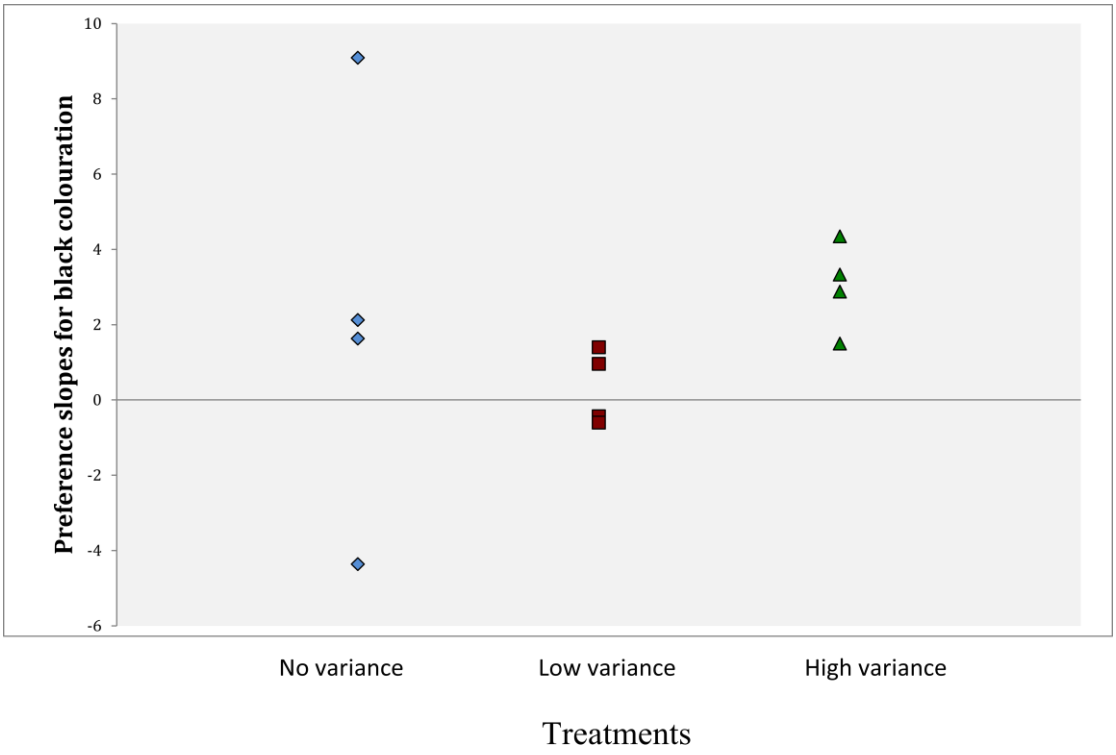

Figure 4.8: Degree of female preference for relative black area across rearing treatments. Each point gives the degree of preference in one observation session.

4.4.2.3. Preference for total colour area

Overall there is a difference in total colour preference between treatments. Females that have experienced different phenotypes during development show a stronger preference for males bearing large amount of colour than females having experienced similar males or females. When treatments are ordered from “no variance” to “high variance”, the correlation between the strength of total colour preference and the degree of variance in male phenotypes experienced as juveniles is not significant (see table 4.3 and fig. 4.9). Further, females from high- and low- variance conditions didn’t experience different values of total colour during ontogeny ($t_{(46)}$= -0.97, p=0.34) confirming the real effect of the treatments.

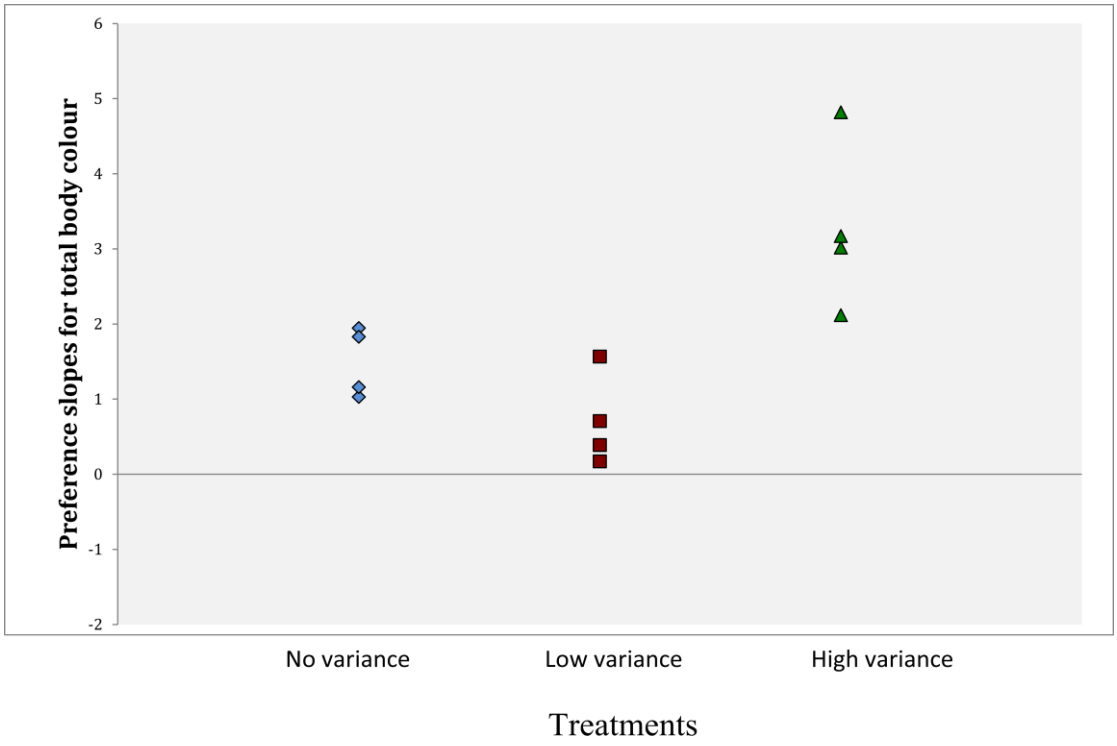

Figure 4.9: Degree of female preference for the total colour area across rearing treatments. Each point gives the degree of preference in one observation session

4.4.2.4. Preference for total body length

Overall there is a difference in female preference for male size between treatments. Females that haven't experienced any male phenotypes during development show a stronger preference for smaller males than females having experienced similar males. By contrast, there is no significant difference between females from the "high variance" condition and females never been in contact with males prior to the test phase (see table 4.3 and fig. 4.10).

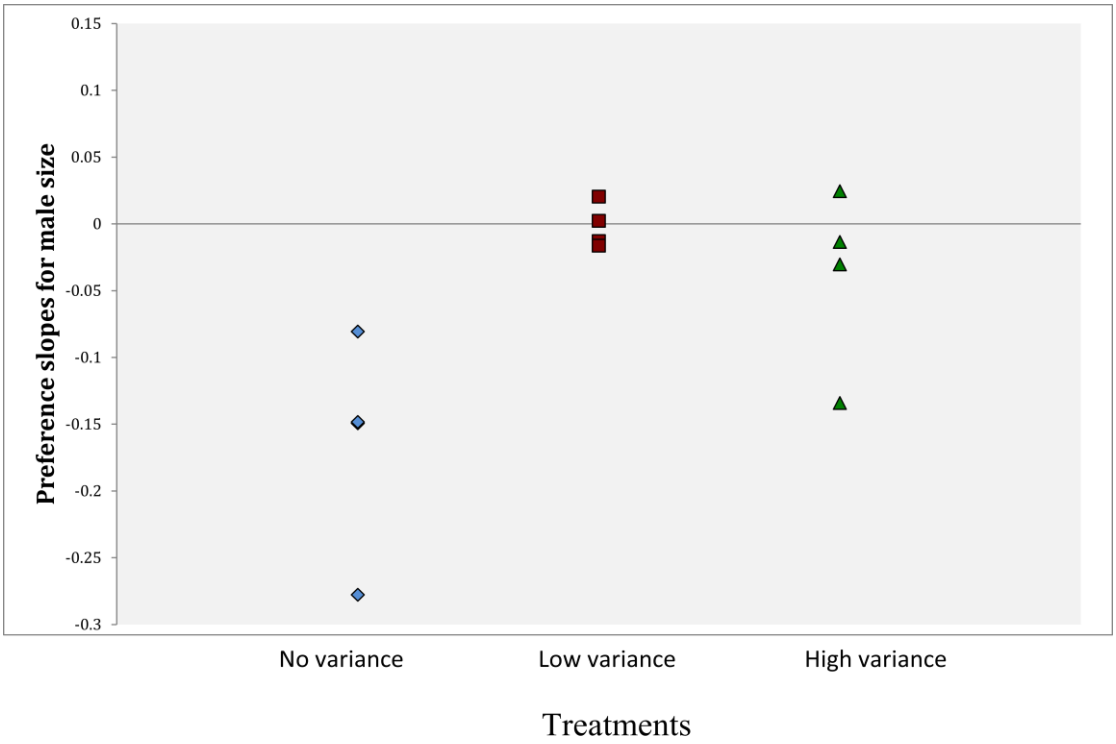

Figure 4.10: Degree of female preference for male total body length across rearing treatments. Each point gives the degree of preference in one observation session.

4.4.2.5. Preference for colour pattern diversity

To my knowledge, there is no previous study that has analysed male phenotype diversity as a potential sexual cue. But it should be important because phenotypic variance was the explanatory variable manipulated across treatments. Hence we can predict that females might discriminate among males based on the level of colour pattern diversity. Overall there is a difference (significant before adjusted significance) in colour pattern diversity preference between treatments. Females that have experienced more diverse phenotypes during development show a stronger preference for males displaying a higher level of colour pattern diversity than females having observed similar phenotypes while maturing; however this result only approaches significance (see table 4.3, fig. 4.11). Again, females from these two conditions didn't experience different values of colour pattern diversity ($t_{(46)}$= 0.09, p=0.93).

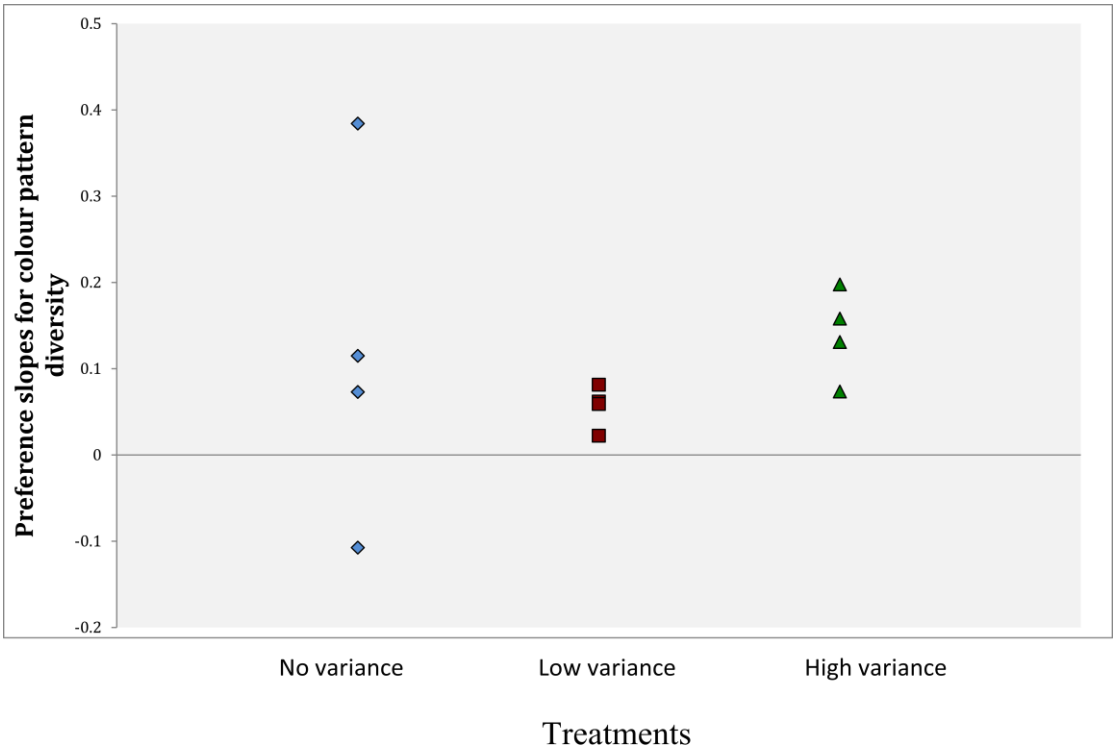

Figure 4.11: Degree of female preference for colour pattern diversity across rearing treatments. Each point gives the degree of preference in one observation session.

Table 4.3: Median of the preference slope for the three different treatments. Each row represents a trait that could affect the linear preference function of females. K-W is the Kruskall-Wallis statistic testing for differences in degree of preference between treatments; multiple comparisons between treatments; r_s is the spearman rank correlation coefficient between preference slope and the treatments ordered from "no variance" to "high variance"; n is the total number of observation session.

	Median of preference slope			Preference Differences		Multiple comparisons			Preference and Treatment	
						No variance - Low variance	No variance - High variance	Low variance - High variance		
	No variance	Low variance	High variance	K-W (df)	*P* –value **	Test statistic + adjusted significance	Test statistic + adjusted significance	Test statistic + adjusted significance	r_s	n
Orange area	1.94	1.14	2.61	3.04 (2)	0.073	-	-	-	0.12	12
Yellow area	2.10	0.37	5.05	8.0 (2)	0.000*	-2.0 p=1.0	5.0 p=0.15	-7.0 **p=0.018**	0.59 ‡	12
Black area	1.87	0.26	3.11	4.77 (2)	0.028	2.0 p=0.47	2.0 p=0.47	8.0 **p=0.014**	0.24	12
Iridescent area	0.24	-0.25	-0.18	0.04 (2)	0.808	-	-	-	0.03	12
Total colour area	1.49	0.55	3.09	8.77 (2)	0.000*	-3.0 p=0.72	4.5 p=0.23	-7.5 **p=0.01**	0.53 †	12
Total body length	-0.15	-0.01	-0.02	6.96 (2)	0.004*	6.5 **p=0.032**	4.75 p=0.19	1.75 p=1.0	0.56 †	12
Simpson's Reciprocal Index	0.09	0.06	0.14	3.85 (2)	0.049	5.0 p=0.49	5.0 p=0.49	1.0 p=0.06	0.3	12

** The significance level is based on the exact distribution of the test statistic providing an exact *p*-value
* Significant after Bonferroni correction for number of tests in column
\- No pairwise comparisons when no overall significant differences across treatments
† $p < 0.1$
‡ $p < 0.05$

4.4.3. Male alternative reproductive tactic

The general linear mixed model analysis, controlling for the effects of the covariates, indicates that males when exposed to females from the “high variance” group attempt more sneak copulations than when exposed to females reared in the two other conditions ($F_{(2;\ 26.7)}$ = 7.21; p=0.003; see fig. 4.12). Moreover, males attempt less sneak copulations towards females reared in the “low variance” condition than in the “no variance” condition. To compensate for a lack of attractiveness owing to non-preferred colour patterns, males might increase their rate of sneak attempts whereby they could get more mating than if performing courtship displays. To control for such a confounding factor, I look for a potential relationship between a male trait and the rate at which they sexually coerced females. It didn’t seem that males varied in their sneak attempts since no correlations were found to be significant after correction (table 4.4).

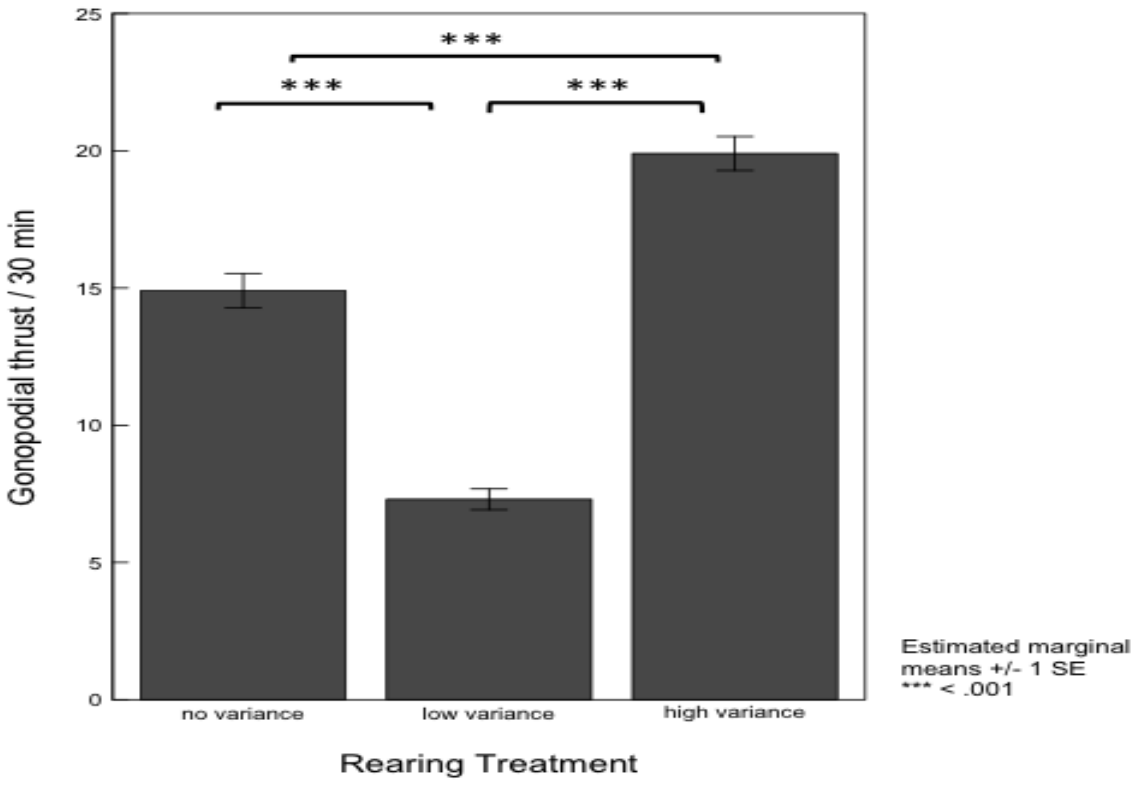

Figure 4.12: Gonopodial thrust per 30 minutes attempted by males to females reared in the 3 different treatments. Values are least square means +/- SE.

Table 4.4: Correlations between sneak attempts and various males traits carried out for each treatment.

Rearing treatments	Male traits	Spearman's rank correlation coefficients	*p-value* (2 tailed-test)
No variance	Orange relative area	0.04	0.89
	Yellow relative area	0.12	0.68
	Black relative area	0.04	0.89
	Iridescent relative area	0.2	0.49
	Total colour relative area	0.35	0.22
	Total body length	-0.62	**0.02***
Low variance	Orange relative area	-0.065	0.76
	Yellow relative area	-0.2	0.36
	Black relative area	-0.16	0.44
	Iridescent relative area	-0.18	0.39
	Total colour relative area	-0.31	0.14
	Total body length	0.2	0.34
High variance	Orange relative area	0.14	0.53
	Yellow relative area	0.25	0.27
	Black relative area	0.2	0.37
	Iridescent relative area	0.17	0.45
	Total colour relative area	0.33	0.14
	Total body length	-0.05	0.82

* Significant before sequential Bonferroni correction

4.5. Discussion

My findings demonstrate for the first time that variation in female mate preferences arise through experiencing variance in phenotypes as a whole during ontogeny. Females differ in both aspects of mate preferences under scrutiny, that is, responsiveness and preference functions for various male traits, after exposure to three different level of phenotypic variance during the whole period of development. I present evidence that female guppies decrease their responsiveness as level of phenotypic variance during development increases. Moreover, the strength of preference for male traits such as yellow body colouration or total colour area increases for females having experienced higher level of phenotypic variance. In response, males shift their reproductive tactics, augmenting the rate of forced copulation in the presence of females who were exposed to the highest level of phenotypic variance during maturation.

4.5.1. Phenotypic variance and responsiveness

Females' responsiveness in guppies is influenced by previous experience since females from the "no variance" treatment are more responsive to males' solicitation than females from the two other treatments.

Other factors than the ontogenetic experience can account for differences in female responsiveness such as their condition (Syriatowicz & Brooks 2004) or their age (Gray 1999). However, the experimental females were reared in the same laboratory conditions ruling out any differences in conditions. Moreover, females were tested at the same age excluding any age-related variation. Courtship display intensity can also influence responsiveness but in this study males were displaying at the same rate towards females reared in the different treatments (Fig. 4.6; table 4.2).

On the other hand, I did not find any evidence of difference in responsiveness between females having been exposed to the "low" or the "high" variance treatment (figure 4.5; table 4.2). This result contrasts with previous studies demonstrating that female guppies are more likely to respond sexually to the displays of males with rare colour pattern over males with common colour patterns (Zajitschek & Brooks 2008; Hampton et al. 2009). Even though different level of rarity in Zajitschek & Brooks (2008) and level of low and high variance in my study could be assimilated in terms of frequencies of males colour pattern, there are fundamental differences between the questions addressed in the two studies and thus different behavioural mechanisms involved. Zajitschek & Brooks (2008) and Hampton et al. (2009) investigated how sexually mature females accommodate their preferences to different level of colour pattern rarity (common or redundant vs. unique vs. novel colour pattern) found within their social environment. In my study, I analysed the effect of different level of colour pattern rarity (named in my study level of phenotypic variance) experienced during ontogeny on the acquisition of mate preferences. Once adult, experimental females were tested with new males (e.g. colour patterns), making all colour patterns as novel. Nevertheless, the difference in responsiveness could be explained by the nature of the fish used to make up different level of phenotypic variance as the "no variance" treatment is composed

of females and the two other treatments of males. Hence, seeing mature male for the first time could increase female responsiveness relatively to females having grown in visual and olfactory contacts with males. It appears that the gender of the fish composing the social environment experienced before maturity override the potential effect of the variance found in male phenotypes. Having not seen any males during maturation may mean that males apparently represent a limited resource in the local environment, urging females to augment the willingness to respond positively (e.g. being more responsive) and thus engaging more into sexual behaviours relatively to other activities such as foraging or vigilance against predators.

Experience-mediated plasticity in responsiveness might, thus, be an adaptive strategy allowing females to adjust their sexual activity to the social environment. Further, Deacon (2010) demonstrated the remarkable ability of a single pregnant female to routinely establish viable populations. Higher responsiveness of females having grown in the absence of mature males is a behavioural mechanism potentially helping successful settlement of viable population. My result also supports a previous study that showed that female field crickets *Teleogryllus oceanicus* reared in silent conditions (comparable to my “no variance” treatment) are more responsive to playbacks than females reared with male song (comparable to my “low” and “high” variance treatment). Since the level of male phenotypic variance experienced during development does not modify female responsiveness, there is little scope here for sexual selection to operate.

4.5.2. Phenotypic variance and preference functions

Preference functions have been measured for seven male traits that are known to be good predictors of male mating success among different Trinidadian guppy populations (Endler & Houde 1995). Female genetic preferences (e.g. innate preferences) in the lower Aripo population have been established in a previous study (see chapter 2 and chapter 3 of this thesis) and my results, here, support them in part. In table 4.3, I present data on female preferences having

been reared in the three different treatments. Remarkably, there are no differences between treatments in the direction of preferences for the different traits under investigation (no difference in the sign of the median preference slopes) except for iridescent colors area that is not a cue on which lower Aripo females based their choice (Table 4.3). Accordingly, females are applying the same ranking criteria independently of their early experience and male attractiveness is not varying as a function of the amount of phenotypic variance that females observed during development.

By contrast, there are clear-cut differences in the strength of preferences (magnitude of the slopes) between treatments (Table 4.3; Fig. 4.7, 4.8, 4.9, 4.10). Overall, females tend to increase their degree of preferences for sexual cues as the level of phenotypic variance, experienced as juveniles, increases. The results are statistically significant (after Bonferroni correction) for yellow area and total colour area and marginally significant (significant before Bonferroni correction) for black area and diversity of colour pattern. Multiple comparisons between treatments show that females from the “high variance” condition have stronger preferences for greater amount of yellow and greater amount of total colour relatively to females from the “low variance” condition. No other between-treatments comparisons yielded significant differences. In other words, the level of phenotypic variance displayed by males during female’s maturation influence female choosiness (measured here as the magnitude of preference slopes).

Only one trait (yellow) showed a significant correlation between ordered treatments and a preference for that trait (table 4.3; Fig. 4.7). Such a relationship suggests that females increase steadily their level of choosiness as the level of variance manipulated by the experimenter increases. Being exposed to similar phenotypes during ontogeny makes females choosier than exposed only to females but less choosy than if exposed to unique male phenotypes. This result is not backed up by the comparisons between “no variance” and “low variance” or “no variance” and “high variance” treatments. In addition, we do not find such correlations for the other cues used by females in the process of mate choice (total colour, black, pattern diversity, body length). Hence, variance in phenotypes

experienced during development mediates choosiness when it occurs in the chosen sex.

Even if rather uncommon, females may base their choice on male size in some guppy populations (Reynolds & Gross 1992; Endler & Houde 1995; Magellan et al. 2005), favoring larger males (but see Endler & Houde (1995) for Paria river). Here, I report some results showing that females are indifferent to male size when reared in their contact (even if “high variance” females tend to prefer shorter males) but strongly prefer shorter males when having experienced only females (“no variance” treatment). Since males are smaller than females, experience-mediated plasticity in female preference for male body length might represent an adaptive strategy to tell sexes apart in the absence of colour. If such a hypothesis was experimentally proven, it would further highlight the importance of the social environment experienced as juveniles in the acquisition of female preferences.

4.5.3. Implications for sexual selection

My results suggest that phenotypic variation found in the social environment during development is unlikely to alter the direction of sexual selection, as females from the three different treatments tend to use the same sexual cues (no differences in preference function). It may, however, change the strength of sexual selection since choosiness increases when developing females had experienced more variation in male phenotypes.

Developmental plasticity in the degree of choosiness can generate dynamic fluctuations in the selection exerted on male traits following this scenario: in a population where phenotype diversity is relatively high, females increase their choosiness, leading to more variance in mating success between males. After a while the diversity in sexual phenotypes is eroded by the strong directional selection imposed by females that are now experiencing less diversity during ontogeny. Less variance in males’ phenotypes drives females to be less choosy, decreasing the threshold at which they accept males, which in turn relax sexual

selection on male traits, allowing for more or different sexual phenotypes to spread in the population. The importance of this feedback loop between plasticity in female preferences, environmental variation and selection can explain the maintenance of phenotypic variation within population besides other mechanisms such as frequency-dependent selection or antagonistic pleiotropy for fitness-related traits.

Moreover, when dynamic fluctuation in sexual selection due to early social experience interacts with other contexts- or conditions- dependent selection pressures (e.g. viability selection), the circumstances are set for allopatric divergence, initiating reproductive isolation between populations and potentially speciation (Ritchie 2007; Maan & Seehausen 2011). From a female perspective, plasticity in choosiness as a function of male phenotypic variance could represent an adaptive strategy diminishing the cost associated with the process of mate choice. Indeed, widening the range of accepted stimuli (e.g. decreasing choosiness) when the variance in male phenotypes is low allows females to spend time and energy on other activities than searching and assessing potential partners.

The effect of plasticity in female preferences is balanced, to some extent, by plasticity in the reproductive tactics adopted by males (Fig 4.12). In response to females being choosier, guppy males perform more sneaky copulations (i.e. forced or unsolicited copulations (Houde 1997)). High relative rate of sneak copulation diminish the importance of mate choice as a determinant of male mating success (Kelly, Godin & Wright 1999; Magurran 2001) potentially decreasing the strength of sexual selection. This finding supports previous work showing that the relative importance of sneak attempt versus courtship display within population depends on environmental factors (Farr 1976; Endler 1987; Godin 1995; Jirotkul 1999; Gamble et al. 2003) and morphological characteristics (Karino & Kobayashi 2005).

The role and importance of sneak copulation in guppies could hamper the influence that variation in female mate preference has on male traits evolution and on population divergence. Magurran (1998, 2005) argued that the importance of sneak copulation in guppies (Matthews & Magurran 2000; Evans, Pilastro & Ramnarine 2003) undermines any isolating effect of divergence in mating

preferences explaining the absence of speciation in spite of ecological gradients selecting for population differentiation. Other factors such as high level of gene flows (Endler 1995) and a rare-male effect (e.g. novel or rare phenotypes are more attractive to females)(Farr 1977; Zajitschek & Brooks 2008) have been put forward to account for absence of speciation in guppies.

More surprisingly, my results suggest that males surrounded by females from the “low variance” treatments attempt significantly less gonopodial thrusts than males being with females from the two other treatments. Two rationales might explain such observations. Firstly, Guevara-Fiore (2012) demonstrated that male guppies were performing relatively more unsolicited copulations when reared only with females (hence less sneak attempt when reared only with males) when Evan & Magurran (1999) showed the contrary, that is, males attempting more sneak copulation when reared only with males. Either way, it is unlikely that the early social experience of the males used in my experiments explain my results, as they were collected in housing tanks where both sex are represented. Secondly, the females from the “low variance” treatment have been exposed to similar males closely related to each other per se. I cannot rule out that being exposed to half- or full-siblings affect female behaviours (for other reasons than their similar phenotypes and not observable with human eyes) that would in turn tune the rate at which males perform their gonopodial thrusts. Eventually, any differences in forced copulations towards females from different treatments cannot be explained by differences in male size (Houde 1997; Becher & Magurran 2004) or male attractiveness, as they were no correlation between different colours classes used as sexual cues and the rate of thrust.

4.5.4. Conclusion:

To conclude, I have demonstrated for the first time that phenotypic variation displayed by males independently of the value of the sexual signal itself affect female mate preferences; females being choosier when reared with more diverse male phenotypes. Such variation in female mate preferences induces variation in

the relative strength of sexual selection. However, traditional sexual selection models assume constant selection and a strong genetic basis for variation in mate preferences. Even though existing models remains an important theoretical backgrounds generating testable predictions, it is important to include in these models the fluctuating nature of selection.

The large differences in behavioural, morphological and life-history traits found between guppy populations (Magurran 2005) arising through a wide array of selective pressures makes it difficult to generalise findings from one population to another. Accordingly, it would be worth investigating whether the levels of developmental plasticity, due to experience with mature conspecifics, vary across population. Further, it will be interesting to analyse the effect of other aspects of the environment such as the level of predation experienced during ontogeny and examine how they interact together in the acquisition of mate preferences. Such studies will make a step forward in the understanding of the complex field of mate preference plasticity.

5. Chapter V

Learning foraging preferences during ontogeny and female mate preferences in guppies

5.1. Abstract

Studies investigating the mechanisms by which females learn mating preferences during ontogeny have generally focused on the influence of the social environment. However, other aspects of the environment experienced by immature individuals can modify the acquisition of preferences. The sensory and neural systems have shown to be very sensitive to developmental conditions and any variation in the physical environment experienced while maturing could have long-term effects on the processing of incoming signals. These changes could presumably alter female mate preferences. To test this hypothesis, I examined, in guppies, whether a foraging preference, associated with a specific colour, acquired during ontogeny could be transferred into a mating context and altered female mating preferences. Such effects could result from a pleiotropic link between different behavioural contexts or being an adaptation of the visual system owing to direct exposure to the spectral properties of objects found in the environment. My results showed that independently of the colours experienced while feeding, females displayed a bias toward violet objects. However, they didn't suggest that females learnt to associate food with any colour proposed. On the other hand, females raised in a situation where food was associated with yellow tended to prefer yellower males compared to females raised with other colours. Although not compelling, these results open a field of investigation that might be promising in the future. Further, if it appears that females develop mating preferences in such a way, it might have important consequences for the study of sexual selection and reproductive isolation.

5.2. Introduction

A growing body of evidence suggests that individual phenotypic plasticity in female mate preference is found across animal kingdom and could have significant evolutionary consequences (Jennions & Petrie 1997; Widemo & Saether 1999; Brooks & Endler 2001b; Brooks 2002). This variability is due to genetic differences, developmental history and environmental factors. Non-heritable causes of variation in mate preference include perceived predation risk (Johnson & Basolo 2003; Kim et al. 2009), the physical signalling environment (Endler 1991; Gordon & Uetz 2011), the female age (Coleman et al. 2004) or the time and cost of sampling males (Milinski & Bakker 1992). The social environment is also an important external factor and includes the operational sex ratio (Jirotkul 1999), mate copying (Mery et al. 2009), eavesdropping and the "audience effect" (Witte & Nöbel 2011; Clark, Roberts & Uetz 2012), the "previous-male effect" (Bakker & Milinski 1991) and prior female experiences of males (Rutledge et al. 2010; Bailey 2011). Yet, little is known about variation in mate preferences due to individual differences in the cognitive system (i.e. sensory and neural system) involved in the mate choice process.

Preferences are necessarily influenced by the sensory and neurological circuitry involved in detecting and processing sexual signals. Much of this circuitry is used in other ecological contexts such as evading predators, food detection or habitat choice and how selection, in these contexts, influences the psychosensory system may in turn influence preferences. For example, in guppies (*Poecilia reticulata*) the visual system is involved in locating food, choosing mates or inspecting predators. Interestingly, Rodd et al. (2002) tested the sensory bias hypothesis in guppies and found a strong relationship between the strength of female preference for orange males and attraction to orange objects (interpreted as an indicator of foraging preference) among populations. Such results supported the hypothesis that female preferences evolved as a by-product of natural selection in a non-mating context. Guppies would have evolved the ability to spot nutrient-rich orange fruits falling in the rainforest stream of Trinidad and female preference for carotenoid colouration may have arisen as a pleiotropic effect mediated through

the visual system. However, the evolutionary order of the events is challenged by Grether et al. (2005) who proposed instead that the sensory bias has been modified as a correlated effect of selection on the preference. In any case, a genetic correlation in the form of pleiotropy exists between these two behaviours that share a common sensory system. Alternatively, orange foraging preferences and orange female mating preferences may have evolved separately, in which case the among-population correlation reflects genetic disequilibrium and not pleiotropy. Mate choice would have evolved following an indicator model mechanism - orange colouration in males being an indicator of foraging skills (Karino, Shinjo & Sato 2007), swimming abilities (Kodric-Brown & Nicoletto 2005) and/or immunity (Houde & Torio 1992) all of which improving viability – and foraging preferences through natural selection.

The extent to which the link between the sensory bias and mating preferences is plastic remains largely unknown. Several studies put forward the importance of the developmental environment in the maturation of the neuronal circuitry affecting later sensory processing (Nowicki, Searcy & Peters 2002; Grubb & Thompson 2004; Ronald, Fernandez-Juricic & Lucas 2012) but we need explicit experimental evaluations of the connection between development, sensory functioning and mate preference. Variation in individual female preference owing to variation in foraging preferences induced by the rearing environments in which the visual system develop is investigated here using guppy as a model system. To do so, I implemented an operant conditioning framework in which developing females learnt to associate a specific colour with some food. I predict that female mate preference should vary in response to the colour experienced while foraging. Such outcomes could also occur in the absence of a pleiotropic link between foraging and mating, directly resulting from variation in individual ontogenetic trajectory of the visual system due to exposure to specific colours. The experimental setting used in this study should help disentangle the two alternative hypotheses. Evolutionary implications of developmental plasticity in the sensory system such as the effect on reproductive isolation will be considered in the discussion.

5.3. Method

The experimental protocol entails several steps: rear maturing individual females in a controlled lighting environment and experimentally manipulate the colours used in the associative learning task; test mate preferences and foraging preferences of the experimental fish for different coloured cues; examine the relationship between mate preferences and foraging preferences. Each of these steps is described in detail below.

5.3.1. Experimental treatments and experimental settings

Guppies are descendants of individuals collected in Trinidad in March 2008 (N 10° .39.031; W 61°13.404; 37m altitude). Neonates are collected from stock housing tanks soon after birth and split randomly across the rearing groups. They were fed once a day with pale light brown flakes (obtained from Angel Plus: http://www.angelsplus.com/) following the procedure described here below.

The experiment consisted of rearing groups of maturing juveniles in five treatment conditions from birth until maturity. There were three different experimental conditions and two control conditions.

In the experimental conditions, fry learnt to associate a coloured cue with achromatic food flakes (to prevent any potential learning of the flake's colour), following an operant conditioning protocol. The colours used were a subset of the colours borne by males and displayed as sexual cues. They were chosen according to the outcome of innate female mate preference experiments such that one colour is liked (i.e. yellow), one not genetically preferred but important in juvenile early social experience (i.e. orange, see chapter two) and one to which females are indifferent (i.e. violet). Food was introduced in a device in which the juveniles need to enter to eat (see fig. 5.1, fig. 5.2). The device is made of two plates: a front wall pierced with two holes leading to two compartments divided by the second plate. For each experimental condition, one of the holes is framed with a plastic coloured sheet behind which the flakes were poured by the experimenter (see fig.5.1 and 5.2). There was no way to pass from one compartment to the

other. The apparatus was introduced daily, around 10:00am, in the tank just before feeding the fry and removed two hours later. Each day the location of the coloured hole was changed in a pseudo-random manner in order to avoid that the holes' positions becoming the positive reinforcer instead of the colour itself (see fig.5.1). Hence, the hole fitted with a coloured sleeve can occupy four different positions that is top-left, top-right, bottom-left, bottom-right. This precaution is worthwhile since spatial learning can outweigh visual discrimination learning (personal observation) and competing learning mechanisms could bias the internal validity of the experiment and/or the interpretation of the results.

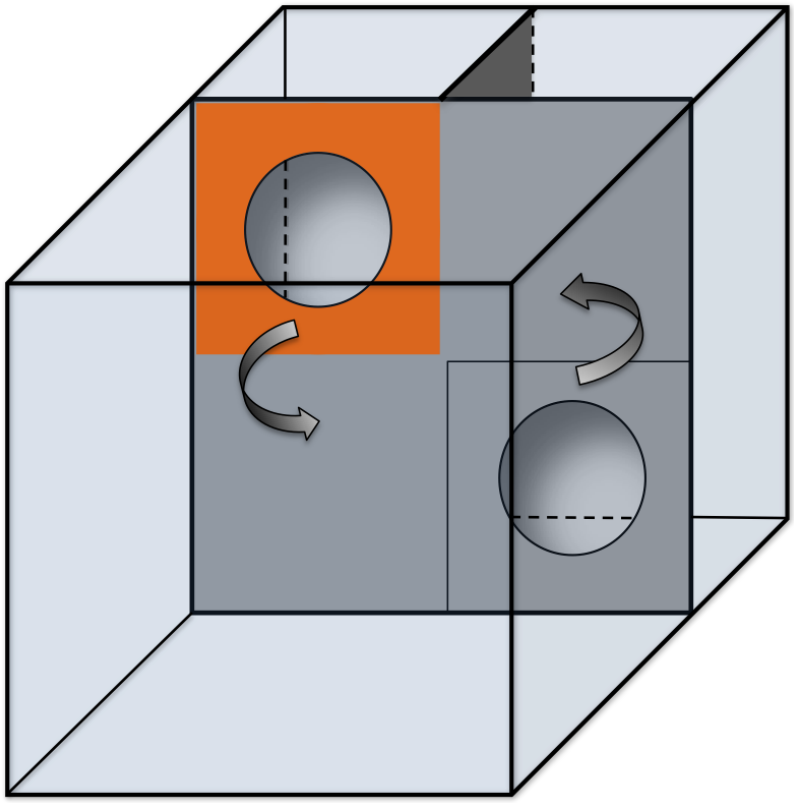

Figure 5.1: Front view of a rearing tank and operant conditioning apparatus. The change of location of the coloured cue (here an orange cue) is represented. Rearing tanks are 28cm X 18cm X 17cm.

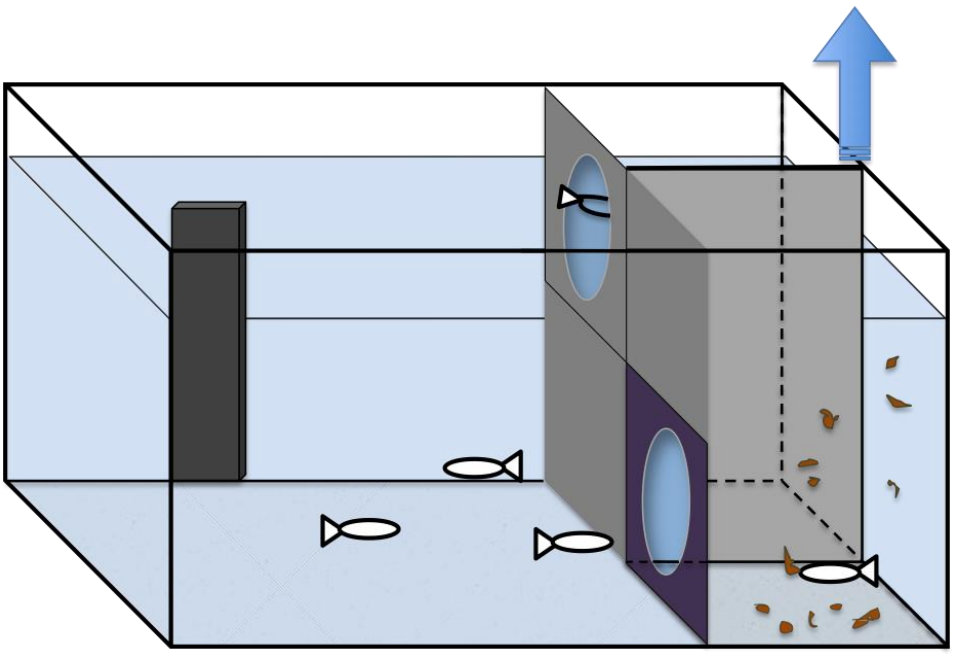

Figure 5.2: Side view of a rearing tank and the operant conditioning apparatus introduced daily and removed after feeding. This diagram represents a group of fish trained to associate a violet cue with food reward.

In the two control conditions, the rewarded hole was wrapped with a grey plastic sheet slightly darker that the grey value of the apparatus (see Fig. 5.3). Sensory systems have evolved to match the average characteristics of the local conditions (Endler 1992) but can deviate from the population norm due to developmental plasticity (Fuller & Noa 2010). For instance, development under different lighting conditions can alter different aspects of the visual system such as oil droplets (Hart, Lisney & Collin 2006), retinal filters (Cheroske, Barber & Cronin 2006) or opsin expression (Fuller et al. 2005a) and presumably female mating preferences. The setting of the two control conditions tested for the possibility that individual variation in mate preference was due to the effects of variation in the spectral content of the rearing tank on the development of vision, outside a foraging context, instead of being the result of plasticity in pleiotropy between foraging and mating. Hence, the two control conditions varied in the nature of a slab that is left in the tank (namely outside of the apparatus) throughout the whole duration of the rearing period (see Fig. 5.3). One control condition had a plastic slab covered with the same grey as the one used to wrap the rewarded hole whereas the other control condition had a slab covered with orange. A grey slab

was added to the experimental conditions to control for its presence in the rearing tanks (see fig.5.2).

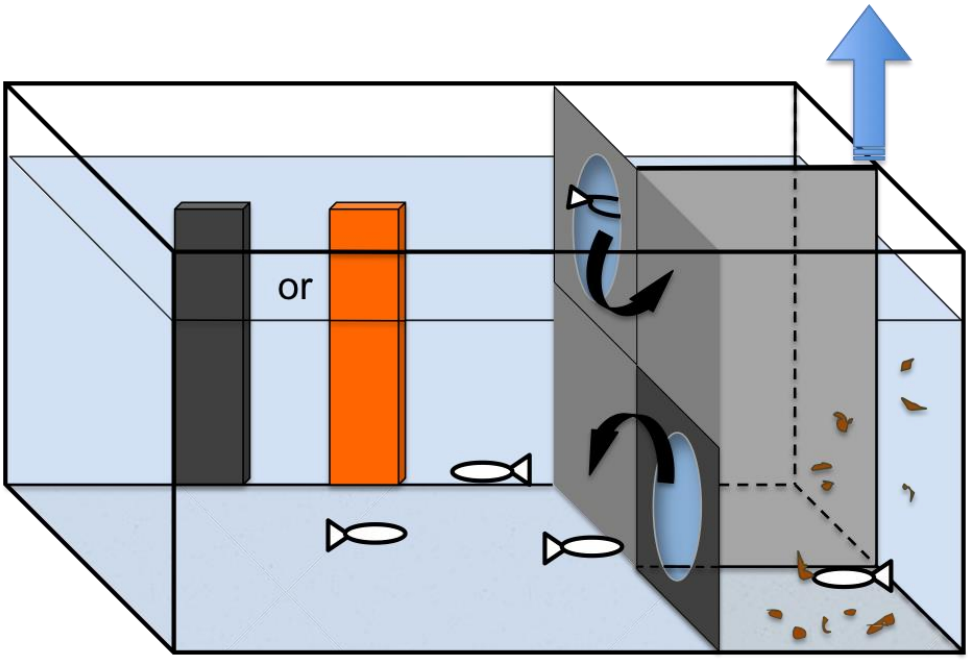

Figure 5.3: Side view of a rearing tank used for the control conditions. The slabs make up for different spectral contents in the ontogenetic environment.

Within the groups of fry, to prevent any fertilization of the experimental females, males were removed before reaching sexual maturity, that is, before the gonopodial hood extends beyond the tip of the fin (Reznick 1990)

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5.3.2. Colour measurements

5.3.2.1. Choice of the colour stimuli used as positive reinforcers in the conditioning protocol and utilized in the coloured disk attraction test

The categories of the colour stimuli used as positive reinforcers were orange, yellow and violet. A single colour category identified by a human eye as orange, for example, varies in its spectral properties. To determine accurately which coloured

sheets should be used as reinforcers, I obtained average reflectance spectra for each relevant colour (i.e. yellow, orange and violet) of male guppies. Next, I chose the coloured plastic sheet that best matched the average guppy reflectance spectra. Reflectance spectra were calculated with “ColourWorker” (details and application to be downloaded found at: http://www.chrometrics.com/colourWorker.html). Osorio, Anderson and Rawlinson from the University of Sussex, UK, developed a patented method that make precise estimates of colour and spectral reflectance from ordinary digital photographs. Figure 5.4 represents an example of a photograph on which measurements were made. For each colour to be chosen, twenty photographs were analysed and on each photograph the measures involved the spots of the colour of interest on a guppy male (compiled into one measure), a selection of the plastic coloured filters with different spectral properties and a colour chart used to calibrate the software. The coloured filter with an average reflectance spectrum that best overlap the average reflectance spectrum of the corresponding colour measured on the guppies was chosen as a positive reinforcer for the associative learning task. The photographs (eight millions pixels set) were taken following the standard method described below to analyse male colour patterns. The coloured plastic sheets were provided by “Rosco Supergel” (details found at http://www.rosco.com/filters/supergel.cfm) and the reference numbers for the chosen filters are 15 for orange, 312 for yellow, 52 for violet and 398 for grey. The same coloured filters were used for the food preferences tests (see below).

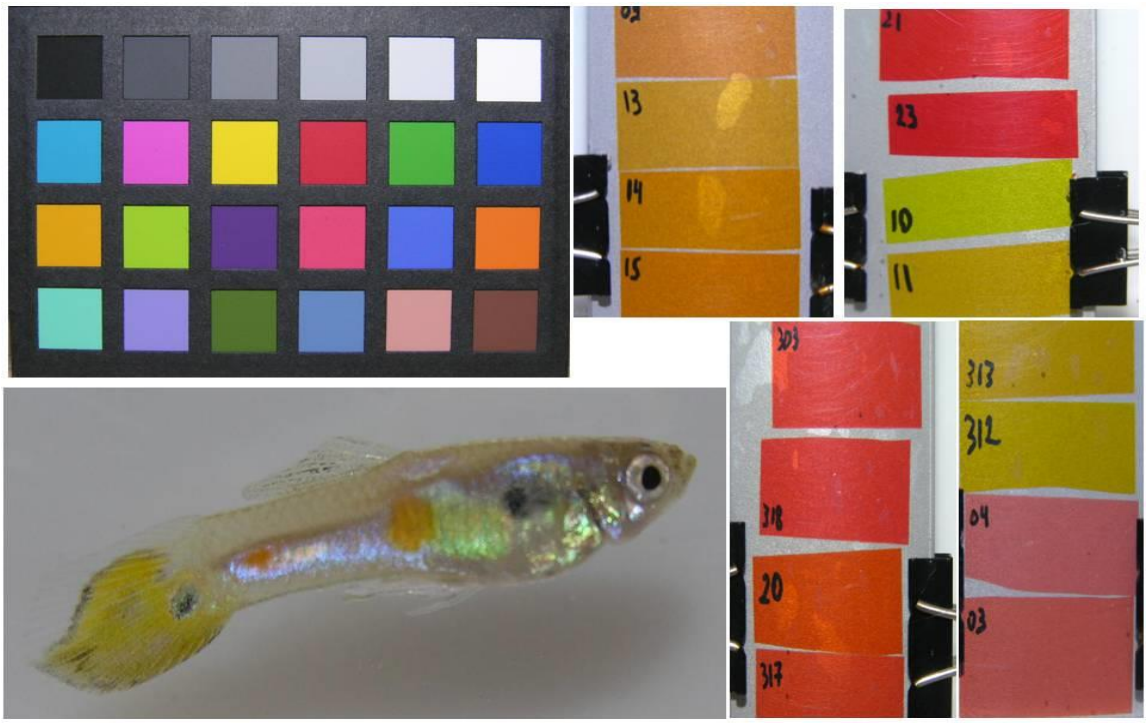

Figure 5.4: Setting of a picture of a guppy male, various colour filters representing different spectral parameters of orange and yellow and a colour chart used for calibration. This picture setting is imported in Colourworker, which in turn allows estimating the reflectance spectra of the chosen colours.

Guppies are tetrachromatic and have a class of cones that absorb UV radiation. However, UV vision could not introduce any bias in my study because the artificial lights used in the rearing room and in the experimental room did not emit any UV (shown by spectrophotometer measurements).

5.3.2.2. Male trait analysis

Male colour patterns were photographed with a digital camera (Nikon coolpix 8800) in a perspex box filled with a small narrow volume of water where fish are free to swim. All the pictures were taken under the same light conditions when the fish was parallel to the front clear face of the box. Both sides of each guppy were photographed and the images analysed with the UTHSCSA ImageTool program (developed at the University of Texas Health Science Center at San Antonio, Texas and available from the Internet at http://compdent.uthscsa.edu/dig/itdesc.html). Colour patches were grouped into the

following colour classes: black, orange (including red), yellow, iridescent (including silver/white, blue, violet, and bronze-green) and total colour area. The colour classes were measured as relative total area (relative to the body + caudal fin) and number of spots per colour class. The data for each male consists of the mean of the right and left sides of the body for both relative area and of the right side for the number of spots.

The total body length (from the tip of the snout to the tip of the longer lobe of the caudal fin) of each male was recorded using a digital caliper.

A measure of the diversity of the colour pattern was calculated for each male. This male trait, which is rarely examined in the study of female preference, was computed with Simpson's Reciprocal Diversity Index. The values span from 1 to X with X being the number of categories being used (for example if there are five colour classes, the highest possible value is X=5 when each of the 5 colours have equal areas on the guppy). The lower the value the less diversity and vice versa (see appendix).

5.3.3. Behavioural trials

5.3.3.1. Mate choice test

Following Houde (Houde 1987; 1988c; Houde 1997) and Grether (2000; 2005), mate choice is measured by observing females' response to males' courtship in a 40 litre open aquarium. Female preferences were analysed in terms of variation in male attractiveness (see appendix),, that is, by calculating the effect of particular male traits on females' responsiveness. It is a standard method used in the study of mate choice in guppies and is biologically relevant as it allows the expression of the full repertoire of sexual behaviours between males and females (Houde 1997).

Each experimental group consisted of the same numbers of individuals of both sex (even sex ratio: in general 5 males and 5 females). Within a group, males

were randomly drawn from different stock tanks where they developed and grew in physical contact with mature females. They displayed different colour patterns and different sizes. Females in a given test were from the same rearing treatment. The day before the trial, virginity of the tested females was eliminated by the presence of non-experimental males to ensure the full expression of their preference, as naive females are known to show little mate discrimination in their first mating (Endler & Houde 1995; Houde 1997; Hughes et al. 1999; Brooks & Endler 2001b). To do so, they were placed individually in 4-l plastic tanks, containing gravel and a plastic plant, for approximately 5 hours with one male that had not been used in the further experiments. On the day of the trial, females were released in the testing tank two hours before the observation started to let them acclimatize to the new environment.

Observation sessions involved 5 min focal observation of each male in turn, in random order. Three sessions in the morning and three sessions in the afternoon were carried out. At the end of the last afternoon session, I continued to observe males for 20 min (focal of 30 sec/male) to ensure that all females have been visited. After the daily experiment, females were replaced in the rearing tanks (to be further tested for their coloured disc attraction) and males were kept in the observational aquarium to be reused until all the females from the same replicate had been tested. Male colour patterns were sketched to help recognize individual males but it was in general easy to tell them apart. After all the females of one replicate (females from the five different treatment conditions) were tested, experimental males were changed.

During each focal observation, I recorded each of the male's sigmoid displays (Houde 1997) and the female's response to these courtship displays. The relative attractiveness of a given male to females in an experimental group is estimated as the proportion of his displays that elicit at least a "glide" response (the "fraction response" D; see table 5.1 for details of the female sexual response). Individual females are not distinguishable so D represents an aggregate measure of the preference of all females in the experimental group for that particular male. D is a reliable predictor of male mating success (Houde 1987, 1988a). The degree of

preference for a sexual trait is calculated as the regression of D on that trait for all the males used in that session. The slope of the regression is a measure of the overall degree of female preferences for that sexual trait in a given observational trial. Males that perform less than five displays throughout the observation sessions are excluded from the analysis. Male displays were recorded only if they were directed towards a particular female, if other males do not interrupt them and if they started after the male becomes the focal male.

The tests were conducted in a windowless room presenting the same lightning characteristics as the room in which the fry were reared. The observation aquarium was covered with natural gravel on the bottom and opaque paper on three sides; the observations were made from the fourth side. The aquarium was illuminated with daylight spectrum fluorescent tube and one incandescent bulb placed above the tank (40 W) yielding a light intensity at the water surface of roughly 900 lux.

Table 5.1: Measure of preference **= D^1 =**∑ (score ≥ 2) / ∑ (all male display)

Score	*Female and Male behaviour*
0	No response; Female ignores male
1	Female orients toward male but does not move closer
2	Female glides toward the male
3	Male and female circle around each other
4	Copulation attempt; mate thrust and makes gonopodial contact
5	Copulation; gonopodial contact followed by male jerk

[1]aggregate measure of preference of all females in the group
[1]relative attractiveness of a given male to all females in the group

5.3.3.2. Coloured disc attraction tests

The attraction to an inanimate coloured object (here coloured discs) is interpreted as an indicator of a foraging preference for food of the same colour (Rodd et al. 2002). The colour attraction test was carried out in a test tank (46 X 46 X 25 cm^3) in which four 7 cm diameter coloured discs, evenly spaced, were stuck

to the bottom of the aquarium that was otherwise covered with light brown sand. The discs were constructed with the same grey material used for the conditioning apparatus and were covered with the same plastic colour filters used in the rearing treatments. Hence, the four colours utilized in the attraction tests presented the same spectral properties as the one employed in the rearing treatments even though I could not rule out the possibility that fish would perceive slight differences owing to a different angle of the incident light. A 13 cm diameter preference zone was established by placing a glass ring around each disk and its upper boundary was delimited by the 6 cm height of the water filled in the aquarium. Females were tested individually and their foraging preferences were measured as the duration of time spent in each zone during a 7-min focal sampling. A fish was considered in a preference zone once its snout crossed the perimeter delimited by the ring and out of the same zone once its caudal fin was found outside the ring. The evening before the trial, the tested individual was placed in the experimental tank to acclimatize. During the acclimatization period, the grey discs were not covered with coloured filters. The following day, before the trial started, the four different filters (i.e. yellow, orange, violet and grey) were randomly assigned a disc. Meanwhile, the female was kept in a plastic cylinder in the middle of the tank for 5 min to decrease the level of stress occasioned by the manipulation. Data were collated with a video camera placed above the tank. The tested females were not fed during the whole duration of their presence in the testing arena.

5.3.4. Statistical analyses

The variables measured as proportions (the fraction response D and the relative areas of males' colour patterns) were angular transformed (arcsine square root transformation) to meet parametric assumptions. I tested for treatments differences in male attractiveness and in the strength of female preferences for different male characters using a linear mixed model with D as the dependent variable and treatments, and male characters as fixed effects. If significant treatment effects were detected, post-hoc tests were performed. Both Bonferroni and Sidak (less conservative than the Bonferroni procedure) corrections were

applied. The covariates have been chosen given the colours used for the treatments and according to the genetic preferences found in the guppy population used. The effects of the treatments on the strength of female preferences were measured from the interactions between treatment and the colour of interest. Because of the possible non-independence of females and males behaviours within an observation tank and the correlated errors associated, behavioural data are analysed using linear mixed models for repeated measures implemented with the “Subjects” and “Repeated” options within the MIXED procedure in SPSS (IBM SPSS statistics release 19.0.0). Within a single group (an observation session), the different males are considered as repeated measures on the dependent variable.

Within a treatment, a multiple regression was used to determine which of the male traits most accounted for male attractiveness. The partial regression coefficients were measures of the preference slope for the trait to which they were associated. The mixed procedure revealed that the residual errors within each observational tank were correlated with each other as shown by the value of the covariance parameter (0.47 +/- 0.22; $Z=2.13$, $p=0.03$). To remove variation in male attractiveness associated with experimental group, male traits were regressed simultaneously on residuals of male attractiveness. Attractiveness residuals were obtained from an ANOVA procedure per treatment with experimental group as a factor.

The data of the coloured disc attraction tests were analyzed with non-parametric procedures.

5.4. Results

5.4.1. Female preferences for different male characters

The colours that females were trained to associate with a food reward significantly influenced how females responded to male sexual displays (Table 5.2, Fig. 5.5). In multiple comparisons tests, females conditioned with yellow cue were significantly less responsive than females reared in the control condition with grey plate ($p=0.011$, fig. 5.5) and than females reared in the control condition with the orange plate ($p=0.011$, fig.5.5), although, the differences were not significant anymore after corrections for multiple comparisons. The treatment-by-colour interactions, which measured the differences in the strength of female preferences across treatments, were significant for violet (that I also call blue-violet) preference and marginally significant for orange and yellow preferences (Table 5.2). The interaction estimates reveal the difference in preference slopes for a certain colour between a specific treatment and the control condition with the grey plate (Table 5.3). Similarly, females raised from birth with orange in their tanks showed lesser preference for blue-violet compared with females raised with no colours. On the contrary, females conditioned to associate orange with food showed stronger preference for blue-violet compared with female of the control “grey plate” condition. No other effects were noted regarding the violet preference. Besides, as predicted, females reared in the yellow treatment demonstrated stronger preferences for yellow coloration borne by males relatively to females from the control condition (Table 5.3). Generally, the results suggest that orange preferences were not influenced by the treatments (Table 5.3). Finally, total body length influenced male attractiveness, females being more attracted by shorter males.

Table 5.2: Sources of variation in male attractiveness. The treatments x male colour pattern (orange, yellow, violet) terms test for treatment differences in the strength of female preferences. Male attractiveness is a measure of individual female responsiveness (see method).

	F	*Df*[1]	*p-value*
Intercept	10.4	1, 15.9	**0.005**
Treatments	13	4, 13.8	**<0.001**
Orange covariate	0.49	1, 17	0.5
Yellow covariate	2.31	1, 15.5	0.15
Violet covariate	0.16	1, 21.6	0.69
Black covariate	1.47	1, 17.7	0.24
Total colour covariate	0.56	1, 18.1	0.47
Total body length	9.4	1, 16.7	**0.007**
Simpson index of diversity	3.46	1, 17.1	0.08
Treatment x Orange	2.5	4, 14	0.09
Treatment x Yellow	2.59	4, 16.2	0.08
Treatment x Violet	4.47	4, 15.4	**0.01**

[1]The denominator degrees of freedom are not integers (values obtained by a Satterthwaite approximation) because the statistics are not based on exact F distributions.

Table 5.3: Estimates of the fixed effects influencing male attractiveness. The fixed effects of the different treatments are represented relatively to the control condition with grey plate. The covariates control for the effects of different males characters experienced during the behavioural trials on females' responsiveness. Interaction estimates tell the difference in slope relatively to the control condition with grey plate.

Factors	Estimates	*se*	*df*	*t*	*P-values*
Intercept	1.02	0.3	15.9	3.4	**0.003**
Orange in the control condition	0.22	0.1	14.7	2	**0.07**
Orange cue (experimental condition)	-0.34	0.1	13.3	-2.9	**0.01**
Yellow cue	-0.46	0.1	14.8	-4.3	**0.001**
Violet cue	0.07	0.1	14.3	0.59	0.57
Orange covariate	1.1	1.9	16.4	0.57	0.58
Yellow covariate	1.65	1.4	15	1.18	0.26
Violet covariate	-0.33	1.4	14.9	-0.24	0.81
Black covariate	-1.82	1.5	17.7	-1.21	0.24
Total colour covariate	0.61	0.8	18.1	0.75	0.47
Total body length	-0.04	0.01	16.7	-3.1	**0.007**
Simpson diversity Index covariate	0.09	0.1	17.1	1.86	0.08
Orange control x Orange covariate	-1.51	1.7	13.1	-0.89	0.39
Orange cue x Orange covariate	0.02	1.5	14.2	0.02	0.99
Yellow cue x Orange covariate	1.51	1.3	13.6	1.15	0.27
Violet cue x Orange covariate	-0.76	1.4	13.6	-0.56	0.59
Orange control x Yellow covariate	1.29	1.1	14.6	1.22	0.24
Orange cue x Yellow covariate	-0.64	1.7	17.4	-0.38	0.71
Yellow cue x Yellow covariate	3.54	1.4	14.9	2.59	**0.021**
Violet cue x Yellow covariate	-0.37	1.1	15.2	-0.32	0.75
Orange control x Violet covariate	-4.1	1.9	14.2	-2.1	**0.05**
Orange cue x Violet covariate	8.3	3.6	14.6	2.3	**0.04**
Yellow cue x Violet covariate	2.8	1.7	14.3	1.7	0.11
Violet cue x Violet covariate	-2.2	1.8	14	-1.3	0.23

The multiple regressions carried out for each rearing condition and, including various male traits as predictors, did not result, overall, in a significant degree of prediction of male attractiveness in spite of a large amount of variation explained by the models (table 5.4). The individual contribution of the different male characters was not clear, as the partial regressions coefficients were not significant.

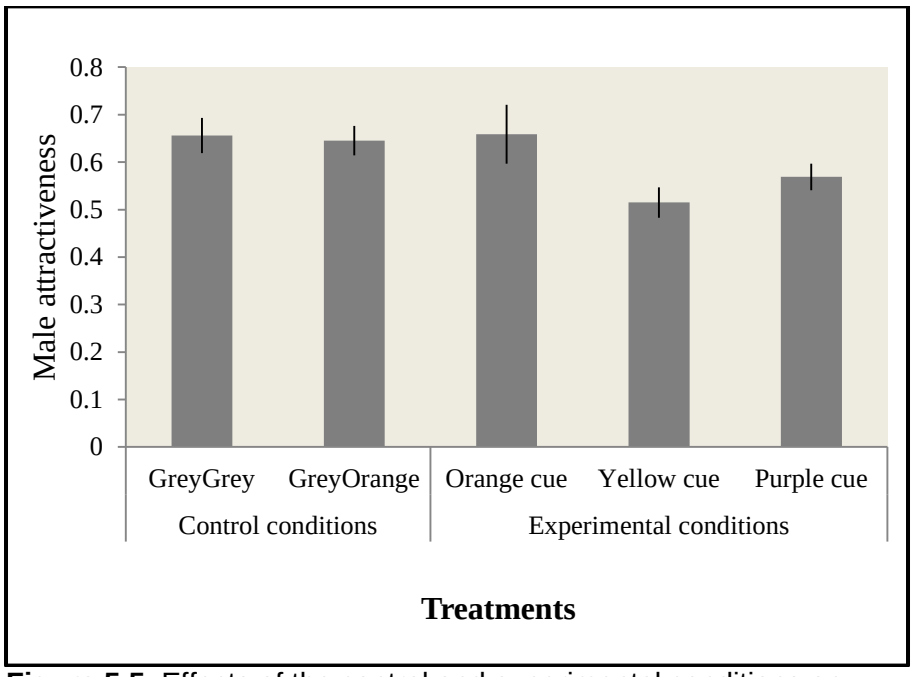

Figure 5.5: Effects of the control and experimental conditions on male attractiveness. Bars represent least square means (+/- SE) from the analysis of variation in table 5.2.

Table 5.4: Multiple regressions of male attractiveness residuals on various male traits. Standardized partial regression coefficients and R^2_{adj} are represented.

Rearing conditions	Orange area	Yellow area	Blue-violet area	Black area	Total colour area	Total Body length	Simpson Diversity Index	R^2_{adj}	N
Grey control	0.39	0.54	-0.37	-0.61	0.05	-0.53	1.02	45.8	10
Orange control	-2.78	-3.93	-1.97	-1.67	5.22	-0.61	2.79	50.6	10
Orange cue	-2.16	-0.88	0.15	-1.58	3.68	-1.59	-0.14	42.6	8
Yellow cue	1.01	1.58	1.86	0.01	-0.71	-1.42	-0.18	71.7	10
Violet cue	-1.24	-1.29	-0.8	-0.91	2.37	-0.58	1.39	40.7	12

5.4.2. Female preferences for coloured discs

The colour attraction test showed that, overall, females differed in their preference for coloured discs, independently of the conditions they experienced as juveniles (Friedman's ANOVA, p=0.022). Multiple comparisons (adjusted for the number of tests) indicated that females were significantly more attracted to violet discs compared to yellow discs (p=0.007).

The preferences for coloured discs between treatments were also analysed, using the median of duration of visits in the preference zone as the dependent variable. Kruskal-Wallis statistics carried out for each colour separately revealed that females differed across treatments in their preference for the grey disc (p=0.052), for the orange disc (p=0.054) and for the violet disc (p=0.032) but not for the yellow disc (Figure 5.6, Table 5.5). Multiple Mann-Whitney tests (adjusted for the number of tests conducted) were used to detect which treatments differed significantly (Table 5.6). Because of the adjustment due to the *post-hoc* procedure and the resulting reduced critical value for significance, not all possible comparisons were made. They were made against the treatment group that involved the colour of interest (e.g. when testing difference in yellow attraction, comparisons were made between control grey and yellow treatments, control orange and yellow treatments, etc.). Despite significance found with the Kruskal-Wallis tests, none of the pairwise comparisons showed significant differences between treatments in the attraction to the different coloured discs (fig. 5.6, table 5.6). However, females trained with violet were marginally more attracted to violet disc than females reared in the control group (with orange plate, table 5.6).

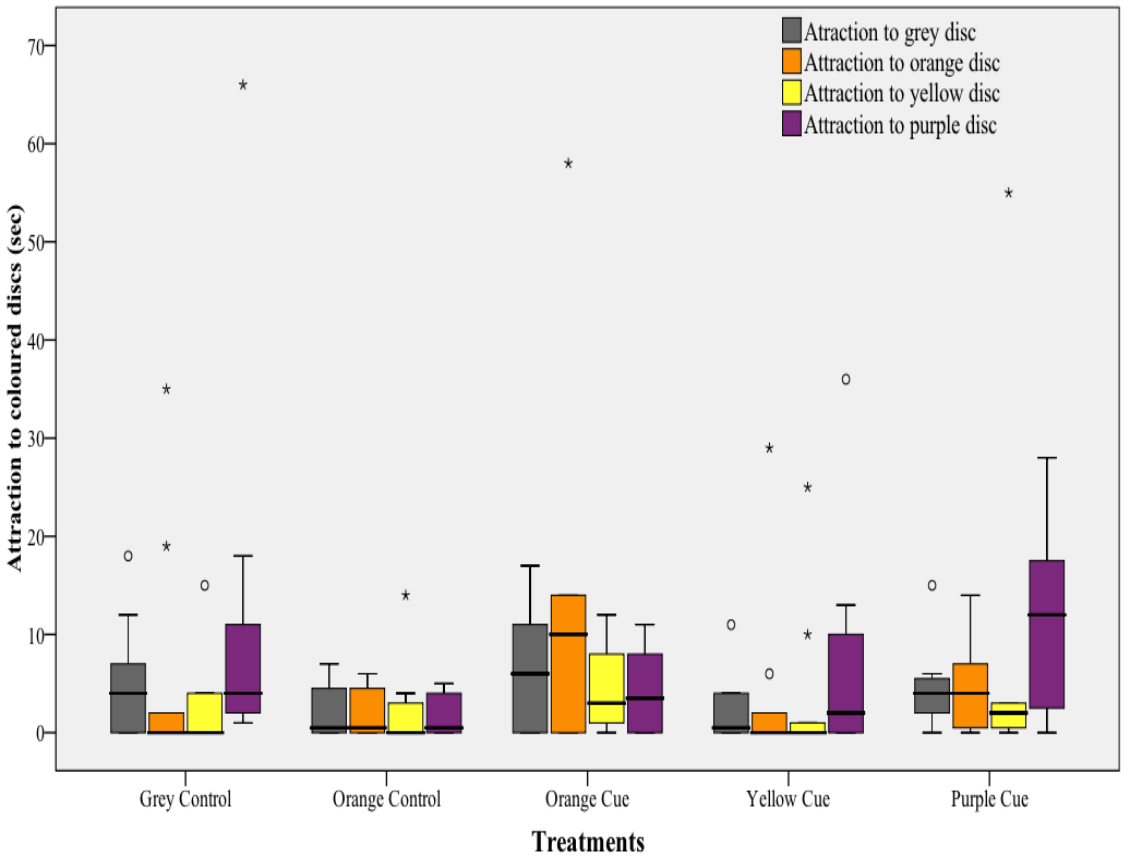

Figure 5.6: Effects of experimental and control conditions on female attraction to different coloured discs. For each treatment, the boxplots indicate how the duration of visits (measured in seconds) in the preference zones of each coloured discs is distributed. Symbols over the boxplots represent outliers.

Table 5.5: Kruskal-Wallis statistics testing for differences in the duration of visit to the different coloured discs across the rearing treatments. The significance of the K-W test is calculated exactly.

Attraction to coloured discs	H statistic	*df*	Exact significance
Grey disc	3.72	3	0.052
Orange disc	3.36	3	0.054
Yellow disc	2.63	3	0.106
Violet discs	4.48	3	**0.032**

Table 5.6: Pairwise comparisons performed with Mann-Whitney statistics and testing for differences in colour preferences between treatments.

Attraction to coloured discs	Comparisons between treatments	U-statistic	Exact significance
Grey attraction	Control grey – Control orange	25	0.3
	Control grey – Orange cue	24.5	0.8
	Control grey – Yellow cue	29.5	0.2
	Control grey – Violet cue	30.5	0.9
Orange attraction	Control orange – Control grey	35.5	0.98
	Control orange – Orange cue	12	0.12
	Control orange – Yellow cue	34.5	0.6
	Control orange – Violet cue	18.5	0.3
	Orange cue – Control grey	19	0.4
	Orange cue – Yellow cue	16	0.1
	Orange cue – Violet cue	15	0.4
Yellow attraction	Yellow cue – Control grey	42	0.8
	Yellow cue – Control orange	40	1
	Yellow cue – Orange cue	17	0.16
	Yellow cue – Violet cue	23	0.2
Violet attraction	Violet cue – Control grey	28	0.7
	Violet cue – Control orange	12.5	0.07
	Violet cue – Orange cue	12.5	0.2
	Violet cue – Violet cue	25	0.3

Following the predictions of potential pleiotropy between foraging preferences learned during ontogeny and subsequent mate preferences, I would expect that females trained to associate yellow with food, for example, would develop a foraging preference for yellow food items and, in parallel, would show a stronger preference for yellow male compared to other females. To examine a potential relationship between the strength of female sexual preference for a certain colour and the attraction to a coloured disc of the same colour, I carried out Spearman's rank correlations between treatments. Contrasting with the predictions, the correlations were negative for orange colouration ($r = -0.67$, $p=0.22$) and for yellow colouration ($r = -0.34$, $p=0.58$) and also highly non-significant. In support of the prediction, sexual preferences and foraging preferences for violet were positively correlated although non-significant ($r = 0.53$, $p=0.36$). Attraction to coloured discs were represented by the median of the duration spent in each preference zone (fig. 5.6). Measures of the female preferences strength were the preference slopes (partial regression coefficients) found in the multiple regression carried out to

determine which traits accounted for most of the variation found in male attractiveness within a treatment (Table 5.4). Due to the uncertainty associated with these coefficients, I also used preference slopes that resulted from simple regression of each colour of interest on male attractiveness but it didn't change the results of the correlations.

5.5. Discussion

The sensory bias hypothesis states that female mate preferences evolve as correlated responses of selection in a non-mating context due to pleiotropy (Endler 1992; Endler & Basolo 1998; Fuller, Houle & Travis 2005b). For instance, in a foraging context, the visual system is naturally selected to locate a particular type of food that will in turn determine the female mate choice and its evolution. Yet, the extent to which learning food preferences during ontogeny modify subsequent mate preferences remains totally unexplored although having important evolutionary implications. To investigate the possibility that variation in female mate preference originates in different foraging preferences acquired while maturing, I set up an operant conditioning protocol where females learnt to associate specific colours with food, acting as positive reinforcer. Several predictions ensued. First, outside a mating context, females ought to develop a preference for the colour they were conditioned with. Second, females should have a stronger preference for males displaying the colour they learnt to associate with food. Third, due to the bias in the sensory or neural system arisen during development, a relationship between the strength of female preference and the level of attraction to the corresponding coloured disc should be detected. Overall, my results didn't satisfy the different predictions.

Independently of the rearing conditions, females tended to be innately attracted to violet objects, although I cannot unequivocally attribute the spectral bias to variation in stimulus hue rather than to variation in stimulus brightness. Nonetheless, it is worth noting that an unconditioned bias for shorter wavelengths has been found in other species inhabiting shallow waters such as goldfish (Muntz & Cronly-Dillon 1966), turtle (Mrosovsky & Carr 1967), anuran tadpoles (Jaeger &

Hailman 1976), frogs (Muntz 1964) and zebrafish (Colwill et al. 2005). Some of the typical spatial and temporal habitats used by guppies have ambient light spectra where blue (woodland shade) and violet (early or late in the day) predominate (Endler 1991, 1993a). In such conditions, short- and medium- wavelength-sensitive cones (S and M cones) are strongly stimulated generating familiarity with short relative to long wavelength light and potentially inducing the spectral bias observed. However I cannot ascertain such reasoning, as I am unaware of the spectral properties of the environment in which the guppy population I tested has evolved. Furthermore, when blue-violet irradiance prevails, guppies have less sensitive S and M cones and more sensitive long-wavelength-sensitive cones, which increase the visual contrast between males colour patterns and make orange and red spots more conspicuous to females (Gamble et al. 2003). The sensory drive model predicts that the visual system would evolve to match the local environmental conditions and maximize signal efficiency (Endler 1992, 1993b). Accordingly, females should have evolved genetic preferences for orange and reddish colour spots but such preferences has not been found in my population. Hence, the spectral bias for violet in the Lower Aripo population and its adaptive significance remains unclear.

With respect to the first prediction formulated here above, my results didn't suggest that the conditioning to a particular colour during ontogeny shaped female interest for coloured objects later in life and by extension foraging preferences. The single evidence of differences between treatments in the predicted direction comes from females trained with violet that are marginally more attracted to violet discs compared to females from the control group although such observation could be an artefact of the innate attraction to violet previously discussed. An alternative view is that growing females were not capable of individual associative learning. Despite large accounts for social learning in guppies (Laland & Williams 1997; Lachlan, Crooks & Laland 1998; Reader, Kendal & Laland 2003; Chapman et al. 2008b), evidence for individual learning is scarce or nonexistent. In a study not presented in this thesis, I demonstrated learning of a visual discrimination task in adult females. They learned to choose one of two colours (identical to the orange and violet used in this study) for a food reward in an experimental setting somewhat

similar to the one utilized in this study. Even though the same experiment was not performed with juveniles, it is reasonable to infer that maturing individual also have the sensory and neural systems enabling them to learn, ruling out learning inabilities as a explanation for the absence of results in coloured discs attraction tests. By contrast, social learning could have hampered the association between the coloured hole and the food reward through a “local enhancement” process where the behaviour of one animal draws the attention of a second animal to a particular stimulus in the local environment (Thorpe 1963). Following this scenario, few individuals would have learnt individually (e.g. asocially) to locate the food becoming demonstrators for the majority of naïve individuals. Given the propensity of guppies to shoal, naïve individuals would approach and follow the few demonstrators and ignore the colored cue. Moreover, olfactory cues released by the flakes or by some fish having eaten and met close to the entrance of the feeding compartment could indicate the location of food. In such a case, foraging fish wouldn’t need to associate a specific colour with food. Eventually, the reduced statistical power owing to small sample size could account for the difficulty to detect any variation in foraging preferences between rearing groups.

Regarding the second prediction made, a treatment effect was observed in the degree of preference for blue-violet colour patterns but the change in preference concerned females trained to associate orange with food and females exposed to orange during ontogeny (orange control). This result was unexpected and all the more difficult to explain as the preferences went in opposite directions. Females conditioned with yellow colour displayed a stronger preference for yellow males relative to females raised in the other conditions. This result is promising as it shows for the first time that acquiring colour preference outside a mating context can mediate the strength of sexual preferences even if it is important to put the outcome into perspective. The absence of preference for yellow discs and consequently the absence of relationship with sexual preference strength complicate the interpretation because of the impossibility to clarify the origin of the increase in yellow preference. The existence of plasticity in a pleiotropic link between foraging preferences and mate preferences or direct developmental effect of exposure to a colour could both lead to the observation just described.

Furthermore the control condition with orange that was introduced in the experiment to discriminate between these two alternative hypotheses didn't provide any help as no variation in orange preference with the group of females reared with the orange cue was observed.

Overall the absence of clear-cut results raises some issues in the study and potentially highlights the necessity to modify the experimental protocol. First of all, from a statistical point of view, the small sample size reduced the ability to detect treatment effects. A high mortality of juveniles during the development in addition to poor condition of some adult females (that were not tested) significantly reduced the number of tested females. Modification of inherent features of the protocol might also have yielded better results. For instance, to investigate possible plasticity in the development of foraging preferences, it might have been better to employ directly coloured food items for the rearing treatments. The use of Daphniidae (cladoceran crustaceans) would be particularly suitable as they are naturally transparent and easily dyed with food colorants. Likewise, during the test phase, using dyed daphnia is more relevant than using coloured discs even though Rodd et al.(2002) demonstrated the validity of attraction to coloured discs as a proxy for foraging preferences. The control condition with orange included in the experiment to test for possible modification of the visual system due to direct exposure to a colour outside a behavioural context might also require some change. A bigger stimulus might be more efficient at triggering variation in the development of the visual and/or neural systems in charge of processing colours. Finally, the experimental design should include a control coloured condition for each colour utilized as an experimental cue.

To conclude, the preliminary results of this study call for more investigations as it seems that the physical environment experienced during development could contribute to variation in female mate preference. As a consequence it might maintain high levels of polymorphism in male colour patterns, impose fluctuating selection pressures on males leading to the coexistence of multiple signals and accelerate population divergence. Contrary to a mechanism such as imprinting on non-parental adults that might inhibit reproductive isolation in its early stages,

variation originating in the local physical environment would precipitate pre-zygotic isolation between two geographically close populations differing in their abiotic conditions.

6. Chapter VI

General discussion and future directions

In this thesis I aimed at expanding the understanding we had of factors that shaped individual female mate preferences. More specifically, I investigated the influence of ontogenetic environments on variation in mate preference in a species that lacks parental care. Despite the crucial importance of development in a multitude of physiological, morphological and behavioural processes (West-Eberhard 2003; Monaghan 2008), very little research had been conducted in this area with species not relying on sexual imprinting. To do so, I used guppies, as there was already some evidence of early learning in mate choice, which paved the way for more research. Throughout the thesis, I examined how mate preferences varied in response to different aspects of social and ecological conditions experienced while growing. Changes in the social context consisted of varying the distribution of male phenotypes exposed to juveniles. Modifying the colours associated to a foraging context made up for the various ecological conditions. Learning is a form of phenotypic plasticity (Verzijden et al. 2012) and in this discussion the terminology “plasticity in mate preference” and “learned mate preference” refer to equivalent processes. The key findings are listed and discussed in more details in the next sections.

6.1. Take-home findings

My thesis strongly suggests that variation in the early social environment of female guppies has a profound impact on the acquisition of mate preferences in the form of oblique imprinting. Integrating the early social life as a key determinant in the broad study of sexual selection would allow a better understanding of the evolutionary trajectories of female mate preferences and male sexual traits. On the other hand, variation in the early physical environment yielded preliminary results, which, if they are not conclusive, at least call for more research.

6.1.1. Early social environment and female mate preference

6.1.1.1. Effects of variation in specific sexual cues and of timing of exposure

In this thesis, I considered the effects of variation in the phenotypic distribution of a male's trait known to be a good predictor of mating success in the population (chapter three; total colour pattern is a genetically preferred trait) and in the phenotypic distribution of a male's trait not primarily involved in the process of mating decision (chapter two; little genetic additive variance in orange preference). Both of which mediated the expression of choosiness (responsiveness and discrimination) and preference functions. Remarkably, not only preference function for the trait to which females were exposed was affected but also preference functions for other sexual traits. Contrary to some species where an interaction between different aspects of sexual behaviours was detected in the mate choice production (Bailey 2008), preference functions and choosiness were, here, independent. A rare male effect whereby females tended to avoid to associate with males bearing some sexual traits experienced as juveniles was identified in both cases. It is the first time this phenomenon is emphasized following early social experience and reinforced previous research in guppies that showed the same effects based on prior adult experience (Eakley & Houde 2004; Zajitschek & Brooks 2008). Favouring rare or novel phenotypes over common ones might increase survivorship and thus provide direct benefits to females because of the reduced risks involved in courtship with rare males – predators tending to prey upon common morphs more often due to a "search image" process (Olendorf et al. 2006). Further, choosing to mate with non-familiar individuals might be a strategy to reduce the costs associated with inbreeding in species lacking kin recognition mechanism such as guppies.

The timing of exposure also played a very important role in the learning of mate preferences and worked either by itself or in interaction with the effects of the sexual traits. Its influence was noticeable after exposure to the different orange treatments and concerned both responsiveness and preferences. However, the

effect of the duration of exposure was particularly striking in treatments where the amount of total colour varied since short-exposed females didn't rely anymore on colours to discriminate among males but, instead, used other sexual cues. Surprisingly, depending on the type of stimuli (orange or total colour that is, non-genetically- or genetically-preferred trait) females reacted differently to short- versus long-exposure. The importance of the period of stimuli exposure in the determination of mate preference recalls filial imprinting, a learning process that occurs during a sensitive period. Once thought to be sharply timed and irreversible, recent findings showed that there are multiple varieties of, and mechanisms underlying, changes occurring in sensitive periods (Thomas & Johnson 2008). The effects of experience operate within the constraints imposed by genetics on neural circuits and only certain kinds of stimuli are able to shape particular circuits. Within that range of stimuli, some are preferred over others and might determine the length of experience-driven plasticity in the brain (Knudsen 2004). Hence, different patterns of connectivity (resulting in different behavioural expressions) are the results of the predisposition of a circuit to be plastic and of the relevance of the stimuli experienced. This could account for the differences females displayed after having experienced different types of male stimuli. However more research is needed to ascertain whether the same mechanisms underpinning the development of circuits' architecture are at play between oblique and filial imprinting.

6.1.1.2 Effects of variation in the phenotypic variance of the social environment on female and male sexual behaviours

Different levels of phenotypic variance independent of the values of sexual traits on which females based their choice are also a source of variation in both female preferences and responsiveness. To my knowledge, it is the first time that the relevance of overall phenotypic variance in the chosen sex is investigated as a possible cause of mate preference plasticity in the discriminating sex. No new preferences were acquired but a strong increase in the degree of preference for yellow, black and total colour traits was observed in females that experienced a social environment with males all different compared to females reared in

conditions with less variability in the phenotypes experienced. Further, these females were also less responsive to males' solicitations.

Taken together, these results showed that variance in male mating success increased because of females being choosier in a context where the phenotypic variance among males increased. As a consequence fewer males have access to females prompting them to adapt their mating tactics. Depending on the ecological conditions, guppy males commonly alternate between two mating tactics that are forced copulations and courtship displays (Endler 1987; Godin 1995; Magurran 2005). Here, I demonstrated that in response to an increase in females' choosiness, males switched towards more sneaky attempts hindering, to some extent, the strong sexual selection exerted by female choices. Furthermore, experiments were conducted in a controlled environment with females in good conditions and it is unlikely that the selection pressure imposed by female choice on male traits would be as intense in nature, if, for instance, they varied in their individual condition (Cotton et al. 2006). Hence, in a given population, even though high levels in male phenotypic variance can induce strong female preferences, extraneous factors (see introductory chapter) might reduce the number of females actually exhibiting such level of preferences.

6.1.2. Early physical environment and female mate preference

Variation in the early physical environment has not yielded conclusive results about whether it shapes female mate preferences. Adopting a sensory approach, I predicted that learned preferences during ontogeny in a foraging context would influence preferences in a mating context later in life. No correlations between attraction to coloured objects and strength of preference for the corresponding colour had been uncovered dismissing the experimental hypothesis. Beside plasticity in pleiotropy between behaviours sharing a same sensory system, I also controlled for the possible alteration of the visual system (presumably affecting mate preference) following direct exposure to an orange object. A large body of studies investigated plasticity in the visual systems of vertebrates describing how

the spectral sensitivity of visual pigments could be tuned to the spectral qualities of the light environment (reviewed in Bowmaker (2008) and Bowmaker & Hunt (2006)). Conversely, less work has been carried out on how photoreceptor cones develop in the presence of objects with different reflectance spectra. My experiments didn't provide any evidence of such effects. Due to experimental weaknesses such as little sample size, it is difficult to decide whether the effects sought were undetectable or non-existent.

6.2. Evolutionary consequences

We saw that determining the exact effects of the social environment on the direction and strength of female preferences is difficult because of the multiple types of male phenotypic distributions involved in their acquisition and subsequent expressions. Moreover, each component of a female sexual behaviour varies in response to the social environment and to a lesser extent to the physical environment, adding to the complexity of the role that variation in preferences plays in evolutionary processes. The evolution of male sexual traits, female preferences themselves and population divergence are considered in the light of these findings.

6.2.1. Are female mate preferences acquired during ontogeny adaptive?

Adaptive behavioural plasticity can play an important role in buffering environmental heterogeneity by allowing individuals to cope with new or altered conditions (West-Eberhard 2003). In the context of mate choice, a female reaps benefits for herself (direct benefits) or for her offspring (indirect benefits) when choosing an attractive male in one set of conditions. This mate choice might not be optimal anymore in a different environment or if her offspring disperse to a new location (Greenfield & Rodriguez 2004; Bro-J¯rgensen 2010) but flexibility in mate preference may solve this problem. Adaptive plasticity has been reported in

previous studies (Qvarnstrom, Part & Sheldon 2000; Grether et al. 2005; Chaine & Lyon 2008) and my work offered support to the adaptive value of preference plasticity in guppies. Both choosiness and preference have proven to be responsive to the variability found in the social environment. As briefly mentioned earlier, learning to favour rare/novel phenotypes over those experienced as juveniles can improve females' fitness when it reduces the predation pressure exerted on common phenotypes (search image mechanism) or when it promotes outbreeding. Further, rare male phenotypes might be less susceptible to diseases (Lively & Dybdahl 2000) which, if carried, could be transmitted during mating. Becoming choosier could also be an adaptive mechanism to breed in a context of high parasitism since the fewer males a female mates with the fewer parasites are passed. More generally, previous experience provides information on the variance in the quality of available males in the population. This allows a female to adjust her level of choosiness to the genetic benefits she can retrieve (or not). In a context of low-variability among potential mates, females ought to be less choosy to mitigate the costs associated with mate choice and ensure genetic variation among offspring. On the contrary, if male phenotypic variance is high then they should be choosier to benefit from the best quality males. My results showed such pattern as, females were more responsive after exposure to a low variance treatment (e.g. LNS treatment, chapter three) but strongly increase the strength of their preference when exposed during ontogeny to high level of male phenotypic variance (chapter four). Consequently, strong selection pressures exist to favour the evolution of plasticity in the form of learned mate preference within a population experiencing variable environments.

However, some constraints might limit the extent to which individuals evolve the ability to adjust perfectly to all environments encountered (DeWitt, Sih & Wilson 1998). For instance, in response to stronger preferences, guppy males switched mating tactics and attempted more sneak copulations, which incur some costs to females, opposing the evolution of plasticity in preferences. From a mechanistic point of view, learning involves sensory and neural constraints in addition to energy costs, which limit the range of behavioural flexibility and thus the potential for evolutionary changes.

Moreover, *genotype by environment interaction* (GxE), being a measure of within-population variation in plasticity and describing how different genotypes display different reaction norm slopes across environments, might also come into play with the evolution of learning performances. Depending on the genetic variation that exists between individuals in the capacity to exhibit plasticity in preferences, the opportunity for selection to act on this variation will vary. In figure 6.1, I explore different scenarios of GxE in learned preference and how evolution can operate within the framework of ontogenetic changes following social experience. In this thesis, I examined how the social environment shaped female mate preference, thereby illustrating plasticity in preferences although not explicitly testing for GxE.

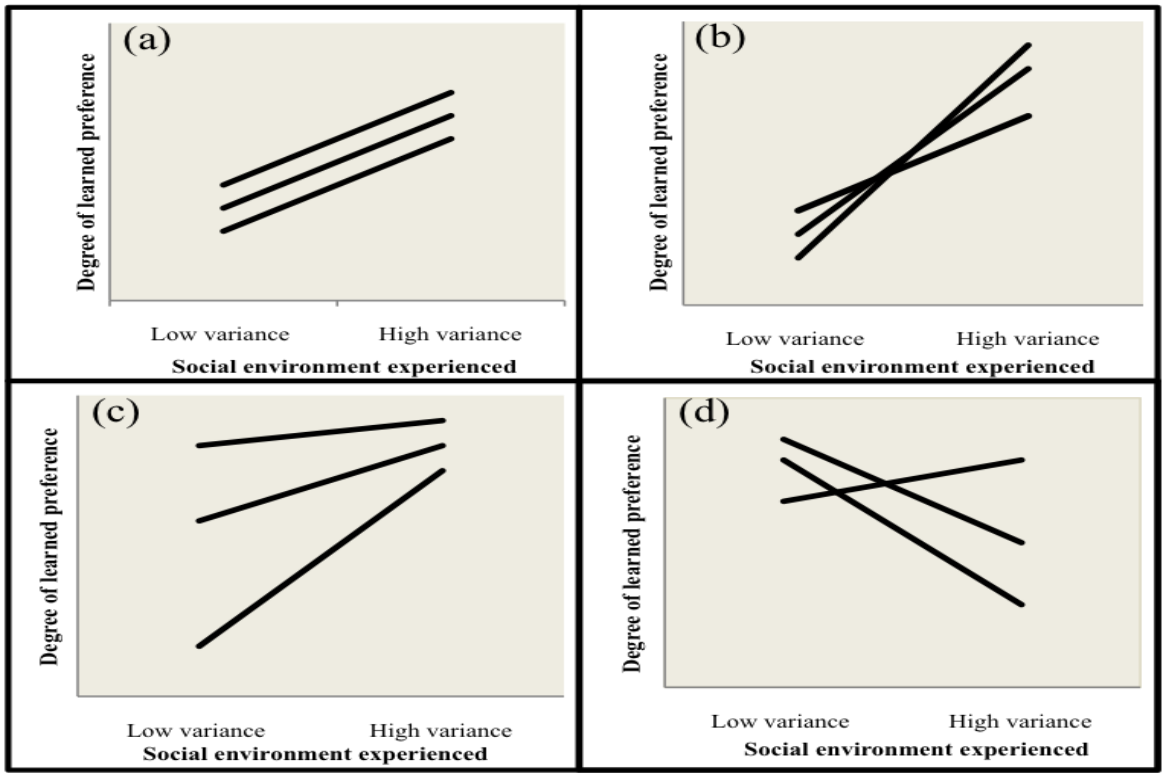

Figure 6.1: Reaction norms for three females displaying different learned preferences after exposure to two different social environments defined by the level of phenotypic variance exhibited by male traits. (a) No GxE. The effect of environmental variation on learned preferences (phenotypic plasticity) is indicated by the non-zero gradient between environments and additive genetic variance is indicated by the differences in trait expression within environment; in this case there is no interaction between the two, and the effect is the same for all individuals as shown by the parallel slopes. (b) GxE where,

even if the rank order of phenotypes changes, the effects of environments remain qualitatively similar. The scale of genetic variation between learned preferences is relatively constant after exposure to both type of social environment. (c) GxE where the scale of genetic variation changes between environment and not the rank order of phenotypes. (d) GxE where early social experience has opposite effects on the expression of preferences between individuals and the scale of genetic variation varies as well between environments.

Like with any other traits, evolutionary changes in learned preferences depend on genetic variation between individuals and response to selection (direct or indirect) as a consequence of fitness. In situation (a), genetic variation being the same between environments (individuals respond in the same way), the evolutionary trajectory of preferences depends essentially on whether there is selective advantage provided to females when learning. In scenario (b), different individuals have different performances across environments but overall, the environment causes a predictable increase in preference and genotype variation didn't differ much within environments. As in situation (a) evolutionary changes depend on the relative gain in fitness associated with learned choices but are limited due to low genetic variation. In scenario (c), although environments drive preferences in the same direction, individuals responded very differently between low and high variance situations and GxE affects the intensity of selection on learned preferences. The relative strength of selective advantage is stronger in low variance conditions. In scenario (d), the strength and direction of environmental effect vary across environment. Benefits of learned preference are little and GxE influences both the strength and direction of selection on learned preferences.

6.2.2. Theoretical models and the evolution of mate preferences acquired through imprinting

Mathematical models have analysed the spread of learned mate preference acquired through an imprinting mechanism (Laland 1994; Aoki, Feldman & Kerr 2001; Ihara, Aoki & Feldman 2003; Ihara & Feldman 2003). As in classical sexual selection models where mating preferences are passed on through genetic mechanisms (see below), preferences in these models evolve due to associations formed between the learned preference and the genetically inherited trait affecting fitness, mimicking the runaway sexual selection process. The study from Ihara & Feldman (2003) is particularly relevant to the guppy mating system and to my findings. They modelled the evolution of disassortative and assortative mating preferences imprinted during early ontogeny on a genetically transmitted trait. Disassortative mating could be assimilated to the preference for rare phenotypes found in my study (see chapter three and chapter two) and as they didn't assume any particular type of imprinting (paternal, maternal or oblique) it leaves open the

possibility that females acquired preferences via non-parental phenotypes as in guppies. Incorporating in the model an inbreeding depression mechanism, which is a putative cause for rare male preference in guppies, they showed that disassortative learned preference could evolve even if initially rare. Tramm & Servedio (2008) identified the “imprinting set” (i.e. the set of phenotypes that are imprinted) as a crucial factor in determining whether a gene for a specific learned preference will evolve - the spread of the gene being facilitated when a lot of individuals in the imprinting set carry a trait with high fitness. Moreover, the evolution of paternal imprinting was found to be more likely than maternal or oblique imprinting. This outcome raises a question: is the little evidence of oblique imprinting (for references see the different introduction of the thesis) found in nature an illustration of the theoretical model or simply the result of a lack of research?

6.2.3. Influence of the social environment on mate preference and classical model of sexual selection

Classical sexual selection models such as Fisherian models (Fisher 1930; Lande 1981) and good-genes models (Zahavi 1975; Grafen 1990b, a; Iwasa, Pomiankowski & Nee 1991) both assume heritable variation in male traits and female mate preferences resulting in a positive genetic covariance between ornaments and preferences in the offspring generation. The ornaments evolve as a consequence of sexual selection favouring genes coding for attractiveness (and/or viability) and the preference evolves as a correlated response to selection on male traits (Lande 1981; Kirkpatrick 1982). However, these genetic models completely ignore plasticity in mate preferences although being frequent. While evidence for heritability of preferences, a critical assumption of traditional models, remains scarce (Schielzeth et al. 2010), several authors have questioned some key predictions of these models such as the presence of genetic benefit for mate choice and the evolution of female mate choice by indirect selection (Kirkpatrick & Barton 1997; Houle & Kondrashov 2002; Hall et al. 2004; Kokko et al. 2006; Qvarnstrom, Brommer & Gustafsson 2006; Kotiaho & Puurtinen 2007). Indeed, if

the environment that parents and offspring experience varies, the benefits of the parental genes can be modified precluding an increased viability and/or fecundity in offsprings. Females adjusting their choice to the distribution of male phenotypes experienced during ontogeny, linkage disequilibrium between a specific sexual trait and its associated preference might be difficult to arise or to be maintained in a changing social environment. Outcomes of traditional models assuming a constant selection over time and space are thus only applicable to a fine temporal and spatial scale presenting homogeneous features. To have a more realistic and integrated view, they have to be refined and take into account environmental components of variation in mate choice. Drawing on this, Bailey & Moore (2012) modelled the influence of the social environment on the evolution of preferences and demonstrated that runaway sexual selection could proceed in the absence of genetic correlation. To do so, they used an interacting phenotype approach that incorporates indirect genetic effects (IGEs, see appendix). The social environment was determined by the trait value of the sexual cues with which females interact. Hence, female preferences could also evolve without linkage disequilibrium if the influence of the social context reaches a certain level.

6.2.4. Variation in female mate preference and evolution of male sexual traits

The influence of variation in female mate preference on the evolution of male sexual traits has been largely discussed in the light of my results throughout the different discussions of previous chapters and only the main points will be reiterated here.

Different types of early social environments shape both choosiness and the strength and direction of multiple preference functions, which in turn impose sexual selection on multiple traits simultaneously. Accordingly, prior social experience is a key determinant of the multivariate sexual selection operating on each trait both directly and indirectly via genetically correlated trait(s). Given how the different components of mate choice function and given the complex nature of covariance

between male traits, the response to selection might not be understood with a univariate approach of phenotypic evolution within populations and equivocal to extrapolate across populations.

Depending on whether the social environment is relatively variable and/or whether growing females are constantly exposed to males during development, fluctuating selection promotes polymorphism in colour patterns. Sufficient gene flow and different predation regime are crucial elements to provide the variability required. As a consequence of flexible mate choice and the resulting dynamic selection, it is important in the future to focus on the most appropriate time (and spatial) scale to understand the effect of sexual selection by mate choice. When selection fluctuates across years (or habitat), a longer time (or a wider habitat range) frame than the usual short-term studies is more suitable for predicting male trait evolution. Conversely, understanding selection operating on female mate preference requires a shorter time frame in order to detect any plasticity and its potential adaptive significance.

6.2.5. Learned preference, population divergence and reproductive isolation

Recently, the extent to which learned preferences participate in the dynamic that drives population divergence, reproductive isolation (barrier to gene flow) and ultimately speciation has received much attention (Verzijden et al. 2005; Servedio et al. 2009; Svensson et al. 2010; Verzijden et al. 2012). It is frequently suggested that mate choice imprinting facilitate reproductive isolation (Irwin & Price 1999). Using a mathematical model, Verzijden et al. (2005) investigated how the ontogeny of female mate preference influences sympatric speciation and demonstrated that the mechanism for the inheritance of mate choice and thus the set of imprinting was decisive. Imprinting on one's parental phenotype leads to assortative mating where similar individuals preferentially mate with each other. When a new trait appears in the population, it might rapidly lead to pre-mating isolation even if it is initially rare (Servedio et al. 2009). Empirical support came from the collared

flycatchers as females imprinted upon males artificially provided with a novel ornament (Qvarnstrom et al. 2004) and from cross-fostered great tits that preferred to mate with the foster species (Slagsvold et al. 2002) or copied its song (Johannessen, Slagsvold & Hansen 2006).

By contrast, early preference learning from non-related individuals hinders pre-mating isolation and thus speciation (Verzijden et al. 2005). Oblique imprinting hampers the build-up of a genetic correlation between preferences and traits essential to the process of divergence. My different findings are in line with such analysis and indicate that the conditions are not gathered to favour pre-mating isolation in guppies. The large effects of early social experience on the formation of the different components of mate choice in general and the preference for rare male in particular would prevent sexual isolation. Early social imprinting comes along with other factors such as multiple mating (Becher & Magurran 2004), sneak copulations (Matthews & Magurran 2000), gene flow (Endler 1995) and phenotypic/genetic variation in preferences that would impede speciation because of the likelihood of mating between two divergent population that would come into secondary contacts. Hence, even if guppies show rapid evolution when facing new conditions (Reznick et al. 1997), differentiated populations are not inevitably a step towards speciation (Magurran 1998).

6.3. Future avenues

Developmental plasticity has long-term effects on morphology, physiology and behaviours but these effects are not always irreversible. Parental imprinting implies that once the sensitive period has ended, the preferences are formed and stable throughout life – the imprinted individual being consistent in his/her choice of partner. However, it is not established that the proximate mechanisms underpinning parental and oblique imprinting are the same, leaving open the possibility that individuals imprinted upon non-parental adults are less consistent in their choices during lifetime. Longitudinal studies would allow assessing whether preferences acquired during development are maintained through life and how "resistant" they are to preferences that could be formed at later life stages through

other kind of social learning. This involve learning after reaching maturity (Magurran & Ramnarine 2004; Zajitschek et al. 2006), eavesdropping and audience effect (Danchin et al. 2004) and mate choice copying (Dugatkin 1992), all of which are particularly evident in guppies (Witte & Nöbel 2011). In addition to socially-based preferences, most species have genetically-based preferences (Bakker & Pomiankowski 1995) that also mediate the final mate choice. Hence, the potential interactions and optimization between these different forms of preferences (genetically- versus culturally- inherited), the ecological context and female's conditions in which some are favoured over the others offer exciting avenues for research in the future. Level of consistency in learned preference and its relative importance compared to other kind of preferences has far-reaching consequences for the evolutionary fate of individuals and the population to whom they belong as strength and direction of sexual selection depend upon it.

Another interesting route for future investigation would focus on the female sensory system. Examining individual variation in its development is a pivotal field since selection pressures are likely to be greatest during early life history (Dangles et al. 2009). Although the role of the early social environment has proven to be essential in mate choice for guppies, we do not have yet, any information on whether it alters some vision components. As a result of variation in the spectral properties of the imprinting set, we can hypothesize that individuals would vary, once adult, in their visual sensitivity or even in the pace at which visual signals are processed. Further, following the idea of compensatory sensory plasticity (Monaghan 2008; Chapman et al. 2010), little information provided by sexual traits perceived in one modality such as vision (little variance in traits or little trait values) would redirect growth allocation towards other sensory modalities, lowering the ability of an individual to rely on the deprived system. Such variation will affect the direction and intensity of selection operating on females sensory modalities (Ronald et al. 2012) and, accordingly, on male sexual signals.

Once a signal has been perceived by the sensory machinery, it is transferred to the central nervous system where it is processed. As for the sensory system, it would be very interesting to look at the influences of different ontogenetic environments on the neural processes and their genetics, subsequently involved in sexual behaviours. In recent years, progress in molecular biology has delivered the tools required to examine how the brain governs behaviour at a molecular level. Researchers have begun to identify the dynamic genomic response involved in mate choice, widening the horizon of sexual selection studies (Andersson & Simmons 2006; Cummings 2012). Using the Poeciliid family for their marked differences in mating systems and for the ease with which sexual behaviours can be observed, they characterized genes functioning in the brain of females when engaged in mate choice compared to other social activities (Cummings et al. 2008; Lynch, Ramsey & Cummings 2012). In swordtails, *Xiphophorus nigrensi,* they showed correlated patterns between the expression of candidate preference genes and female sexual response towards males (Cummings et al. 2008). Further, at a functional level, many of these putative candidate genes are linked to synaptic plasticity and synaptogenesis, which are the neural basis for learning and memory. It is then expected that experience-mediated preferences detected at the behavioural level would affect, at least partly, the same genomic pathways. My results suggested then, that guppy would be a good candidate model to extend these prior studies and orientate the investigation towards the effect of the early social environment on the described genetic pathways. The importance of developmental stages on brain plasticity and the phylogenetic proximity between these species reinforced the idea of fruitful discoveries in future studies. Looking at how the genes are activated or inhibited in a mating context will shed light on the way early social conditions shape the genetic architecture underpinning the learning and memory processes at play. The understanding of the mechanism underlying the absence of attraction to any colour patterns for short-exposed females (chapter three), the increase in preference strength when exposed to high-variance males (chapter four), or the possible pleiotropic links between choosiness and preference functions would greatly benefit from a functional genomic approach.

Future research should also occur at a group level. Animals living in social contexts present complex non-random patterns of associations between members of a group (Croft, James & Krause 2008) determining the social network structure of populations. The structural organization of groups determines the strength and the direction of interactions between individuals that will in turn mediate the potential of learning from conspecifics. When females shape their preference through experience pre- and post- maturity or through mate copying for instance, the social organization of a group may have a strong influence on the expression of preferences. As a consequence it seems particularly important to integrate in the study of mate preference and its evolutionary implication the quantitative framework offered by social network theory.

In this thesis, effects of the ontogenetic environment have been exclusively analysed from a female perspective. Just as there are strong selection pressures on females to adjust their preferences according to the local conditions, males could maximize their fitness tuning the development of reproductive characters to environmental cues encountered. Incidentally, plasticity in male sexual traits owing to their social status (Cornwallis & Birkhead 2008) or previous pairing situations (Badyaev & Duckworth 2003) has been reported, among other causes. However, the extent to which sexual signals in adults mediate the development of sexual traits in juveniles remains poorly understood. The conspicuousness of such signals provides a cheap source of information on the number and quality of future competitors that growing males could use to adaptively allocate resources to the production of attractive traits. To my knowledge, Bailey et al. (2010) were the first to demonstrate such phenomenon using field crickets, *Teleogryllus oceanicus.* To secure copulation, males rely on two alternative mating tactics: the production of a long-range calling song obtained by rubbing their forewings together or an alternative tactic whereby silent males behave as “satellites” and parasite calling males. When reared in the presence of calling song (relatively to males reared in silence), males were less likely to exhibit satellite behaviours, were in better condition and invested more in reproductive tissues. Hence, social experience in

the form of a sexual signal was sufficient to stimulate adaptive plastic changes in sexual behaviour and morphology.

Recently in guppies, two elegant studies highlighted the importance of the early social environment on the development of male sexual behaviours (Guevara-Fiore 2012; Guevara-Fiore et al. 2012), showing that the experience gained during ontogeny with either males or females mediated the use of courtship display versus sneak attempts. Thus, as with females, a large part of the reproductive behaviours are learned (before and after sexual maturity) and not genetically fixed**.**

Following up with the studies aforementioned, I propose to examine whether male reproductive tactics could also be shaped by early experience with different levels of attractiveness displayed by adult males. Sperm competition theory predicts that males should adjust their mating tactics to cues that provide information about the risk of sperm competition. Sexual signals are such cues as they indicate the abundance and quality of rivals and are then good candidates to promote variation in male mating behaviours.

Models of sperm competition also predict that males should increase their reproductive investment as sperm competition increases but that the investment in one reproductive trait comes at the expense of another reproductive trait (Parker et al. 1997; Birkhead & Møller 1998; Evans 2010). In parallel to my previous hypothesis, I suggest that the development of primary and secondary sexual characters could also be dependent on the sexual cues present in the early environment. Depending on the male phenotypic distribution, developing individuals might maximize their fitness investing differentially in gaudy ornaments or sexual organs such as the length of the gonopodium or the spermatozeugmata (i.e. tissues producing sperm). Allocating more resources to colour patterns will increase male attractiveness and give an advantage in pre-copulatory episode of selection but on the other hand, investing more in tissues or organs that can increase the number, the quality or the transfer of sperm would provide advantages in post-copulatory selection.

6.4. CONCLUSION

Previous works, carried out essentially in wolf spiders and Poeciliids fish, laid the ground for the investigation of the effect of early experience on the formation of mate preferences. Broadening the contribution of these studies, my thesis has demonstrated the crucial impact of the set of imprinting and of the timing of exposure to shape female mate preferences in a species lacking parental care. My findings add to the growing body of evidence emphasizing the importance of non-genetic information in the transmission of mating preferences and widen the scope of cultural transmission in evolution. Consequently, in order for progress to be made in the understanding of processes such as the coevolution of sexual traits and preferences or reproductive isolation, it is essential to integrate the study of sexual selection with the study of the influence of the environment in which individuals develops. More generally, the next step would integrate various causes of variation into a unified framework and pay a special attention to their mutual interactions.

Appendices

Appendix to Chapter 2, 3, 4, 5 - Simpson Diversity index: a measure of colour pattern diversity

Simpson's diversity index (aka Species diversity index) is one of a number of diversity indices, used to measure diversity. In ecology, it is often used to quantify the biodiversity of a habitat. It takes into account the number of species present (richness) as well as the relative abundance of each species (evenness). I adapted this index to evaluate the diversity of colour patterns of individual guppy males. Instead of considering the number of plant species and their relative abundance, I measured the number of colour classes (comparable to richness) and the area of each class relatively to the total body colour area (comparable to evenness, see below). The term "Simpson Diversity Index" (named D) refers, actually, to any one of three closely related indices. I chose to use the "Simpson's reciprocal Index" (see below). The values span from 1 to N with N being the number of category being used (for example if there are five colour classes equally distributed, the higher value is X=5). The lower the value the lesser diversity and vice et versa.

$$D = \sum_{i=1}^{N} (p_i)^2$$

N= Number of colour class(es)

p_i= proportion of colour i relatively to the total colour area

Simpson's reciprocal index = 1/D

Appendix to chapter 2, 3, 4, 5 – Male attractiveness

In this thesis, attractiveness is used in two different but related ways. On the one hand, it refers to a conceptual notion defining how males, based on their phenotype, entail females' sexual interests and/or responses. Depending on a female preference, a male is more or less attractive, which determines ultimately his mating success. On the other hand, variation in male attractiveness defines a variable used to measure female preferences, that is, by calculating the effect of particular male traits on the responsiveness of females. Depending on the experimental setting, female responses were not calculated in the same way. In chapter two and three, responses were measured individually and were described as the proportion of time spent in the preference zone of the different male present in the mate choice arena. In this case, individual preference functions are described by the regression coefficients of the female's responses on the male trait of interest. In chapter four and five, the relative attractiveness of a given male to females is estimated as the proportion of his displays that elicit a female sexual response also known as the "fraction response". With this setting, individual females are not distinguishable so the fraction response represents an aggregate measure of the preference of all females present in the experimental group for that particular male. The slope of the regression of the fraction response on a given male trait is a measure of the overall degree of female preferences for that trait.

Appendix to chapter 2-3: The genetic basis of ornamental traits in guppies:

There is extensive genetic evidence supporting a heterogametic XY sex determination in guppies (Winge 1922a, b). Alleles involved in phenotypic variation of ornamental traits (including patterns of variation in colour, size, position and shape of spots) are sex-linked and to a lesser extent located on autosomal loci (Winge 1922a, b; Winge 1927; Yamamoto 1975; Houde 1992; Brooks & Endler 2001a). Particular colour spots are under the control of genes located on the non-recombining region of the Y-chromosome (tightly linked with the sex-determining loci) but also on genes that recombines between the X and the Y chromosomes and genes harboured by autosomes (Lindholm & Breden 2002). However, the fact that males and their fathers have usually very similar colour patterns suggests that X-linked genes and autosomal genes might not be normally expressed in males. Furthermore, quantitative genetic studies of the inheritance of male colour patterns (Houde 1992; Brooks & Endler 2001a) showed that estimates of heritability for these colours were mainly attributable to variance among sires. Such asymmetry between sire and dam additive genetic contribution to a sexual trait is consistent with Y linkage of much of the genetic variation in the trait. Recently, a quantitative trait loci analysis (Tripathi et al. 2009b) revealed the complex nature of the genetic basis of colour variation in guppies. All the colour spots analysed (essentially different black and orange spots) showed a significant contribution of a combination of QTL situated on different linkage group (i.e. chromosomes) although sex-linked QTLs were over represented in frequency and were accounting for more variance in most of the colour spots than the other QTLs. Consequently, quantitative genetic studies and QTL analysis demonstrate that on one hand one colour spot is the product of many genes (situated on loci that are physically linked or not) and on the other hand a single gene can control more than one colour spot. Physical linkage of a set of genes responsible for male sexual traits with the sex determining locus (SDL) on the non-recombining region of the Y chromosome (Tripathi et al. 2009a) is consistent with evolutionary theory predicting that sexually antagonistic genes (genes coding for weaponry or gaudy traits) that

benefit the heterogametic sex are more likely to evolve on sex chromosomes and particularly close to the SDL on the Y chromosome.

Appendix to chapter 6: Interacting phenotypes and indirect genetic effects

Like any other trait, the expression of a behavioural trait can be partitioned into a genetic component (g) and an environmental component (e) following a standard quantitative genetic approach (z=g+e). Many behaviours involve interactions among individuals and, as a consequence, are influenced by conspecifics' traits (i.e. behaviours or other type of traits). *Interacting phenotypes* is the term coined by Moore, Brodie and Wolf (1997) to designate these traits expressed, influenced by or involved in social interactions. They differ from other traits in terms of underlying genetic models and evolutionary dynamics as explained here below. Extending the basic quantitative genetic framework, the environmental component is further partitioned into social and non-social effects:

$$z = a + e_n + e_s$$

Where z = phenotypic value of the trait
a = additive genetic effect (heritable component)
e_s = effect of the social environment on trait expression
e_n = all other sources influencing the expression of the trait including non-additive genetic effects and non-social environmental effects

In the simplest case, the effect of the social environment is attributed to a measurable trait *t'* borne by a single social partner. Hence, e_s have a genetic basis, evolve and contribute to the evolution of the *interacting phenotypes* (Moore et al. 1997; Chenoweth, Rundle & Blows 2010; Wolf & Moore 2010). Replacing the generic term for social environment e_s with the effect that a specific trait *t'* has on the expression of a trait *p* in a focal individual (z_p), the precedent equation becomes:

$$z_p = a_p + e_p + \Psi_{pt}\, z_{t'} \quad \text{(Wolf \& Moore 2010)}$$

Where
a_p = direct additive effect representing the effect of the focal individual's genotype on its own phenotype
e_p = random environmental effect other than the social environment
$z_{t'} = a_{t'} + e_{t'}$ = phenotypic value of the trait *t'* borne by the social partner and decomposed into heritable ($a_{t'}$) and non-heritable component ($e_{t'}$)
$\Psi_{pt'}$ = *interaction effect coefficient* defines the extent to which the expression of a trait p is influenced by the expression of another trait t' in a social partner. It can be viewed as a measure of how important social interactions are for the expression of a trait. The coefficient takes any value from -1 to 1 and zero indicates that there is no effect of the social environment.

Substituting, in the equation, $z_{t'}$ by its components we obtain:

$$z_p = a_p + e_p + \Psi_{pt}\, a_{t'} + \Psi_{pt}\, e_{t'} \quad \text{(Wolf \& Moore 2010)}$$

where $\Psi_{pt}\, a_{t'}$ represents the *indirect genetic effect* (IGE) and corresponds to the effect that the genotype of a social partner has on the expression of *p* in the focal individual. $\Psi_{pt}\, e_{t'}$ represents indirect environmental effects. Both direct and indirect additive genetic effects contribute to the evolution of trait *p* and, in some cases, a correlation between the genotypes of the interacting individuals is possible.

In the context of sexual selection, the *interacting phenotypes* framework provides a method to analyse the evolutionary dynamics arising when male sexual display *t'* ($a_{t'} > 0$) alters female preference *p* (Bailey & Moore 2012). In this context, the interaction coefficient Ψ indicate the degree to which female preference is enhanced or diminished after experiencing a male sexual trait t'. For instance, Ψ is positive if females increased their level of choosiness in response to the distribution of male trait value and on the contrary, a negative Ψ indicates a social experience that decreased choosiness in the population and led females to mate more randomly (Bailey & Moore 2012). Bailey and Moore (2012) demonstrated that while the line of equilibrium found in classical models of traits/preference co-evolution (Lande 1981) is not altered by the social environment, the conditions for

runaway are strongly affected. Positive Ψ tends to exaggerate evolutionary changes whereas a negative Ψ retards sexual trait elaboration driven by female preference. Incorporating feedback from the social environment in the model enable preferences to evolve even in the absence of genetic correlation with male traits. When dealing with sexual selection, the interacting phenotypes approach is in its early stages and, to date, only two studies have quantified Ψ (Chenoweth et al. 2010; Bailey & Zuk 2012). More investigations will provide a better understanding of the genetic architecture (direct and indirect genetic effects) of female preferences and male traits and clarify the conditions under which the Fisher process occurs. Given the importance of the social environment in the expression of female preference, guppy would be a suitable model system for future studies.

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