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Sexual selection by female choice in guppies (Poecilia reticulata)

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mainly when I address the question of why males have several secondary sexual characters. For the main part, I have only answered why male cues are exaggerated, and not what information the cues provide to the female.

Guppies are small ovoviviparous fish native to streams of northeastern South America and the nearby islands, including Trinidad (Houde 1987, 1988 b). For this study, fish were drawn from a feral population existing in a small (0.5-2 m wide, 75 cm to 20 cm deep at centre) stream about 500 metres upstream from the Beachwood mangrove swamp in suburban Durban, South Africa. There is some doubt as to the origin of this population, but it is likely that Trinidadian guppies were introduced to rivers and streams near human habitation in Durban and north to Empangeni as an attempt to control mosquitoes (Mr K Farquharson, personal communication).

I have structured the investigation as a series of papers which have been submitted to journals for publication. Taken together, they are intended to form a coherent and thorough investigation of the possibility that the secondary sexual characters, most notably colour spots, of male guppies have evolved and are maintained by female choice. Whilst why males bear ornaments at all is not beyond controversy, several new theoretical problems are raised by the fact that males of many species, including guppies, bear several ornaments. I have tried to interpret the results of this investigation in the context of theories dealing with the presence of multiple secondary sexual characters (see Møller & Pomiankowski 1993 a, Pomiankowski & Iwasa 1993, Iwasa & Pomiankowski 1994).

The first chapter is intended to reproduce a mate choice scenario similar to that faced by females in the wild, with the minimum of experimental manipulation.

Guppies (*Poecilia reticulata*) are possibly the most thoroughly studied species with regard to mating behaviour and the interplay of natural selection and sexual selection (Andersson 1994). Despite this, we still do not have a clear understanding of how or why the preferences of female guppies or the ornaments of males evolve and are maintained. There are remarkable inter-population differences in female mating preferences and male ornamentation, with most male secondary sexual characters related to female response in at least one population (see summarised data in Table A). This is thought to be a result of opposing selection for crypsis to avoid predators and for conspicuousness in attracting mates. The strength of these types of selection varies between populations according to local predation pressure (Endler 1980, Breden & Stoner 1987, Stoner & Breden 1988), background gravel patterns (Endler 1980, 1983, 1987), lighting conditions (Endler 1987, Reynolds & Gross 1992) and stream turbidity (Luyten & Liley 1991).

In this thesis I investigate female choice as a selective pressure for the multiple secondary sexual characters of males in a feral South African guppy population. I also investigate some aspects of the inheritance and ontogeny of the female preferences in this population, including the intriguing notion that female preferences may be transmitted culturally, by copying the choice decisions of other females, rather than genetically (Dugatkin 1992), rather than genetically. What drives the evolution and maintenance of female choice is a more difficult and controversial subject, especially in animals such as guppies in which there are no direct benefits to choosing a given male (Kirkpatrick & Ryan 1991). I can only make oblique contributions to the debate on the nature of the benefits to females of choosing,

will refer to from now on as ornaments and displays. Whilst female preference and the preferred male character may be strongly correlated within populations (Breden & Stoner 1987, Stoner & Breden 1988, Houde & Endler 1990) or individuals (Houde 1994), it is important not to conflate the processes selecting for the female preference and the selective pressures those preferences exert on the male character (Kirkpatrick 1987). This second selective process is the primary focus of this thesis.

Table A: The number of populations in which a relationship has been shown between each male guppy character and female response.

Character	Populations	References
Orange area	10	2, 3, 5, 6, 8, 10
Orange intensity	3	7, 9, 10
White area	4	2, 3, 8, 10
Yellow area	2	3
Number of black spots	5	3
Blue/green	4	3
Pattern complexity	1	12
Brightness contrast	1	3
Colour contrast	7	3
Body size	5	3, 13
Dorsal fin size	1	1
Caudal fin size	4	1, 3
Display rate	5	1, 4, 10, 11, 12
Male dominance	1	10

References: 1- Bischoff et al. 1985, 2- Endler 1983, 3- Endler & Houde 1995; 4- Farr 1980 a, 5- Houde 1987, 6- Houde 1988 a, 7- Houde & Torio 1992, 8- Kodric-Brown 1985, 9-Kodric-Brown 1989, 10-Kodric-Brown 1993, 11- McMinn 1990, 12- Nicoletto 1993, 13-Reynolds & Gross 1992.

From the production of the gametes to the provision of parental care, it is usually females that invest more, and whose lifetime reproductive output is therefore more constrained. As the requirements of parental care constrain lifetime number of offspring, so an individual is likely to gain more from choosing her mate. Trivers' insight explains why mutual choice occurs in monogamous species in which there is biparental care, and why promiscuous or polygynous species show female choice only.

Female mating preferences are phenotypic characters, expressed during choice decisions (Heisler *et al.* 1987), which may evolve for one or both of two broad types of reasons. Firstly, they may be selected for in an ecological context other than mate choice and thus provide a sensory bias which favours some males over others (Basolo 1990, Ryan & Rand 1990). Secondly, a females preferences may be adaptations to choose males that will contribute more to her fitness than other males. There are several such contributions that males can make, from relatively direct benefits such as the failure to transmit ectoparasites during copulation (Hamilton 1990), male parental care (Searcy 1982) and a high quality territory (Alatalo et al. 1986), to indirect and somewhat more controversial genetic benefits such as attractiveness of male offspring to females (Fisher 1930) and 'good genes' which increase offspring viability (Pomiankowski 1988, Grafen 1990 a, Hamilton & Zuk 1982).

The second important consequence of female mate choice is the selection that female preferences impose on the male traits and displays that are preferred. The most obvious consequence of this is the exaggeration of these male traits, which I

Preface

"There is a second agency at work in most (bisexual) animals .....

it does not require the death of the less successful, but gives to them fewer descendants"

With these words, Darwin (1858) in his joint paper with Alfred Russel Wallace in which they introduced the concept of natural selection to the Linnaean Society and the world, first described what was later to become known as sexual selection. Sexual selection has recently blossomed into one of the richest fields of research in behavioural ecology (see Bradbury & Andersson 1987, Andersson 1994 for overviews, and Cronin 1991 for an accessible yet thorough introduction). A phenotypic character will be sexually selected if there is a non-random association between it and some component of mating success (Darwin 1871, Møller 1994 a). In a wide variety of sexual organisms, there is intense albeit sometimes subtle intra-sexual competition for opportunities to mate. However, processes other than the acquisition of mates such as the mate's genetic quality, sperm competition, infanticide and parental effort may also be sexually selective (Møller 1994 a).

Trivers (1972) accounted for the fact that in species where there is mate choice, it is almost always the female that does the choosing, and where both sexes choose it is usually females that are the more particular. His explanation drew on the difference between the sexes in parental investment in each individual offspring.

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For my parents

Ben and Patti Brooks

who always let me decide

Abstract

I investigated some evolutionary implications of female mate choice in a feral population of guppies (*Poecilia reticulata*). I performed correlative and manipulative experiments to establish the relationship between female mate choice and other sexually selective forces including intermale aggression and sneak copulation, and the exaggerated secondary sex characters (ornaments) of male guppies. Orange (carotenoid) ornamentation is the best predictor of male attractiveness and mating success, a relationship which held under manipulation. Black (melanin) is sometimes correlated with male mating success. Manipulating the black area of males reveals that it is important to male attractiveness, and this appears to be in the form of a signal amplifier of male orangeness. This is the first experimental evidence for a visual signal amplifier. For both orange and black areas, the relationship between absolute area and fluctuating asymmetry is positive and significant, suggesting that neither is a signal of male condition. These results are interpreted in the context of theories of the evolution of multiple male ornaments. Females are able to express their preference for orange with the first mature male they ever encounter, suggesting an important role for genetic determination in female preferences. This is corroborated by high repeatability of female mate choice behaviour. Experience modifies a female's preferences as she is able to adjust her response to a male in relation to the ornamentation of (at least) the previous male she saw. Mate copying has no measurable effect on the outcome of female mate choice decisions.

Declaration

I declare that this thesis is my own, unaided work unless specifically acknowledged in the text. It is being submitted for the Degree of Doctor of Philosophy in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.



22 January 1996

Sexual selection by female choice in guppies (*Poecilia reticulata*)

Robert Clinton Brooks

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hypothesis, no negative relationship between asymmetry and size is anticipated (Møller & Pomiankowski 1993 b, but see Balmford et al. 1993).

Fluctuating asymmetry of orange and black colour areas was also examined as a potential cue for female choice because if it signals male quality it could be a particularly informative cue for mate choice (Møller 1992 b; Swaddle & Cuthill 1994).

METHODS

We used guppies collected from a feral population in Beachwood, Durban, South Africa in February and April 1993. Males bore a close superficial resemblance to Trinidadian wild guppies (from photographs in Endler 1983). Males all had orange (mean ± S.D; 8.0% of body area ± 0.5%) and black (3.5% ± 0.2%) spots but these differed widely in number, position, shape and area between males. Some males had iridescent (white/silver, blue, green and violet) (44.1% of sample) and yellow (30.5% of sample) patches. None of these colours other than the occasional small black spot were seen in females.

Fish were held in single sex stocks of about 40 per 100 l aquarium, the temperature was kept at 25°C by controlling room temperature and with thermostatically controlled heaters. Water was filtered through air-driven box filters filled with activated charcoal, filter wool and Siporax filter medium. A 12:12 light:dark cycle was maintained with dawn at 0900 hours. The fish were fed every morning on Tetramin flake for tropical fish.

different aspects of the bearers condition. (ii) The "redundant signal hypothesis" assumes that individual ornaments are noisy indicators of condition such that several condition-dependent traits would be more reliable indicators of vigour than one. (iii) The "unreliable signal hypothesis" predicts that most ornaments of multiple signal systems do not reliably signal condition but are maintained because they are relatively uncostly and there is a weak preference for them.

Our primary aim was to experimentally identify mate choice cues in a feral guppy population. Secondly, like Møller & Pomiankowski (1993 a), we used fluctuating asymmetry (FA) to attempt to discriminate amongst their three hypotheses. FA is a consequence of an individuals inability to develop identically on both sides of the body due to environmental stress and genomic disruption (Van Valen 1962). Condition-dependent ornaments which signal male quality by imposing a handicap (sensu Zahavi 1975) on the male will not only be largest in males of "high genetic quality", but also most symmetrical (Møller & Höglund 1991; Møller & Pomiankowski 1993 b, Watson & Thornhill 1994). Møller & Pomiankowski (1993 a) use the expected negative correlation between ornament size and asymmetry to test their three hypotheses. The first two (multiple messages and redundant signals) predict that multiple ornaments evolve because together they reliably signal male condition, and thus negative correlations between asymmetry and ornament size are expected, at least for some characters (others may not be signalling condition at that time, and under these conditions, the negative correlation is not expected). The unreliable signal hypothesis predicts that several relatively uncostly ornaments evolve in a so-called runaway manner (Pomiankowski & Iwasa 1993) and thus under this

Endler & Houde 1995) melanin and yellow (Endler & Houde 1995). The shape and size of the caudal and dorsal fins is highly variable and often quite exaggerated (Bischoff et al. 1985; Nicoletto 1991) and has been shown to be important in female choice (Bischoff et al. 1985), as has body size (Reynolds & Gross 1992).

In addition to the obvious advantages to a male in having a functionally effective intromittent organ, such structures are also often a cue for female mate choice (Eberhard 1985, 1993). We investigate the possibility that the gonopodia (intromittent organ, comprising the modified and elongated anal fin) of male guppies act as a cue for female choice.

There are several hypotheses that might explain the multiplicity of sexually selected characters in male guppies. Female choice can be based on assessment of different criteria at different times in the sampling period. Female fiddler crabs (*Uca annulipes*) discriminate amongst males on the basis of size, however they then choose amongst these larger males on the basis of burrow quality (Backwell & Passmore unpublished). Female Jackson's widowbirds (*Euplectes jacksoni*) cue on display rate and male tail length in making choice decisions, but the quality of a male's display court (which he constructs) needs to exceed a certain threshold if he is to mate (Anderson 1991). Our experimental design is sensitive to the possibility that females use different criteria to assess males at various stages in the series of behaviours between male display and mating.

Møller & Pomiankowski (1993 a) put forward three hypotheses explaining the maintenance of multiple sexual ornaments related to their ability to signal male condition. (1) The "multiple message hypothesis" holds that different ornaments signal

INTRODUCTION

There is wide acceptance that sexual selection (Darwin 1871) explains the presence of exaggerated male secondary sexual characters in many species. There is, however, little clarity as to why males of many species have more than one exaggerated secondary sexual character, especially when none is used in overt contests between males. The presence of multiple secondary sexual characters appears to be related to the intensity of sexual selection (Møller & Pomiankowski 1993 a). For example, polygynous or lek-mating bird species tend to have multiple sexual ornaments (eg. Zuk et al. 1992) whilst monogamous birds tend not to (Møller & Pomiankowski 1993 a, but see Burley 1981)

Guppies (*Poecilia reticulata*) are highly promiscuous, with a heavily male-biased operational sex-ratio due to the short period of female receptivity (Kodric-Brown 1993). The most successful type of mating (display-acceptance, Farr 1980) requires female cooperation, and the female receives no direct benefits because of her choice of mate. These features, together with the bright colour patterns of males, have made guppies a popular subject for studying the role of female choice in sexual selection.

Mature males bear conspicuous coloured spots and patches due to the presence of several different pigments: melanin (black/brown), carotenoids (orange) and less-prevalent structural pigments for iridescence (silver/white, blue-green, violet) and yellow coloration. All of these colours are important to male attractiveness or mating success in one or more populations: orange (Houde 1987; Long & Houde 1989; Kodric-Brown 1985, 1989), iridescence (Kodric-Brown 1985; Endler 1983;

Chapter One :

Female choice in a feral guppy population: are there multiple cues?

Brooks & Caithness 1995 a *Animal Behaviour* 50: 301-307

ABSTRACT

Mating behaviour in a feral guppy (*Poecilia reticulata*) population from Durban, South Africa was examined. Males bear carotenoid (orange) and melanin (black) spots and low levels of iridescent and yellow pigment. Areas of colour, body size and lengths of the dorsal fin and intromittent organ (gonopodium) were studied as female mate choice cues. Attractiveness was positively related to the degree of orange coloration in all tests, and with the length of the gonopodium in some tests. We investigate asymmetry of colour spots, and conclude that either orange and black spots are not condition-dependent ornaments or the expected negative relationship between expression of condition-dependent ornaments and fluctuating asymmetry does not occur in these guppies. Several hypotheses regarding the maintenance of multiple secondary sexual characters are discussed.

field trips, Shirley Hanrahan and the Department of Zoology for employing me as a junior lecturer, and Neil for letting me antagonise the second years with molecular evolution and for his open and unique approach to the teaching and learning process.

I would like to thank my parents Ben and Pat and my sister Leonie for their wisdom, support and the having the confidence to let me develop a mind of my own. Wendy not only brings out the best in me, but her example and common sense provide purchase on the real world. Whilst the thesis is dedicated to my parents, I want to share it with her.

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Thanks to my supervisors Robin Crewe and Neil Caithness for giving me the space to define my own direction, and the support and advice to know that it wasn't all tangential. Neil showed me what science should be about, and Robin provided me with place in a laboratory full of stimulating people, and the material support necessary to perform the work involved via grants from the Communication Biology Research Group of the University and the FRD. I also received financial support in the form of bursaries from the Foundation for Research Development and the University.

This work was performed under the following University of the Witwatersrand animal ethics screening committee clearance certificates 93-32-1, 94-49-4, 94-82-3. Thanks to Rob Veale for advice on designing my experiments in a form that was ethically acceptable.

Several of the people I have already acknowledged helped me to grow immensely by showing confidence in my abilities, even when it wasn't merited. I can't mention them all, but would like to thank Pat and Mike for involving me in their thoughts and treating me as an equal, Graham for letting me teach on the second year

squares to measure colour areas in Chapter Seven. Matthew Sledge, Theresa Wossler, Dave Horne and Pete Hawkes babysat the fish when I was out of town. Beverly Kramer not only let me use her digital image analysis system, but patiently showed me how to use it.

I owe a great deal to all those who helped direct, shape or clear the way for my thoughts or who listened as I beat those thoughts into shape. Endless discussions of the Honourable Order of Cheese & Graça were always stimulating even if signal was often drowned by noise. The number and vocality of those postgraduates and young staff members who understand the spirit of CAG appear to be thinning out in the Department of Zoology. I hope that this is not a sign of intellectual desolation, as this department has been a stimulating, friendly and supportive place to work. Graham Alexander, Pat Backwell, Mike Jennions, Anne Houde and Dan Polakow gave me superb intellectual support, didn't let me get too lazy, read several drafts of papers and let me see their unpublished work. John Endler, Andrew Pringle and Steven Snyman also read drafts of one or more papers and gave helpful suggestions. The referees and editors did their jobs, thanks for being gentle. Molly Morris introduced me to the freeze-branding technique, and helped me to get to grips with it. Evenings spent at the PG club and at cricket helped make working in the department pleasant.

I was fortunate enough to present my work at three conferences during this study, the Ethology Group Meeting of the ZSSA at Spioenkop in January 1994, the ZSSA symposium in Grahamstown in July 1995 and the joint meetings of the Society for the Study of Evolution, Society for Molecular Evolution, American Society of Naturalists and Society for Systematic Biology in Athens, Georgia in June 1994. My

inheritance and experience to choice behaviour.

Chapter Seven addresses the question of preference heritability in a different way. The repeatability of behavioural traits such as preferences is a convenient estimate of the upper limit to which the trait may be heritable. I have examined the repeatability of female choice behaviour and how repeatability measures are influenced by the time interval between choice trials. I have also addressed the controversial topic of mate copying (see Gibson & Höglund 1992 for a review), and its implications for repeatability of female choice, the way in which preferences are inherited (culturally or genetically), and how choice can select for male ornamentation. This chapter asks important questions about how female preferences develop in individuals and evolve within populations.

I have kept the chapters intact as they were published except for stylistic standardisation, and the references and acknowledgements which are presented once (at the end of the thesis and the end of this preface respectively) for convenience and continuity. My supervisor up to the beginning of 1995 (and subsequently my cosupervisor), Neil Caithness, was second author on the earlier papers to be submitted. If the reader bears in mind that some of the papers were submitted by two authors, it will be clear why I have left the corresponding chapters in the second person.

I have several people to thank for the role they played, directly or indirectly in this investigation. Graham Alexander told me where to find the fish, and helped me to think my way into the project. Marcus Byrne and Herman du Preez gave useful technical advice, especially at the beginning of the study. Herman also counted grid-

reanalysis, and discusses the concept of signal amplification which may go a long way to resolving the paradox of multiple ornamentation, both generally and in the specific case of guppies.

In the final two chapters I have asked somewhat different questions. Rather than focusing on the relationship between male ornamentation and attractiveness, I have tried to resolve some aspects of the nature of female preferences. The decision-making processes and decision-rule used by females are very poorly understood, with only a handful of theoretical models (Janetos 1980, Janetos & Cole 1981, Real 1990) and almost as few empirical studies (eg Milinski & Bakker 1992) existing in the literature. Furthermore, the factors which influence the development of a female's preference, such as genetic inheritance (Majerus et al. 1982, 1986, Wilkinson & Riello 1994, Houde 1994), the effect of (the ornamentation of) previous males (Bakker & Milinski 1991) and copying the mate choice of other females (Losey et al. 1986, Pruett-Jones 1992, Gibson & Höglund 1992) are very poorly understood, despite their obvious importance to the form and intensity of sexual selection.

In Chapter Six I have addressed the problem of whether or not female guppies are able to act upon their mating preferences without having experience of other males to use as a referent in choice decisions. This addresses the question of whether preferences can be expressed without previous experience such as learning from other females or sampling the levels of ornamentation in the males of the population. It is also important to know whether females can adjust their preference in relation to their previous experience of males. The two questions addressed in this chapter then form an initial contribution to any debate about the relative contributions of genetic

eliminates the possibility that the observed relationship (say between cue magnitude and male attractiveness) is due solely to mutual correlation with a third variable (e.g. Collins et al. 1994). The third and fourth chapters are short manipulative experiments designed to test relationships between male attractiveness and ornamentation observed in the earlier parts of the investigation. In Chapter Three I test the "preference" observed for orange (Chapters One and Two) in a manipulative experiment, using the ingenious technique of changing the colour of incident light, developed by Long and Houde (1992). This eliminates the possibility that the correlative results observed in the first two chapters are spurious consequences of a correlation between orange and some other determinant of "attractiveness". Similarly, the relationship between mating success and black area documented in Chapter Two is examined in a manipulative experiment (Chapter Four). More importantly, in the fourth chapter I have dealt with the question of whether or not a seemingly non-preferred male ornament can be maintained by female choice. This is of interest because it is difficult to resolve whether female choice or other selective forces are responsible for the maintenance of these apparently non-preferred characters.

After the paper that comprises Chapter Four had been accepted for publication, I encountered Hasson's (1989, 1990, 1991) concept of signal amplification. This notion allows for the possibility that characters that are not themselves the object of female preferences may function to enhance (amplify) the signal contained in other sexual ornaments. Hasson's theoretical work made explicit predictions about the outcome of manipulating amplifiers or displays, which I was able to test using the data from Chapter Four. Chapter Five presents the results of this

This chapter serves three purposes in the investigation: First, it provides an introduction to the study animals and a rationale for the decision to study sexual selection in these guppies. Second, it serves as a preliminary attempt to document the relationships (if any) between male attractiveness and secondary sexual characters. Lastly, it introduces the topic of multiple secondary sexual characters and attempts to discriminate between several hypotheses for their origin and maintenance by means of the relationship between character size and fluctuating asymmetry (after Møller & Pomiankowski 1993 a).

Chapter Two is central to the investigation in that it examines the importance of female choice and other sexually selective processes, and of secondary sexual characters to mating success. Darwin's (1871) sexually selective processes, male-male competition and female choice, have proved notoriously difficult to disentangle in many cases (Houde 1988 b, Møller 1994 a). Present function is not enough to infer the selective history of a male trait. It is possible for male-male competition to select for ornaments that have no function in aggressive encounters simply due to the incidental effects testosterone has on other parts of the body (Ligon et al. 1990). In this chapter I try to build an understanding of what it is that confers success in mating on males in this population by pursuing three lines of evidence: male-male aggression and dominance interactions, female response to males and male secondary sex characters.

Biologists cannot claim to understand the causative relationship between sets of phenomena simply by documenting observed association, they must show that the proposed relationship holds under experimental manipulation (Dawkins 1989). This

is no consensus in the literature as to whether or not the latter two processes influence differential reproduction of males (Farr 1980; Gorlick 1976; Houde 1988; Kodric-Brown 1992, 1993; Reynolds et al. 1993).

Male guppies have several exaggerated secondary sexual characters including orange (carotenoid), black (melanin), iridescent and yellow colour patches and exaggerated dorsal and caudal fins. Sexual selection is an obvious possible explanation for the maintenance of exaggerated secondary sexual characters (Møller 1994 a). However, behavioral studies of this population (Brooks & Caithness 1995 a) have shown only that orange coloration consistently relates to measures of male "attractiveness". We investigate the relationship between secondary sexual characters in males (orange coloration and other characters not preferred in behavioral experiments) and mating success in order to infer the importance of these traits to the sexually selective processes at work.

METHODS

We used a pairwise design for simplicity in statistical analysis. Twenty pairs of males and the same number of females were haphazardly chosen from wild caught (from a feral population in Beachwood, Durban; described more fully by Brooks & Caithness 1995 a) single-sex laboratory stocks (approximately 40 fish/100 l tank). Fish were kept in a temperature controlled (25^0 C) room and fed daily on Tetramin flake food for guppies. Lighting was provided by florescent tubes on a 12:12 h light:dark cycle, with no variation in lighting intensity during the "day".

INTRODUCTION

In formulating the concept of sexual selection, Darwin (1871) characterised female choice and direct intrasexual contests as its principle mechanisms. Sneak copulation is a third phenomenon which may influence the mating success of individuals. Sexual selection has recently become a highly productive field of study in behavioral ecology (see Bradbury & Andersson 1987 for an overview), but it is often difficult to distinguish which sexually selective processes are at work in the focal taxon (Møller 1994 a). In this paper we examine the relative importance of sneak copulation, male-male dominance interactions and female choice to male mating success in a feral guppy (*Poecilia reticulata*) population.

Studies of female mate choice often estimate the strength of preferences by scoring female behaviours other than mating (eg. Houde 1987; Swaddle & Cuthill 1994). Proximate measures of male attractiveness used in female choice studies must be shown to be useful predictors of mating success for two reasons. Firstly, arbitrary behaviours might be reified to the status of sexual responses by the observer (for a discussion of such difficulties, see Lewontin 1984). More importantly, even if female sexual response is really being measured, it must contribute to variation in male mating success to be important to sexual selection (Houde 1988).

We explore the ability of several female-response measures to predict mating success in a feral guppy population. We also studied behavioral interactions between males, and other male-female interactions so that we could infer the relative importance of female choice, overt male-male aggression and sneak copulations ("gonopodial thrusts", Farr 1980) to mating success. This is an important goal as there

Chapter Two:

Female choice, male-male competition, sneak copulation and sexual selection in guppies.

ABSTRACT

To test the relative importance of female mate choice, sneak copulation and male dominance to male mating success in a feral guppy population, we conducted a paired-male mating experiment using the colour patterns of male progeny to score paternity. Sneak copulation and male-male aggression had no noticeable effect on male mating success which was strongly related to the frequency of female behavioral responses to both displaying and non-displaying males. Males with a greater relative area of orange coloration and more complex colour patterns were more successful at mating. This substantiates the observed behavioral preference for this trait, and strengthens our claims regarding the importance of female choice to male mating success. Males in trials in which both females failed to produce offspring showed lower levels of black and possibly yellow coloration, suggesting a threshold criterion of mate "choice" for a cue (or cues) that are not the focus of discrimination between males. Females appear to make complex mate-choice decisions based on the assessment of at least two different criteria.

coloration) (unpublished data : Appendix of this thesis). However, this argument does not preclude the possibility that less prevalent colours (iridescence, yellow) are present in some individuals due to pleiotropy with the ornament of choice.

The choice situation presented to females in this experiment represents only one of several ways in which sexual selection could be maintaining male secondary sexual characters. Models of multiple sexual ornaments that attribute their origin and maintenance in a population to sexual selection in different ways and at different times need to be generated.

The absence of a negative correlation between colour area and asymmetry (in fact it is significantly positive) suggests (in terms of Møller & Pomiankowski's argument) that neither orange nor black area are condition-dependent ornaments. However, orange spots have been shown to be exemplary condition-dependent traits (Kodric-Brown 1989, Houde & Torlo 1992). Either we must, like Møller & Pomiankowski (1993 a, for multiple ornamented birds), propose a Fisherian mode of evolution for multiple ornaments in guppies, or we must reject the universality of a strong negative correlation between ornament size and asymmetry in condition-dependent ornaments (Møller & Höglund 1991; but see Balmford et al. 1993). However, the surplus carotenoids provided by commercial flake food might eliminate the condition dependence of orange spots in this experiment. Also, although orange intensity is known to be condition-dependent (Kodric-Brown 1989, Houde & Torlo 1992) less is known about the condition-dependence of orange area. Orange area is known to be under considerable genetic control (Houde 1992), and it is possible that it behaves as the non condition-dependent trait suggested by the asymmetry analysis.

Because only orange was consistently related to male "attractiveness", the results of this study do not favour an explanation for the multiplicity of male guppy ornaments in which more than one ornament is assessed at one time such as the multiple message and unreliable signal hypotheses of Møller & Pomiankowski (1993 a) and the "overall brightness" hypothesis of Endler (1980) which postulates that other colours are maintained as dishonest signals. Ornaments are expected to be individually sexually selected because variation in their expression cannot be attributed to correlated expression with the ornament of preference (orange

As shown in figure 1.1, there was a significant positive correlation between relative area and asymmetry (corrected for body size) in both orange ($r_s = 0.324$, $n = 73$, 2-tailed $p = 0.01$) and black ($r_s = 0.328$, $n = 73$, $p < 0.01$) coloration. There was no significant correlation between female preference and the symmetry (corrected for body size) of black (orient response: $r_s = -0.035$, $n = 34$, n.s.; glide response: $r_s = -0.000$, $n = 34$, n.s.) or orange (orient: $r_s = 0.069$, $n = 56$, n.s.; glide: $r_s = 0.004$, $n = 34$, n.s.) coloration of males. If absolute asymmetry was used, without correction for body size, the results of the correlations were similar.

DISCUSSION

Orange is the most frequently reported preferred character in male guppies (Endler 1983, Kodric-Brown 1985, 1993; Houde 1987). In this study there is insufficient evidence to claim that females prefer different traits at the orientation and the glide-toward stages in their response. Gonopodial length as a cue for female choice is worthy of further investigation, because the correlation between it and female response was not due to a mutual correlation with relative orange area. A preference for longer gonopodia may evolve because of the enhanced mating success conferred on a female's son in sneak copulations by a longer gonopodium (Reynolds et al. 1993).

A weakness of the study exists with regard to the measurement of black area as this varies greatly over a time scale of minutes (Baerends et al. 1955). The single "snapshot" method of measuring cues gives an unreliable estimate of the information available to the female during choice experiments (Sullivan 1990).

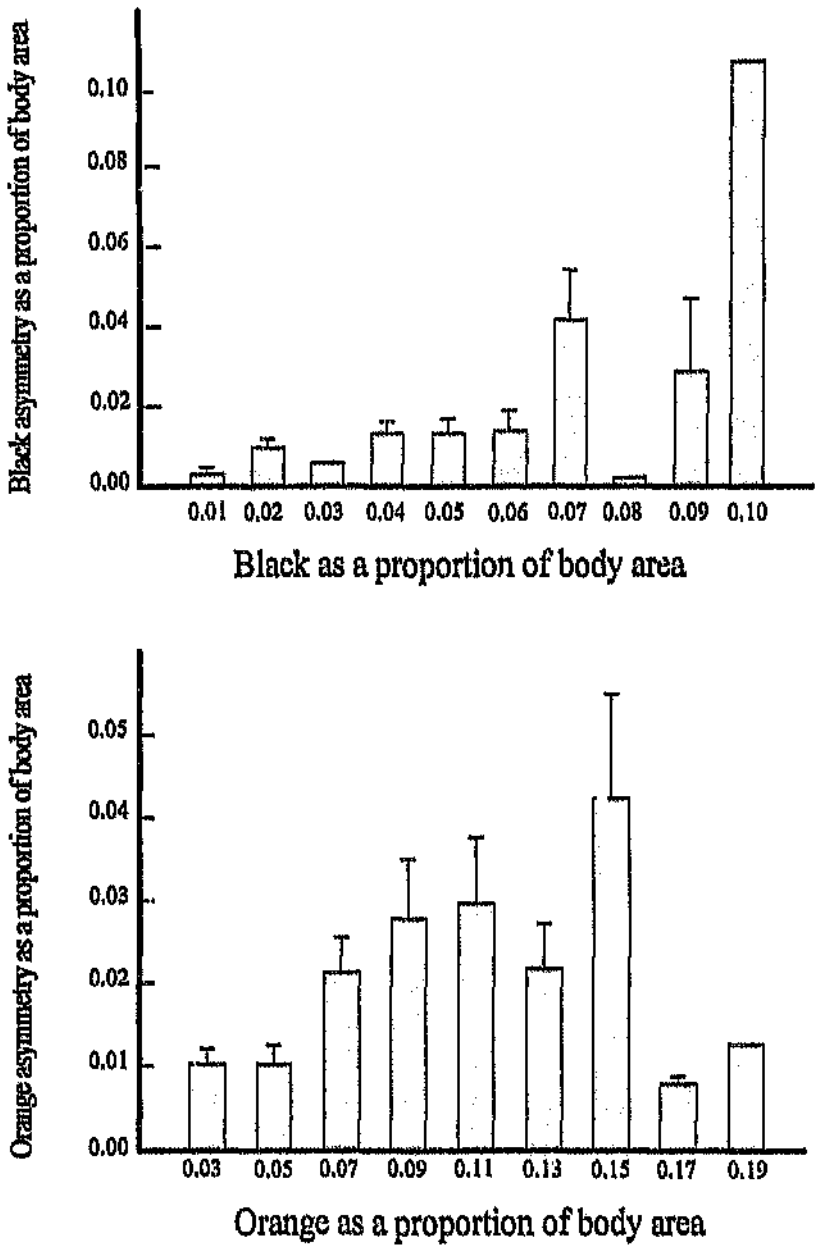


Figure 1.1. Fluctuating asymmetry in colour areas of black (a) and orange(b) spots in relation to the proportion of total body area covered by each colour. Values are means ± one SD.

with the rest of the population in terms of the measures of attractiveness. There was no difference between those with and those without these colours in either proportion "orient" response (Mann-Whitney U: iridescence z= -1.33, n=37, n.s.; yellow z=-1.00, n=37, n.s.) or "glide" response (Mann-Whitney U: iridescence z=1.11, n=37, n.s.; yellow z= -1.69, n=37, n.s. [1-tailed]). Similarly, correlations between these colours and response measures for all individuals, including those without the colours are presented in table 1.1. The relationship between female response and variation in iridescence and yellow in only those individuals in which these colours are expressed is also not significant. Orient response: iridescence r_s=-0.249, n=16, n.s.; yellow r_s=-0.184, n=9, n.s.. Glide response: iridescence r_s=-0.131, n=16, n.s.; yellow r_s=-0.009, n=9, n.s.

Table 1.1. Correlation between morphological and female response measures

		Orient response		Glide response	
Potential Cue	n	r_s	1-t P	r_s	1-t P
body area	37	0.120	n.s.	0.034	n.s.
relative orange	37	0.405	< 0.01	0.352	< 0.02
relative black	37	0.158	n.s.	-0.056	n.s.
iridescence	37	0.157	n.s.	0.075	n.s.
yellow	37	-0.146	n.s.	-0.286	n.s.
gonopodium	22	0.449	< 0.02	0.257	n.s.
dorsal fin	26	0.072	n.s.	-0.070	n.s.

conform to the assumptions of parametric tests or could not easily be transformed to normality, and thus non-parametric statistical analyses were used.

Spearman rank-order correlation coefficients and Kendall's partial rank-order correlation coefficients and their associated significance tests (Siegel & Castellan 1988) were used. Significance tests were one-tailed to test the hypothesis that ornaments are maintained by female preference. The alpha level of acceptance for all tests was 5%.

RESULTS

Only relative measures (proportion of body area) of orange and black are reported in Table 1.1 because relative orange was a better predictor of mating success than an absolute measure in all cases and there was no difference in the predictive power of relative and absolute measures of black. Female "orient" response was significantly and positively correlated with relative orange area and gonopodium size as was female "glide" response with relative area of orange in individual significance tests. Using Kendall's partial correlation analysis to examine the correlations of gonopodial length and of orange area with orient response shows that the significance of the relationships remain when the effects of their inter-correlations with orange area are partialled out (Kendall $\tau_{\text{GonopodiumOrient.Orange}} = 0.200$, n =23, one-tailed $P < 0.05$; $\tau_{\text{OrangeOrient.Gonopodium}} = 0.305$, n = 23, one-tailed $p < 0.015$).

Because only some individuals had iridescent and/or yellow patches, correlations may not accurately reflect the relationship between these variables and others. For this reason we compared individuals in which these colours were present

and her response was noted as described above. Attention was then shifted to another male, and so on until each male had displayed ten times. This took between 30 minutes and 90 minutes.

The proportion of male displays that elicit at least a "glide" response has been used in other studies (Houde 1987, 1988 a, b; Houde & Endler 1990; Houde & Torio 1992; Nicoletto 1993; Endler & Houde 1995) to measure male attractiveness, and reliably predicts male success in siring litters by the responding female (Houde 1988 b, Chapter Two of this thesis). In this experiment, frequency of orient response was used as an additional measure of attractiveness as it is conceivable that females assess different male cues at different phases during courtship. The proportion of displays that elicit at least an orient response is a reliable indicator of male reproductive success (Chapter Two of this thesis).

Data from both observation sessions for each male were amalgamated. This gave two different behavioural scores for each male: proportion "orient" response and proportion "glide" response. Only one mating was observed during the experiment, preventing analysis at this level. To correct for the fact that some males did not complete 20 displays, scores were divided by the total number of displays. Two males who did not display more than twice were discarded from analysis, and one male died before he could be photographed.

Statistical Analysis

Statistical analyses were performed using SYSTAT for Windows version 5 (SYSTAT Inc 1992) software. Behavioural variables and measures of asymmetry did not

area, was used in analysis. 36 individuals not used in the behavioural component of this experiment were included when fluctuating asymmetry was compared with cue size to increase the sample.

Measuring Female Preference

The degree of female preference was measured using variations of the method described by Houde (1987) in which the initial female response indicates male "attractiveness". Female guppies respond to displaying males in a sequence of well defined behaviours which may lead to mating (Baerends et al. 1955). The female may ignore a displaying male, or respond to him by ceasing her previous activity and orienting unambiguously toward him (orient response). Following this, the female may break off the interaction or swim toward the male with a smooth gliding motion using her pectoral fins, often with her tail curved up and to one side (glide response). Lastly, in a few cases, the interaction may continue until the female and male rapidly circle one another and the male inserts his gonopodium into the female's genital opening, this is often followed by "jerking" by the male (mating).

Four males of varying ages and five female[illegible]ht and isolated very young, and thus probably virgins) were placed in an aquarium (900 mm × 315 mm filled to 270 mm) which had brown paper stuck to three sides to standardise background colour, and left for 24 h (after Houde 1987). A female biased operational sex ratio was used to minimise any possible effects of male-male competition for access to females (after Houde 1987). The following two mornings, before feeding, observations were carried out. A male was observed until he displayed to a female,

Measuring Potential Cues

Fish were placed in a clear plastic tissue-culture bottle (23 mm by 39 mm filled to 40 mm) and the left and right sides of each fish were photographed against a white background using Fujichrome 100 ASA slide film under tungsten lighting. Colour spots, with the possible exception of black did not appear to vary with handling or with the change in lighting or from a brown (used in the experimental tanks) tc a white background. A ruler with graduations in millimetres was included in each photograph for calibration. These slides were projected onto paper and the outline of the fish as well as the dorsal fin, gonopodium, outlines of all visible colour spots and a 5 mm section of the ruler were traced. The digitiser board of a personal computer running Videoplan 2.1 digital image analysis software was used to measure the following from the tracings: body length, body area, area of each colour spot, dorsal fin length and gonopodium length. Measurement error (M.E.) was calculated after Yezerinac et al. (1992) and was not significant for length measures (%M.E. = 0.19, $F_{[1,23]}$=0.002, n.s.) or area measures (%M.E. = 0.26, $F_{[1,73]}$=0.003, n.s.).

Because fluctuating asymmetries are defined as ran' 'm, normally distributed deviations from bilateral symmetry (Van Valen 1962), we used a Kolmogorov-Smirnov test to compare the distribution of the differences between areas of a colour on either side with a normal distribution about a mean of zero. Only black and orange were expressed sufficiently in the population to perform tests using asymmetry. Neither deviated significantly from a normal distribution. Møller's (1992) measure of absolute asymmetry (the absolute value of the difference between the amount of colour on left and right sides), corrected for size of the individual by dividing by body

success in this population makes it difficult to explain how the[illegible] maintained in the population. The apparent threshold requirement of black pigment before a female will mate with a male is interesting, more so because once males reach this minimum requirement, other cues become the focus of female choice. Backwell and Passmore (in press) showed that female fiddler crabs (*Uca annulipes*) sample larger males but, within that sample, mate (independently of male size) on the basis of a threshold criterion of burrow quality. The possibility that females guppies mate differentially with oranger, more complex males within a sample of males who meet a minimum criterion in another suite of cues further supports the notion that females make complex mate-choice decisions based on assessment of different criteria at different times in the sampling period.

The mating advantage and positive female response to males with more complex patterns is intriguing. Pattern complexity in another population (Nicoletto 1993) varies with male condition and swimming performance. It is possible that females somehow assess pattern complexity as an assay of male quality. Alternatively, elements of the colour pattern other than orange may serve to emphasise the orange elements of a male's pattern. This provides an alternative explanation for our finding that females fail to mate with males with a small relative area of black. Otherwise attractive (large relative area of orange) males may fail to appear attractive due to the lack of black area to emphasise the orange patches. Endler and Houde (1995) constructed the variables "colour contrast" and "brightness contrast" in order to measure colour pattern in terms of the contributions of chroma, value and hue of each spot to the overall "mosaic" of colour perceived by the female.

(Bischoff et al. 1985; Farr 1980; Kennedy et al. 1987). Our study provides no evidence for this in this population in that the number of male displays per unit time does not relate to mating success. However, the length of the display may be under scrutiny during the sequence of female responses, suggesting a possible reason for the evolution of this behaviour. Courtship can be energetically very expensive (Höglund et al. 1992; Klump & Gerhardt 1987; Vehrencamp et al. 1989, but see Wells & Taigen 1984). If the sigmoid displays of male guppies impose high energetic costs, only the most vigorous males can bear the handicap (sensu Zahavi 1975) of repeated, sustained, displays. If this is the case, the length of the sigmoid display could be a means for the female to assess a male's genetic quality, as suggested in the case of costly arthropod, avian and anuran courtship (Halliday 1987; Höglund et al. 1992; Klump & Gerhardt 1987). However, female choice as measured by the swim-toward response of females is also a significant predictor of mating success. This implies that what makes a male "attractive" to a female is (to some degree at least) independent of male sigmoid display performance.

In this population, males with a greater area of orange pigment not only elicit positive female sexual responses (Brooks & Caithness 1995 a), but they are more successful at obtaining matings than males with a smaller area of orange. This corroborates many female-choice studies performed to date using guppies (Endler 1983; Houde 1987, 1988; Kodric-Brown 1985, 1989, 1993). We are therefore justified in our claim that the orange elements so characteristic of male colour patterns have been sexually selected by female choice.

The fact that other characters are not significantly related to male mating

and to predators, resulting in a corresponding diurnal cycle in male mating behaviour (Endler 1987). The relative importance of sneak copulation and courtship based on displaying visual cues thus varies directly with light intensity (Endler 1987; Reynolds et al. 1993). Similarly, male-male competition may be important under conditions present at some point in the diurnal cycle, but not in the constant laboratory conditions imposed during this experiment.

The initial (orient) female response and the second (glide) response (which is always preceded by an orient response) may reliably be used as indices of female choice. This amounts to a progressive chain of behaviours, throughout which the female has a chance to assess the displaying male and either continue toward copulation or break off the interaction. Studies of other populations (Endler & Houde 1995; Houde 1987, 1988) used the glide response as their measure of female response. The further along the progression of responses used as a measure of female sexual response, the more accurate it is as a measure of mating success. However, the sampling effort required to observe sufficient display-response interactions in order to preserve this accuracy increases. It is also possible that females assess several different criteria at different times during the courtship-response interaction. This favours the use of as many behavioral variables as available in studies of female choice in order to be sensitive to these differences.

We predict that the time from the onset of male display until the female breaks off the interaction relates strongly to mating success. The sigmoid display rate of male guppies is related to male condition (McMinn 1990; Nicoletto 1993) and is thus the type of character that should be chosen by females, as demonstrated in several studies

not significantly influence male mating success. The implications of this are twofold. First, it is possible to design experiments in which several females and several males interact with one another, without concern about the effects of male-male interactions (see Nicoletto 1993) in circumventing female choice. Secondly, and more importantly, it is possible to study the basis of female choice knowing that it is an important evolutionary force.

Some authors working on other populations have shown that male-male interactions are not important to differential reproduction amongst males (Farr 1980; Houde 1988). However, others have shown that male aggression and dominance (Gorlick 1976; Kodric-Brown 1992, 1993) and sneak copulations (Reynolds et al. 1993) are correlated with male reproductive success. Dominance hierarchies appear more likely to be established in small aquaria or when males are kept at high stocking densities especially when the same males are kept together for a long time (Endler, personal communication). However, our stock aquaria were of a similar size and our test aquaria were smaller than those used by Kodric-Brown (1993), and our breeding trials lasted for a full week. Alternatively, one could invoke the profound documented differences between guppy populations in female mate preferences (Houde & Endler 1990; Stoner & Breden 1988). The relative importance of female choice, intermale aggression and sneak copulation may vary between populations due to differences in local conditions. Also, the relative importance of different sexually selective processes may vary with time. The standardized laboratory conditions in our study might negate the importance of intermale aggression and sneak copulation. Diurnal variation in lighting conditions influences conspicuousness of males both to females

Table 2.3. "Winning" and "losing" males in paired mating trials compared in terms of secondary sexual characters.

	d.f.	Winner	Loser	*t*	*p*
% Orange	13	10.30 (4.55)	4.67 (3.26)	5.278	0.000*
% Black	13	3.28 (1.44)	3.71 (1.81)	0.834	n.s.
% Yellow	13	0.54 (0.82)	0.51 (0.83)	0.112	n.s.
% Iridescence	13	1.34 (1.42)	0.66 (0.62)	0.593	n.s.
Complexity	13	67.04 (17.3)	51.71 (20.3)	2.392	0.016
Body area (mm^2)	13	102.2 (17.5)	103.5 (21.7)	-0.030	n.s.
Dorsal fin (mm)	9	7.36 (1.83)	6.71 (2.52)	0.742	n.s.
Gonopodium (mm)	9	5.71 (1.18)	5.96 (1.68)	-0.845	n.s.

t = paired sample Student's *t*
* = Significant ($p < 0.05$) after Bonferroni readjustment

DISCUSSION

A common difficulty faced in sexual selection studies is how to separate the importance of female choice from that of male-male interactions (Houde 1988; Møller 1994 a), and of mating tactics that circumvent female choice. By showing the low frequency of male-male interactions and sneak copulations and their unrelatedness to the paternity of litters, we have shown that in this population, they do

(Brooks & Caithness 1995 a) did not deal with pattern complexity, we estimated the correlation between complexity and female response. Complexity was significantly correlated with the proportion of displays eliciting an orient response (Spearman r_s = 0.480, n = 38, 1-t $p < 0.002$) and the proportion glide response (r_s = 0.330, N = 38, 1-t $p < 0.025$).

Table 2.2. "Winners" of paired trials compared with "losers" in terms of male behaviours.

	Winner	Loser	z	p
Display	29.06 (10.7)	26.43 (12.3)	0.85	0.39
Gonopodial thrusts	1.313 (1.25)	1.813 (1.64)	-1.12	0.264
Nip female	4.750 (3.62)	4.125 (2.42)	0.25	0.80
Nip male	0.063 (2.5)	0.313 (0.79)	-1.13	0.257
Chase male	0.313 (0.60)	0.375 (0.80)	0.07	0.94
Display to male	0.500 (0.89)	0.250 (0.78)	0.816	0.414

N = 16 pairs in all cases
z = Standard normal approximation of Wilcoxon rank-sum test statistic

frequency (12, 8 and 14 times in 800 minutes of observation). As far as could be tested, these interactions did not affect mating success.

Table 2.1. "Winning" and "losing" males in paired mating trials compared in terms of female responses to displaying[1] and non-displaying[2] males.

	Winner	Loser	test statistic	*p*
Orient[1]	13.00 (6.93)	9.38 (6.80)	1.51[z]	0.066
Orient/displays[1]	0.443 (0.14)	0.313 (0.16)	2.58[t]	0.005[*]
Glide[1]	3.56 (2.48)	1.938 (2.02)	2.33[z]	0.010[*]
Glide/displays[1]	0.128 (0.09)	0.070 (0.08)	3.01[t]	0.001[*]
Swim toward[2]	3.38 (3.00)	1.75 (1.57)	2.06[z]	0.020[*]

N = 16 pairs in all cases.
p = One-tailed Probability
z = standard normal approximation of the Wilcoxon signed ranks test statistic.
t = Student's t-test for paired samples
* = Significant ($P < 0.05$) after Bonferroni readjustment

Cues for Female Choice

Only percentages of total body area covered by each colour are presented in table 2.3 because it was a better predictor of mating success than the absolute measure in orange, and there was no difference of any consequence for the other colours studied. Both orange and pattern complexity were significantly higher in successful males than in unsuccessful ones, but complexity was not significant after the Bonferroni readjustment. Because our earlier study relating morphology to female response

three cases neither female produced any offspring. We compared the secondary sex traits of the six unmated males with the remaining 34 males to see if they were ignored by females due to an absolute preference for a particular cue. Of the ten morphological characters studied in this experiment, the relative areas of black and yellow were significantly smaller in the ignored males (2-sample Students t using separate variances: black $t = 2.963$, $d.f. = 15.5$, individual one-tailed $p = 0.005$, yellow $t = 2.418$, $d.f. = 33.4$, one-tailed $p = 0.011$). The first of these results is significant (one-tailed $p = 0.045$) and the second is not (one-tailed $p = 0.105$) after the sequential Bonferroni readjustment.

Female "Response" to Males

Both "orient" and "glide" responses of females to displaying males appear sound predictors of male mating success (Table 2.1). In both cases these behaviours are more closely related to mating success when corrected for display rate. Also, the female "swim toward" (a passing non-displaying male) behaviour is significantly more common for males that are successful at fertilizing females, and would seem a genuine female sexual response.

Other Behavioral Interactions

Male behaviours are reported in table 2.2. The number of male sigmoid displays per unit of time, attempted sneak copulations and the frequency of nipping at the female's gonopore did not predict mating success. Male directed behaviours such as displaying to the other male, nipping at his gonopore and winning chases occurred at a very low

& Rohlf 1987) was used to transform relative areas of orange and black and the proportion of male displays eliciting a glide or an orient response. Other behavioral variables could not readily be transformed to normality, and I used non-parametric statistical procedures (Seigel & Castellan 1988) when this was the case.

I analysed the results using SYSTAT 5.0 for Windows (1992) statistical software. For each mating trial, the male who was more successful in terms of number of matings was compared with the less successful male using paired sample *t*-tests for variables conforming to the assumptions of parametric tests or else Wilcoxon's signed ranks test for matched pairs using a corrective equation for tied ranks. The null hypothesis was no difference between the "winner" and the "loser", and tests were one-tailed except in the case of the frequency of male-male interactions where no directionality was anticipated. In addition, we compared males who did not sire sons with males who sired sons by one female and by both females using the Jonckheere test for ordered alternatives (Seigel & Castellan 1988) including the data from pairs where males did not differ in mating success. The results of this analysis did not differ substantially from the results of pairwise analysis and are therefore not presented here.

We adjusted the 0.05 α-level of acceptance for the number of tests presented in a table using a sequential Bonferroni readjustment (Rice 1989).

RESULTS

In four of the 20 trials (20%) neither male enjoyed an advantage in the number of females mated with. Once, both males sired sons by both females, and in the other

Measuring Potential Cues

The method of measuring morphological characters follows that of Brooks & Caithness (1995 a). Briefly, each male was placed in a clear-plastic bottle (23 mm by 39 mm filled to 40 mm) and photographed on both sides against a white background under tungsten lighting. We traced the outlines of the fish and all colour spots from the projected slide onto A4 paper. The digitiser board of a computer (Videoplan 2.1 digital image analysis software) was used to calculate the area of the body and of each spot and the length of the gonopodium and the dorsal fin of each male. A short section of a plastic ruler included in each photograph was used for calibration. This method of measurement shows less than 0.3% error (Brooks & Caithness 1995 a). We used the mean value for the two sides in all bilateral traits.

Some studies suggest that spot number rather than the area of some colours may be important to females. We performed all analyses using the number of spots and the total area for each colour, but the two types of measure showed similar results, and so only results for area measures are presented. However, we did use spot number data to create a measure of pattern complexity (after Nicoletto 1993) by ranking each male for the number of spots or patches of each colour (orange, black and iridescent) and then summing those three ranks. This provides a measure of a male's complexity relative to the other males in the experiment (Nicoletto 1993).

Statistical Techniques

All morphological variables conformed to the assumptions of parametric tests. Some required transformation to their natural logarithms. The angular transformation (Sokal

at his gonopore and dominance (i.e. ended up with the female) in chase/standoff interactions (Houde 1988). The latter two have been used by Kodric-Brown (1993) as measures of male dominance.

After seven days the females were isolated in glass and netting enclosures (90 mm x 157 mm) of a 100 l tank. We inspected all enclosures between one and three times a day until the first fry were found for each female, or until three months had elapsed (8 of the 40 females used in the experiment) in which case that female was considered not to have been fertilised. Fry were re ired in the enclosures in which they were born and mothers were returned to a stock tank. Between five and six months after birth, we sexed offspring and established the paternity of each male by comparing his colour patterns with those of the two potential sires. Male guppy colour patterns are highly heritable (Endler 1978), and in this population are very similar between father and son, particularly in the size, shape and placement of orange spots (personal observations). In all cases but three (from different broods, those individuals were ignored during analysis), paternity could be clearly established. Each male was considered to have mated with a female if he sired at least one son by her. Thus, males could obtain a score of zero, one or two for the number of matings obtained. This method is not a precise measure of male mating success for two reasons: First because in two cases only daughters were produced, and it is possible that in other litters where all the sons were sired by one male, the other male sired one or more of the daughters. Secondly, and less important to mating success, a male producing one son in a litter scores the same whether he mated with the female once or several times.

Behavioral Measurements and Mating

We used females that were immature when caught, and isolated as soon as the "gravid spot" became apparent, and were thus probably virgins. Each pair of males was placed with two females in an aquarium (290 mm x 215 mm filled to 140 mm) covered with brown paper on three sides to standardise background colour. The mating trials lasted seven days, with behavioral observations on the first two mornings of the trial. For each male, these comprised two ten-minute sessions of observation, ten minutes apart. We combined behavioral data from both days. We scored the female response to every sigmoid display of the focal male as follows (described more fully by Brooks & Caithness 1995 a, Houde 1987): No response, "orient response" (female ceases previous activity and orients unambiguously toward male), "glide response" (female "orients" and then swims toward male with smooth, gliding motion using pectoral fins) or mating (female "orients", 'glides" and then male and female rapidly circle one another and male inserts gonopodium into female genital opening). Mating was infrequently seen (9 times in 800 minutes of observation), which is one reason breeding experiments are required to quantify mating success. We used both the absolute number of each type of female response, and the proportion of the total number of displays by the male that they comprised as response variables. We also used the "swim-toward" response (Houde 1987) in which a female swims unambiguously and smoothly toward a non-displaying male as an attractiveness measure. Nipping at a female's gonopore and attempted sneak copulations ("gonopodial thrusts") were noted.

Male-directed male behaviours include displaying to the other male, nipping

METHODS

We used first and second generation laboratory-bred guppies from a feral population in Durban, South Africa. Due to the high heritability of male colour patterns (Endler 1978), full sibling male guppies resemble one another in size and placement of colour patches. One male from each of 25 sibling pairs of males was assigned to the experimental group and the other to the control. Each male was placed individually in a 10 l tank with three females. On the following morning, female response to each male was scored in two five-minute observation sessions, 15 minutes apart. Female response to a displaying male was scored as positive if she stopped her previous activity and unambiguously "oriented" toward him (after Houde 1987). The proportion of displays eliciting this response (hereafter referred to as his "attractiveness score") reliably predicts male mating success in this population (Chapter Two of this thesis).

Male guppies' black areas comprise some relatively permanent spots, and others that are expressed occasionally such as during courtship (Baerends 1955, Endler & Houde 1995). One of the relatively permanent black spots on each side of each experimental male was removed by freeze-branding with dry ice under MS-222 anaesthetic (after Hert 1986). Control males were anaesthetized and freeze-branded on an unpigmented part of the body of similar area. After a recovery period of ten days in water containing Tetramin general tonic (to prevent infection), a second round of behavioural trials was performed as above. No males died within 30 days of freeze-branding, but some males failed to display to females after treatment and where thus removed from analysis. When studying female preference for an ornament such as

relationship between the extent of natural melanin pigmentation and female response (Brooks & Caithness 1995 a). Instead, females consistently respond to and mate with males on the basis of relative area of orange (carotenoid) pigment (Brooks & Caithness 1995 d, Chapter Two of this thesis). However, we did find that males that did not obtain matings possessed less black than those that successfully mated (Chapter Two of this thesis). A threshold requirement or a weak general female preference for black pigmentation is one but by no means the only explanation for this result. Alternatively, the low mating success of males with small black area may be due to a relationship with male infertility, or the intensity of male courtship. The latter explanation is supported by the fact that black area of a male varies dramatically over time, reaching its greatest expression when a male actively courts a female (Baerends *et al.* 1955, Endler 1983).

Manipulation of male ornaments is the most satisfactory test for a female preference for that trait (eg. Andersson 1982). Additionally, manipulation serves to distinguish the contributions of different ornaments to the choice decision (eg. Collins *et al.* 1994). It is conceivable that weak, relatively uncostly female mating preferences could maintain ornaments such as black that are not the obvious focus of choice (Iwasa & Pomiankowski 1994). Unless preference can be demonstrated, alternative explanations for the presence of an ornament cannot be eliminated. These include crypsis (Endler 1983, 1987), pleiotropy (Zuk *et al.* 1990), thermoregulation (Endler 1983) and signalling in contexts other than courtship. We test for such a preference in a feral guppy population by reducing the area of black colouration on males and measuring the corresponding change in their attractiveness to females.

INTRODUCTION

Despite wide acceptance that female choice selects for exaggeration of male secondary sexual characters (ornaments) (see Andersson 1994 for review), it is uncertain whether current models of sexual selection can account for the evolution of the multiple ornaments borne by individual males of several species. It has been suggested that female choice can select for several male ornaments (Møller & Pomiankowski 1993, Pomiankowski & Iwasa 1994), but in many species, one ornament is the only consistent predictor of female preference.

Frequently, if only a subset of the ornaments borne by males of a species are shown in a particular study to be important to female choice, the evolution and maintenance of other male ornaments is attributed to natural selection pressures imposed due to the presence of the "cue" ornament/s (Endler 1983), or phenotypic correlation with the cue/s (Collins *et al.* 1994). The possibility that weaker, or less frequently expressed female preferences are maintaining the seemingly non-preferred ornaments is seldom investigated.

Male guppies (*Poecilia reticulata*) bear conspicuous colour patterns comprising black (melanin) and orange (carotenoid) patches and (in some individuals) structural pigments which produce a variety of iridescent and yellow areas. The only study demonstrating female choice for males with high melanin showed, using multivariate statistical techniques, that a high black spot number was preferred in five of the eleven populations studied by (Endler & Houde 1995), and in all five cases there was a stronger relationship between female preference and another cue. In previous studies on the South African population we found no significant

Chapter Four:

Manipulation of a seemingly non-preferred male ornament reveals a role in female choice.

Brooks & Caithness 1995 b. *Proceedings of the Royal Society of London* Series B 261: 7-10.

ABSTRACT

Past studies of a feral South African population have shown that females use the area of orange colouration borne by males as a criterion for mate choice. However, males bear spots of other colours, the most noticeable of which is black. We investigate whether female preference can maintain the black spots of male guppies despite the apparent unimportance of black to female choice in correlative studies. When part of a male's black pigmentation is removed, his "attractiveness" to females decreases. This supports the hypothesis that in male animals with several ornaments, seemingly non-preferred ornaments can be maintained by female choice. These findings are discussed in the context of several models which account for the evolution of multiple male ornaments.

of choice. However, this is difficult to distinguish from two possible effects of orange light on female perceptions of male patterns. Firstly, because unpigmented areas and orange spots both reflect all wavelengths of incident light, causing males to appear uniformly orange, it should be impossible for females to choose using orange. Alternatively, males may appear uniformly lacking in orange due to visual adaptation and bleaching (by overstimulation) of female's orange-red photoreceptors (see Long & Houde 1989 for a fuller discussion). This could lead to a failure to meet a threshold criterion of mate choice. If female guppies failed to choose on the basis of orange area for either of these reasons, the redundant signal hypothesis predicts that one or more other characters will be used for choice (Møller & Pomiankowski 1993). At this point, orange area is the only feature for which I have convincingly convincingly demonstrated the presence of a female preference.

DISCUSSION

Our finding that females prefer males with a greater relative area of orange under white light, but not under orange light (when males look uniformly orange) supports the notion that carotenoid patches are the cue females choose. However, under blue light, which contains little or none of the "orange" wavelengths reflected by orange spots (causing them to appear black), females show significant preference both for orange (as Long & Houde found) and for black area. Both spot colours would then appear black to females, making it possible that females from this population use the complexity (Nicoletto 1993) or visual contrast (Endler & Houde 1995) of males' colour patterns rather than orangeness *per se* in mate choice. Pattern complexity is related to mating success in this population (Chapter Tw' of this thesis), but was unrelated to male attractiveness in this experiment. It is unlikely (as in Long & Houde 1989) that the blue globe eliminated all orange wavelengths, and it is still possible that "orangeness" of a male's colour pattern contributed to his attractiveness. However, in sticklebacks, green light (complimentary to red) eliminated the importance of red colouration to female choice (Milinski & Bakker 1990).

The redundant-signal hypothesis (Møller & Pomiankowski 1993) for the presence of multiple secondary sexual characters postulates several cues which advertise phenotypic quality to females. As Zuk et al. (1992) manipulated the combs of red jungle fowl cocks, hens switched to other secondary sexual characters to choose mates. The low female receptiveness despite sustained male courtship intensity under orange light may account for our failure to detect a switch in the cue

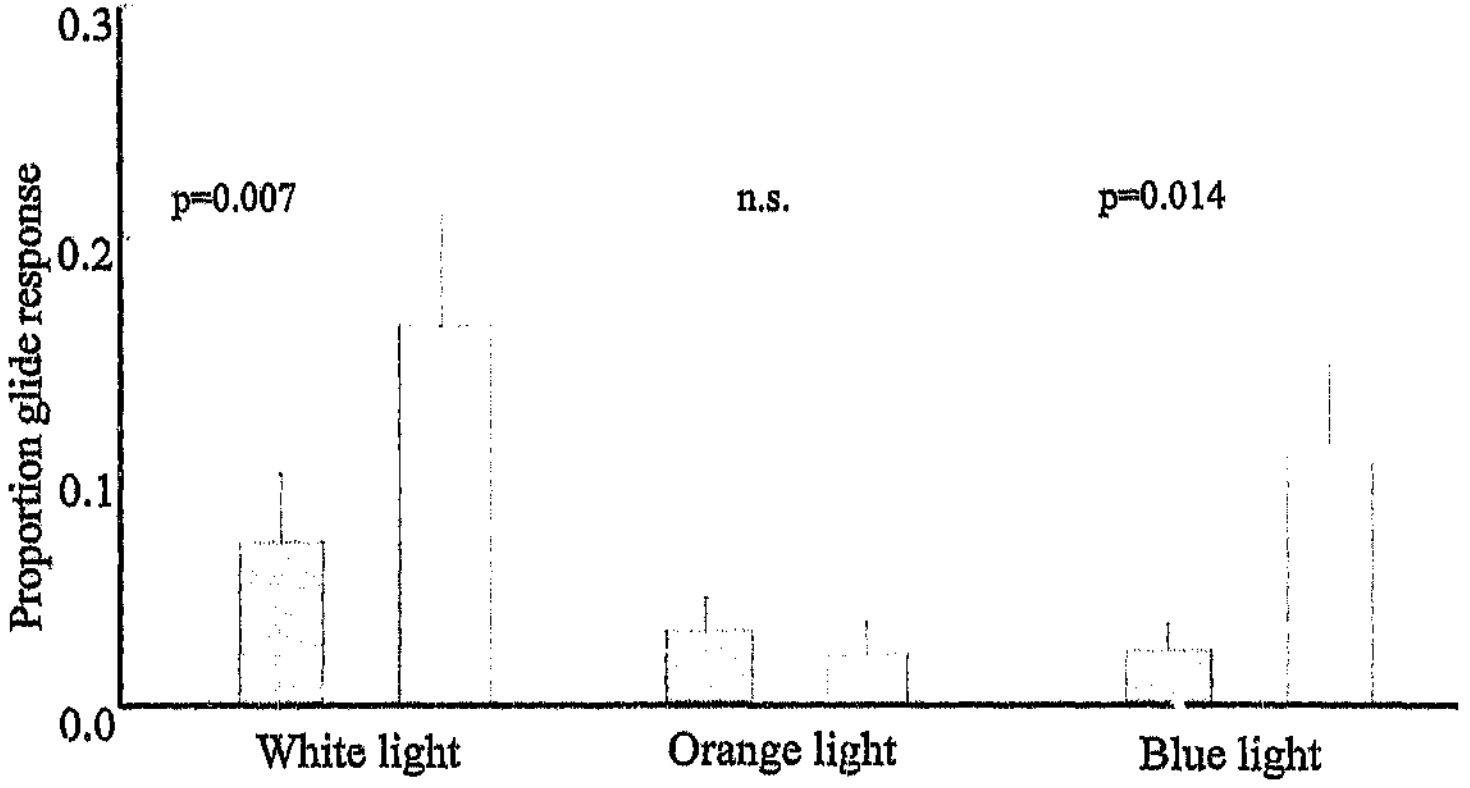


Figure 3.1. Female response (± 1 SD) to males with smaller (shaded) and greater (unshaded) relative areas of orange.

RESULTS

Females responded more positively to the oranger male of a pair under white and to a lesser extent under blue light, but not under orange light (Figure 3.1).

Female response did not differ significantly between males with greater and lesser expression of any other secondary sexual character, including "pattern complexity", measured under orange light as follows: black area ($z = 0.447$, n.s.), white area ($z = 1.069$, n.s.), iridescent blue ($z = 0.000$, n.s.), yellow ($z = -1.000$, n.s.) and complexity ($z = 0.0535$, n.s.). Females also did not respond signifcantly more to males with greater expression of any other secondary sexual character under any of the other lighting conditions with one exception: black area under blue light ($z = 1.820$, 1-tailed $p = 0.035$).

There was significant systematic variation in female responsiveness (proportion "glide" response) between light colour treatments (Kruskal-Wallis test statistic = 7.38, $p = 0.025$). Responsiveness was lowest under orange light (0.024 ± 0.06) and highest under white light (0.170 ± 0.19). There was no significant systematic variation in the frequency of any of the male sexual or aggressive behaviours studied between lighting treatments.

"orient" towards male, "glide" towards male or "mate" (described by Brooks & Caithness 1995 a). The "swim toward" (a non-displaying male) response (Houde 1987) was also noted. This response, the proportion (of displays that received an) "orient response" and the proportion "glide" response all significantly predict of mating success (Houde 1988, Chapter Two of this thesis). Only results for glide response are presented here as it is the best predictor of mating success and other measures did not provide substantially different results.

The colour patterns (under white light) of each male were drawn onto standard guppy outlines (after Houde 1987) and relative area of each colour spot was estimated using a grid of squares. This method of quantifying colour patterns, despite reduced precision is sufficient for discriminating which male of a pair had a greater relative area of a pigment. Also, it provides a better measure of structural colour areas which do not show up well on photographic film. We measured pattern complexity (after Nicoletto 1993) by ranking each male for the number of spots of each colour (orange, blue, white, black and yellow) and then adding the five ranks to give a measure of male pattern complexity relative to the other males in the sample.

Non-parametric statistical tests (Siegel & Castellan 1988) were used because behavioural measures could not be transformed to normality. We examined the three treatments (orange, blue and white light) for systematic variation in the frequency of the various behaviours studied using a Kruskal-Wallis one-way analysis. Wilcoxon rank-sum tests were used to check if the male with a greater relative area of a particular colour was preferred over the other within treatments.

Previously, we found a significant positive correlation between orange area of males and both female preference (Brooks & Caithness 1995 a) and mating success (Chapter Two of this thesis) in a feral guppy population from Durban, South Africa. We designed the following experiment both as a manipulative test of this trend and an examination of the effect of other secondary sexual characters when the cue of choice is factored out.

METHODS

We used wild-caught or first generation laboratory-bred guppies. Two males (paired to vary dramatically in the percentage of their surface covered by orange spots) and two virgin females were placed in a tank (900 mm x 315 mm filled to 270 mm, with brown paper on three sides to standardise background colour) at noon, and behavioural trials took place on the next three mornings. 10 h of light from fluorescent tube lighting was provided beginning at 12h00 each day, preceded by two hours of either orange, blue or white light such that each colour was presented on one morning of the trial. The lighting (orange, blue or white) during these two hours was provided by two 60 Watt bulbs suspended 60 cm above the surface of the water. The order in which the lighting colours were presented was random in each of twenty replicates.

After a fifteen-minute adjustment period, we watched each male for two ten minute periods, 15 minutes apart. Gonopodial thrusts, nipping a females' gonopore, chasing the female, and chasing, nipping and displaying to the other male were all noted. We scored the response of a female to a displaying male as either: no response,

Chapter Three :

Female guppies choose oranger males when they can.

1995. *South African Journal of Zoology* 30 (4): 1-4.

INTRODUCTION

Evidence of female mate choice using visual cues is often correlative, failing to show that the character studied is the object of preference rather than a correlate of the real cue. Distinguishing between correlation and causation is especially important when males have several secondary sexual characters, each of which may be sexually selected. One solution is to experimentally manipulate the ornament in question (eg. Andersson 1982), but fish colour patterns are not as easily manipulated as the tails of birds. Long & Houde (1989) devised an ingenious test of whether the correlation observed between orange (carotenoid) area and female preference in a Trinidadian guppy population indicated a real preference for orange males. Instead of altering the colour patterns of males, they varied the colour of incident light, thus altering female perception of these patterns. We show that female guppies' preference for males with a greater area of orange disappears when their ability to discriminate between males using this character was hampered under orange light. Because the importance of female choice to mating success changes with lighting conditions (Reynolds et al. 1993), we examined the effects of light colour on other behaviours such as attempted sneak copulations and male-male interactions.

The importance of measures such as contrast and complexity of a pattern is that they allow for the possibility that female preferences can coevolve with a complex of independently expressed morphological characters, rather than invoking individual evolutionary histories for each secondary sexual character. Endler & Houde (1995) found that females choose males with higher colour contrast. Recent studies have tried to explain the presence of multiple secondary sexual characters by treating them as mate-choice cues that are individually assessed in the process of choice (Møller & Pomiankowski 1993; Brooks & Caithness 1995 a). We propose a "composite ornaments" rather than a multiple ornaments explanation for the presence of multiple secondary sexual characters. Such ornaments may be composite morphological traits, comprising several secondary sexual characters under separate genetic and ontogenetic control.

Chapter Six:

Does a male's attractiveness to a female depend on her previous experience?

Brooks & Caithness 1995 c. *South African Journal of Science* 91: 156-158.

ABSTRACT

Female guppies from a South African feral population display a strong mating preference for males bearing large areas of orange pigmentation. We provide substantiation for this in pairwise choice trials. We show that naïve virgin females are capapble of acting upon their mating preferences in the absence of prior experience of males on which to base this choice. This is evidence for a 'fixed internal standard' of preference. We also show that this standard of attractiveness is modified by a female's previous experience.

networks (Enquist and Arak 1993) has shown that the combination of colours in a signal is often more important than the expression of individual colour pattern elements, this experiment demonstrates practically for the first time the presence of a visual signal amplifier. Such empirical support for the theoretical prediction that ornaments and displays can evolve to amplify (Hasson 1989, 1990, 1991) or improve detectibility of signals (Schluter & Price 1993) suggests that theoretical biology is providing ever more useful models for understanding complex sexual signalling systems and the effects of sexual selection on them.

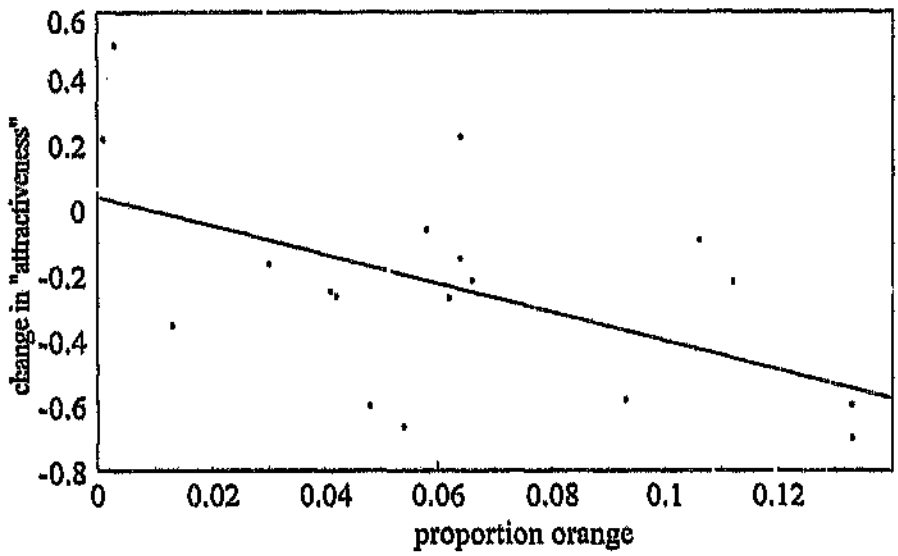


Figure 5.1 Linear regression of the change in proportion "orient" response before and after males had part of their black (melanin) colouration removed by freeze branding on relative area of orange (carotenoid) pigmentation, a male ornament positively related to female choice. $Y=-4.400X + 0.036$. $R^2 = 0.289$, $N = 18$, $p = 0.011$.

Reducing the expression of a pure signal amplifiers is not expected to reduce male attractiveness on the whole within the population (Hasson 1991) as I have shown for this data set (Brooks & Caithness 1995 b). It is possible that melanin pigment has become an amplifying indicator (Hasson 1991), and is thus to some degree at least a cue for female choice. However, the non-significant relationship between natural levels of melanin and male attractiveness is not expected under a model of amplifying indicators.

Whilst recent innovative work using both animals (Endler 1991) and neural

females choose using orange as a cue. Similarly, I used the relationship between male attractiveness and percentage black area as a measure of the importance of black area to choice.

RESULTS AND DISCUSSION

Using analysis of covariance (ANCOVA) I showed that the relationship between orange and attractiveness is stronger before than after treatment (before: slope = 3.62, $R = 0.575$; after: slope = 0.079, $R = 0.0282$; $F_{[1,32]} = 5.84$, $p = 0.022$). The decrease in attractiveness of individual males was significantly related to the area of orange borne by those males and not to the amount of black removed from a male (figure 5.1). Black was unrelated to attractiveness throughout this experiment.

However, females in this population have been shown to use black when exercising mating preferences (Brooks & Caithness 1995 b). This paradox is resolved if one argues that ornaments or displays can be important to female choice without being the object of a female preference. One possibility is that black coloration is a signal amplifier which has evolved because it improves the accuracy with which females discriminate between males based on the extent of their orange coloration. Our findings that female sexual response to males was related more strongly to orange before than after black coloration was reduced and that the decrease in male attractiveness with treatment is negatively related to the male's orange area are explicitly predicted by Hasson's theoretical work on signal amplification (Hasson 1991). The increase in attractiveness of males with low proportions of orange is predicted because female discrimination against these males becomes less acute as the amplifier is manipulated.

amplifier is expected to effect mating success of males with greater expression of the "quality" signal more negatively than those with less (Hasson 1991), even to the point at which males with smaller "quality" signals increase in attractiveness when the amplifier is reduced. I test these predictions post hoc using data from an earlier study (Brooks & Caithness 1995 b), to empirically establish whether black coloration behaves like an amplifier of the orange area signal. Detail on methods used that are not available in this paper are given in the report on the earlier study (Brooks & Caithness 1995 b).

MATERIALS AND METHODS

I placed males individually in a 10 l tank with three females. The next morning, I scored male "attractiveness" in two five-minute observation sessions, 15 minutes apart. I used the proportion of a male's displays eliciting the "orient" response as his "attractiveness score", a measure which reliably predicts male mating success in this population (Chapter Two of this thesis). I photographed the males under tricaine methanosulphate (MS-222) anaesthetic and then used a grid of squares to measure body area and the area of colour patches from the projected slides. From this information, I calculated areas of black and orange in relation to body area. I removed one black spot on each side of each male by freeze-branding (Hert 1986) with dry ice under anaesthetic. After a recovery period of ten days, I performed a second round of behavioural trials.

I used the relationship between male attractiveness and the percentage of the total body area that was covered by orange as a measure of the accuracy with which

comprising black (melanin), orange (carotenoid) and a variety of iridescent and yellow areas. In guppy populations (including in the feral population from Durban, South Africa) studied in this experiment), the extent of orange coloration is the most frequently preferred male ornament (Kodric-Brown 1989, Houde 1987, Brooks & Caithness 1995 a). It is also an indicator of male condition (Houde 1987), foraging ability (Nicoletto 1993) and parasite resistance (Houde & Torio 1992), and is thus quite possibly a signal of male quality.

Understanding the evolution of black coloration is more difficult. Natural variation in melanin area is not correlated with female response in this population (Brooks & Caithness 1995 a), but in a recent manipulative experiment (Brooks & Caithness 1995 b), I showed that when melanin area is reduced, male attractiveness decreases. A male ornament can theoretically coevolve with female choice without being attractive to females itself if it accentuates the pattern of expression of another ornament which is preferred because it is a signal of male quality (Hasson 1989, 1990, 1991). Such an ornament is known as a signal "amplifier". The black areas of male guppies may well be amplifiers as they often "outline" orange areas and appear (to the human eye, personal observations) to make the areas of orange pigment more conspicuous.

Theories of signal amplifier evolution make three important predictions. First, they predict that variation in mating success of males will (initially at least) not be related to the expression of the "amplifier" (Hasson 1989, 1991). Secondly, it is argued that females will choose more accurately in relation to the cue of quality before the levels of the amplifier are reduced than afterwards. Lastly, manipulating an

Chapter Five:

Melanin as a visual signal amplifier in male guppies.

1996 *Naturwissenschaften* 83: 39-41.

INTRODUCTION

According to "good genes" models of sexual selection (Zahavi 1975, Pomiankowski 1988, Grafen 1990 a, b), male secondary sex characters (ornaments) evolve as signals of male genetic quality, and female preferences for them are favoured because offspring derive enhanced vigour from the genetic quality of their sire. In an ideal (error-free) signalling system, only one male ornament should evolve to signal male quality (Johnstone & Grafen 1992, 1993). However, males of many species bear multiple ornaments. Inaccurate female perception is one source of error which may favour the evolution of multiple ornaments, either as redundant sets of "quality" signals which reduce the overall probability of error (Zuk et al. 1992, Møller & pomiankowski 1993a) or as signal amplifiers which reduce perceptual error (Hasson 1989, 1990, 1991). I show that manipulating a seemingly non-preferred ornament (melanin pigment) in male guppies reduces the accuracy with which females make mate choice decisions based on a signal of male quality (carotenoid pigment), and that this manipulation reduces the attractiveness of males bearing high levels of the quality cue more than those with low levels.

Male guppies (*Poecilia reticulata*) bear conspicuous colour patterns

guppies' ornaments do not appear to be redundant signals used in the same choice process.

Theoreticians warn that multiple female preferences are unlikely to evolve by the handicap process (Pomiankowski & Iwasa 1993, Iwasa & Pomiankowski 1994) but rather that preferences for multiple Fisherian traits evolve in tandem with one handicap preference. In this population of guppies there is no evidence regarding the relationship between male quality and cue magnitude. However, in other populations, the intensity (Kodric-Brown 1989, Houde & Torio 1992) and area (Nicoletto 1993) of orange ornamentation is condition-dependent, as is pattern complexity (Nicoletto 1993) to which orange pigmentation contributes. Other male ornaments have not been shown to be indicators of male condition or quality. Our findings, and the knowledge we have of condition-dependence of male ornaments fit the theoretical prediction (Iwasa & Pomiankowski 1994) that secondary preferences can evolve as Fisherian traits or as signal "amplifiers" (Hasson 1989) together with one "handicap" preference - orange (carotenoid) colouration, but this support requires critical test.

alternatives to measuring individual components of colour patterns. "Pattern complexity" not only predicts male condition and vigour (Nicoletto 1993) but also predicts male attractiveness and mating success in the guppy population we studied (Chapter Two of this thesis). Petrie and Halliday (1994) show that peahens assess the number of spots in a peacocks train in order to choose a mate, providing an analog of female preference for more complex male colour patterns in fish. Unfortunately we found it impractical to increase pattern complexity whilst reducing black area in order to separate the effects of black area from those of pattern complexity on female choice. The overall contrast or conspicuousness of a male may be more important than individual colour areas (Endler & Houde 1995), such that the efficacy of orange areas as mate-attractant signals is mediated by the expression of black areas.

Females may scrutinise ornaments separately, with the strength of the relationship between cue size and attractiveness revealing the strength of the preference. Zuk *et al.* (1992) showed that when comb size, which is the primary determinant of mating success in red jungle fowl, is manipulated, females revert to another ornament as their cue for choice. Our findings provide support to their argument that female choice can select for an ornament that is at first apparently not the object of preference (Zuk *et al.* 1990, 1992). However, in our experiment, the apparent preference for black was exerted in a situation in which the primary trait (orange area) was not changed in any way. Likewise, when a female's ability to discriminate between males on the basis of orange area is impaired (by keeping the fish under orange light), she does not revert to another ornament as a substitute primary object of choice (Brooks & Caithness 1995 d). Thus, unlike jungle fowl,

DISCUSSION

This is (as far as we are aware) the first direct manipulation of a colour pattern element in guppies (Long & Houde 1989 used light colour manipulation to show preference for orange males). If this technique proves successful for manipulating other colour pattern elements, a clearer picture of the relative contributions of different elements to male attractiveness must emerge.

By manipulating a secondary sexual character which is not the principle focus of female choice, we have shown that it is used by females exercising mating preferences. This supports the idea that in guppies, female choice is a subtle process involving the assessment of several different criteria (Kodric-Brown 1993), and eliminates non-choice hypotheses for the maintenance of black ornamentation in this population. More generally it is insufficient to claim that ornaments play no role in female mate choice if they are not correlated with measures of attractiveness upon first inspection.

Female choice explanations for multiple male ornaments fall into two broad groups: those suggesting that females scrutinise a composite trait to which several male characters contribute (Chapter Two of this thesis), and those postulating that separate female preferences select for each ornament (Pomiankowski & Iwasa 1993, Iwasa & Pomiankowski 1994). Our findings are consistent with explanations in both of these categories.

Composite measures of male colour patterns such as colour contrast (Endler & Houde 1995) and pattern complexity (which is a function of the number of spots of each colour in a male's pattern, Nicoletto 1993) are beginning to provide useful

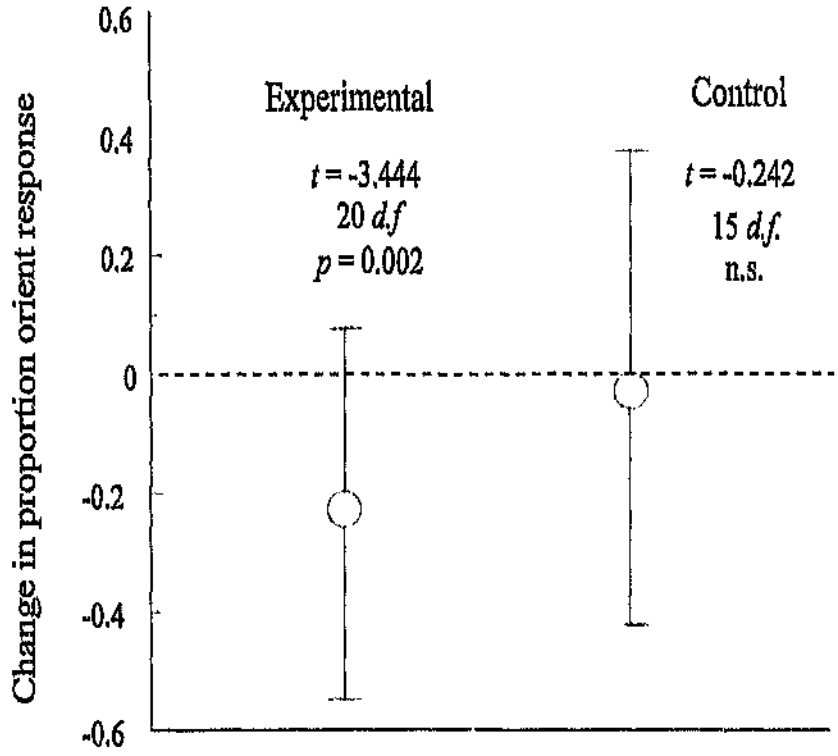


Figure 4.1. Change in female orient response to males with black (experimental) and unpigmented (control) areas freeze-branded. Values are means ±1 SD

RESULTS

Males that have had part of their black colouration removed by freeze branding are significantly less attractive to females than before branding in terms of female orient response (t = -3.444, $d.f.$ = 20, adjusted p = 0.006), but this reduction in attractiveness is not seen in males freeze branded on an unpigmented area of similar size (t = 0.242, $d.f.$ = 15, n.s.) (figure 4.1). The decrease in "orient" response was significantly greater in males with reduced black area than full sibling control males (Paired-sample t = 2.194, $d.f.$ = 12, adjusted p = 0.049).

melanin pigmentation which varies over the sampling period, the investigator should take both the females sampling period and the information available to her throughout that period into account (Sullivan 1990). The strength of our design is that by removing one of the relatively permanent spots, we reduce both the maximum and minimum area of black that a male can show, whilst keeping all other morphological variables the same.

Male attractiveness scores before and after treatment served as paired comparisons, and the control served to distinguish the effects of anaesthetic and freeze branding *per se* from the effects of manipulating black area. Whilst male attractiveness scores (per session) were neither normally distributed nor transformable to normality, the differences in female orient and glide response to a male before and after treatment in both experimental and control groups did not differ from a normal distribution (control: Lilliefors test $p = 0.718$; experimental: $p = 0.647$). This permits the use of a paired-sample t-test as this test involves the comparison of differences between paired samples with a normal distribution with a mean of zero. Paired-sample *t*-tests were used in this way to test the null hypothesis of no decrease in male attractiveness after experimental or control freeze-branding. A paired-sample *t*-test was also used to compare the change in attractiveness of siblings in the experimental and control groups. To correct for the increased possibility of type I error because this approach requires three t-tests, we adjusted the 0.05 α-level of significance for three tests using a sequential Bonferroni readjustment (Rice 1989).

INTRODUCTION

Despite the popularity of sexual selection, and particularly female mate choice as a field of study (see Andersson 1994 for a thorough overview), the inheritance and ontogeny of female mating preferences are still poorly understood. Models of female choice have traditionally treated female preferences as characters that are entirely determined by genes (Lande 1981, Grafen 1990 a, b). Whilst this served to simplify some difficult questions regarding how male displays and female preferences for those displays might evolve, we still lack insight as to how female preferences are inherited and how they are shaped during growth. Recently, the forces influencing female mating behaviour and preferences have begun to receive more attention (see Rosenqvist & Berglund 1992). In particular, the genetic basis for female preferences (Majerus et al. 1982, 1986, Bakker 1993, Houde 1994, Wilkinson & Rlelo 1994) and the effects of female experience in sampling males (Janetos 1980, Brown 1981, Bakker & Milinksi 1991), imprinting on early experiences (ten Cate & Bateson 1988, 1989, Laland 1994 b), and using information about the choices made by other females when choosing mates (Losey et al. 1986, Wade & Pruett-Jones 1990, Gibson & Höglund 1992, Kirkpatrick & Dugatkin 1994, Laland 1994) have enjoyed increased theoretical and empirical attention.

In this chapter I address two processes fundamental to the way preferences evolve in populations and develop within individual females: (i) the repeatability of choice behaviour - which is an indicator of the heritability of mating preferences, and (ii) the importance of mate copying, by which mating preferences can be transmitted culturally rather than genetically. This p. therefore represents a first attempt at

Chapter Seven:

Copying and the repeatability of mate choice

ABSTRACT

Models of sexual selection by female choice require heritable variation in female mating preferences in order for sexual selection to operate. However, recent theoretical work shows that female preferences which are transmitted non-genetically can result in exaggeration of male ornamentation. Guppies exhibit both mate copying and considerable heritable variation in female preferences. I studied the importance of these phenomena by measuring repeatability of female mate choice, which acts as an estimate of the upper limit to which a feature may be heritable, and the incidence of mate choice reversal in paired-trial binary mate choice experiments. Mate choice was significantly repeatable except in the treatment where females were given the opportunity to copy a female that contradicted their original choice. Otherwise, I found no evidence that females copy the mate choice of others. The differences between males in ornamentation had no effect on the consistency of female mate choice or the probability that they would reverse their original choice decision (in both controls and the copying experiment). The interval between choice trials did not influence repeatibility significantly, indicating that the independence of choice decisions is not related to the time interval between them.

to act on their preferences in the absence of experience of males? Bakker & Milinski (1991) provide evidence in favour of this, although their females could have had experience of males before they were collected from the wild. Our results demonstrate for the first time in a vertebrate (Moore & Moore 1988, showed this in an insect) that female guppies possess fixed internal standards of male attractiveness which enable them to express their mating preferences in the absence of experience of males.

The adaptive significance of decision-rules based on experience of past males is twofold. Firstly, if standards of acceptability are reduced with decreasing marginal value of prolonging the search period, a female can avoid missing an entire breeding cycle if she has the misfortune to sample several relatively drab males. Secondly, a female can take advantage of a distribution of male ornaments that is higher than 'expected', and mate with the most highly ornamented of those males. We suggest that stochastic decision-rule models are more biologically meaningful than optimality models of mate choice criteria.

response to him would exceed that expected due strictly to his ornamentation. Secondly, the change in ornamentation from the first to the second male a female encountered is significantly correlated with the change in her response, suggesting that females do compensate in such a manner.

Bakker & Milinski (1991) found that female three-spined sticklebacks (*Gasterosteus aculeatus*) use a stochastic decision rule based on the relative attractiveness of the present and previous males (previous male effect). In our study, the previous male observed by a female influences her response to the present male. However, due to the nature of our experimental design, our findings cannot be used to infer the decision rule used by female guppies. We do not argue, however, that females base their response to a given male only on the ornamentation of the previous male she sampled. In fact, our results show that under a strict previous-male decision rule, accuracy of choice is likely to be extremely poor. Male guppies can decide on which male conspecifics to associate with based on information that has been remembered for at least 4 h (Dugatkin & Alfieri 1991). If similar cognitive capacities are available to females for mate discrimination decisions, they may be able to base their standard of attractiveness on the ornamentation of several males. If this is the case, we predict that the accuracy of her response relative to a male's ornamentation in the population will improve as more males are sampled. Statistical solutions to problems of choice based on the 'quality' of previously sampled males abound (Ferguson 1989), and we suggest that models of female choice among two consecutively sampled males will benefit from reference to these solutions.

Do females have a fixed internal standard of attractiveness which allows them

second male presented to each female was positively and significantly correlated with the change in her responsiveness ($r_s = 0.387$, $n = 56$, 1-tailed $p < 0.0025$). In the two-male trials, male attractiveness was significantly and positively correlated with orange ornamentation ($r_s = 0.270$, $n = 56$, 1-tailed $p < 0.025$). Females were no more or less likely to respond positively to a the first male than the second male she encountered (Wilcoxon signed ranks test: orient - $Z = -0.369$, $n = 55$, n.s.; glide - $Z = -0.536$, $n = 56$, n.s.).

DISCUSSION

In this guppy population the 'calibration' of female mate preferences is, to some degree at least, based on standards of attractiveness that are independent of a female's past experience of males. However, we also showed that a female's experience is important to her response to a given male. We base this on two inferences from the rank-order correlations between orange colour area and the second part of the single male trials. In the second part, the significant positive relationship between orange and male attractiveness disappears. This appears counterintuitive to the notion that females use experience as a basis for deciding whether or not to respond to a given male, however we could not infer this unless the significant relationship disappeared. The 'noise' introduced to the mate-choice situation by the females experience of just one male could suffice to make a female's assessment of a given male less accurate than if she had no experience of males at all. For example if a given female saw two males that were below the population mean (or some other biologically meaningful standard), and the second was better endowed in terms of the cue of choice, her

RESULTS

Percentage of male body area that was orange was the only male character that varied systematically between winners and losers of paired male trials (Table 6.1). Likewise, it was the only character positively correlated with female response to males in any of the phases of the experiment, and so is the only character discussed with reference to the importance of fixed internal standards of attractiveness and female experience.

Table 6.1. Difference between "winners" and "losers" of mixed male mating trials in magnitude of secondary sexual characters.

		orient response	
Potential Cue	*d.f.*	test statistic	1-t *p*
body area	27	0.458[t]	n.s.
relative orange	27	3.754[t]	0.001
relative black	27	0.073[t]	n.s.
iridescence	20	0.859[z]	n.s.
yellow	16	0.024[z]	n.s.
gonopodium	13	0.910[z]	n.s.
dorsal fin	16	0.267[t]	n.s.

t = Student's *t* (paired sample test)
z = normal approximation (Wilcoxon signed ranks test)

The correlation between relative area of orange and male attractiveness to naïve virgin females was positive and significant (r_s=0.309, n = 56, 1-tailed $p < 0.025$). When males were switched, this relationship became weaker, and was no longer significant (r_s = 0.066, n = 56, n.s.). However, the difference between the first and

pairwise tests were used to test a null hypothesis of no difference in cue magnitude between the winner and the loser. Paired sample Students *t*-tests (Sokal & Rohlf 1987) were used in the case of measures that conformed to the assumptions of parametric tests, otherwise Wilcoxon's signed ranks tests (Siegel & Castellan 1988) were used.

In order to infer the relative strengths of female's internal standards of choice and the effects of experience on her choice, we performed a series of Spearman rank-order correlations, as response measures could not be transformed to normality.

In the first part of the single male trials, we test the null hypothesis that the responsiveness of naïve virgin females is randomly associated with the expression of an ornament for which there is known to be choice (relative orange area, Brooks & Caithness 1995 a, Chapter Two of this thesis). Similarly, we calculated the correlation between female responsiveness to a male in the second part of the single male trials and the relative area of his body that is orange. We then calculated the change in orange ornamentation experienced by each female when the males were switched and related this to the change in her responsiveness. Lastly, we compared the mean attractiveness of the male in paired-male tests with his degree of orange colouration as a basis for comparison with the strengths of the other relationships. Our arguments relating the importance of experience to the expression of female preferences are based on the relative strengths of the various correlations. All tests of significance are one-tailed tests of the null hypothesis of no positive association between ornament magnitude and female response. The α-level of significance was 0.05.

Two-male trials

Both females and both males were then placed together with a third female in a tank and left until the following morning. Each male was watched for two five minute periods and the responses to his displays scored as described above. This part of the experiment enabled females to directly and simultaneously compare males.

Measuring cues

Each male was placed in a clear plastic bottle (23 mm x 39 mm filled to 40 mm) and photographs of his left and right sides were taken using Fujichrome 100 ASA slide film under tungsten lighting. We projected the slides onto white paper and traced the outlines of the body, dorsal fin and gonopodium as well as all visible colour spots and a 5 mm section of the ruler which was included in the photograph for calibration. The digitiser board of a personal computer was used to measure the following from the tracings: body length and area, length of the gonopodium and of the dorsal fin and area of each colour spot. Measurement error using this method is very low (0.3%) and not significant (Brooks & Caithness 1995 a).

Statistical Analysis

Statistical tests were performed using SYSTAT for Windows 5.0 software. When total area of a colour was divided by body area to remove the effect of body size, these variables were arcsine transformed (Sokal & Rohlf 1987). Measures of length were transformed to their natural logarithms to satisfy normality. To investigate the relationship between female choice and male ornaments in the mixed male trials,

Single male trials.

Females were raised in mixed sex cohorts of the same age and removed to single-sex stocks as soon as they showed evidence of the "gravid spot" which signifies sexual maturity. Males were removed from these cultures as soon as their anal fins showed signs of differentiating into gonopodia. Males were chosen by one of us to differ in relative orange area were paired. Paired males were put into different tanks (900 mm x 315 mm filled to 270 mm) and each was introduced to a virgin female that had never been exposed to mature males (i.e. naïve virgin female). We gave the fish five minutes to adjust and then watched the female for two five minute periods with five minutes in between (part one). Males were then switched and once again females were watched for two five minute periods (part two). Males were presented to females in random order. Part one allows us to ask whether or not females are able to act on their mate preferences when they have no previous experience of mature males and no other males are present for the purposes of comparison. Part two is a trial in which the females have prior experience of one male, the phenotypic attributes of which are known (see "measuring cues" below), and the change in her response can be attributed to the differences between males.

Female response to each display was scored in terms of the proportion of sigmoid displays eliciting "orient", "glide" and "mating" responses from females (described by Houde 1987, Brooks & Caithness 1995 a). Only the proportion of orient responses was used in analysis as this provides a larger behavioural sample than either of the other two measures, but is still positively related to male mating success (Chapter Two of this thesis).

favours a model of mate choice in which females do not have fixed internal standards of attractiveness and are thus only able to make choice decisions after sampling a number of males. We use a unique inductive technique to assess whether females are able to act on their mate preferences without experience of other males and the role of comparative information in making choices. We study only the first two males encountered by naïve virgin females to resolve the question of whether or not females use fixed internal standards, experience of other males or both to make mate-choice decisions.

Guppies are ideal animals for the study of female response to a sequence of males because a males attractiveness to any receptive female can be quantified as a continuous variable which approximates the probability of her mating with him, without mating necessarily taking place (Houde 1988 b, Chapter Two of this thesis). Moreover, the well documented relationship between orange colouration and male attractiveness (Kodric-Brown 1985, Houde 1987, Brooks & Caithness 1995 a, d, Chapter Two of this thesis) provides a convenient yardstick of a females ability to choose males based on ornamentation.

METHODS

We used first generation laboratory-bred individuals from a feral population in Beachwood, Durban, South Africa. They were kept at a density of approximately 20/fish on a 12:12 light:dark photoperiod in a temperature controlled (25°C) room. We provided Tetramin flake food once a day.

exert their mating preferences without any prior experience of males.

Fixed internal standards can, in both threshold and comparative choice situations, lead a female to mate nonrandomly with males based on cue magnitude, and thus sexually select for exaggeration of those cues (sensu Darwin 1871) even when she has no experience of males with which to compare the focal male. However, in a population of larger-than-average cued males, a female would often end up with a relatively drab male unless she were able to adjust her standard of attractiveness to account for the distribution of cue magnitude in the population (Bakker & Milinski 1991).

The best strategy for choosing in the absence of costs associated with searching for males is to sample several, and then mate with the best of those (the 'best of n' rule, Janetos 1980, Janetos & Cole 1981). However, often there are costs which increase throughout the sampling period (eg. the impending last time of spawning or larval release, Bakker & Milinski 1991, Backwell & Passmore submitted; energetic costs of moving between males, Milinski & Bakker 1992, or predation risk, Hedrick & Dill 1993) or if it is impossible for a female to return to a male once she has moved on to another male. In these cases, other sequential decision-making rules are often optimal (Real 1990, eg. Brown 1981). Also, simple stochastic decision-making processes which return fitness benefits slightly below those of optimal solutions are expected to be common in nature (Janetos & Cole 1981).

Naïve virgin female guppies (*Poecilia reticulata*) mate very soon after first introduction to males, which may be due to low initial choosiness (Houde 1987). This

INTRODUCTION

Female choice is a major selective pressure for the exaggeration of male secondary sexual characters (Møller 1994 a), but less is known about how female preferences evolve. One controversial aspect of this problem is how females decide whether or not to mate with a given male, especially when there is no opportunity for simultaneous comparison with other males (Milinski & Bakker 1992). This is not problematic when fixed threshold choice criteria are used by females, because females are expected to exert mating preferences when courted by those and only those males that meet the threshold requirement (eg. Zuk et al. 1990). Several models have been formulated to explain non-threshold (comparative) female response across the range of variability in a male cue. Lande (1981) distinguished two such choice processes, absolute choice, in which a female compares available males directly, and chooses the better endowed without regard to it's absolute cue magnitude, and relative choice, in which females compare a given male with a standard of attractiveness (eg. the population mean for cue expression) which she holds. By far the more perplexing of these two phenomena is relative choice. Even when preferences are "open ended", they must be "calibrated" so that females are choosy enough to derive benefit from choosing, but not so choosy that they fail to find a mate. Two types of standard may be used in the "calibration" of a female's preferences: fixed internal standards of attractiveness which are independent of past experience, and the female's experience of males. Female's have been shown (Milinski & Bakker 1992, Brown 1981) to respond to males based on their previous experience of males. Only one account (Moore & Moore 1988, on cockroach mate choice) shows that females are able to

treatments (figure 7.3). The increase from part 1 to part two was not significantly greater in the copying treatment than in the two day treatment ($t = 0.076$, $d.f. = 39$, n.s.).

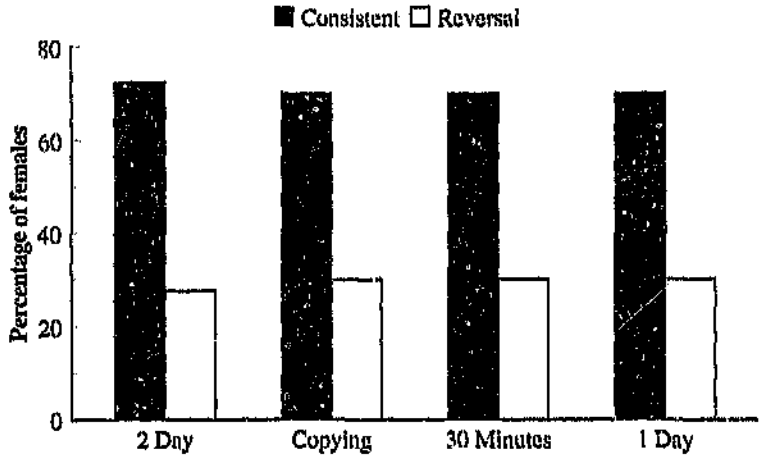


Figure 7.2. The percentage of (focal) females choosing the same male (consistent) and reversing their choice (reversal) between the first and second trial in each experiment. In all four treatments, significantly more females consistently prefer the same male than change their choice of male. This consistency is significantly different from a random model of choice for all experiments (2 day $G_{adj} = 8.295$, $d.f. = 1$, $p < 0.01$; copying $G_{adj} = 6.501$, $d.f. = 1$, $p < 0.02$; 30 minutes $G_{adj} = 4.857$, $d.f. = 1$, $p < 0.05$; 1 day $G_{adj} = 4.857$, $d.f. = 1$, $p < 0.05$). The number of females reversing their choice in the copying treatment did not differ significantly from the pattern shown in the 2 day treatment ($G_{adj} = 0.625$, $d.f. = 1$, n.s.) or the 30 minute treatment ($G_{adj} = 0.063$, $d.f. = 1$, n.s.).

Table 7.1. Results of one-way ANOVA's of the proportion of time a female spends with one (randomly picked) male in a binary choice trial, and the repeatability estimate and standard error thereof for each of the four treatments.

		ANOVA					Repeatability	
Treatment	Source of variation	SS	df	MS	F	p	r	SE
2 day	Between females	6.499	39	0.169	2.088	0.011	0.352	0.052
	Within females	3.191	40	0.080				
Copying	Between females	4.321	39	0.111	1.114	0.367	0.056	0.012
	Within females	3.977	40	0.099				
30 Minute	Between females	5.199	29	0.179	2.057	0.027	0.346	0.059
	Within females	2.614	30	0.087				
1 Day	Between females	3.899	29	0.134	1.924	0.040	0.315	0.050
	Within females	2.096	30	0.070				

In all four treatments, females chose the same male significantly more often than expected under a random model of choice (figure 7.2). Furthermore, in the copying treatment, the relative frequencies of consistency and reversal did not differ significantly from those observed in the 2 day treatment or the 30 minute treatment, as predicted if females reverse their choice due to copying. Whilst females did not copy the "model" female, they did not avoid copying her either.

Although females did not reverse their preference in the copying treatment, the proportion of the total time near males that they spent with the male that had been near the model female (as opposed to their originally preferred male) increased (figure 7.3). However, significant increases in time spent with the loser of the first choice trial also occurred from the first to the second trial in all three of the other

obtained in the copying treatment with the ratios observed in the 2-day treatment (in which the same female was used with each pair of males) and in the 30-minute treatment (in which the same time elapsed between choice trials).

To compare the increase in proportion of time a female spent with the loser of the first trial in the first and second trials of a treatment, I used paired-sample Students *t*-tests.

I used a Mann-Whitney *U* test to compare the magnitudes of the difference in various secondary sex characters between the two males for replicates in which the female reversed her choice versus those in which she chose the same male in both trials. Lastly, I estimated the correlation between the above differences and the absolute change in the measure of female preference in a replicate using Spearmans rank-order correlation coefficient.

Where tables of results are presented, I have used the sequential Bonferroni readjustment (Rice 1989) to correct the 0.05 α-level of acceptance for the number of test performed.

RESULTS

The repeatability with which a female chooses one male over the other is significantly greater than zero in all treatments except the copying treatment (Table 7.1). None of the repeatability estimates differed significantly from any of the others, the largest difference being between the 2-day and copying treatments ($Z = 1.34$, $\sigma = 0.232$, 1-tailed $p = 0.090$).

different results from those obtained for the separate colour dimensions, and is therefore the only colour measure presented.

Statistical analysis

I analysed data using Statistica for Widows (v 4.5, Statistica inc, 1993) statistical software. I tested all variables for normality using Lilliefors' test, and used the angular transformation (Sokal & Rohlf 1981) before using proportional data in parametric tests. Where normality or other assumptions of parametric tests were not satisfied, I used non-parametric tests.

I estimated repeatability after Falconer (1981), Lessels and Boag (1981) and Boake (1989), using a one-way analysis of variance (ANOVA) to partition the total variance in female choice of one (randomly chosen) male in a pair over the other into the added variance due to differences between females (S^2_A) and the variance within individuals (S^2 or error variance). The ratio of between-individual variance (S^2_A) to total phenotypic variance ($S^2 + S^2_A$) is equivalent to r, the coefficient of intra-class correlation which is a measure of repeatability. I used Becker's (1984) method to calculate the standard error of the repeatability measure. I compared the repeatibilites of different pairs of treatments using Zar's (1984) method for comparing correlation coefficients.

I used the G-test (Sokal & Rohlf 1981) to compare the ratio of females reversing:remaining consistent in their "decision" between trials with different models of choice. I compared all treatments with a model of no choice (half the females switch their preference and half are consistent). I also compared the ratios

performed for the first two treatments, but unfortunately, during the period between the first two and the last two treatments, many of the males contracted fin-rot or died. If a male had visible fin-rot, I did not use him in a trial in case females avoided him to prevent contracting the infection themselves, thereby influencing the outcome of the trials. Therefore of the original 40 pairs of males used in the first two treatments, only 24 remained intact. 12 healthy fish from the 16 "broken" pairs were arbitrarily paired up to make the sample for these two trials up to 30 pairs.

The design of this experiment is therefore similar, except for superficial differences in choice tank design, to other experiments investigating female choice (Bischoff et al. 1985, Kodric-Brown 1985) and in particular repeatability of female choice (Godin & Dugatkin 1995) and female copying (Dugatkin 1992, Dugatkin & Godin 1992, 1993) in guppies.

Measuring male ornamentation

I measured ornamentation by taking slide photographs of male fishes under tungsten lighting and then tracing the projected slides onto white A3 paper, including part of a section of plastic ruler which I included in the photographs for calibration. This enabled me to measure the area of each fishes body and of each colour patch using a grid of squares. The orangeness of each male was estimated visually using Munsell colour chips. The hue, value and chroma of the colour chip that most closely resembled each colour patch was noted. In analysis, all three colour dimensions were used, and in addition an "orangeness" variable was constructed by scoring hue from 1 (2.5 Y) to 6 (10 R) and multiplying this by chroma. This did not provide substantially

female choice behaviour have been used before, and shown to accurately predict success in mating encounters (Kodric-Brown 1985).

For each treatment, I swopped the side of the tank that m ·es were in between trials in half of the replicates, and left males in the same tubes in the other half. There was no significant side preference either generally or within males. The time between choice trials varied between treatments. In treatment 1 ("2-day") 48 hours elapsed between choice trials. In treatment 2 ("copying") the second choice trial from the 2-day treatment made up the first part of the treatment, and the second trial followed 30 minutes later. The difference between this and the other treatments was that during the first eight minutes of the 10-minute "viewing time" (when the focal female was confined to her tube) a second, "model" female was placed in a (similar) glass tube adjacent to the male that had "lost" the first trial. I removed the model female and her tube after 8 minutes and 2 minutes later released the focal female to swim freely for ten minutes during which I documented the time . The model female was larger than any of the focal females used in the experiment (model = 36.66mm , focal = 32.54 - 20.48 mm total body length) , as a female is more likely to copy a larger (and thus older) female than one smaller than herself (Dugatkin & Godin 1993).

I used the same m. .le pairs in treatment 3 ("30-minutes"), in which 30 minutes elapsed between choice trials, between 10 and 30 days after treatments 1 and 2. Whilst in treatments 1 and 2 I used the same female with a given male pair, I used a different focal female in the 30 minute treatment for a given pair of males than in the first two. I then used yet another different female in treatment 4 ("1 - day"), in which 24 hours passed between the first and second choice trial. Forty replicates were

METHODS

This study comprised four treatments. In each treatment two choice trials were conducted, using one female and two males. Choice trials were conducted in a 100 ℓ aquarium as shown in figure 7.1.

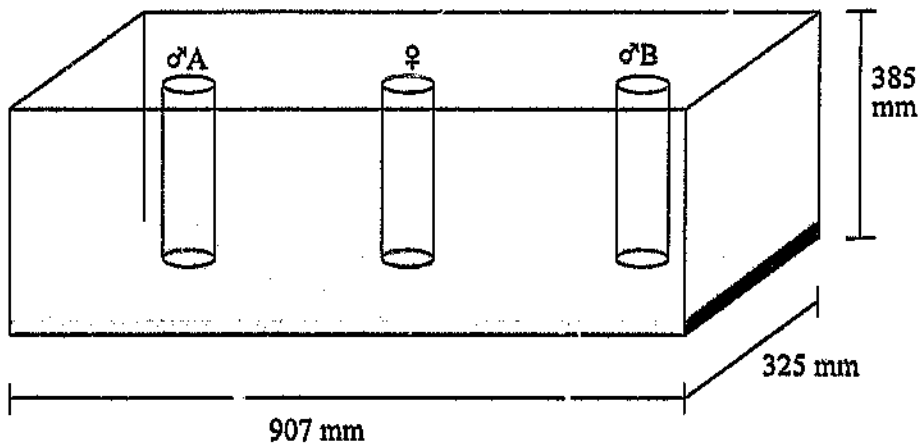


Figure 7.1. The aquarium used in choice trials. Background and bottom colour were standardised by placing 2 cm of fine light brown river sand on the bottom of the tank and tan paper on three sides (the long side facing the observer was left clear). Three open ended glass tubes 90 mm in diameter and 350 mm in length were placed in the longitudinal midline of the tank. The tube (♀) which contained the focal female was in the centre of the tank, with tubes bearing males (♂A & ♂B) 290 mm away (centre to centre).

I placed a male in each of two "end" glass tubes and a female (the "focal" female, after Dugatkin 1992) in the central glass tube. After ten minutes, I slowly lifted the central tube until the female swam clear, and then placed the tube gently down in the tank again. The female was watched for 10 minutes, and the time spent within two body lengths (horizontally) of each male's tube and within three centimetres (vertically) of the male in that tube was recorded. Similar measures of

Kirkpatrick and Dugatkin (1994) argued that widespread copying is unlikely to favour the origin or maintenance of multiple sexual ornaments, but will rather favour the most common male ornament at the expense of others. The multiple ornaments borne by male guppies contradict this prediction, such that Kirkpatrick and Dugatkin were forced to invoke some unidentified form of selection as the agent favouring multiple ornaments in opposition to the effects of mate copying.

Virgin female guppies from the population I used in this study are able to exercise their mating preference for more orange males (Brooks & Caithness 1995 c). This precludes the possibility that female mating preferences are transmitted entirely by culture as in the models by Laland (1994) and Kirkpatrick and Dugatkin (1994). However, I have also shown that a female's previous experience of males is important to her choice decisions (Brooks & Caithness 1995 c) in that a female adjusts her response to a male based on her experience of the level of ornamentation in the population.

I have therefore attempted, in this chapter, to understand the extent to which female mating preferences could be heritable by quantifying the repeatability of a female's choice between two males. I have further examined some aspects of copying behaviour in order to understand if female preferences could be, at least partially, culturally transmitted. Lastly, I have looked at how male ornamentation is related to the repeatability of choice and the likelihood of mate copying.

the likelihood of copying.

Guppies are well suited to attempts to resolve the repeatability of female mating behaviour, and the effects of copying on the outcome of choice decisions, for several reasons. They are something of a model organism in studies of sexual selection due to their highly promiscuous, non resource-based mating system and the importance of female choice to paternity. Male attractiveness can be measured as a continuous, rather than discrete, variable by measuring the time a female spends near each male in a choice trial (Bischoff et al. 1985, the approach used in this study), or the proportion positive female responses a displaying male elicits (Houde 1987). Female choice is responsible for the evolution and maintenance of several of the ornaments borne by male guppies including orange (carotenoid) pigment (Houde 1987, Kodric-Brown 1985, 1989, Brooks & Caithness 1995 a), black (melanin) pigment (Endler & Houde 1995, Brooks & Caithness 1995 b, Brooks 1996) iridescent (structural) pigments (Kodric-Brown 1985, Endler & Houde 1995) exaggerated fins (Bischoff et al. 1985) and enlarged body size (Reynolds & Gross 1992, Endler & Houde 1995). There is evidence from selection experiments that there is additive genetic variation in (at least) the female preference for orange spots on males (Houde 1994, but see Breden & Hornaday 1994). Studies on guppies have produced the best evidence yet for the joint evolution of female preference and male ornament within populations (Breden & Stoner 1987, Houde 1988 a, Stoner & Breden 1988, Houde & Endler 1990, Endler & Houde 1995).

To date, the best experimental evidence for female mate copying has been obtained using guppies (Dugatkin 1992; Dugatkin & Godin 1992, 1993). However,

1979). It also raises the possibility that if preferences are entirely culturally (as opposed to genetically) transmitted, a female preference and the corresponding male ornament or display can coevolve rapidly (Kirkpatrick & Dugatkin 1994, Laland 1994). Furthermore, the spread of a preference is likely to be faster than under strictly vertical (genetic) inheritance because preferences can also be transmitted diagonally or horizontally. This may result in more rapid exaggeration or fixation of the male ornament on which the preference is based than is the case with preferences that are only inherited genetically (Laland 1994), and even the loss of all but the (initially) most common male traits and female preferences (Kirkpatrick & Dugatkin 1994, but see Findlay et al. 1989).

The effects of ornamentation on repeatability of choice and the operation of mate copying have not been examined previously. A functional explanation is that copying reduces costs associated with the time taken to assess males and that some females are poorer at discriminating between males than others (Dugatkin & Godin 1993, but see Gibson et al. 1991), and that it is these females who have most to gain from copying. In a mixed copying / independent choice strategy, there may be a tradeoff for females between following their own choice decision and copying others when the two are contradictory. I therefore asked whether copying is stronger or weaker than independent choice, and whether the similarity of the ornamentation of males contributes to a female's decision to copy or stick with her original choice. When males are most similar, choice is expected to be at its least repeatable and copying is expected to be common. I relate, for the first time, the differences in ornamentation between males in binary choice trials to the consistency of choice and

Mate copying is a form of nonindependent choice which occurs if the probability of a female choosing a particular male increases if he has already been chosen by other females and decreases if he has not (Pruett-Jones 1992). Apparent copying behaviour has been documented in several taxa. In many nest-building fishes, females prefer to spawn in nests which already contain eggs (Ridley & Rechten 1981, Marconato & Bisazza 1986, Unger & Sargent 1986, Kraak & Groothuis 1994). Females of the marine isopod *Paracerceis sculpta* prefer to live on sponges which contain other females (Shuster & Wade 1991). Lekking birds (Lill 1974, Höglund et al. 1990, Gibson et al. 1991) and fallow deer (Clutton-Brock et al. 1989) also show apparent female copying. However, in at least fallow deer (McComb & Clutton-Brock 1994), and quite probably some other cases (see Gibson & Höglund for a more thorough discussion) females are not copying but associating with other females. Females may also be responding to the enhanced display rates of males that have already mated, although this has recently been ruled out in nesting fishes (Kraak & Groothuis 1995). In guppies, however, these confounding possibilities have been eliminated (Dugatkin 1992) and copying has been demonstrated to such an extent that females will reverse their original c males in binary mate-choice trials to copy the choice of another female (Dugatkin 1992, 1993).

The possibility that there is a cultural (after the analogy with human cultural transmission of behaviour by observing the behaviour of others) component to the transmission of mating preferences has important evolutionary consequences. Copying is likely to result in an increased variance in male mating success (Wade & Pruett-Jones 1990), and therefore increased opportunity for sexual selection (Wade

trying to understand the relative contributions of genetic and cultural determinants to female choice behaviour in a single species (*Poecilia reticulata*).

Despite the importance of additive genetic variation in female mating preferences to most models of sexual selection (Lande 1981, Grafen 1990 a, b, Pomiankowski et al. 1991, Iwasa et al. 1991) only a handful of experiments have shown evidence for this (Majerus et al. 1982, 1986, Moore 1989, 1990, Bakker 1993, Houde 1994, Wilkinson & Riello 1994). The repeatability of a behavioural trait such as a mating preference describes the contribution of within-individual variability in the trait (which obviously cannot be heritable) to the total variability within the population (Boake 1989). Therefore repeatability estimates an upper limit of the extent to which a trait can be heritable (Falconer 1981, Boake 1989). In fact, repeatability is thought to overestimate heritability (Boake 1989). This may be because not all between-individual variability is the result of heritable differences between females, because social and developmental differences between females are not factored out. Also, within individual variability may be underestimated if trials conducted on the same female within the time constraints of a normal experiment are nonindependent. This may occur if preference expression is influenced by factors such as condition and recent experience (eg Bakker & Milinski 1991, Brooks & Caithness 1995 c). In this paper I test the influence of the interval between mate choice trials on the measureable repeatability of female preference by varying the time interval between choice trials in different treatments. I also examine the effects of one social phenomenon - the opportunity to copy the mate choice of others, on repeatability.

relationship between male quality and carotenoid-based pigment expression. If males bore several cues each of which revealed quality under different environmental stresses (multiple message hypothesis), these handicap traits would not all be expected to reliably reveal male quality simultaneously (Møller & Pomiankowski 1993 a).

Very little is known about whether black colouration and structural pigments signal male quality in a fashion similar to the way orange area and intensity do (Endler 1983, Kodric-Brown 1989, Nicoletto 1993), which counts against the "redundant signals" hypothesis. However, the dearth of knowledge as to whether and how male ornaments signal male quality (if such evidence can be found for any animal) limits our ability to discriminate the three condition-dependent signalling hypotheses of Møller & Pomiankowski (1993 a).

If males possess redundant suites of cues for signalling condition, these cues should not become the focus of choice until the primary cue has been factored out (as shown by Zuk et al. 1992). However, I showed that when a female's ability to discriminate on the basis of orange area was removed, females did not switch to any other ornament as their cue for choice (Chapter Three). Moreover, by manipulating an ornament that does not appear to be related to choice (black area), that chan. in ornament magnitude revealed a corresponding change in male attractiveness (Chapter Four). This constitutes a weak refutation of the redundant signals hypothesis in th. case, but reinstates the possibility that females scrutinise several cues simultaneously (see "Composite traits or single preferences?" below), but the effects of changes in a males visual contrast should be examined before this can be claimed..

relationship between male quality and carotenoid-based pigment expression. If males bore several cues each of which revealed quality under different environmental stresses (multiple message hypothesis), these handicap traits would not all be expected to reliably reveal male quality simultaneously (Møller & Pomiankowski 1993 a).

Very little is known about whether black colouration and structural pigments signal male quality in a fashion similar to the way orange area and intensity do (Endler 1983, Kodric-Brown 1989, Nicoletto 1993), which counts against the "redundant signals" hypothesis. However, the dearth of knowledge as to whether and how male ornaments signal male quality (if such evidence can be found for any animal) limits our ability to discriminate the three condition-dependent signalling hypotheses of Møller & Pomiankowski (1993 a).

If males assess redundant suites of cues for signalling condition, these cues should not become the focus of choice until the primary cue has been factored out (as shown by Zuk et al. 1992). However, I showed that when a female's ability to discriminate on the basis of orange area was removed, females did not switch to any other ornament as their cue for choice (Chapter Three). Moreover, by manipulating an ornament that does not appear to be related to choice (black area), that change in ornament magnitude revealed a corresponding change in male attractiveness (Chapter Four). This constitutes a weak refutation of the redundant signals hypothesis in this case, but reinstates the possibility that females scrutinise several cues simultaneously (see "Composite traits or single preferences?" below), but the effects of changes in a males' visual contrast should be examined before this can be claimed..

Condition dependent signalling

Møller & Pomiankowski (1993 a) offered three alternative hypotheses for the presence of multiple male ornaments, drawing on the ability of ornaments to signal male condition and thus male genetic quality: the "redundant signals", "multiple messages" and "unreliable signals" hypotheses. I have provided no direct evidence regarding the ability of male ornaments to signal male condition, however there are several lines of inference that may be followed to gain a broad understanding of the importance of these three processes to the evolution of multiple male ornaments.

The only evidence I have presented with respect to the condition-dependence of ornaments is the assessment of fluctuating asymmetry (Chapter One). The significant positive correlation between ornament size and asymmetry for both black and orange pigmentation suggests that neither is condition dependent (according to Møller & Pomiankowski 1993 a). However, this line of reasoning must be viewed with extreme scepticism because orange brightness, at least, is a condition-dependent trait (Kodric-Brown 1989, Houde & Torio 1992), and there is evidence that orange area and pattern complexity are similarly condition-dependent (Nicoletto 1993), in other guppy populations. Amongst other ornaments and displays, only body size (Reynolds & Gross 1992) and display rate (Nicoletto 1993), neither of which are related to male attractiveness in this population, are known to reflect male quality. If the ornaments are not condition-dependent in their expression, they are unlikely to be maintained by female choice for a handicapping trait (sensu Zahavi 1975), unless some cues are simply not *presently* signals of male quality. For example, in laboratory stocks of guppies, carotenoids are expected to be abundant, and this may disrupt the

artifact of laboratory conditions (J.A. Endler, personal communication) as such hierarchies have been observed in other guppy populations (Gorlick 1976, Kodric-Brown 1992, 1993). Therefore it is conceivable that sneak copulation and male dominance are significant contributors to male fitness under conditions other than those in this study and that they therefore may select for some male ornaments.

FEMALE CHOICE AND MALE ORNAMENTATION

Female choice is the only potentially sexually selective phenomenon that significantly influenced male mating success in this study (Chapter Two). It is clear that the area of orange colouration borne by a male is an important cue for female choice (Chapters One and Three), and an important determinant of male mating success (Chapter Two). It is less obvious whether or not other male ornaments have evolved or are maintained by female choice.

By showing that black, which does not normally correlate with female response is important to female choice under manipulation, I show how apparently non-preferred traits can be present due to female choice (Chapter Four). One way in which this can occur is for the non-preferred ornament to act as a signal amplifier for another ornament or display (Hasson 1989, 1990, 1991). Black (melanin) pigmentation apears to act as an amplifier of orange (colouration) in this population (Chapter Five).

In order to answer how the multiple ornaments of male guppies can evolve by female choice it is necessary to examine my findings in the context of some of the more successful models for the evolution of female choice.

Conclusion

In this thesis, I have investigated several different aspects of female mate choice behaviour and its importance to the evolution of male secondary sexual characters and courtship behaviour. I have addressed two principle questions: "How important is female choice as an explanation for exaggerated male secondary sexual characters?" and "How important are genetic inheritance and experience to the expression of female mating preferences?" I have attempted to answer these questions bearing in mind that male guppies have several exaggerated ornaments. In this conclusion I will attempt to review the possible explainations for why male guppies have multiple secondary sexual characters, highlighting the role of female choice.

NON-CHOICE EXPLANATIONS FOR MULTIPLE ORNAMENTS

Firstly, it is useful to first discuss the possibility that female choice is not responsible for the evolution of some or all male ornaments, as this should be the null hypothesis for the investigation.

Neither male dominance nor sneak copulation relate significantly to male mating success (Chapter Two). However, the caveats voiced in that chapter need to be reiterated before we can eliminate these sources of sexual selection from consideration. Most notably, male mating strategy changes with lighting conditions which influneces the costs and benefits of the courtship-display strategy (Endler 1987, Reynolds et al. 1993). Also, the failure to observe dominance hierarchies may be an

ornament, as only the most common ornament will be selected for. The apparent lack (or extreme weakness) of mate copying in this population allows for the possibility that female choice is the agent selecting for the multiple ornaments of male guppies in this population (see Brooks & Caithness 1995 a, b, Brooks 1996).

Since Fisher (1930) it has been accepted that female preferences evolve (and coevolve with male ornamentation) genetically. It seems possible from recent models (Laland 1994, Kirkpatrick & Dugatkin, 1994) that similar results could be obtained even if the female preferences were inherited in a strictly cultural fashion. At this point, the evidence points to a role for both genetic inheritance and experience (see also Brooks & Caithness 1995 c) but not cultural determination, in shaping female choice behaviour in this guppy population.

is the reason for our failure to find strong evidence for copying, it may be true that previous studies overestimated the importance and strength of imitative behaviour in determining the outcome of female mating decisions. This would favour the hypothesis that copying is an important aid to females having trouble discriminating between males on the basis of their ornamentation (see Gibson & Höglund 1992 for a fuller treatment of this hypothesis and its alternatives).

The above explanation is unsubstantiated, however, as males in all treatments including the copying treatment in replicates where females reversed their choice decision were no more or less similar to one another than in replicates where females chose the same male twice. Likewise, the change in female behaviour was not significantly correlated with the difference in male ornamentation. These two types of analysis were used because they were expected to reflect the "certainty" with which a female chooses a male. If males do not differ a great deal in the cues that a female uses for choice, and we know that the females in this population use at least orange (Brooks & Caithness 1995 a) and black (Brooks & Caithness 1995 b, Brooks 1996), I predicted there would be larger changes in preference scores and a higher incidence of choice decision reversal than if the males are extremely different. This is because the chances of receiver error and failure to discriminate effectively (Schluter & Price 1993) are higher if the males on offer are similar than if they are very different. It seems that within-female variability in mate choice behaviour cannot be explained by receiver error or inability to effectively discriminate between males.

Kirkpatrick and Dugatkin (1994) argue that the nature of sexual selection if copying is widespread will not favour the evolution or maintenance of more than one

seen in this study. This prediction remains to be tested.

The time between choice trials does not appear to influence the repeatability of choice. The slightly (but not significantly) lower repeatability in the 30 minute and 1-day treatments than in the 2-day treatment may well be due to the reduced sample in the former treatments due to illness and death of fishes. This lends credibility to measures of repeatability in which the repeated measures are separated by a relatively short time interval, such as that obtained by Godin & Dugatkin (1995). However, the time intervals used in this study are all relatively short, and certainly several orders of magnitude smaller than a female's reproductive lifespan. I suggest that repeatibilities should be estimated using longer intervals between measures before firm conclusions are drawn in this regard.

Ironically, whilst the copying treatment shows such low repeatability, there is no direct support for any claim that females in this population copy the mate choice of others. Females do not reverse their choice decision when faced with a female that chooses another male, as shown by Dugatkin and Godin (1992, 1993) in a wild Trinidadian guppy population. Similarly, although females in the copying treatment pay relatively more attention to the male that they had observed with the "model" female, this increase is not significantly larger than the increase in attention paid to the "loser" of the first trial in other treatments. If copying does occur, it is certainly much weaker than independent mate choice. The predominance of individual mate choice over copying (if it occurs) may be due to the fact that I did not match male pairs for similarity in size and colouration as was the case in experiments where females did copy one another (Dugatkin 1992, Dugatkin & Godin 1992, 1993). If this

(except in the copying treatment). The repeatability of a female's preference for one male over another is the maximum to which a female preference for any particular male trait may be heritable, because it is unlikely that, even in the simplest mate choice systems, one factor (the cue) alone is the only determinant of a female's response to a male. The repeatabilities obtained in this study are higher than the non-significant values for preferences for specific ornaments in flour beetles ($r = 0.0$, Boake 1989) and barn swallows ($r = 0.152$-0.241, Balbura 1992), but not as great as those obtained in sticklebacks ($r = 0.65$, Bakker 1993) and, interestingly, another study of barn swallows ($r = 0.57$, Møller 1994 b). The repeatability measures that I obtained in this study are lower than the repeatability of (Quaré river, Trinidad) female guppies' preference for brighter males (0.577 ± 0.107) reported by Godin & Dugatkin (1995), but not significantly so (compared with the 2-day trial: $Z = 0.125$, $\sigma = 2.32$, 2-tailed $p = 0.216$) except for the copying treatment ($Z = 0.259$, $\sigma = 2.32$, 2-tailed $p = 0.011$). Therefore in this population, as in others, female choice behaviour shows significant repeatability, a result necessary but not sufficient to claim that additive genetic variation for female mate preferences is present in the population.

Repeatability of mate choice behaviour is reduced when a female observes another female contradicting her mate choice decision. The negative effect on repeatibility that was seen in the copying treatment is not necessarily the same as the effect expected in a wild situation. This is because, if females in a population share similar preferences, copying is more likely to corroborate a female's independent decision than contradict it. I therefore predict that if there is copying and it has any effect on repeatability in the wild, it increases repeatability rather than decreasing it as

Table 7.3. Correlations of the absolute value of the difference between males in each potential cue with the absolute value of the difference between preference for one (randomly chosen) of the males in the first and second parts of the treatment.

r_s
(N)
p

	2 - day	Copying	30 minute	1 - day
Body size	-0.030	0.071	-0.244	-0.483
	(30)	(30)	(19)	(19)
	> 0.1	> 0.1	> 0.1	0.02
Orange area	-0.424	-0.069	-0.054	-0.123
	(30)	(30)	(19)	(19)
	0.01	> 0.1	> 0.1	> 0.1
Orangeness	0.172	0.095	-0.037	0.217
	(34)	(34)	(28)	(28)
	> 0.1	> 0.1	> 0.1	> 0.1
Black area	-0.083	0.210	0.000	0.269
	(30)	(30)	(19)	(19)
	> 0.1	> 0.1	> 0.1	> 0.1
White area	-0.322	-0.107	-0.022	0.211
	(30)	(30)	(19)	(19)
	0.04	> 0.1	> 0.1	> 0.1
Pattern complexity	0.301	-0.262	0.043	0.153
	(30)	(30)	(19)	(19)
	0.05	> 0.05	> 0.1	> 0.1

DISCUSSION

I have shown that not only do females prefer the same male significantly more often than they reverse their choice decision, but that the variability between females in their mating behaviour is significantly greater than the within female variability

Table 7.2. Results of Mann-Whitney *U* tests in which the absolute value of the difference between males in each potential cue was compared for trials in which females reversed their choice versus those in which females chose the same male both times. None of the differences are significant after the sequential Bonferroni adjustment.

U
Z_{adj}
1- tailed p

	2 - day	Copying	30 minute	1 - day
Body size	75 (1.044) > 0.1	85 (0.316) > 0.1	47 (1.415) > 0.05	36 (2.038) 0.028
Orange area	76 (1.001) > 0.1	83 (0.406) > 0.1	61 (0.623) > 0.1	46 (1.472) > 0.05
Orangeness	100 (0.924) > 0.1	104 (0.497) > 0.1	84 (0.456) > 0.1	79.5 (0.684) > 0.1
Black area	91 (0.348) > 0.1	69 (1.04) > 0.1	68 (0.226) > 0.1	64 (0.453) > 0.1
White area	97 (0.087) > 0.1	85 (0.316) > 0.1	55 (0.963) > 0.1	59 (0.736) > 0.1
Pattern complexity	96 (0.131) > 0.1	87 (0.203) > 0.1	55 (0.963) > 0.1	62 (0.567) > 0.1

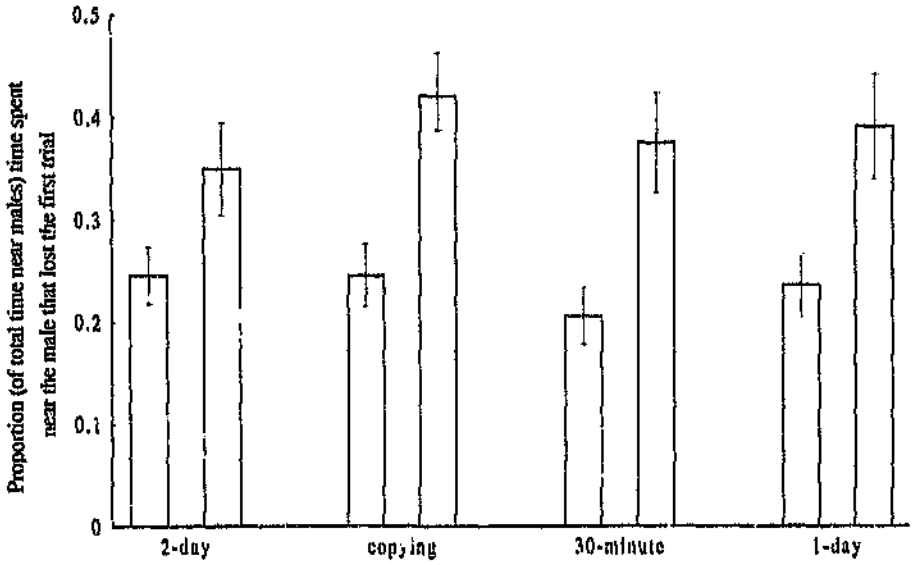


Figure 7.3. The proportion (of the total time a female spent near both males) time that a female spent with the loser of the first trial of each treatment in the first (left bar) and second (right bar) trials of that treatment. Differences are all significant (2-day: paired-sample $t = 1.893$, $d.f. = 39$, 1-tailed $p = 0.033$; copying: $t = 4.076$, $d.f. = 39$, $p = 0.000$; 30-minute: $t = 3.219$, $d.f. = 29$, $p = 0.002$; 1-day: $t = 2.566$, $d.f. = 29$, $p = 0.008$).

Males in trials where females reversed their choice decisions were neither more nor less similar to one another than in trials when females remained consistent in any of the potential cues measured (table 7.2). Similarly, the absolute value of the change in female preference between trials did not relate consistently to any of the potential cues (table 7.3).

The possibility that the model female influences the outcome of behaviour trials because females are more likely to investigate the male she was "with" first than they are the other male is refuted as the focal female went first to the side where the model female was only 16 times out of 40 ($G_{adj} = 1.161$, $d.f.=1$, $p > 0.1$).

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least) the previous male they encountered. This is probably an adaptation to allow females to mate with the best males on offer regardless of the mean levels of ornamentation in a population. There is only the most equivocal evidence that mate copying has any effect on the outcome of female choice decisions (Chapter Seven), despite robust experimental evidence for it in other populations (Dugatkin 1992, Dugatkin & Godin 1992,1993). If copying does occur in this population, it is certainly far weaker than independent choice. This gives me reason to doubt the importance of cultural transmission to the spead of female preferences in this population.

There can be little doubt that female choice is an important source of epigamic selection in this population, thereby selecting for at least some of the multiple ornaments borne by males. It appears that females are able to choose based on genetically inherited preferences that are modified by recent experience.

The above three classes of explanation are not mutually exclusive, but I have tried to contrast three very different selective phenomena. I predict that by viewing multiple male ornaments as a single pattern, the way females and predators see it, we can break new gound in learning how male ornamentation evolves. However, it is often still useful to study the different ornaments as indpendent characters as well.

INHERITANCE AND ONTOGENY OF FEMALE PREFERENCES

The second major aim of this investigation was to learn about the inheritance and ontogeny of female preferences, subjects which have not been priorltised sufficiently until recently (Rosenqvist & Berglund 1992).

The fact that naïve vigin females are able to respond to the orangeness of a single male (Chapter Six) indicates that female preferences are genetically determined, and that experience can only modify preferences if it plays any role at all. Similarly, the significant, and relatively high repeatibility of female mate choice behaviour (Chapter Seven) suggests significant additive genetic variation in female mating preferences. Only selection and cross-cultural experiments will reveal the true extent to which female preferences are heritable, but it appears that the genetic variation in preferences that is required for their biological evolution and coevolution with male ornaments (Lande 1981, Pomiankowski 1988, Grafen 1990 a,b) is present. This study does not, however, address the question of the heritability of preferences for particular male traits.

Chapter Six also reveals a role for experience in female choice. Females appear to adjust their response to males based on the degree of ornamentation of (at

"good-genes" models of sexual selection. Endler (1983, 1987) suggests that secondary male ornaments have evolved to exploit the female preference for an honest signal - orangeness in the case of guppies. This implies that females assess male ornamentation imperfectly, therefore permiting cheating, as has been shown to be theoretically plausible by Johnstone and Grafen (1992, 1993). Receiver error can have two effects on the evolution of ornamentation: it can select for amplifiers as discussed above, to reduce reciever error, or it can permit other ornaments to "hitchhike" on the original signal, by stimulating the female in similar ways. The amplifier option is intuitively more likely to be stable and widespread as it benefits the female and the fittest males, and not just the least fit males (although formal modelling is necessary to develop confidence in this assertion). Endler (1983) hints at a role for cheating in maintaining structural pigments, a possibility which can neither be refuted nor corroborated by my work. If dishonest signals have evolved to exploit preferences for honest advertisements of male quality, these signals are expected to be less prevalent than the honest signal. In wild guppy populations (Endler 1983) and this feral population (Chapter One) all males bear orange spots, but far fewer bear structural pigments.

3) **Pleiotropy** between different ornaments in male guppies is a well studied phenomenon (see Endler 1978). However, if non-preferred ornaments are present due to pleiotropic expression with the ornament of choice, there should be a strong correlation in expression between the ornament of choice and the focal ornament (Falconer 1981). There appears to be no such correlation (Chapter One and Appendix).

be assessed by a female in making her choice decision. Endler (1987) describes a tradeoff between sexual selection for contrast with the background gravel and predation-mediated natural selection to cryptically mimic that gravel background. Therefore contrast may be the important parameter when investigating the evolution of the signal.

Composite measures of male patterns have recently proved to be very successful indices of male ornamentation, and are not only good predictors of male attractiveness (Nicoletto 1993, Endler & Houde 1995), but of male vigour too (Nicoletto 1993). The significant relationship between pattern complexity and male mating success in this population (Chapter Two) indicates that there may be a real female preference for males with more complex patterns. My caveat is that complexity is (by definition) correlated with both orange and black colouration and ingenious tests are required to disentangle the effects of complexity and individual ornaments.

Constructing composite trait variables is a useful, but should not be the exclusive, way to understand the importance of ornamentation to female choice. The evidence I presented in Chapter Five that black pigmentation functions to amplify orange as a signal is evidence that two independent traits can be the result of preference for only one of them - orange. The manipulation did effect composite trait measures such as complexity and contrast, but considered apart, and manipulated independently, these traits taught us a great deal more about how signals evolve than they would have, had they been amalgamated into one composite measure.

2) **Dishonest signalling** is the traditional thorn in the side of advocates of

and black *have* evolved by this route. I have shown convincingly that orange is the object of preferences, and by showing a role for black under manipulation, probably as an amplifier rather than a signal, I have demonstrated that other ornaments *can* evolve by female choice despite an apparent lack of relationship between choice and ornament size.

Composite traits or single preferences?

In Chapter One, I argue that, because only one cue consistently predicts male attractiveness, this does not favour explanations for multiple ornaments in which several ornaments are assessed simultaneously. These explanations include the "multiple messages" and "unreliable signals" hypotheses, as well as the "overall brightness" and "dishonest signals" hypotheses (Endler 1983, 1987). So far in this discussion, I have treated male secondary sexual characters as distinct characters under separate genetic and ontogenetic control and with distinct selection histories. This assumes multiple female preferences have been responsible for multiple male ornaments, a mini-paradigm taken to its logical endpoint by Tomlinson and O'Donald (1989) in their model in which preferences and ornaments evolve in a "gene-for-a-gene" fashion. However, there is a group of explanations which do not require multiple preferences for multiple ornaments to evolve. It is possible that a single female preference can lead to the evolution of what we classify as several male characters, and it is almost certain that black and orange at least are favoured by one female preference.

1) **Brightness, contrast or complexity** of the males entire colour pattern may

The Fisher process

Theoretical biologists have made several positive strides toward understanding how female preferences for multiple male criteria can evolve. Pomiankowski and Iwasa (1993) demonstrated that multiple preferences can evolve by Fisher's (1930) classical self-reinforcing runaway process of sexual selection if the costs of choosing according to several criteria do not substantially exceed those associated with exerting a single preference. Iwasa and Pomiankowski (1994) then showed that multiple preferences for handicapping traits are theoretically implausible, but that it is quite possible for one handicap preference to evolve in tandem with several, relatively uncostly, Fisherian preferences. The predictions of both of these theoretical studies are consistent with the results of this investigation. It is difficult at the best of times to discriminate empirically between Fisherian and "good-genes" models, and I can build no strong argument regarding the relative strengths of these two processes. However, of the ornaments borne by male guppies, the strongest case for a signal of male quality can be made for orange colouration (Kodric-Brown 1989, Houde & Torio 1992, Nicoletto 1993, but see Reynolds et al. 1993 who argue for body size). If orange is the single, strong "handicap" preference, it is quite possible that other characters are maintained by weak Fisherian preferences in the fashion suggested by Iwasa and Pomiankowski (1994). However, the preferences for them would have to be so weak as not to have been detected in the various correlative chapters (one, two, six and seven) of this thesis.

Although I am discussing male ornaments as though they could all evolve due in some part to female choice, I am not arguing that any characters other than orange

of secondary sexual characters other than the correlations that are due to allometry. If apparently non-preferred characters are not sexually selected in some way, the extent to which they are influenced by the same genes is expected to be high because any other genes for non-preferred characters should be eliminated by natural selection. In such a case, the correlation between the two characters within individuals should be high (Falconer 1989).

Appendix

Correlations between morphological variables

	Body area	orange area	relative orange	black	relative black	yellow	iridescence	gonopodium
orange	0.556 (59) 0.000*							
relative orange	0.139 (59) n.s.							
black	0.546 (59) 0.000*	0.333 (59) 0.005	0.142 (59) n.s.					
relative black	0.024 (59) n.s.	0.007 (59) n.s.	0.038 (59) n.s.					
yellow	-0.154 (59) n.s.	-0.300 (59) n.s.	-0.271 (59) n.s.	0.167 (59) n.s.	0.237 (59) n.s.			
iridescence	-0.025 (59) n.s.	-0.126 (59) n.s	-0.146 (59) n.s.	-0.147 (59) n.s.	-0.168 (59) n.s.	-0.094 (59) n.s.		
gonopodium	0.726 (23) 0.000*	0.518 (23) 0.006	0.326 (23) n.s.	0.134 (23) n.s.	-0.151 (23) n.s.	-0.069 (23) n.s.	-0.304 (23) n.s.	
dorsal fin	0.260 (41) 0.050	0.376 (41) 0.008	0.331 (41) 0.017	0.134 (41) n.s.	-0.069 (41) n.s.	0.560 (11) n.s.	0.123 (41) n.s.	0.167 (41) n.s.

Pearson *r*
(*n*)
1-tailed P

* P<0.05 after sequential Bonferroni adjustment

This data is referred to in Chapter 1 as weak evidence that there is no pleitropic expression

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