Sexual Selection:

Symmetry, Inbreeding, and Mate Choice in Trinidadian guppies

Letitia Anne Devoy Sheridan

University College London

Abstract

This thesis investigates whether asymmetry has a role in sexual selection in guppies. This is potentially interesting because it might illuminate whether the cues used in female mate choice correlate with male quality (the "good genes" hypothesis) or purely "aesthetic" choice. Guppies are well suited to investigations of mate choice as males have prominent sexual pigments which females use in mate choice decisions. Theory predicts that the symmetry of secondary sexual characters could indicate the quality of the male, as it is widely thought that fluctuating asymmetry (FA) is a sensitive indicator of developmental stress.

Sexual coloration in guppies was found to be sensitive to genetic stress caused by-inbreeding. Several traits showed indications of inbreeding depression. Pigment areas and numbers of spots and colours all decreased with inbreeding. The response of different pigments was highly correlated with their importance to females in each population. Display rate and swimming performance also showed signs of inbreeding depression. The evidence on these traits is consistent with the “good genes” hypothesis. But there was no difference in pigment FA between inbred and control fish. This provides no evidence that genetic stress is reflected particularly well by FA.

Females did not prefer outbred males, but preferred high display frequency above other cues. However, if display rate was controlled, females of both populations preferred males with symmetrical patterns when given choices between males differing in spot symmetry - but this is unusual FA as this concerned unpaired characters. Spot asymmetry showed no response to inbreeding and thus was a poor indicator of male condition. This cue appears to support the "aesthetic choice" hypothesis.

Overall, this evidence suggests that most (but not all) traits preferred by females are good indicators of male quality. There was no evidence that any form of symmetry correlated with male condition.

Table of illustrations

Chapter 1: Introduction

OBJECTIVE:

To investigate whether symmetry has a role in sexual selection in guppies. This is potentially interesting because it might help to illuminate whether female mate choice is related to male genetic quality. This could help to assess the relative importance of "Fisherian" and "good genes" sexual selection theories in the evolution of female choice in the guppy (*Poecilia reticulata*).

Genetic stress was induced by inbreeding guppies, allowing assessment of several preferred male traits for correlations with vigour, in males of differing genetic backgrounds. Particular reference was made to symmetry in the measurement of these sexually-selected traits, in order to test the claim that fluctuating asymmetry (FA) is a highly sensitive indicator of genetic stress. The relationships of symmetry and trait size with inbreeding, display, and swimming endurance were analysed for evidence on this hypothesis.

1.1 Sexual selection theory

Darwin's theory of evolution identified two forms of selection that occur in the wild: natural selection, involving the adaptation of a population to its environment, and sexual selection, resulting from competition for reproductive access to the other sex (1859,1871). Darwin developed the concept of sexual selection to account for the presence of exaggerated traits in animals (such as the spectacular plumage of male peafowl or the antlers of male deer) which cannot provide a survival advantage and thus seem incompatible with natural selection.

Differential reproductive success arises because of competition for mates (usually by males) and choice of mating partners (usually females selecting males) - these differences in success and thus fitness cause selection on organisms just as powerful as the other forces of natural selection (e.g. predation). It should be stressed that sexual selection is just the component of natural selection relating to mating success. This has resulted in the evolution of a huge variety of secondary sexual characters exaggerated to a degree that lowers fitness in contexts other than mating. Male competition has led to the evolution of sexual dimorphisms such as large male size, adornments for increasing apparent size to intimidate rivals, and a huge variety of weapons used for fighting. Female choice appears to explain the evolution of conspicuous colours and displays used by males in courtship.

The evolution of male weapons has never been controversial - their function and advantage is usually self-evident. Female choice on the other hand was largely neglected by biologists for nearly a century following Darwin's work and has since been the subject of fierce debate. Initially the debate focused on whether female choice could possibly exist at all. Wallace (1870) disagreed with Darwin and believed that female choice had nothing to do with the evolution of outlandish male traits. More recently (since evidence of female choice in the natural world became incontrovertible - see section 1.2) there has been a major resurgence of work on this aspect of sexual selection. The adaptive reasons for female choice are not well understood. There are cases in which a female may receive direct benefits through mate choice (e.g. a territory, male parental care, courtship feeding, avoidance of diseased males). For example, the condition of the sexual trait may reflect the parenting ability of the male (Heywood 1989;

Hoelzer 1989) or infection by ectoparasites that can be transmitted to the female or her offspring (Freeland 1976). The selective forces driving the evolution of choice with such benefits are obvious - females which can choose males with better parenting ability or territory or without transmittable parasites will benefit directly. Mate choice which simply ensures mating with a member of the correct species also has obvious benefit, as females choosing males of the wrong species are likely to have sterile or inviable offspring. However, when females get no resources by mate choice among conspecifics, then explanations are far less obvious. This is a common situation among polygynous species (though not limited to them), most obviously when males gather at display grounds, or leks. Females appear to gain nothing from the male except sperm (found in a huge range of species from peafowl to guppies to frogs) Female choice is still a powerful force in these situations judging by the elaborate ornaments and behaviours males produce. This prompts investigation of the reasons behind the evolution of female choice in species where the male contributes little or nothing more than sex cells. This is where the controversial issues of female choice lie.

This discussion on female choice centres on a lively debate concerning two main classes of mechanisms for the evolution of choice. The most contentious issue is whether male traits are simply attractive or act as indicators of male vigour (Pomiankowski 1988). The "good genes" theories postulate that selection drives females to evolve mate choice for the best adapted genotypes, and the "non-adaptive" theories suggest that preferences cause male traits to evolve in ways that are not adaptive for their ecological environment.

1.1.1 Fisher

Fisher's model of sexual selection suggests that secondary sexual characters evolve as a result of the coevolution between mating preference and the sexual character (Fisher 1930; Lande 1981; Pomiankowski, Iwasa &Nee, 1991). The Fisherian theory of sexual selection centres on the premise that male sexual traits are simply attractive (and have no other function). An exaggerated male sexual trait that has evolved by the Fisher process has no correlation with male vigour. The trait may have an initial correlation with a survival advantage, but this can become uncoupled by the action of the "runaway process". A "Fisherian" sexual trait is essentially non-adaptive with respect to survival, but confers a mating advantage. Females choose a signal that may reduce viability, and that is not correlated with other components of viability.

Fisher first proposed the runaway theory of sexual selection in 1930 (though also mentioned in Fisher 1915). The runaway theory proposes that initially a female genetic preference arises by chance that discriminates differences in a male genetic trait that is correlated with selective advantage. For Fisherian sexual selection the male trait must initially be correlated with a selective advantage, as a necessary initial condition for the evolution of sexual characters that are not so correlated.

Once such a preference has arisen, choosy females benefit because their offspring will not only have a slight survival advantage, but their sons will also enjoy a mating advantage. Sons will not only inherit the genes for the preferred trait, but also the genes for the preference which will be passed on to their daughters, thus causing an increase in frequency of the preference genes. This genetic covariance or linkage disequilibrium is an important consequence of

Fisher's model - resulting in the coevolution of the mating preference and the sexual trait. The balancing effect of natural selection on ornamented males can cause correlated evolution of the female preference (a good illustration of this is Houde & Endler's (1990) study on guppies, in which females from high predation rivers preferred the drab males of their own population to the brighter males of low predation rivers and vice-versa - evidence of coevolution). Both preference and trait can spread through the population by the runaway process - once the preference is established the link between male trait and viability is no longer necessary to drive up female preference. Choosy females still benefit from choice of males with exaggerated sexual traits because their "sexy" sons have a mating advantage and their daughters inherit the preference. The joint evolution of the preference and the preferred trait produces the "runaway process", increasing rapidly with time, and selects the sexual trait to become exaggerated to the extent that fitness can be lowered in contexts other than mating.

Female preference selects for males that are different from those that natural selection favours. There is conflict between the forces of natural and sexual selection acting on males, causing them to evolve to a compromise state that is a trade-off between them. The compromise state is maladaptive with respect to what natural selection alone would produce (as the drab camouflage of most female birds whose mates are lurid illustrates). The runaway process can proceed until balanced by natural selection; in Fisher's words, "until checked by severe counter-selection in favour of less ornamented males"(Fisher 1930). In other words, the perfect mating advantage is useless if the owner can't stay alive long enough to use it. Conversely, the most viable of all males will fail to pass on any genes if all the other males are sporting the ornament females prefer. This illustrates the disadvantage of using the term “fitness” when discussing this issue - by definition when a female chooses a male as a mate she increases his fitness, so it is circular to ask whether she chooses the most fit male. Terms such as viability, survivorship and mating success are much clearer in this context.

Fisher’s model for the evolution of female mating preferences and male display characters was verbal. More recently formal mathematical versions of the model (e.g. O’Donald 1962, 1967, 1980; Lande 1981; Kirkpatrick 1982) have established that the self-reinforcing process can work.

1.1.2 Good genes

The good genes school of thought suggests that the signals females choose are connected with viability in some way. Confusion often arises due to Fisher’s comments that an *initial* correlation of the preferred male sexual trait and viability is necessary for the runaway process to *begin* - this is *not* the same as the “good genes” hypothesis. A “Fisher” trait is *not* correlated with viability (but may have been in the past). A “good genes” trait *is* correlated with overall viability.

The most widely discussed mechanism for the good genes hypotheses was first proposed by Zahavi (1975) - the “handicap” principle. There is much evidence that male sexual traits decrease survival (see section 1.3) - by their very nature, conspicuous signals increase predation risk, and large ornaments reduce the ability to flee effectively. Zahavi argued that bright males (or long-tailed, or whatever the exaggerated trait might be) are usually also vigorous (high viability) with respect to other characters, otherwise they would not have survived to maturity. The effect of the male trait decreasing survival is an important part of this (rather than a side-effect, in Fisherian traits). Only high quality males can afford to produce a trait that handicaps them and survive long enough to breed. If a low quality male can easily survive with the handicap, then the trait provides females with no information about male viability. Therefore genuine handicap traits are "uncheatable" signals. Females are selected to mate with the bright (or otherwise handicapped) males, because by choosing them she will also tend to be choosing other genes for high viability, which will be passed to her offspring.

There are several models of the handicap theories suggesting that secondary sexual characters are reliable signals of male quality (Zahavi 1975, 1987; Andersson 1982a, 1986; Kodric-Brown & Brown 1984; Nur & Hasson 1984; Pomiankowski 1987, 1988). If the costs of producing a larger ornament are greater for males in worse condition, then the optimum ornament expression is an increasing function of quality (Grafen 1990; Iwasa, Pomiankowski & Nee 1991). There have been different approaches: the revealing handicap (Hamilton and Zuk 1982) and the condition-dependent handicap (West-Eberhard 1979). The revealing handicap theory proposes that the handicap trait is expressed to varying degrees depending on the viability of the male and so reliably reflects parasite resistance (since only healthy individuals are able to produce the most extravagant sexual traits). The condition dependent handicap theory (also proposed by Williams 1966, Zahavi 1977 and modelled by Andersson 1986) proposes that only males above a certain threshold value of viability develop the handicap at all. These are similar arguments all detailing ways in which the expression of the male trait is in some way conditional on the overall genetic "quality" of the male. Physiological mechanisms can then cause the trait to become more exaggerated (or present) in individuals that carry good genes.

All these interpretations conclude that female preferences for these exaggerated male phenotypes receive an evolutionary advantage by becoming associated with adaptive (high viability) genotypes. This is the fundamental difference between the good genes and Fisherian theories. A female with a preference for a Fisher signal receives an evolutionary benefit solely due to her son's mating advantage and daughter's choosiness. A female with a preference for a good genes signal receives the benefit in the form of higher viability offspring.

There have been significant objections to the handicap principle. Foremost is the problem of depletion of variability - females selecting fitter males will drive the alleles for increased fitness to fixation. Population genetics theory predicts that populations close to equilibrium will have zero or very low levels of additive variance in fitness (Falconer 1960). This means that mate choice could not result in fitter offspring. However, there are several ways to overcome this objection. Fitness variation can be maintained by recurrent mutation (both deleterious and beneficial), migration and spatially varying selection pressures, and temporally varying selection pressures (Iwasa *et al.*1991). In particular, Hamilton & Zuk's parasite theory (1982) can explain consistent fitness correlations between offspring and parents by cycles of coadaptation between hosts and parasites.

There are other objections due to models in which the handicap principle could not operate (Maynard Smith 1976,77; Bell 1978; Andersson 1982a). The major difficulties involved choice being costly to the female; this caused several models to fail. These have been supplanted by models which have shown that the handicap principle (including costly female choice) can work under certain conditions, such as a directional effect of mutation and a direct relationship between viability and expression of the handicap (Iwasa & Pomiankowski 1991).

1.2 Evidence of sexual selection by female choice

1.2.1 General

The days when scientists refused to believe female choice could exist are gone. All sexual selection was once thought to be due to forms of male competition (e.g. Wallace 1870). Times have changed. It was noted that mating is usually less costly for males (in terms of parental care or even gamete size) and so it is in a female's interest to choose a good quality partner (Parker 1983). This leads to the expectation that female choice will be observed in many species for adaptive reasons. The sex which invests more reproductive effort in offspring will generally be the more choosy sex (and this is usually females). This is traditionally held to be because they have more wasted investment in a failed breeding attempt, particularly if they provide parental care; however under closer investigation it seems likely that it is actually because they have more to gain (Reynolds 1996). Also both the costs and benefits of choice for the lower-investing sex increase the more the opposite sex invests, due to higher variation in the quality of contributions to the young (Johnstone *et al* 1996). Modelling has shown that costs of choice are likely to be more important than quality variation of the higher-investing sex, so this returns to the prediction that that the less competitive sex will commonly be more choosy (and this is generally the females) (Johnstone *et al* 1996).

Experimental studies have shown directly which cues females are using in mate choice in many species. One type of strong evidence for the existence of female choice comes from experiments in which males are restrained so they cannot physically interact, and females are allowed to choose between them. Females have been shown to have consistent preferences among restrained males in ring doves (*Streptopelia risoria)* (Morris & Ericson 1971); rock doves (*Columba livia)* (Burley 1977, 1981); pupfish (*Cyprinodon variegatus)*(Itzkowitz 1978) and house mice (*Mus musculus)* (Yanai & McClearn 1972). Even stronger evidence comes from experiments in which the preferred character is abstracted and presented to females without the confounding presence of males - such as playback experiments using male calls to elicit female mating behaviour. Female brown-headed cowbirds (*Molothrus ater)* respond to the calls of dominant males with copulatory display (West *et al.* 1981); female *Physalaemus pustulosus* neotropical frogs prefer the calls of larger males (Ryan 1981) and more complex calls in the Tungara frog (Ryan 1985); Pacific tree frog females (*Hyla regilla*) prefer call leaders and their call bouts (Whitney & Krebs 1975); female preference favours the odours of dominant males in a cockroach, *Nauphoeta cinera* (Breed *et al.* 1980); female field crickets prefer males of longer calling-bout endurance (Hedrick 1986).

An increasing number of careful field studies have directly demonstrated sexual selection by female choice in natural populations. A famous and elegant example is Andersson's widowbird manipulations (1982b): by surgical enhancement or reduction of male widowbird tails, conclusive demonstration of female preference for longer-tailed males was produced. In many species for which there is no experimental evidence of mate choice, observation of courtship and mating supports the hypothesis that mate choice occurs. In territorial species it is common for females to visit several territories before choosing a mate, and males seem unable to coerce unwilling females to remain on their territory (e.g. Cottidae fish (Brown 1981); bullfrogs (Emlen 1976); black grouse (Kruijt & Hogan 1967); toads (Licht 1976) prairie warblers (Nolan 1978); green frogs (Wells 1977)). Females often show the capacity to reject mating attempts by males (e.g. *Drosophila* (Merrell 1949) wood frogs (*Rana sylvatica*) (Howard 1980) cockroaches (Breed *et al.* 1980) and beagles (Beach & LeBoeuf 1967). Mating may occur only if the female first actively solicits it (e.g. village indigobird (*Vidua chalybeata)*(Payne & Payne 1977)). Female choice has been found to occur widely in fish and other groups of animals (Kodric-Brown 1990).

1.2.2 Sexual selection in guppies

Sexual selection in guppies appears to be mediated through female choice rather than intermale competition. Male guppies have conspicuous colour patterns which are displayed to attract females; differences in these colour patterns affect mate choice by females (Endler 1983; Kodric-Brown 1985; Houde 1987, 1988a,b; Long & Houde 1989; Houde & Endler 1990). Fertilisation is internal and offspring are born live (rather than hatching from eggs as in most fish). Guppies are ovoviviparous and do not nourish their embryos after fertilisation (Thibault & Schultz 1978). Males do not defend or provide any resource, but engage almost continuously in courtship behaviour (Magurran & Nowak 1991). Most displays are ignored (Endler 1987) as females are only receptive when virgins or for short periods following the birth of a brood (Liley 1966; Houde 1988), when they may choose several males. Females may mate with many different males during their lifetime (Farr 1989). The proportion of receptive females at any one time is very low, which creates a strongly male-biased operational sex ratio. These conditions create strong sexual selection on males to display frequently (Haskins *et al.* 1961; Liley 1966; Reznik & Endler 1982). These factors all make the guppy an ideal species for the study of mate choice.

In guppies, males can use a mixture of strategies - both courtship displays which encourage female receptivity (Farr 1980a,b; Bischoff *et al.* 1985) and also direct thrusts which may circumvent female choice. (Farr 1989). Courtship display is very conspicuous to predators (Endler 1987) while the alternative mode, called gonopodial thrusting (Baerends *et al.* 1955), rape (Farr 1980b), or sneak copulation (Endler 1983) is inconspicuous both to females and predators. However these "sneaky" mating attempts carry a very low success rate for fertilisation owing to female evasiveness and the likelihood of sperm being completely displaced by a subsequent full copulation (Clark & Aronson 1951; Liley 1966; Grove 1980). This is a poor option in terms of reproductive success for the male. Obviously it is in the female's interests to avoid forced copulation with a male that does not display the preferred traits (Houde 1988; Houde & Endler 1990). Most thrusts do not result in contact as females can usually control whom they mate with (though there is evidence that a small proportion of sneak attempts that contact the female also result in insemination (Liley 1966; Farr 1980a)). Males that are chosen by females for full copulations obtain far greater paternity.

Female choice has been demonstrated to be more important than male competition in guppies (Farr 1980a; Houde 1987). Under natural sex ratios and densities (both in the wild and in the laboratory) fights or other forms of dominance are rare in most guppy populations (Reynolds *et al.* 1993). There is little evidence of intermale competition in nature (Luyten & Liley 1991) or in an artificial laboratory stream (Crow 1981). Although several studies have documented male aggression in the laboratory (Farr 1976, 1980a,b; Gorlick 1976; Martin & Hengstebeck 1981) these have all used domestic fish rather than wild ones. However some male-male competition has been observed in laboratory stocks (Liley 1966; Ballin 1973; Houde 1988b) descended from the wild. Such behaviour is mostly seen at high population densities (Warren 1973; Farr & Herrenkind 1974). The enclosed conditions of laboratory tanks appear to encourage intermale competition - more natural stream conditions (even artificial ones) offer males little opportunity to benefit from aggression. They cannot monopolise females or resources that females need under these conditions by driving away other males, and this is assumed to be the reason why intermale competition is so unimportant in the natural guppy mating system (unlike most other Poeciliids) (Farr 1980a). On the other hand, male courtship display is common both in nature and the laboratory (as many as 2.6 displays per minute per male in some natural populations - Farr 1975) (e.g. Baerends *et al.* 1955; Liley 1966; Farr 1980a, 1983, Endler 1980, 1983).

It should be noted that Magurran & Seghers (1991) found high levels of aggression in first and second generation laboratory guppies from several populations (and also observed it in the wild). However in the laboratory these were hungry single-sex groups of strangers given access to profitable and defendable food patches - all of these factors could increase aggression, and females showed the same levels of aggression as males (also in Houde 1988b). In addition, when heterosexual agonistic encounters occurred, females emerged as the dominant sex (also Schröder 1983). This all suggests male competition for reproductive success may be independent of aggression for a feeding site. Males display far more vigorously when in the presence of other males (Farr 1976); though even when alone with females, male display rate compared to female response rate is extremely high. This element of male competition arises as a response to the female preference for the most actively displaying males (Farr 1980a). Intra-male competition to inseminate females, in terms of competing display rates (rather than direct fighting), can be intense (particularly in domestic guppies kept at unnatural densities).

However, males of wild populations have been shown to prefer approaching females not already attended by a courting male (Houde 1988b), and display once or twice to most females before moving on to court another female. Occasionally a male attempts to court a female already attended by a male, and the intruding male is usually "fended off" by the original male darting between them. One male (almost always the intruder) leaves the other male to court the female, sometimes after a short attempt to jockey for position. The intruder is virtually never directly attacked or threatened, and if both males persist in courting, the female almost always swims away. Males appear to be attempting to remain closest to the female rather than direct confrontation with the other male (Houde 1988b).

For one of the populations studied in this thesis, the Lower Aripo, Houde (1988b) found that male aggression during courtship was relatively uncommon and did not explain mating success. For all populations, the lack of observations of aggressive intermale competition in the wild has led to widespread conclusion that female choice is more important in the guppy mating system (Luyten & Liley 1991). Accordingly interest has centred on the role of female choice, or epigamic, sexual selection in the evolution of sexual display and colour pattern in the male guppy.

1.3 Evidence of male traits being maladaptive

The oldest argument that natural and sexual selection commonly oppose one another follows the logic of Darwin, who first suggested the concept of sexual selection to account for traits that appeared to be inconsistent with "survival of the fittest". Darwin built his case on evidence from comparative data. There are observations that sexual display traits are restricted typically to one sex, not expressed in juveniles, and behaviours and often physical display traits only appear (or are much enhanced) during the mating season. All of these suggest that these traits are not advantageous under natural selection alone. Later observations have all added to this evidence (e.g Eberhard 1985, Payne 1984). Particularly interesting are the cases of female-limited mimicry in butterflies - because this suggests female mate choice prevents males from evolving adaptive mimetic colour patterns (Belt 1874, Turner 1978).

Several studies have directly demonstrated that natural and sexual selection are opposed. The most dramatic and obvious examples concern male displays attracting not only females but also predators. This is the case in guppies. Male guppies contrast visually with their environment and are more likely to be eaten by visually hunting predators (but brighter males are preferred by females) (Endler 1980, Endler 1983, Haskins *et al* 1961). Bright male sticklebacks (which females prefer) are also more likely to be eaten (Moodie 1972, Semler 1971). Experiments using playbacks of the calls of male tungara frogs have demonstrated that a predatory bat uses the same components of their calls to locate the males that female frogs use (Ryan 1985). Similarly, the luminescent flashes that male fireflies use to attract females are used to the advantage of predaceous fireflies of another species (Lloyd & Wing, 1983). Experimental manipulation of the tail feathers of scarlet-tufted malachite sunbirds (*Nectarinia johnstoni*), which are used to attract females, has shown that long tails severely disrupt flying and decrease the efficiency of catching aerial insects (Evans & Thomas 1992). A similar effect has been reported after manipulation of barn swallows (*Hirundo rustica*) (Møller 1989). Measures of the energetics of courtship, both in frogs (Ryan 1988) and sage grouse (Vehrencamp *et al.* 1989) show that display is the most costly act a male performs.

In guppies, when predators are present or at high light levels when predation risk is greatest, courtship display decreases (Luyten & Liley 1985; Endler 1987; Magurran & Seghers 1990). Males show sensitivity to predation risk in their courtship - larger and more conspicuous males display less under high light intensity (Reynolds *et al.* 1993). Male display attracts predators as well as females and so is a very costly act. Much field and laboratory data indicates that the patterns of male guppies have evolved to a compromise state as a result of the trade-off between natural selection (by predators for crypsis) and sexual selection (by females favouring conspicuous coloration) (Haskins *et al.* 1961; Endler 1978, 1980, 1983, 1987; Reznik & Endler 1982). Males cannot have colour patterns which are too conspicuous, or they will be consumed by predators; but they cannot be too inconspicuous, or females will choose other males (Fisher 1930; Haskins *et al.* 1961; Gandolfi 1972; Greene 1972; Farr & Herrenkind 1974; Farr 1976, 1977; Gorlick 1976; Endler 1978). Male body size is also subject to conflicting selective forces - in a population with high predation on adults, females prefer larger males (Reynolds & Gross 1992). Strong evidence that colour patterns make the male vulnerable to predation is provided by studies which show that in the absence of predation, male conspicuousness and tail length increases (Endler 1978, 1983).

This interpretation of the guppy situation is further supported by the observations that male coloration varies from population to population depending on levels of visual predation *and* female choice criteria. Male guppies from low visual predation environments are bright, while males from high predation environments are comparatively dull; corresponding female preferences are heritable and population specific (Houde & Endler 1990; Houde 1988a; Breden & Stoner 1987; Stoner & Breden 1988). Females who prefer males significantly brighter than the population mean will tend to have sons that are too conspicuous to avoid predation - therefore predation pressure selects indirectly to balance female preference. Estimating the copulation success of competing males by labelling their sperm with radioisotopes, Luyten & Liley (1991) found that males had highest success with females from their own population. Population-specific female preferences remained after several laboratory-bred generations, making it likely that they had a genetic basis (Houde & Endler 1990). Joint genetic evolution of female preference and male ornamentation could have occurred by

Fisherian, good genes or other processes and this evidence does not support any one theory (Pomiankowski 1988).

The finding that there is parallel variation in male traits and female preferences in different environments may be evidence of genetic covariance. Covariance between female preference and preferred male trait in different populations has been found in several species (guppies, bushcrickets, corn borers, turnip moths, fruitflies, cricket frogs and house finches - see review in Bakker & Pomiankowski 1995). Guppy population studies have provided some of the strongest evidence for joint evolution of female preference and male trait. A prerequisite of sexual selection models is that genes for sexiness and choosiness become genetically correlated. Non-random mate-choice should cause genes that influence expression of a male ornament to be passed to offspring that also have genes regulating female preference, creating co-inheritance. Both runaway and good genes models require strong genetic correlations for coevolution of trait and preference. Selection experiments on male ornaments alone should give correlated changes in female preference if such coevolution exists. Studies have shown this effect in stalk-eyed flies (Wilkinson & Reillo 1994), sticklebacks (Bakker 1993) and guppies (Houde, 1994); though a similar selection experiment in guppies (Breden & Hornaday 1994) did not find this. Differences in technique (such as removal of female choice, weaker population preferences and several generations of laboratory conditions) may explain why the latter study did not find the expected correlated change in female preferences (Pomiankowski & Sheridan 1994a, b). Houde's (1994) study used Paria guppies which show very strong female preferences and very bright males as they come from a very low visual predation river. Breden & Hornaday's (1994) study used Lower Aripo guppies from a very high predation river - these males are comparatively dull and females prefer much less bright males. This may explain why no response was found in their selection experiments. These two contrasting populations of guppies are the subjects of the experiments in this thesis.

1.4 The importance of symmetry

The reason why fluctuating asymmetry (FA) is attractive as a tool for sexual selection work is the fact that the ideal state is known: the perfect individual is perfectly symmetrical. The ideal is rarely known for other measurements. FA provides an appealing measure of "developmental noise" or minor environmentally induced departures from some ideal development programme, due to this *a priori* knowledge of the ideal.

Fluctuating asymmetry (FA) is the term used for the small random deviations from symmetry in paired traits. FA is one of three kinds of asymmetry found in bilateral traits. The other two are antisymmetry and directional asymmetry. Antisymmetry refers to distributions in which symmetrical individuals are rare (or absent) but the asymmetries show no directional bias (e.g. the giant signalling claws of male fiddler crabs, *Uca lactea*, where either left or right claws are enlarged). Traits with directional asymmetry show a mean strongly biased to the left or right - one side is consistently larger (e.g. ear positioning in owls (Strigidae); bird beaks (*Loxia* spp., *Anarhyncus* spp.); handedness in humans, bird testes, human testes). Some of these other kinds of asymmetry have been shown to be advantageous (e.g. the examples in fiddler crab claws, owl ears and bird beaks - Leary & Allendorf 1989). Antisymmetry and directional asymmetry will not be mentioned again (though statistical tests were used to check that they were not present in these guppy studies).

Fluctuating asymmetry is defined as departures from perfect bilateral symmetry which are random with respect to side and normally distributed (Ludwig 1932). This applies to traits in which the normal state is symmetry and there is no tendency for one side to be larger (Van Valen 1962; Palmer & Strobeck 1986; Leary & Allendorf 1989; Parsons 1990). The frequency distribution of left-minus-right character values shows a normal distribution around a mean of zero (Palmer & Strobeck 1986).

The reason why fluctuating asymmetry is interesting is the possibility that it may be a measure of stress during development. Because both sides of a bilaterally symmetrical animal are a product of the same genome, random deviations between left and right indicate how accurately that genome is able to control the process of development. FA results when development is not identical on both sides of the body, and this inability to achieve perfect symmetry is a measure of developmental homeostasis.

1.4.1 Environmental stress

Many different environmental stressors during development have been shown to cause elevated FA in morphological traits. These include various chemicals such as pesticides (fish *(Leuresthes),* Valentine & Soulé 1973; *Drosophila,* Hoffmann & Parsons 1989a,b); food deficiency (quality or quantity)*(Drosophila,* Parsons 1990); audiogenic stress (rats, Sciulli *et al.* 1979); and extreme temperatures *(Drosophila melanogaster,* Parsons 1962; *Mus,* Siegel

& Doyle 1975; rats, Sciulli *et al.* 1979; 2 species of *Lacerta* lizards, Zakharov 1989).

FA as a good indicator of environmental stress can be most clearly seen in the field in ecologically marginal situations (Parsons 1992). Individuals showed increased FA at higher altitudes (*Coenonympha tullia* butterflies, Soulé & Baker 1968); the extremes of species ranges (*Lacerta agilis* lizards, Zakharov 1989); and in recently introduced species in a new habitat (*Gasterosteus aculeatus* three-spined sticklebacks, Zakharov 1989). Severe nutrient deprivation of pregnant mothers causes bristle number asymmetry in *Drosophila* offspring (Parsons 1990) and skeletal asymmetries in rat offspring (Sciulli *et al.* 1979). It is not known whether any of these factors have different effects on FA in sexually selected characters as opposed to other traits (Møller & Pomiankowski 1993).

Pathogens and parasites have also been demonstrated to reduce symmetry in paired traits. Møller's 1992(a) study demonstrates a differential response of FA to stress in sexual and non-sexual characters in barn swallows. Ectoparasite load manipulation was followed by changes in the following year's feather growth. Male tail ornaments became more asymmetrical compared to the homologous female trait after experimentally increased mite load preceding the previous moult. Other comparable non-sexual feather traits show no effect on FA and no difference between the sexes. In humans, many diseases are known to cause distortion of an otherwise symmetrical appearance (e.g. Bailit *et al.* 1970; Livshits & Kobylianski 1991).

1.4.2 Genetic stress

Inbreeding has been associated with increased FA. This could either be due to exposure of recessive deleterious alleles or increased levels of homozygosity (Lerner 1954). This has been observed in *Mus, Drosophila*, bivalves and other animal and plant species both in the laboratory and in natural populations. Homozygous individuals have been shown to be less developmentally stable than their heterozygous counterparts in several studies (Leary, Allendorf & Knudsen 1983, 1984, 1985a; Clarke & MacKenzie 1987;Clarke *et al.* 1986) and many studies have also shown this at the population level (Lerner 1954; Soulé 1979; Kat 1982; Vrijenhoek & Lerman 1982; Biémont 1983; Leary, Allendorf & Knudsen 1985b; Livshits & Kobyliansky 1991; review in Mitton & Grant 1984). Individuals heterozygous for one or more proteins generally develop less FA and have higher fitness in terms of growth, fecundity and physiological performance (Mitton & Grant 1984; Palmer & Strobeck 1986). In other words, for reasons not yet clear, increased heterozygosity appears to provide increased "buffering" against environmentally disturbed development (Lerner 1954).

However, there have been several cases of hybrids showing higher FA than either parental species, despite their increased heterozygosity. This is seen in hybrids between species or populations that are normally reproductively isolated (Tebb &Thoday 1958; Zakharov 1981; Graham & Felley 1985; Leary, Allendorf & Knudsen 1985b). A natural experiment where habitat disturbance has brought two previously isolated populations of sunfish into contact has shown that FA increased in the hybrids (Graham & Felley 1985). In contrast, crosses between inbred stocks usually yield hybrids with lower FA (Mather 1953; Thoday 1955;

Reeve 1960). Offspring arising from crosses between strains show much lower FA than inbred, highly homozygous and relatively unfit mice (Leamy 1984).

The data on hybrids do not conflict with the general pattern of the degree of FA being negatively correlated with fitness. Inbreeding increases the amount of homozygosity and so exposes more deleterious recessives. Outbreeding protects against rare deleterious recessive alleles and so may raise fitness if there is a heterozygote advantage. Therefore theory predicts that hybrids from *inbred* stocks will show lower FA than parental stocks. This is found to be true. In contrast, crosses between species (or reproductively isolated populations) disrupt genomic adaptations and hence reduce fitness. Thus hybrids between previously isolated parental stocks show increased FA. Whatever canalising role heterozygosity may play within species, such canalisation appears to be disrupted by the mixing of "coadapted gene complexes" in interspecies hybrids (Dobzhansky 1970).

One study neatly illustrates the importance of those coadapted gene complexes. Clarke & McKenzie's (1987) study on the Australian sheep blowfly *Lucilia cuprina* showed differences in FA associated with adaptation over time - long-established insect resistance genes showed low associated FA, though when resistance genes first appeared they showed high associated FA. This effect was proved to be due to a general adaptive shift in the genome rather than changes in the resistance gene itself by backcrossing - the resistance gene in susceptible flies (without its background of coadapted genes) caused very highly elevated FA. The fitness increase associated with resistance was initially coupled to increased FA because this major mutation had disruptive effects on development.

Subsequent selection on resistant flies has ameliorated this developmental instability.

Major genomic changes can cause elevated FA; in addition to the previous examples of major mutation, inbreeding and hybridisation there have also been examples of increased FA due to responses to directional selection (*Drosophila,* Thoday 1958) and upsetting the balance between chromosomes (human Down's syndrome Townsend 1983).

Despite this wealth of correlational evidence, uncertainty still surrounds the suggestion that FA increases with inbreeding and the degree of homozygosity. Carefully controlled and well-replicated experiments have not shown an association of FA with inbreeding (Clarke *et al.* 1992; Fowler & Whitlock 1994) using known levels of inbreeding and large numbers of lines. Other studies have also found no correlation (Houle 1989; Leary *et al.* 1984; Kieser & Groeneveld 1991; Whitlock 1993). The previous correlational evidence may be misleading due to technique - sampling a few allozymes may not give a realistic measure of genomic heterozygosity. This is a problem common to many analyses of enzyme-associated variability. Predictions of total heterozygosity based on measures at a handful of loci will be unreliable for species which are typically outbred (Chakraborty 1987). Fowler & Whitlock (1994) avoided these problems by manipulating stocks of known relatedness, so there was no need for problematic estimation of heterozygosity from isozyme analysis. They found no increase in FA in inbred *Drosophila* and no evidence of heritability of FA. The single other study of this type also found no correlation between degree of inbreeding and FA (Clark *et al.* 1992). It is likely that other data showing negative results has gone unreported. The conclusion is that heterozygosity does not necessarily allow a more stable development.

1.4.3 Symmetry and fitness

Several studies have shown a correlation between natural levels of asymmetry and components of fitness. Symmetric males have been shown to have advantages in mating (Thornhill 1992a,b,c; Radesäter & Halldórsdóttir 1993; Liggett *et al* 1993; Markow & Ricker 1992), fighting (Thornhill 1992a; Radesäter & Halldórsdóttir 1993) and survival (Thornhill 1992c; Packer & Pusey 1993). A survival advantage for females was also found (Thornhill 1992c) but in lions (*Panthera leo*) females with symmetric muzzle spots die younger (Packer & Pusey 1993). Symmetry in insects has been shown to correlate with male condition (earwigs, Radesäter, & Halldórsdóttir 1993) and in scorpionflies these fitness advantages were heritable in terms of fighting and mating success (Thornhill & Sauer 1992). This suggests that symmetric males of these species are better foragers or more resistant to environmental stress. However, in the barn swallow, females with symmetric tail ornaments did not show increased reproductive success despite arriving in breeding habitat earlier and laying eggs earlier (Møller 1994a). Nonetheless, other advantages have been shown for symmetric barn swallows - asymmetric individuals (natural and manipulated) showed decreased manoeuvrability (Møller 1991) as predicted by aerodynamic modelling, particularly in longer tails (Balmford & Thomas 1992). Male swallows with higher mating success, reproductive success and survival consistently had lower FA (Møller 1994b). A substantial body of evidence shows that FA in male sexual traits is indicative of condition and also is used by females in their mate choice (Møller 1992a,1994; Swaddle & Cuthill 1994a,b). It has also been suggested that damage due to wear or injury (causing asymmetry) is more likely to be shown by lower-quality individuals (Alatalo *et al.* 1991). Certainly in the case of male weaponry, asymmetry can help conspecifics assess fighting ability (Møller 1992b). Stags are known to develop severe asymmetries in antlers in response to body wounds acquired during fights in previous years - ability to channel resources evenly during growth is impaired (Brown 1984). This illustrates the point that conspecifics may be incapable of distinguishing between developmentally caused asymmetry and asymmetry due to damage - and this may not matter. Non-developmental sources of asymmetry may also reveal quality.

The pattern of FA in ornaments is different from that of ordinary morphological traits. The absolute magnitude of FA is considerably larger in secondary sexual traits than comparable non-sexual traits (e.g. barn swallow tail ornaments Møller 1990). Average FA in male feather ornaments was five to ten times larger than homologous traits in conspecific females in sexually dimorphic species, and ornament FA was also five to ten times higher than that found in the homologous traits of both sexes in closely related monomorphic species without ornaments (Møller & Höglund 1991). Species without ornaments showed similar FA levels in tail and wing feathers. Absolute FA in ornaments, by contrast, is often large enough to be determined by the naked eye (e.g. an average of almost 5cm in the exaggerated ornaments of the quetzal (*Pharomachrus mocinno*) Møller & Höglund 1991). One study has also shown that FA is a more sensitive measure of stress than other measures (such as trait size) (Møller 1992a).

The distribution of FA in ornaments may often be different to that of non-sexual traits. Typical non-sexually selected traits show a U-shaped distribution (may be flat or positive, often due to sampling missing the extremes of the distribution) when FA is plotted against trait size. Conversely, many ornaments show a negative relation between FA and trait size (Soule 1982; Moller & Hoglund 1991). When sexual traits reliably reflect male condition, individuals that produce the largest traits are likely to have the lowest FA. If the trait reflects condition, a given level of ornamentation is more costly to low than high quality individuals. Therefore, a condition-dependent (good genes) trait will show a negative correlation between trait size and FA, while a dishonest signal (Fisherian trait) will show a U shaped distribution. The negative relationship between fluctuating asymmetry and absolute ornament size is common (particularly in species with a single ornament, whereas in species with multiple ornaments one trait typically shows this distribution while the others do not) (Møller & Pomiankowski 1993). This suggests that many ornaments may reliably reflect individual quality, in support of the good genes hypotheses. This prompts investigation of the distribution of FA and trait size in the guppies studied in this thesis.

However the idea of FA as the answer to sexual selection work should be treated with caution. (See the evidence against at the end of section 1.4.2). The use of FA may shed useful light on the questions of condition-dependence in ornaments, but it should be borne in mind that different species and different traits within species may respond in different ways.

1.5 Preferred traits in guppies

The guppy is one of the most thoroughly studied species as regards sexual and natural selection. Much has been documented on their biology, ecology, behaviour and genetics (reviewed in Endler 1978, 1983). The guppy was the first organism in which a Y-linked inheritance was demonstrated (Schmidt 1920). The species was then still called by its old name *Lebistes reticulatus* (now *Poecilia reticulata).* Dozens of colour genes in domestic guppies were demonstrated in the following decades (Haskins *et al.* 1961), mostly X or Y linked. There are established protocols for measuring female preferences and male display which have been widely used (e.g. Kodric-Brown 1985, 1989; Houde 1987, 1988a). This knowledge base, along with ease of laboratory culture, makes the guppy an ideal organism for the study of female choice.

1.5.1 What do females like?

Haskins *et al.* (1961) suggested that wild female guppies prefer brighter males over those with less conspicuous colour patterns. The major problem with interpretation is the fact that fish which may seem most conspicuous against a laboratory background may actually be quite cryptic in their natural environment. Careful experimentation subsequently showed that females favour males with colour patches which are larger or smaller than those of their natural background, or have colours which are rare in the background (Endler 1980, 1983).

The targets of female preference are population specific. In some populations, females have been shown to prefer males with greater numbers of carotenoid (red, orange, yellow) patches (Endler 1983; Houde 1987, 1988a,b;

Long & Houde 1989; Houde & Endler 1990; Kodric-Brown 1985); also *brighter* carotenoid patches (Kodric-Brown 1989; Endler 1980, 1983; Houde & Torio 1992; Nicoletto 1991) which may indicate male quality. Houde and Endler's (1990) study showed that across seven populations, the degree of female preference for orange was correlated with the population average orange area.

Males with more carotenoid *and* structural (blue, green, silver, iridescent) colour *area* are preferred (Endler 1983; Kodric-Brown 1985, 1989; Brooks & Caithness 1995a; Nicoletto 1993). Melanin pigments have been shown to play a part in attractiveness when other factors are controlled (Kodric-Brown 1985; Endler & Houde 1995; Brooks & Caithness 1995b). Pattern complexity (numbers and colours of spots) has also been shown to be preferred (Endler 1980, 1983; Nicoletto 1993). Females favour more total spots (Endler 1983), males with rare colour patterns (Farr 1977, 1980a) and domesticated "fancy" strains over wild strains (Kodric-Brown 1985). All these studies suggest a general preference for more conspicuous males.

Many populations have been shown to prefer particular male colours. However, in other populations no discernible preference for *any* particular pigment group can be found (Reynolds & Gross 1992 - Quaré river guppies; Houde 1988b - Lower Aripo guppies)(though in the case of Lower Aripo guppies non-random mating involving conspicuousness has been shown - see below). But colour patterns are not the only cues used by females in mate choice. Morphological and behavioural traits are also important in some populations. The shape and size of the caudal (tail) and dorsal fins are highly variable and often exaggerated (Bischoff *et al.* 1985; Nicoletto 1991) and are important in female choice (Bischoff *et al.* 1985) as is body size (Reynolds & Gross 1992) and absence of parasites (Kennedy *et al.* 1987; Houde & Torio 1992). A preference for longer gonopodia has been shown in a South African population (Brooks & Caithness 1995a). Display frequency of males has been shown to be very important for mating success (Farr 1980a, Bischoff *et al.* 1985; Nicoletto 1993) though others have found a negative correlation of display and mating success (Houde 1988b).

Farr (1980a) working with domesticated strains of guppies, could find little evidence of an effect of colour and pattern brightness on reproductive success and concluded that display rate is the major determinant of male success. Farr proposed that male coloration serves as a tie-breaker among males having similar display frequencies. However the wealth of experimental evidence of the importance of male coloration in mate choice suggests that colour pattern *is* important in many populations.

1.5.2 Preferences in the Paria and Lower Aripo populations

The two populations used in the experiments described in this thesis were the Paria river population and the Lower Aripo river population, both from Trinidad. Paria females come from a low visual predation environment, have particularly strong preference for orange area, and Paria males have particularly large and brilliant orange spots with high carotenoid density (i.e. brighter)(Houde 1987, 1988a; Houde & Endler 1990). Paria males show characteristic melanic spots and bars, and little iridescent pigment. Lower Aripo guppies come from a very high predation environment and males are dull in comparison with Paria males (see Appendix 1 for illustrations). Lower Aripo males show comparatively large amounts of iridescent pigment, similar amounts of melanin, and very small, pale orange spots. The reflectance spectra of carotenoid (orange) spots differ significantly between the two populations (Endler, unpublished data). Aripo females have correspondingly no obvious preference for orange (Houde 1988b); though Kodric-Brown (1989) showed orange preference for descendants of Aripo stock, but they had been allowed to interbreed with the strongly orange-preferring Paria stock so this can probably be discounted. No evidence for genetic differences in courtship behaviour between the two populations has been found (Houde, unpublished data).

However there is some information about preferences in the Lower Aripo guppies. Houde (1988b) found strong non-random mating in the population, though could not determine which pattern elements were important. There is evidence that Lower Aripo females prefer more colourful males overall (Breden & Hornaday 1994) when given choices between brighter and duller males. However when given choices between extremely bright and very dull *models*, Lower Aripo females have been shown to prefer the dull models (Breden & Stoner1987), unlike females from a lower predation river. But Stoner & Breden's (1988) study also tested Lower Aripo guppies along with contrasting low predation regime females (including Upper Aripo) - in this case Lower Aripo females preferred the brighter model when both were stationary (but not when the bright model was moving). It has been objected that the use of these models (which are of similar size to natural guppy predators) confounds examination of mate preference by inducing fright responses in wary high-predation regime females (but not in low-predation females) (Endler 1988). These objections were countered by the observation that females responded to the models in a similar fashion to real males (Breden 1988). Magurran and Segher's (1990) study found a marked decrease in the courtship behaviour under threat in Lower Aripo males compared with Upper Aripo males (which experience low predation), and similarly different schooling tendencies in both sexes. This suggests that Endler's objections to the use of large models may indeed be correct regarding the responses of wary Lower Aripo females.

Further evidence that Lower Aripo females prefer brighter males (but not too bright) is provided by Endler's (1980) field study. Lower Aripo guppies were introduced to similar habitat without dangerous piscivores. In the absence of predators (for 15 generations) males showed an increase in overall diversity of coloration, number of pigmented spots, and total coloration adjusted for body size. Total conspicuousness increased due to female choice when no longer constrained by predation pressure. Laboratory experiment results agreed with the field introduction results (in the reverse situation, presence of predators also led to decreases in conspicousness) (Endler 1980). In the former situation, pigmentation increases were most significant for blue and iridescent spots (by far the most conspicuous - Endler 1980), and least important for black or orange spots. This illustrates how different Lower Aripo guppies are compared to Paria guppies - where orange (and black) are the most significant pigments.

1.5.3 Are preferred traits correlated with fitness?

1.5.3a Carotenoid (orange) pigment

Much interest has centred on the widespread female preferences for orange area and brightness in males. Orange is the most frequently reported preferred character in male guppies (Endler 1983; Kodric-Brown 1985, 1993;

Houde 1987). This has been found in many wild populations (e.g. the Paria River and adjacent streams flowing north from Trinidad's Northern Range mountains, and a feral population in Durban, South Africa) and several domestic populations (e.g. the pure strains called Maculatus and Armatus descended from wild populations, fancy domesticated breeds (red cobra, yellow cobra, blue tuxedo) and common aquarium guppies). This female preference has been shown even in strains where males do not normally express much orange (Kodric-Brown 1985), though this only applies to captive-bred guppies.

The reason why carotenoid pigments are interesting in the context of mate choice is the hypothesis that they reliably reflect male quality. They may be honest advertisements of male phenotypic and genetic quality - because carotenoids cannot be synthesised by fishes, but must be ingested (Rothschild 1975; Fox 1976; Goodwin 1980). They can only be acquired from ingested plants and invertebrates (Harma & Hasegawa 1967; Fujii 1969), and so carotenoid pigment intensity should reflect the quality of the male's diet and hence his foraging ability. This could be an estimate of overall genetic quality. Such characters could potentially provide a reliable basis for females to choose high-quality mates.

The importance of carotenoids in the development of body pigments is well documented in fishes (e.g. salmonids fed invertebrates produce orange skin pigments while deprived individuals do not (Fox 1976). This is a breeding pigment in these species). Astaxanthin and canthaxin are deposited in the flesh and/or skin of salmon, trout, sea bream and goldfish (Fujii 1969; Goodwin 1980; Bauernfeind 1981) and have been used as dietary supplements to enhance the appearance of hatchery-reared salmonid fishes (Foss *et al.* 1987). Active mobilisation of carotenoids to gonads of fishes has been implicated in stimulating spermatozoal activity (Deufel 1975). By choosing to mate with a male with well developed carotenoid pigments, a female may increase the likelihood that she will receive viable sperm. Thus carotenoid pigments may function as an accurate indicator of male physical condition and overall genetic quality as well as his immediate investment in reproduction. In guppies, the presence and intensity of carotenoid pigments accurately indicates a male's foraging efficiency and his competitive ability in environments where high quality food is limited (Endler 1980). Guppies typically live in streams that are very sterile: the forest canopy ranges from 50% to 100% and casts deep shade; so algae and other sources of carotenoids are scarce or absent. The gravel or rock is clean and there is very little sediment in which small arthropods can live (Endler 1980). In these circumstances evidence of foraging ability in a mate could be of great importance to females. This is an unusual situation in that the condition-dependence of carotenoids offers a possible mechanism for the origin of this sexual selection. In other species it is easy to see what maintains sexual selection, but not how it started.

There is an additional complication when considering the evolution of carotenoid display pigments in guppies. Populations such as the Paria which display bright orange spots live in rivers where the main predator appears to be a prawn, *Macrobrachium* (Endler 1978, 1983) which cannot see orange. Populations such as the Lower Aripo live with predatory fish which can see orange and may prey selectively upon guppies with conspicuous orange spots. Endler (1978, 1983) has argued that Paria guppies and those like them have evolved conspicuous orange coloration because their only visual predator cannot see orange. Others have argued that *Macrobrachium* predation pressure is very slight in comparison to that on fish living with characin and cichlid predators (such as the Lower Aripo) (e.g. Magurran & Seghers 1990), as the prawn hunts mainly at night using non-visual cues. However it seems likely that the evolution of orange pigment is constrained by visual predation in the low-orange populations, and not constrained in the prawn-selected populations. This could have evolved due to differing female preferences: a preference for condition-dependant orange would be more likely to spread in the low predation populations, while preferences for other traits would have more initial advantage in high-predation populations.

If female choice simply reflected male conspicuousness, it would be expected that the highly reflective iridescent pigments would be more important than carotenoids. Iridescent pigments can be seen at much greater distances (Endler 1983). This implies that carotenoid pigments are important for other reasons than conspicuousness alone. Further support for this is the finding that in large samples of many wild populations, individuals missing one or more structural colours were common, but individuals missing carotenoid pigments entirely were *never* found (Endler 1983).

There is reason to expect that carotenoid pigment density (i.e. brightness) is condition dependant, but carotenoid *area* may not be - Kodric-Brown's (1989) diet manipulation experiment showed that males fed an enhanced carotenoid diet produced brighter orange spots, but not larger or more numerous spots. Carotenoid pigment density was positively correlated with swimming performance (Nicoletto 1991). However, female preference for area of orange (Houde 1988a) as well as brightness (Kodric-Brown 1989) has been shown. It has been suggested that brightness of orange colour in large spots might be a better indicator of condition than in small ones (Houde 1992), favouring a preference for greater orange area. Alternatively, the preference based on orange area might be an incidental effect of female preferences originally evolved in the context of variation in the brightness of orange spots (Houde 1992). Carotenoid pigment *area* was also shown to be condition-dependant in Nicoletto 1993. Carotenoid area has shown strong father-son heritability in Paria guppies ($h^2 >$ 0.54, Houde 1992). Due to all this correlational evidence, it is plausible that carotenoid pigments reflect male quality - but there is no direct evidence as yet for this in laboratory or wild populations. The effect of genetic stress due to inbreeding on pigment areas (including orange) is investigated in this thesis.

1.5.3b Other pigments

The structural colours and black spots are independent of food (Endler 1988) and formed by guanine crystals (Endler 1983). Structural colours (iridescent e.g. blue, green, silver) are the most reflective of guppy pigments and highly conspicuous (Endler 1980). When a male is displaying he vibrates his body rapidly both vertically and about the long axis, causing reflective pigments to flash. This highly conspicuous flicker increases risk of predation greatly (though they may not increase conspicuousness when escaping - natural selection can act very differently during escape and sexual displays, and the same pigment coupled with different behaviours may be conspicuous to females and cryptic to predators when required (Endler 1989)). However there is still the likelihood that predators will be attracted by flickering reflective colours when a male is displaying. There is evidence that iridescent pigments are reduced in habitats where predation risk is high (Haskins *et al.* 1961; Endler 1978, 1980, 1983). Females preferred males with large areas of iridescence in several studies (e.g. Kodric-Brown 1985, Endler 1983), though when correlations were controlled, carotenoid pigment was more important.

Endler (1983) suggested that structural pigments function by attracting and focusing a female's attention on individual males. Black pigments (melanins) have also been shown to be important but only when carotenoid and iridescent pigment effects were controlled (Kodric-Brown 1985; Brooks & Caithness 1995b). These findings that "less important" pigments are preferred when other factors are controlled lends support to the idea that seemingly non-preferred ornaments can be maintained by female choice (Zuk *et al.* 1990, 1992). There is the possibility that less prevalent colours are present because of pleiotropy with the ornament of choice (Brooks & Caithness 1995b).

There is also the "overall brightness" hypothesis of Endler (1980) which postulates that other colours are maintained as dishonest signals - i.e. they are not condition dependant; low quality males can synthesise them; they may attract females from a distance who may then scrutinise other cues that are honest advertisements (such as carotenoid pigment, body size or perhaps display rate). This implies that both Fisherian and good genes processes may be at work concurrently in the guppy, and have caused the evolution of different aspects of male sexual coloration. This thesis investigates response of pigment area of all colours to inbreeding stress in order to illuminate possible condition dependence.

1.5.3c Morphological traits

Brooks & Caithness (1995a) found a female preference for longer gonopodia independent of orange area. This preference could evolve due to enhanced mating success conferred on a female's sons in sneak copulations by a longer gonopodium (Reynolds *et al.* 1993). The low probability of insemination from this technique probably makes any fitness advantage very slight.

Bischoff *et al.* (1985) found a preference for large tail size. Dorsal and caudal (tail) fins are involved in making male displays more conspicuous and fins may carry pigment spots. These fins are exaggerated in males but not females, and this is assumed to have evolved due to sexual selection. There is evidence that tail size and dorsal fin length may not be condition dependent: Nicoletto (1991) found no correlation between the size of these fins and swimming performance (though a correlation with orange intensity was found). This finding is unexpected: perhaps there is a cost to enlarged fin size in manoeuvrability (c.f. swallows). There is currently no evidence that enlarged fins are condition dependent (though they may be conspicuous enough to attract predators and so may decrease survival in less fit males). These may be a Fisherian trait, though the evidence is inconclusive.

Quaré river guppies have not been shown to prefer any particular pigments, but females are attracted to larger body males (Reynolds & Gross 1992). Male size turned out to have significant father-son heritability, and most interestingly, large fathers sired both sons and daughters with higher growth rates. This resulted in more grandchildren for large fathers, due to the higher growth rates of daughters producing larger body size. This is significant evidence that for this population the preferred male trait (large body size) is a "good genes" trait. Females preferring large male size secure genetic benefits for their offspring. Male size is controlled by both genetic (Reynolds & Gross 1992) and environmental (Reznick 1990) components. Male size is not simply a function of age; while females continue to grow, male size has been shown to be virtually fixed at maturity (Reynolds *et al.* 1993). Female size and fecundity are strongly correlated within populations (Reznick 1983). Populations with high predation pressure show smaller size at maturity (e.g. Reznick & Endler 1982; can also be seen in the two populations studied in this thesis) which suggests that male body size may be subject to conflicting selection from females and predators.

These results are consistent with the predictions of good genes models. However, as other models of sexual selection make few mutually exclusive predictions (Balmford & Read 1990), others are not ruled out (such as "sensory bias" (Ryan 1990) - large males may be simply more conspicuous). However, given the increased reproductive output of offspring, even if sensory bias explained the origin of the preference, it seems likely that selection would have acted to modify female choice subsequently. Mating advantage of sons (Fisherian) and the benefits of offspring growth and reproduction (good genes) could also be acting, and it is possible that direct selection could also play a part (e.g. if larger males were less likely to carry sexually transmissible diseases (Reynolds & Gross 1990)). However the most consistent explanation for the effects of male size on offspring is the good genes hypothesis.

1.5.3d Display

Female preference for higher display rates has been shown in several studies (Farr 1980a, Bischoff *et al.* 1985; Nicoletto 1993). The sigmoid display is the most distinctive feature of the male's courtship behaviour and serves to "show off" the colour pattern in a highly conspicuous fashion. When a male performs a sigmoid display, he first approaches a female, then usually swims to place himself in front of her. The body is then arched in an S-shaped posture and the guppy vibrates his body rapidly both vertically and about the long axis of the body with the unpaired fins (dorsal and caudal) either closed or fully spread (Baerends *et al.* 1955; Liley 1966). Females generally respond only to open displays (with fins spread) (Farr 1977). The closed display is generally of shorter duration, far less conspicuous, and energetically probably less costly. Farr (1980b) hypothesised that the closed display functions to probe the female's receptivity, and that males switch to the full open display if a female does not reject their closed display. Rates of closed and open displays vary with changes in female fertility or receptivity (Liley 1966; Farr 1980b).

Display rate has been shown to be particularly sensitive to parasite load. In fish infested with a gut parasite (the nematode *Camallanus cotti*) or an ectoparasite (*Gyrodactylus* spp.), females preferred males which had relatively fewer parasites, and which displayed at a greater rate than males with more parasites (Kennedy *et al.* 1978). Sexual display rate was suppressed by the presence of parasites - it follows that females may be using display rate as an indicator of the male's parasite loads. However, these results may simply be interpreted as the host animal exhibiting symptoms of general illness; especially as display rates also affect female choice in unparasitised males (e.g. Bischoff *et al.* 1985). The hypothesis that sexual displays are particularly selected to reveal parasite infection can be tested by demonstrating a different effect of parasites on sexual and non-sexual behaviour. This has been done by McMinn (1990).

Females were unable to distinguish between other females with different parasite loads, so the response is not due to a simple parasite avoidance behaviour using non-sexually selected traits (which has obvious advantages if it is possible). Male sexual behaviours were affected by parasitism in a markedly different way to non-sexual behaviours. Sexual behaviour (sigmoid displays and following of females) was significantly suppressed by parasites, whereas general non-sexual activity such as feeding, grubbing and turning while swimming were either slightly affected or actually increased. The most costly element of male courtship, sigmoid display, which is also the most important predictor of female choice in some populations (e.g. Bischoff *et al.* 1985; also both populations in this thesis - see Display chapter) is also the most sensitive to nematode infection. Guppies in the wild commonly have gut parasites. It seems reasonable to infer (at least for this host-parasite system) that some sexually selected displays are particularly selected to reveal parasite infection. Similar findings on roach ornamentation have been found to indicate parasite load (Wedekind 1992).

This is in support of Hamilton & Zuk's (1982) sexual selection model driven by cycles of coadaptation between hosts and parasites. The model proposes that the ever-present risk of parasite infection has led to selection for choice of mates with traits that indicate genetic resistance. The bright ornaments of males may be selected to be particularly vulnerable to disruption by parasites, and so advertise genetic quality.The interactions between host and parasite are cyclic, as parasites typically have far shorter generation time than their hosts and may evolve to counteract resistance almost as fast as resistance evolves. This version of "running to stand still" ensures that the genetic variance of the male's resistance can be maintained (Hamilton 1982).

It seems likely that male display rate may be a good indicator of vigour. This thesis investigates display rate in inbred and outbred guppies for signs of condition dependence under genetic stress. Also the hypothesis that experienced unattractive males compensate with increased courtship effort in the form of display rate is tested. Compensation behaviour was suggested by the findings in Kodric-Brown's (1989) study on dietary manipulation and carotenoids - the carotenoid-deprived males appeared to compensate for their lack of attractiveness by increasing following behaviour and sneak copulation attempts.

There is other evidence that males are aware of their relative attractiveness and adjust their behaviour - e.g. Reynolds 1993 - large (more attractive) males increased their courtship relative to small (unattractive) males under low light levels (when safer from predators and with more to gain (a two-fold advantage) than small males). This was reversed under higher light levels. There is no indication of mechanism (e.g. learning from past female responses, genetic linking, feedback). Under different conditions, Bischoff *et al.* (1985) found a correlation between tail size and display rate. Larger (and more attractive) tailed males displayed more. They hypothesise that this could be due to genetic or phenotypic linking, or proprioceptive feedback provided by the tail. Depending on the costs and benefits, it may sometimes benefit more attractive individuals to court more, and sometimes it may benefit unattractive individuals to try harder. I investigated whether display rate in males of different attractiveness (in terms of pattern symmetry) involved a learning component from female interaction.

1.5.3e Symmetry

The predictions of theory lead to expectations that aspects of symmetry will correlate with viability and/or female choice (see section 1.4.3). Only one study has been published previously on FA in guppies (though J.A.Endler and A. Magurran have considerable unpublished data). Brooks & Caithness (1995a) measured FA in black and orange pigmentation of feral South African guppies. They found no significant correlation of female preference and symmetry, using either relative or absolute asymmetry. The relationship of FA and trait area showed a significant positive correlation for both carotenoid and melanic pigments. The absence of a negative correlation suggests that neither orange nor black area is a condition-dependent ornament. Orange spots, however, have a wealth of evidence showing them to be exemplary condition-dependent traits (Kodric-Brown 1989; Houde & Torio 1992). Either the idea of the universality of a strong negative correlation between ornament size in condition-dependent ornaments (Møller & Höglund 1991) must be rejected, or FA may not be such a good measure to show condition-dependence after all. It is possible that the surplus carotenoids provided by commercial fish food may eliminate the condition dependence of orange spots. Also most of the evidence of the condition dependence of carotenoids concerns pigment intensity rather than pigment area (though see Nicoletto 1993). I investigate the response of FA in all three pigment groups to genetic stress produced by inbreeding (see Sheridan &Pomiankowski 1997).

1.6 Thesis structure

Each experimental chapter is fairly self-contained with separate introductions, analyses and discussions. Chapter 2 describes the elements in common for these chapters such as origin of populations, maintenance regime, photographic techniques and inbreeding pedigrees.

Chapter 3 covers the analysis of FA and pigment area in inbred and control fish and discusses the implications for sexual selection theory in the context of previous FA work involving inbreeding. Significant differences were found in the response of different pigments to inbreeding depression both within and between populations. The results indicate that the degree of pigment area is a good measure of genetic stress, whereas fluctuating asymmetry was not. The response of pigments to inbreeding depression was highly correlated with the pigment's importance in mate choice for that population.

Chapter 4 describes the analysis of a different order of symmetry at the level of colour pattern. Previous FA work has only compared the size of paired characters. These experiments concerned unpaired characters. In guppies a form of symmetry involving entirely missing and misplaced colour spots is very common in some pigment groups. This is a different order of magnitude to the small deviations in paired traits that are called FA. Spot asymmetry was analysed separately to determine possible effects of inbreeding. Very different results to those of paired characters were obtained. No inbreeding effect was visible. However, in Aripo guppies, the numbers of spots and colours decreased in inbred males. Symmetry at the level of pattern showed no sensitivity to stress (like FA) though other aspects of pattern complexity - spot number and numbers of colours - were better indicators of genetic stress.

Chapter 5 contains female choice experiments. Preliminary experiments showed the preferences expected for those populations. Females given choices between males differing only in spot asymmetry (*not* FA) showed consistent preferences for symmetrical males in both populations. The implications regarding the predictions of sexual selection theory are discussed with reference to the findings in chapter 4.

Chapter 6 describes experiments on male display rates and female responses. Inbreeding stress reduced display rates of males. Females overwhelmingly preferred the most displaying male of each group in both populations. This was more important than any other factor including body size and pigmentation. There were interesting results concerning pattern asymmetry. Unattractive (asymmetric) males appeared to be compensating with increased display rates. This was only found in older experienced males, in young males and older naïve males this effect was not present. I argue that this implies a learning component of courtship behaviour and that males learn their relative attractiveness from previous female responses.

Chapter 7 investigates male swimming performance. Males swimming endurance was measured and compared with morphological and sexual traits. The technique was a poor discriminator between males, though an inbreeding effect was detected. Inbreeding depression slightly affected swimming performance - only outbred males were capable of strong sustained swimming. There were also correlations with pigment areas, implying that females choosing on the basis of this cue may be choosing males that are better swimmers. There was a strong age effect - older males being far better swimmers. No correlation with FA was found. There were interesting differences between the older, stronger swimming males and the younger group - the older males had greater proportional colour areas and larger bodies. Females prefer both these traits, and they are sensitive to inbreeding depression.

Chapter 8 contains a summary of the findings of the experimental chapters and relates them to the initial objective - to investigate the role of symmetry in sexual selection in guppies.

1.7 References

Alatalo, R.V., Höglund, J. & Lundberg, A. 1991. Lekking in the black grouse - a test of male viability. Nature (Lond.) 352: 155-156.

Andersson, M. 1982a. Sexual selection, natural selection and quality advertisement. Biol. J. Linn. Soc. 17: 375-393.

Andersson, M. 1982b. Female choice selects for extreme tail length in a widowbird. Nature 299: 818-820.

Andersson, M. 1986. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. Evolution 40:804-816.

Baerends, G.P., Brouwer, R. & Waterbolk, H.T. 1955. Ethological studies on *Lebistes reticulatus* (Peters). Behaviour 8:249-334.

Bailit, H.L., Workman, P.L., Niswander, J.D., & MacLean, C.J. 1970. Dental asymmetry as an indicator of genetic and environmental conditions in human populations. Human Biol. 42: 626-638.

Bakker, T.C.M. & Pomiankowski, A. 1995. The genetic basis of female mate preferences. J. Evol. Biol. 8: 129-171.

Bakker, T.C.M. 1993. Positive genetic correlation between female preference and preferred male ornament in sticklebacks. Nature 363: 255-257

Ballin, P.J. 1973. Geographic variation in courtship behaviour of the guppy; *Poecilia reticulata.* M.Sc. thesis, University of British Columbia, Vancouver.

Balmford, A. & Read, A.F. 1990. Testing alternative models of sexual selection through female choice. Trends Ecol. Evol. 6: 274-276.

Balmford, A. & Thomas, A. 1992 . Swallowing ornamental asymmetry. Nature (Lond.). 359: 487.

Bauernfeind, J.C. 1981. Carotenoids as colorants and vitamin A precursors. New York: Academic Press.
Beach, F.A. & LeBoeuf, B.J. 1967. Coital behaviour in dogs. I.
Preferential mating in the bitch. Anim. Behav. 15: 546-558.

Bell, G. 1978. The handicap principle in sexual selection. Evolution 32: 872-885.

Belt, T. 1874. The naturalist in Nicaragua. London: Murray.

Biémont, C. 1983. Homeostasis, enzyme heterozygosity and inbreeding depression in natural populations of *Drosophila melanogaster*. Genetica 61: 179-189.

Bischoff, R. J., Gould, J. L. & Rubenstein, D. I. 1985. Tail size and female choice in the guppy (*Poecilia reticulata)*. Behav. Ecol. Sociobiol., 17, 253-255.

Breden, F. & Hornaday, K. 1994. Test of indirect models of selection in the Trinidad guppy. Heredity 73: 291-297.

Breden, F. & Stoner, G. 1987. Male predation risk determines female preferences in the Trinidad guppy. Nature 329: 831-833.

Breden, F. 1988. Reply: sexual selection and predation risk in guppies. Nature 332: 594.

Breed, M.D., Smith, S.K. & Gall, B.G. 1980. Systems of mate selection in a cockroach species with male dominance heirarchies. Anim. Behav. 28: 130-134.

Brooks, R. & Caithness, N. 1995a. Female choice in a feral guppy population: are there multiple cues? Anim. Behav., 50, 301-307.

Brooks, R. & Caithness, N. 1995b. Manipulating a seemingly non-preferred male ornament reveals a role in female choice. Proc. R. Soc. Lond. B., 261, 7-10.

Brown, L. 1981. Patterns of female choice in mottled sculpins (Cottidae, Teleostei). Anim. Behav. 29: 375-382.

Brown, R.D. 1984. Antler development in Cervidae. Kingsville: Caesar Kleberg Wildlife Research Institute.

Burley, N. 1977. Parental investment, mate choice, and mate quality. Proc. Natl. Acad. Sci. USA. 74: 3476-3479.

Burley, N. 1981. Mate choice by multiple criteria in a monogamous species. Am. Nat. 117: 515-528.

Chakraborty, R. 1987. Biochemical heterozygosity and phenotypic variability of polygenic traits. Heredity 59: 19-28.

Clark, E. & Aronson, L.R. 1951. Sexual behaviour in the guppy, *Lebistes reticulatus* (Peters). Zoologica 36: 49-66.

Clarke, G.M. & McKenzie, J.A. 1987. Developmental stability of insecticide resistant phenotypes in blowfly: a result of canalising natural selection. Nature (Lond.) 325: 345-346.

Clarke, G.M., Brand, G.W. and Whitten, M.J. 1986 Fluctuating asymmetry: A technique for measuring developmental stress caused by inbreeding. Aust. J. Biol. Sci. 39 145-153.

Clarke, G.M., Oldroyd, B.P., & Hunt P. 1992. The genetic basis of developmental stability in *Apis mellifora:* heterozygosity versus genic balance. Evolution 46: 753-762.

Crow, R.T. 1981. Behavioural adaptations to stream velocity in the guppy, *Poecilia reticulata.* M.Sc. Thesis, University of British Columbia, Vancouver. Pp 1-106.

Darwin, C. 1859 On the Origin of Species by Means of Natural Selection. London: John Murray.

Darwin, C. 1871 The Descent of Man and Selection in relation to Sex. London: John Murray.

Deufel, J. 1975. Physiological effects of carotenoids on salmonidae. Hydrologie 37: 244-248.

Dobzhansky, T. 1970. Genetics of the Evolutionary Process. New York: Columbia Univ. Press. pp 505.

Eberhard, W.G. 1985. Sexual Selection and Animal Genitalia. Cambridge, Mass: Harvard University Press.

Emlen, S.T. 1976. Lek organisation and mating strategies in the bullfrog. Behav. Ecol. Sociobiol. 1: 283-313.

Endler, J.A. & Houde, A.E. 1995. Geographic variation in female preferences for male traits in *Poecilia reticulata.* Evolution 49: 456-468.

Endler, J.A. 1978. A predator's view of animal colour patterns. Evol. Biol. 11: 319-364.

Endler, J.A. 1980. Natural selection on colour patterns in *Poecilia reticulata*. Evolution, 34, 76-91.

Endler, J.A. 1983. Natural and sexual selection on colour patterns in poeciliid fishes. Environ. Biol. Fish 9: 173-190.

Endler, J.A. 1987. Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces:Poecilidae). Anim. Behav., 35, 1376-1385.

Endler, J.A. 1988. Sexual selection and predation risk in guppies. Nature 332: 593-594

Evans, M.R. & Thomas, A.L.R. 1992. Aerodynamic and mechanical effects of elongated tails in the scarlet-tufted malachite sunbird: Measuring the cost of a handicap. Anim. Behav. 43: 337-347.

Falconer, D.S. 1960. Introduction to Quantitative Genetics. Edinburgh: Oliver & Boyd. 3rd Edn. 1989. Longman, Harlow, UK.

Farr, J.A. & Herrnkind, W.F. 1974. A quantitative analysis of social interaction of the guppy, *Poecilia reticulata* (Pisces: Poeciliidae) as a function of population density. Anim. Behav. 22: 582-591.

Farr, J.A. 1975. The role of predation in the evolution of social behaviour of natural populations of theguppy, *Poecilia reticulata* (Pisces: Poeciliidae). Evolution 29: 151-158.

Farr, J.A. 1976. Social facilitation of male sexual behaviour, intrasexual competition, and sexual selection in the guppy; *Poecilia reticulata* (Pisces: Poeciliidae). Evolution 30: 707-717.

Farr, J.A. 1977. Male rarity or novelty, female choice behaviour, and sexual selection in the guppy, *Poecilia reticulata* Peters (Pisces: Poeciliidae). Evolution 31: 162-168.

Farr, J.A. 1980a. Social behaviour patterns as determinants of reproductive success in the guppy, *Poecilia reticulata* Peters (Pisces: Poecilidae). Behaviour. 74, 38-91.

Farr, J.A. 1980b. The effects of social experience and female receptivity on courtship-rape decisions in male guppies, *Poecilia reticulata* (Pisces: Poeciliidae). Anim. Behav. 28: 1195-1201.

Farr, J.A. 1983. The inheritance of quantitative fitness traits in guppies, *Poecilia reticulata* (Pisces: Poeciliidae). Evolution 37: 1193-1209.

Farr, J.A. 1989. Sexual selection and secondary sexual differentiation in poeciliids: determinants of male mating success and the evolution of female choice. In: Ecology and Evolution of Livebearing Fishes (Poeciliidae)(Eds. Meffe & Snelson). Pp 91-123. New Jersey: Prentice Hall.

Fisher, R.A. 1915. The evolution of sexual preference. Eugenics Review 7: 184-192.

Fisher, R.A. 1930. The genetic theory of natural selection. Clarendon, Oxford.

Foss, P., Storebakken, T., Ausstreng, E. & Liaaen-Jensen, S. 1987. Diets for salmonids. I. Pigmentation of rainbow trout and sea trout with astaxanthin and astaxanthin dipalmitate in comparison with canthaxanthin. Aquaculture 65: 293-305.

Fowler, K. & Whitlock, M.C. 1994. Fluctuating asymmetry does not increase with moderate inbreeding in *Drosophila melanogaster.* Heredity 73: 373-376.

Fox, D.L. 1976. Animal biochromes and structural colours. Berkeley: University of California Press.

Freeland, W.J. 1976. Parasitism and behavioural dominance among male mice. Science, Wash. 213: 461-462.

Fujii, R. 1969. Chromatophores and Pigments. In: Fish Physiology. Vol 3. Eds Hoar & Randall. Pp 307-353. New York: Academic Press.

Gandolfi, G. 1972. Sexual selection in relation to the status of males in *Poecilia reticulata* (Teleostei: Poeciliidae). Boll. Zool. 38: 35-48.

Goodwin, T.W. 1980. Comparative biochemistry of the carotenoids. Vol 1. 2nd Edn. Chapman & Hall.

Gorlick, D.L. 1976. Dominance heirarchies and factors affecting dominance in the guppy ; *Poecilia reticulata* (Peters). Anim. Behav. 24: 336-346.

Grafen, A. 1990. Sexual selection unhandicapped by the Fisher process. J. Theor. Biol. 144: 475-516.

Graham, J.H. & Felley, J.D. 1985. Genomic coadaptation and developmental stability within introgressed populations of *Enneacanthus gloriosus* and *E. obesus* (Pisces, Centrarchidae). Evolution 39: 104-114.

Greene, R.J., Jr. 1972. Female preferential selection for males in *Lebistes reticulatus.* Thesis (Biology Department), University of Utah.

Grove, B.D. 1980. An analysis of intraovarian sperm interactions in the guppy, *Poecilia reticulata.* M.Sc. thesis, University of British Columbia, Vancouver.

Hamilton, W.D. 1982. Pathogens as causes of genetic diversity in their host populations. In: Population biology of infectious diseases. Eds. Anderson & May. Springer, Berlin Heidelberg New York, pp 269-296.

Hamilton, W.D. and Zuk, M. 1982. Heritable true fitness and bright birds: a role for parasites. Science 218: 384-387.

Harma, T. & Hasegawa, H. 1967. Studies on the chromatophores of *Oryzias latipes* (teleostean fish): behaviour of pteridine, fat and carotenoid during xanthophore differentiation in the colour varieties. Proc. Japan Acad. 43: 901-906.

Haskins, C.P., Haskins, E.F., McLaughlin, J.J.A., and Hewitt, R.E. 1961. Polymorphisms and populations structure in *Lebistes reticulatus*, an ecological study. In Vertebrate Speciation, ed. W.F. Blair, pp 329-395. Austin: Univ. Texas Press.

Hedrick, A.V. 1986. Female preferences for male calling bout duration in a field cricket. Behav. Ecol. Sociobiol. 19: 73-77.

Heywood, J.S. 1989. Sexual selection by the handicap mechanism. Evolution 43: 1387-1397.

Hoelzer, G.A. 1989. The good parent process of sexual selection. Anim. Behav. 38: 1067-1078.

Hoffmann, A.A. & Parsons, P.A. 1989a. An integrated approach to environmental stress tolerance and life history variation: Desiccation tolerance in *Drosophila*. Biol. J. Linn. Soc. 37: 117-136.

Hoffmann, A.A. & Parsons, P.A. 1989b. Selection for increased desiccation tolerance in *Drosophila melanogaster*: Additive genetic control and correlated responses to other stresses. Genetics 122: 837-845.

Houde, A. E & Torio, A. J. 1992. Effect of parasite infection on male colour patterns and female choice in guppies. *Behavioural Ecology,* **3**, 346-351.

Houde, A. E. 1987. Mate choice based upon naturally occurring colour-pattern variation in a guppy population. Evolution, 41,1-10.

Houde, A. E. 1988a Genetic difference in female choice between two guppy populations. Anim. Behav., 36, 510-516.

Houde, A. E. 1988b The effects of female choice and male-male competition on the mating success of male guppies. Anim. Behav., 36, 888-896.

Houde, A. E. and Endler, J. A. 1990. Correlated evolution of female mating preferences and male colour patterns in the guppy *Poecilia reticulata.* Science, 248, 1405-1408.

Houde, A.E. 1994. Effect of artificial selection on male colour patterns on mating preference of female guppies. Proc. Roy. Soc. Lond. B256: 125-130.

Houle, D. 1989. Allozyme-associated heterosis in *Drosophila melanogaster.* Genetics 123: 789-801.

Howard, R.D. 1980. Mating behaviour and mating success in woodfrogs, *Rana sylvatica.* Anim. Behav. 28: 705-716.

Itzkowitz, M. 1978. Female choice in the pupfish, *Cyprinodon variegatus.* Behav. Proc. 3: 1-8.

Iwasa, Y, Pomiankowski, A. & Nee, S. 1991. The evolution of costly mate preferences 2: The “handicap” principle. Evolution 45: 1431-1442.

Johnstone, R.A., Reynolds, J.D. & Deutsch, J.C. 1996. Mutual mate choice and sex differences in choosiness. Evolution 50: 1382-1391.

Kat, P.W. 1982. The relationship between heterozygosity for enzyme loci and developmental homeostasis in peripheral populations of aquatic bivalves (Unionidae). Am. Nat. 119: 824-832.

Kennedy, C. E. J., Endler, J. A., Poynton, S. L., & McMinn, H. 1987. Parasite load predicts mate choice in guppies. Behav. Ecol. Sociobiol., 21, 291-295.

Kieser, J.A. & Groeneveld, H.T. 1991. Fluctuating odontometric asymmetry, morphological variability, and genetic monomorphism in the cheetah *Acinonyx jubatus.* Evolution 45: 1175-1183.

Kodric-Brown, A. 1985. Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). Behav. Ecol. Sociobiol., 17, 199-205.

Kodric-Brown, A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. Behav. Ecol. Sociobiol., 25, 393-401.

Kodric-Brown, A. 1990. Mechanisms of sexual selection: insights from fishes. Ann. Zool. Fenn. 27: 87-100.

Kodric-Brown, A. 1993. Female choice of multiple male criteria in guppies: interacting effects of dominance, colouration and courtship. Behav. Ecol. Sociobiol. 32: 415-420.

Kruijt, J.P. & Hogan, J.A. 1967. Social behaviour on the lek in black grouse, *Lyrurus tetrix tetrix* (L.). Ardea 55:203-240.

Leamy, L. 1984. Morphometric studies in inbred and hybrid house mice. V. Directional and fluctuating asymmetry. Am. Nat. 123: 579-593.

Leary, R. & Allendorf, F.W. 1989. Fluctuating asymmetry and indicators of stress: implications for conservation biology. Trends Ecol. Evol. 4: 214-217.

Leary, R. F., Allendorf, F. W. and Knudsen, L. K. 1983. Developmental stability and enzyme heterozygosity in rainbow trout. Nature (Lond.) 301: 71-72.

Leary, R. F., Allendorf, F. W. and Knudsen, L. K. 1984. Superior developmental stability of heterozygotes at enzyme loci in salmonid fishes. Am. Nat., 124, 540-551.

Leary, R. F., Allendorf, F. W. and Knudsen, L. K. 1985a. Inheritance of meristic variation and the evolution of developmental stability in rainbow trout. Evolution 39: 308-314.

Leary, R. F., Allendorf, F. W. and Knudsen, L. K. 1985b. Developmental instability and high meristic counts in interspecific hybrids of salmonid fishes. Evolution 39: 1318-1326.

Lerner, I.M. 1954. Genetic Homeostasis. Edinburgh: Oliver & Boyd.

Licht, L.E. 1976. Sexual selection in toads (*Bufo americanus*). Can. J. Zool. 54: 1277-1284.

Liggett, A.C., Harvey, I.F., & Manning, J.T. 1993. Fluctuating asymmetry in *Scatophagus stercoraria*: successful males are more symmetric. Anim. Behav. 45: 1045-1048.

Liley, N.R. 1966. Ethological isolating mechanisms in four sympatric species of poeciliid fishes. Behav. Suppl. 13: 1-197.

Livshits, G. & Kobyliansky, E. 1991. Fluctuating asymmetry as a possible measure of developmental homeostasis in humans: A review. Human Biol. 63: 441-466.

Long, K.D. & Houde, A.E. 1989. Orange spots as a visual cue for female mate choice in the guppy *(Poecilia reticulata)* Ethology 82: 316-324.

Ludwig, W. 1932. Das Rechts-Links Problem im Tierreich und beim Menschen. Springer, Berlin.

Luyten, P.H. & Liley, N.R. 1985. Geographic variation in the sexual behaviour of the guppy, *Poecilia reticulata* (Peters) Behaviour 95: 164-179.

Luyten, P.H. & Liley, N.R. 1991. Sexual selection and competitive mating success of male guppies *(Poecilia reticulata)* from four Trinidad populations. Behav. Ecol. Sociobiol. 28: 329-336.

Magurran, A.E. & Nowak, M.A. 1991. Another battle of the sexes: the consequences of sexual asymmetry in mating costs and predation risk in the guppy, *Poecilia reticulata.* Proc. R. Soc. Lond. B. 246: 31-38.

Magurran, A.E. & Seghers, B.H. 1990. Risk sensitive courtship in the guppy *(Poecilia reticulata).* Behaviour 112: 194-201.

Magurran, A.E. & Seghers, B.H. 1991. Variation in schooling and aggression amongst guppy *(Poecilia reticulata)* populations in Trinidad. Behaviour 118: 214-234.

Markow, T. & Ricker, J.P. 1992. Male size, developmental stability and mating success in natural populations of three Drosophila species. Heredity 69: 122-127.

Martin, F.D. & Hengstebeck, M.F. 1981. Eye colour and aggression in juvenile guppies, *Poecilia reticulata* Peters (Pisces: Poeciliidae). Anim. Behav. 29: 325-331.

Mather, K. 1953. Genetic control of stability in development. Heredity 7, 297-336.

Maynard Smith, J. 1978. The Evolution of Sex. Cambridge: Cambridge University Press.
Maynard Smith, J. 1976. Sexual selection and the handicap principle. J. Theor. Biol. 57: 239-242.

Maynard-Smith, J. 1987. Sexual Selection - A Classification of Models. In: Sexual Selection: Testing the Alternatives. (Bradbury, J.W., and Andersson, M.B. eds.), pp. 9-20, Wiley

Merrell, D. 1949. Selective mating in *Drosophila melanogaster.* Genet. 34: 370-389.

Mitton, J.B. & Grant, M.C. 1984. Associations among protein heterozygosity, growth rate and developmental homeostasis. Ann. Rev. Ecol. Syst. 15: 479-499.

Møller, A.P. & Pomiankowski, A. 1993. Fluctuating asymmetry and sexual selection. Genetica 89, 267-279.

Møller, A.P. 1989. Viability costs of male tail ornaments in a swallow. Nature (Lond.) 339: 132-135.

Møller, A.P. 1990. Fluctuating asymmetry in male sexual ornaments may reliably reveal male quality. Anim. Behav. 40, 1185-1187.

Møller, A.P. 1991. Sexual ornament size and the cost of fluctuating asymmetry. Proc. R. Soc. Lond. B. 243: 59-62.

Møller, A.P. 1992a. Female swallow preference for symmetrical male sexual ornaments. Nature, 357, 238-240.

Møller, A.P. 1992b. Patterns of fluctuating asymmetry in weapons: evidence for reliable signalling of quality in beetle horns and bird spurs. Proc. R. Soc. Lond. B. 248: 199-206.

Møller, A.P. 1994a. Sexual selection in the barn swallow (*Hirundo rustica*). IV. Patterns of fluctuating asymmetry and selection against asymmetry. Evolution 48, 658-670.

Møller, A.P. 1994b. Sexual selection and the barn swallow. Oxford: Oxford University Press.

Møller, A.P. and Höglund, J. 1991 Patterns of fluctuating asymmetry in avian feather ornaments: implications for models for sexual selection. Proc. R. Soc. Lond. B 245, 1-5.

Moodie, G.E.E. 1972. Predation, natural selection and adaptation in an unusual three-spine stickleback. Heredity 28: 155-167.

Morris, R.L. & Ericson, C.J. 1971. Pair bond maintenance in the ring dove (*Streptopelia risoria*). Anim. Behav. 19: 398-406.

Nicoletto, P. F. 1991. The relationship between male ornamentation and swimming performance in the guppy, *Poecilia reticulata*. Behav. Ecol. Sociobiol., 28, 365-370.

Nicoletto, P.F. 1993. Female sexual response to condition-dependent ornaments in the guppy *Poecilia reticulata.* Anim. Behav. 46: 441-450.

Nolan, V. Jr. 1978. The ecology and behaviour of the prairie warbler *Dendroica discolor.* Ornithol. Monogr. No. 26. pp595.

Nur, N. & Hasson, O. 1984. Phenotypic plasticity and the handicap principle. J. Theor. Biol. 110: 275-297.

O'Donald, P. 1962. The theory of sexual selection. Heredity 17: 541-552.

O'Donald, P. 1967. A general model of sexual and natural selection. Heredity 22: 499-518.

O'Donald, P. 1980. Genetic Models of Sexual Selection. Cambridge University Press.

Packer, C. & Pusey, A.E. 1993. Should a lion change its spots? Nature (Lond) 362: 595.

Palmer, A. R., and Strobeck, C. 1986. Fluctuating asymmetry: Measurement, analysis, patterns. Ann. Rev. Ecol. Syst., 17,391-421.

Parker, G.A. 1983. Mate quality and mating decisions. In: Mate Choice. (ed. Bateson) pp 141-161. Cambridge University Press.

Parsons, P. A. 1990. Fluctuating asymmetry: An epigenetic measure of stress. Biol. Rev., 65,131-145.

Parsons, P.A. 1962. Maternal age and developmental variability. J. Exp. Biol. 39: 251-260.

Parsons, P.A. 1992 Fluctuating asymmetry: a biological monitor of environmental and genomic stress. Heredity 68, 361-364.

Parsons, P.A. 1992. Fluctuating asymmetry - A biological monitor of environmental and genomic stress. Heredity 68: 361-364.

Payne, R.B. & Payne, K. 1977. Social organisation and mating success in local populations of village indigobirds, *Vidua chalybeata.* Z. Tierpsychol. 45: 113-173.

Payne, R.B. 1984. Sexual selection, lek and arena behaviour, and sexual size dimorphism in birds. *Ornithol. Monogr.* 33 Am. Ornith. Union.

Pomiankowski, A. & Sheridan, L. 1994a. Linked sexiness and choosiness. Trends in Ecology and Evolution 9: 242-244.

Pomiankowski, A. & Sheridan, L. 1994b. Reply: Female choice and genetic correlations. Trends in Ecology and Evolution 9: 343.

Pomiankowski, A. 1987. Sexual selection: the handicap principle does work - sometimes. Proc. R. Soc. Lond. B. 231: 123-145.

Pomiankowski, A.N. 1988. The evolution of female mate preferences for male genetic quality. Oxford Surveys in Evolutionary Biology 5: 136-184

Radesäter, T. & Halldórsdóttir, H. 1993. Fluctuating asymmetry and forceps size in earwigs, *Forficula auricularia.* Anim. Behav. 45: 626-628.

Reeve, E. C. R. 1960. Some genetic tests on asymmetry of sternopleural chaeta number in *Drosophila. Genet. Res.Camb.* **1**,151-172.

Reynolds, J. D., Gross, M. R., and Coombs, M. J. 1993. Environmental conditions and male morphology determine alternative mating behaviour in Trinidadian guppies. Anim. Behav., 45, 145-152.

Reynolds, J.D. & Gross, M.R. 1990. Costs and benefits of female mate choice: is there a lek paradox? Am. Nat. 136: 230-243.

Reynolds, J.D. & Gross, M.R. 1992. Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata.* Proc. R. Soc. Lond. B. 250: 57-62.

Reynolds, J.D. 1996. Animal breeding systems. Trends Ecol. Evol. 11: A68-A72.

Reznick, D. 1983. The structure of guppy life histories: the trade-off between growth and reproduction. Ecology 64: 862-873.

Reznick, D.A. & Endler, J.A. 1982. The impact of predation on life history evolution in Trinidadian guppies (*Poecilia reticulata).* Evolution 36: 160-177.

Reznick, D.N. 1990. Plasticity in age and size at maturity in male guppies (*Poecilia reticulata)*: an experimental evaluation of alternative models of development. J. Evol. Biol. 3: 185-203.

Rothschild, M. 1975. Remarks on carotenoids in the evolution of signals: In: Coevolution of animals and plants. Eds Gilbert & Raven. Pp 20-47. Austin: University of Texas Press.

Ryan, M.J. 1981. Female mate choice in a neotropical frog. Science 209: 523-525.

Ryan, M.J. 1985. The Tungara frog: A Study in Sexual Selection and Communication. Chicago: Univ. Chicago Press.

Ryan, M.J. 1990. Sexual selection, sensory systems, and sensory exploitation. Oxf. Surv. Evol. Biol. 7: 157-195.

Schmidt, J. 1920. Racial investigations. IV. The genetic behaviour of a secondary sexual character. C. R. Trav. Lab. Carlsberg 14 (8): 1-12.

Schröder, J.H. 1983 . The guppy (*Poecilia reticulata* Peters) as a model for evolutionary studies in genetics, behaviour and ecology. Berichte des naturwissenschaftlichen-medizinischen Vereins Innsbruck 70: 249-279.

Sciulli, P.W., Doyle, W.J., Kelley, C., Siegel, P. & Siegel, M.I. 1979. The interaction of stressors in the induction of increased levels of fluctuating asymmetry in the laboratory rat. Am. J. Phys. Anthropol. 50: 279-284.

Semler, D.E. 1971. Some aspects of adaptation in a polymorphism for breeding colours in the Threespine Stickleback (*Gasterosteus aculeatus*). J. Zool. Lond. 165: 291-302.

Sheridan, L. & Pomiankowski, A. 1997. Fluctuating asymmetry, spot asymmetry and inbreeding depression in the sexual coloration of male guppy fish. Heredity, *in press.*

Siegel, M.I. & Doyle, W.J. 1975. The differential effect of prenatal and postnatal audiogenic stress on fluctuating dental asymmetry. J. Exp. Zool. 191: 211-214.

Soulé, M and Baker, B. 1968 Phenetics of natural populations. IV. The population asymmetry parameter in the butterfly *Coenonympha tullia*. Heredity 23, 611-614.

Soulé, M. E. 1979. Heterozygosity and developmental asymmetry: another look. Evolution, 33, 396-401

Stoner, G. & Breden, F. 1988. Phenotypic differentiation in female preference related to geographic variation in predation risk in the Trinidad guppy (*Poecilia reticulata)*. Behav. Ecol. Sociobiol. 22: 285-291.

Swaddle, J. P. and Cuthill, I. C. 1994a . Preference for symmetrical males by zebra finches. Nature 367, 165-166.

Swaddle, J. P. and Cuthill, I. C. 1994b. Female zebra finches prefer males with symmetrically manipulated chest plumage. Proc. R. Soc. Lond. B, 258, 267-271.

Tebb, G. &Thoday, J.M. 1958. Stability in development and relational balance of X chromosomes in *D. melanogaster*. Nature (Lond.) 174: 1109-1110.

Thibault, R.E. & Schultz, R.J. 1978. Reproductive adaptations among viviparous fishes (Cyprinodontiformes: Poeciliidae). Evolution 32: 320-333.

Thoday, J.M. 1955. Balance, heterozygosity and developmental stability. Cold Spring Harbor Symp. Quant. Biol. 20: 318-326.

Thoday, J.M. 1958 Homeostasis in a selection experiment. Heredity 12, 401-405.

Thornhill, R. & Sauer, P. 1992. Genetic sire effects on the fighting ability of sons and daughters and mating success of sons in a scorpionfly. Anim. Behav. 43: 255-264.

Thornhill, R. 1992a. Fluctuating asymmetry, interspecific aggression and male mating tactics in two species of Japanese scorpionflies. Behav. Ecol. Sociobiol. 30: 357-363.

Thornhill, R. 1992b. Female preference for the pheromone of males with low fluctuating asymmetry in the Japanese scorpionfly (*Panorpa japonica*: Mecoptera). Behav. Ecol. 3: 277-283.

Thornhill, R. 1992c. Fluctuating asymmetry and the mating system of the Japanese scorpionfly *Panorpa japonica.* Anim. Behav. 44: 867-879.

Townsend, G.C. 1983 . Fluctuating dental asymmetry in Down's syndrome. Aust. Dental. J. 28: 39-44.

Turner, J.R.G. 1978. Why male butterflies are non-mimetic: Natural selection, sexual selection, group selection, modification and sieving. Biol. J. Linn. Soc. 10: 385-432.

Valentine, D.W. & Soulé, M.E. 1973. Effect of *p,p*-DDT on developmental stability of pectoral fin rays in the grunion, *Leuresthes tenius.* Fishery Bull. 71: 920-921.

Van Valen, L. 1962. A study of fluctuating asymmetry. Evolution 16: 125-142.

Vehrencamp, S.L., Bradbury, J.W. & Gibson, R.M. 1989. The energetic cost of display in male sage grouse. Anim. Behav. 38: 885-896.

Vrijenhoek, R.C. & Lerman, S. 1982. Heterozygosity and developmental stability under sexual and asexual breeding systems. Evolution 36: 768-776.

Wallace, A. 1870. Contributions to the Theory of Natural Selection. London: MacMillan.

Warren, E.W. 1973. The establishment of a "normal" population and its behavioural maintenance in the guppy - *Poecilia reticulata* (Peters). J. Fish. Biol. 5: 285-304.

Wedekind, C. 1992. Detailed information about parasites revealed by sexual ornamentation. Proc. R.Soc. Lond. B. 247: 169-174.

Wells, K.D. 1977. Territoriality and male mating success in the green frog (*Rana clamitans*). Ecology 58: 750-762.

West, M.J., King, A.P. & Eastzer, D.H. 1981. Validating the female bioassay of cowbird song: relating differences in song potency to mating success. Anim. Behav. 29: 490-501.

West-Eberhard, M.J. 1979. Sexual selection, social competition and evolution. Proc. Amer. Phil Soc. 123: 222-234.

Whitlock, M.C. 1993. Lack of correlation between heterozygosity and fitness in forked fungus beetles. Heredity 70: 574-581.

Whitney, C.L. & Krebs, J.R. 1975. Mate selection in Pacific tree frogs. Nature 255: 325-326.

Wilkinson, G.S. and Reillo, P. R. 1994. Female choice response to artificial slection on an exaggerated male trait in a stalk-eyed fly. Proc. R. Soc. Lond. B. 255: 1-6.

Williams, G.C. 1966. Adaptation and Natural Selection. Princeton Univ. Press.

Yanai, J. & McClearn, G.E. 1972. Assortative mating in mice. I. Female mating preference. Behav. Genet. 2: 173-183.

Zahavi, A. 1975. Mate selection - a selection for a handicap. J. Theor. Biol. 53: 205-214.

Zahavi, A. 1977. The cost of honesty (further remarks on the handicap principle). J. Theor, Biol. 67:603-605.

Zahavi, A. 1987. The theory of signal selection and some of its implications. In International Symposium of Biological Evolution. (Delfino, V.P. ed.) pp 305-327 Adriatic Editrice, Bari.

Zakharov, V.M. 1981. Fluctuating asymmetry as an index of developmental homeostasis. Genetiks (Belgrade) 13: 241-256.

Zakharov, V.M. 1989 Future prospects for population phenogenetics. Sov. Sci. Rev. F. Physiol. Gen. Biol. 4, 1-79.

Zuk, M, Johnson, K. Thornhill, R. & Ligon, J.D.1990. Mechanisms of female mate choice in red jungle fowl. Evolution 44: 477-485.

Zuk, M, Ligon, J.D & Thornhill, R. 1992. Effects of experimental manipulation of male secondary sex characters on female mate preference in red jungle fowl. Anim. Behav. 44: 999-1006.

Chapter 2: Materials & Methods

2.1 Origin of populations

Guppies are small tropical freshwater fish found in the West Indies and South America (Rosen & Bailey 1963). Guppies used in these experiments were descendants of individuals of mixed sexes and ages collected from the wild in Trinidad in 1992. The guppies were collected from two different sites, the Lower Aripo and Paria rivers. These rivers flow down opposite sides of the Northern Range mountains in Trinidad and the populations have been separated for approximately 250,000 years (Fajen & Breden 1992). Large samples (200+ individuals) were collected from each river so as to maintain genetic variation in founding the laboratory stock populations. There was high mortality among Aripo males soon after transport, though large numbers of females survived. Females are often multiply inseminated so probably a large sample of the natural population was maintained. Paria survival was high.

2.2 Biotic environment in the field

In the wild each population coexists with very different predators. The Paria collection site, in common with other headstreams, has few guppy predators. The Paria guppies are sympatric with freshwater prawns of the genus *Macrobrachium. M. Crenulatum* in particular is present in high densities (Endler 1983). *Macrobrachium* prawns are guppy predators (Magurran & Seghers 1990) though it has been suggested that they hunt primarily at night and use olfactory and tactile cues in addition to vision when detecting prey, and so are not an important visual predator of guppies (Magurran & Seghers 1991). Conversely, Endler (1978, 1983) has suggested that the inability of *Macrobrachium* to see orange is a major cause for the evolution of the brilliant orange coloration in guppies typical of north flowing streams such as the Paria; this implies a significant selection pressure by visual predation. Paria guppies are also sympatric with a Cyprinodont fish (or killifish), *Rivulus hartii,* which is a predator but attacks mainly juvenile fish (Seghers 1974; Liley & Seghers 1975). The presence of guppies has been shown to reduce the growth rate and adult size of *Rivulus,* and this has been shown to be an effect of competition between adult guppies and juvenile *Rivulus* juveniles (Gilliam *et al.* 1993). There is also another fish species found in the Paria, a *Sicydium* species of Goby; this is not thought to be a predator of guppies. This low predation environment has allowed the evolution of very bright males and little schooling or other anti-predator behaviour is seen (Liley & Seghers 1975).

The Lower Aripo guppies coexist with several dangerous characin and cichlid predators. Of these the cichlid *Crenicichla alta* is most often used in laboratory studies of anti-predator behaviour (which is very pronounced in Lower Aripo guppies) (Liley & Seghers 1975; Magurran & Seghers 1991). *C. alta,* another cichlid *Aequidens pulcher,* and the several characin fish (*Astyanax bimaculatus, Hemibrycon dentatum, Corynopoma riisei, Steindachnerina argentea,* and *Hoplias malabaricus*) are the major dangerous piscivores preying on guppies. Some other fish are present: *Hypostomus robinii*: Loricariidae; *Rhamdia* spp: Pimelodidae; and *Synbranchus marmoratus* (Magurran & Seghers 1991). The presence of many visually hunting predatory fish has put strong selection on males to have duller colour patterns, and the population has developed strong schooling and predator inspection behaviour. (Magurran & Seghers 1991)

2.3 Summary of population characteristics

Each population is typified by different male sexual displays and corresponding female mate preference (Houde 1988a). Paria males (low visual predation river) have very bright carotenoid coloration, much melanin pigment but little iridescent pigment. Paria females show strong preference for males with more carotenoid (Houde 1988a, Houde and Endler 1990). In contrast Aripo males (high visual predation river) have very little carotenoid, about the same amount of melanin but much greater iridescent pigment. Aripo females do not show any preference for carotenoid pigments (Houde 1988b). Aripo females prefer different males but it has not been shown which elements in the male display are the most important (Houde 1988b), though there is some evidence that they prefer more colourful males under some circumstances (see section 1.5.2).

2.4 Laboratory conditions

The populations were maintained in 22 litre aquaria under a 12:12 hr light:dark regime, and fed Tetramin tropical fish food *ad libitum.* Each tank had aeration and filtration of the "biofilter" sponge type; no gravel or under-gravel filtration was used in any tank. The tanks were illuminated with white fluorescent tubes and temperature was kept at an average 25 degrees but there was variation between 21 and 27 degrees Celsius. Light intensity was about 80 lumens at surface level - far less bright than tropical sunshine (similar to dawn or dusk - the ideal conditions for maximum guppy sexual activity). The light cycle is close to conditions in the wild, but temperature variation is less. This should ensure minimal variation due to accidental environmental effects.

The new environment of dechlorinated London tap water was actually similar to their natural habitat in that mountain stream water is very "hard" i.e. containing many minerals and slightly alkaline with a Ph of around 7.5. The stream water in the wild is very clear (Endler 1991) - therefore the aquaria were treated with a commercial algicide periodically (Algaway). This also helps standardise diet as guppies may obtain some carotenoid intake from algae (after Kodric-Brown 1989). One quarter of the water in each aquaria was replaced weekly plus a standard dose of water conditioner (Tetrasafe) to neutralize chlorine and toxic heavy metal ions and to provide a protective colloidal coating to fish fins and gills.

For healthy aquaria and to provide cover (lack of it in confined laboratory conditions causes stress) each tank contained Java moss *Vesicularia dubyana.* Java moss is a free floating filamentous plant. Its presence, though unlike natural conditions (virtually no plants occur in Trinidad mountain streams, even algae is rare or absent - see section 1.5.3a), reduced stress on fish (particularly in the wary Lower Aripo guppies). In addition, when breeding up stocks young fish used the moss as cover to avoid predation by adults. Broods used in experimentation were collected from breeding traps to prevent the mother or other adults consuming fry.

2.5 Inbreeding scheme

Experimental populations were set up after 5-6 generations in the laboratory. Several virgin females were isolated with single males. Individual broods were collected. Male offspring were removed as soon as there was evidence of sexual differentiation. Offspring were collected over 8 weeks. This collection period was deliberately kept short so that the offspring used in the experiments were a cohort of similar ages.

Pairs of brothers were used as sires, one for the inbred line the other for a matched control. See Figure 2.2 (Aripo) and Figure 2.3 (Paria) for pedigree diagrams. Most male colour pattern genes of large effect are *Y*-linked (Houde 1988b). So standardising the *Y* chromosome across pairs of lines limited the effect of inbreeding to autosomal genes. Each male was placed in a separate aquarium and mated to five virgin females. Males and females were matched for age (6 months). In the inbred line the 5 females were full-sibs to the male. In the control line the 5 females were also full-sibs to eachother but from a family unrelated to the male. A total of 6 matched lines were established, 4 from the Aripo population and 2 from the Paria. The fecundity of Paria females was low so the breeding scheme was repeated for a second generation. Further pairs of males were selected, one from each inbred and control line. Inbred males were mated to 5 sisters and control males were mated to 5 sisters from an unrelated brood. Thus the second generation of the Paria inbreeding pedigree repeated the control design and *further* inbred using the inbred offspring of the previous generation. The design using 5 sisters to each male was intended to remove dam-family effects between unrelated females (shown to be strong in one case - Nicoletto 1995, and small in another - Houde 1992). In all but one case only one foundress in each line produced offspring, so this concern turned out to be of less relevance. The inbreeding coefficients in the inbred lines were F=0.25 in Aripo and F=0.25-0.31 in Paria.

2.6 Data collection

All offspring of inbred and control males were photographed on both sides at 14 weeks. At this age male colour patterns had developed. Males were photographed at the same age as coloration continues to develop through time (Endler, 1987). Photography was done under standard lighting conditions with a

Kodak 18% reflectance grey background. Each guppy was placed in a small plastic spectrophotometer cuvette which had optically clear sides. Anaesthetic was not used as it alters colour patterns (Reynolds et al. 1993, Endler 1978). Each fish was confined for a maximum of thirty seconds before release, and all fish were removed from their tanks for the same total amount of time to standardise the effect of stress on the appearance of pigments (which are partially under voluntary control). Some melanic areas can be maximised for display and minimised when threatened, or on a pale background - (personal observation; Brooks & Caithness 1995b) and can vary dramatically over a time scale of minutes (Baerends *et al.* 1955). Being confined and photographed is a relatively stressful experience and all males would show their minimum melanic area. Therefore the time taken with this procedure was standardised for all fish.

These images were transferred to a Macintosh computer using a black and white CCD camera. Lengths and areas were measured using NIH Image. The resolution power of this system was limited by the pixel dimension of the screen (average fish dimensions 547x210 pixels). Boundaries of colour areas were visible on the captured black and white images. Where contrast was poor the boundaries were pinpointed using the original colour photograph.

Several morphological characters and secondary sexual characters were measured in each male guppy. Morphological characters measured were (Fig. 2.1): total length, body length, maximum depth, total area (including the tail fin), body area (excluding the tail fin), dorsal fin area, caudal fin area and gonopodial length. One paired morphological trait was measured, the length of ten dorsal scales (on left and right sides). The measurement of male colour pattern was broken down into pigment groups: carotenoid (orange), melanin (black) and iridescent (purple, green, yellow, blue and silver). The total area of the pigment groups on each side of the body was estimated. The boundaries between different iridescent colours were too faint to allow each colour area to be measured separately. Pigment areas on the tail and dorsal fin were ignored as they are visible from both sides and thus can not be considered as paired characters. In addition, fins (especially tails) were not always fully spread in photographs (c.f. Reynolds *et al.* 1993) as is typical of non-displaying males. Besides, previous results have shown that tail colours yield similar results to body colours (Reynolds *et al.* 1993). Every measurement was done twice to estimate measurement error. In addition the number and position of spots on each body side was recorded. Spot position was measured by the distance from the x.y centre of the spot area to both the tip of the nose and vertically to the top of the fish. A group of older males (photographed at >5 months) were measured in the same way.

More primitive colour analysis was employed for earlier experiments (see chapters 5 and 6). A grid composed of 1mm square units was used to measure body area and pigment areas for males used in the preliminary trials on display and female choice.

2.7 References

Baerends, G.P., Brouwer, R. & Waterbolk, H.T. 1955. Ethological studies on *Lebistes reticulatus* (Peters). Behaviour 8:249-334.

Brooks, R. & Caithness, N. 1995b. Manipulating a seemingly non-preferred male ornament reveals a role in female choice. Proc. R. Soc. Lond. B., 261, 7-10.

Endler, J.A. 1978. A predator's view of animal colour patterns. Evol. Biol. 11: 319-364.

Endler, J.A. 1983. Natural and sexual selection on colour patterns in poeciliid fishes. Environ. Biol. Fish 9: 173-190.

Endler, J.A. 1987. Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces:Poecilidae). Anim. Behav., 35, 1376-1385.

Endler, J.A. 1991. Variation in the appearance of guppy color patterns to guppies and their predators under different visual conditions. Vision. Res. 31(3): 587-608.

Fajen, A. and Breden, F. 1992. Mitochondrial DNA sequence variation among natural populations of the Trinidad guppy, *Poecilia reticulata*. Evolution, 46, 1457-1465.

Gilliam, J.F., Fraser, D.F. & Alkins-Koo, M.A. 1993. Structure of a tropical stream fish community: a role for biotic interactions. Ecology 74 (6): 1856-1870.

Houde, A. E. 1988a. Genetic difference in female choice between two guppy populations. Anim. Behav., 36, 510-516.

Houde, A. E. 1988b. The effects of female choice and male-male competition on the mating success of male guppies. Anim. Behav., 36, 888-896.

Houde, A. E. 1992. Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces: Poecilidae) (guppies). Heredity, 69, 229-235.

Houde, A. E. and Endler, J. A. 1990. Correlated evolution of female mating preferences and male colour patterns in the guppy *Poecilia reticulata.* Science, 248, 1405-1408.

Kodric-Brown, A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. Behav. Ecol. Sociobiol., 25, 393-401.

Liley, N.R. & Seghers, B.H. 1975. Factors affecting the morphology and behaviour of guppies in Trinidad. In: Function and evolution in behaviour. Eds Baerends, Beer & Manning. Clarendon Press, Oxford. Pp 92-118.

Magurran, A.E. & Seghers, B.H. 1990. Risk sensitive courtship in the guppy *(Poecilia reticulata).* Behaviour 112: 194-201.

Magurran, A.E. & Seghers, B.H. 1991. Variation in schooling and aggression amongst guppy *(Poecilia reticulata)* populations in Trinidad. Behaviour 118: 214-234.

Nicoletto, P. F. 1995. Offspring quality and female choice in the guppy, *Poecilia reticulata.* Anim. Behav., 49, 377-387.

Reynolds, J. D., Gross, M. R., and Coombs, M. J. 1993. Environmental conditions and male morphology determine alternative mating behaviour in Trinidadian guppies. Anim. Behav., 45, 145-152.

Rosen & Bailey 1963 Rosen, D.E. & Bailey, R.M. 1963. The poeciliid fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. Bull. Amer. Mus. Nat. Hist. 126, 5-176.

Seghers, B.H. 1974. Schooling behaviour in the guppy (*Poecilia reticulata*): an evolutionary response to predation. Evolution 28: 486-489.

Figure 2.1

Standard morphological measurements of a male guppy

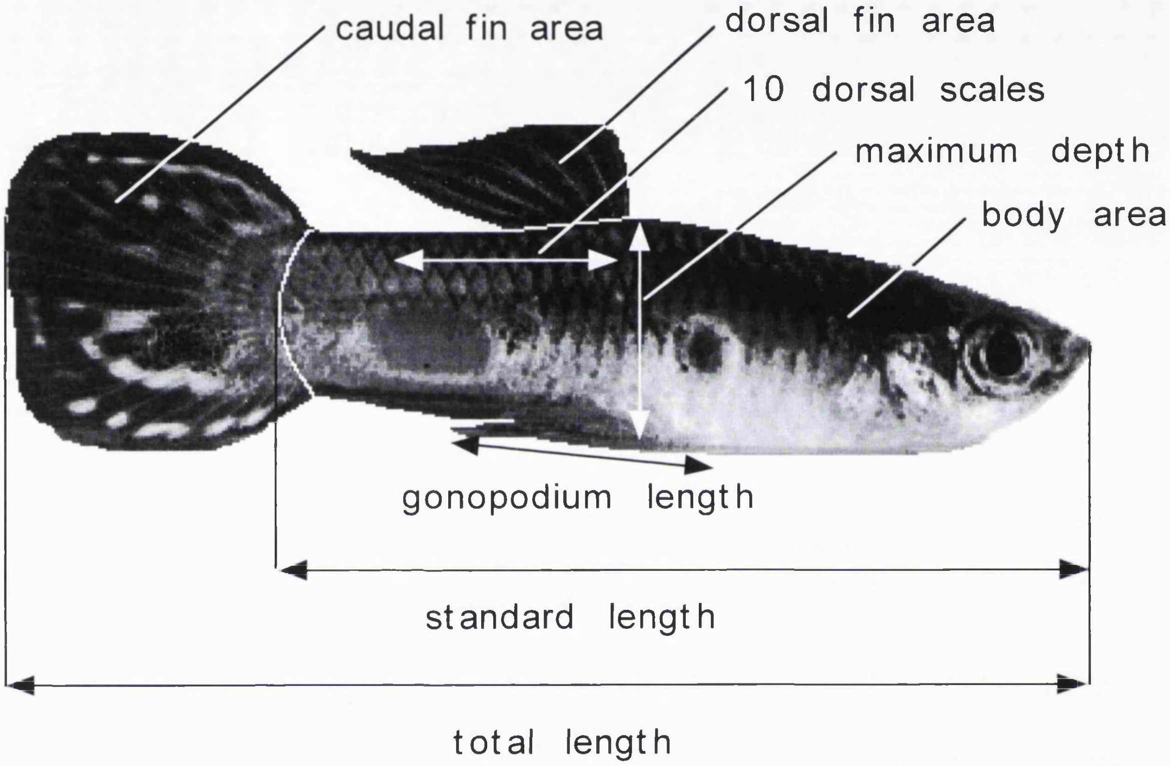
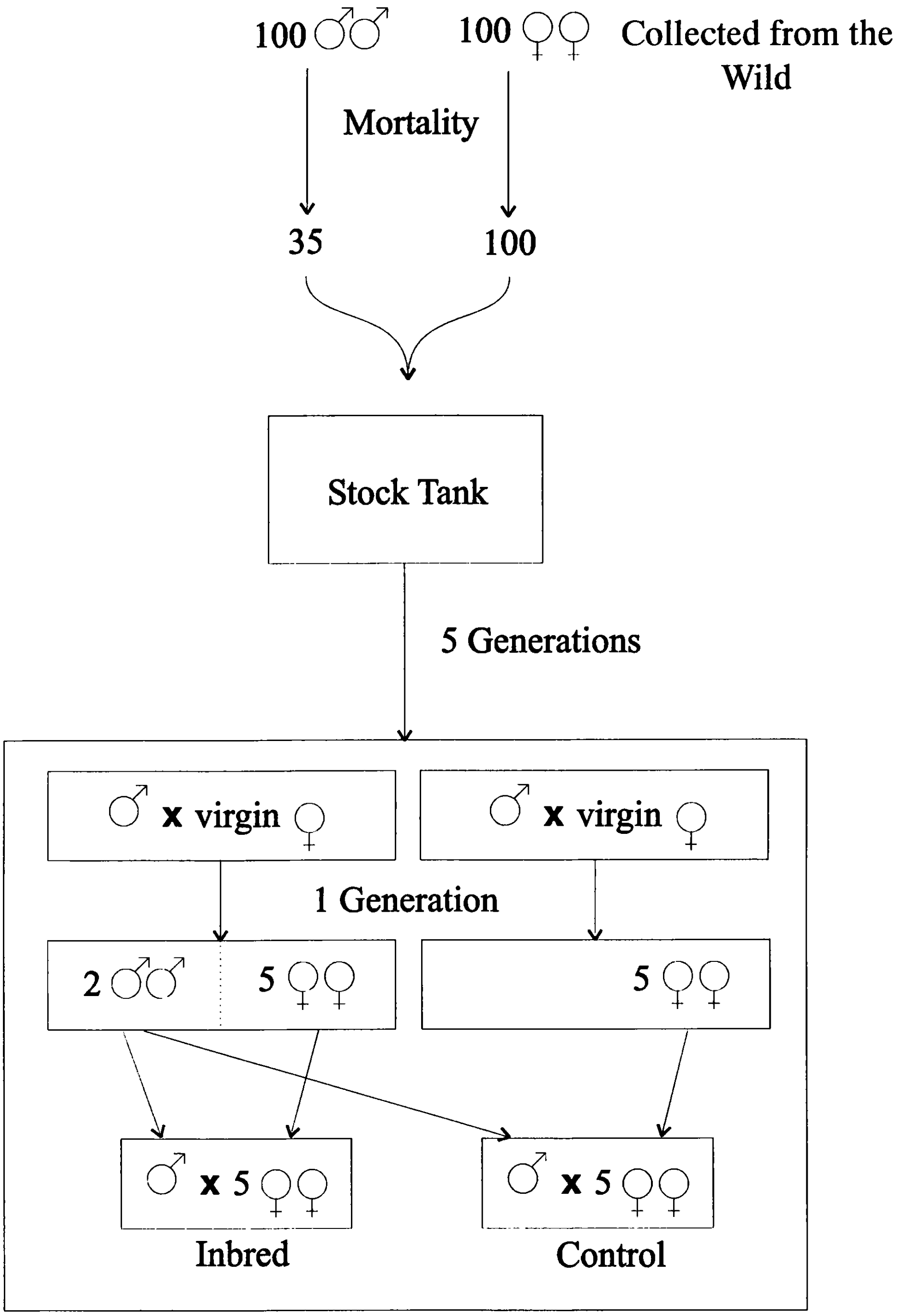
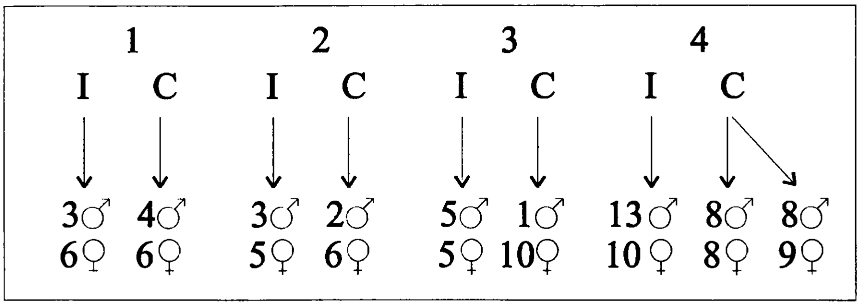


Figure 2.2 :
Inbreeding Pedigree

Aripo



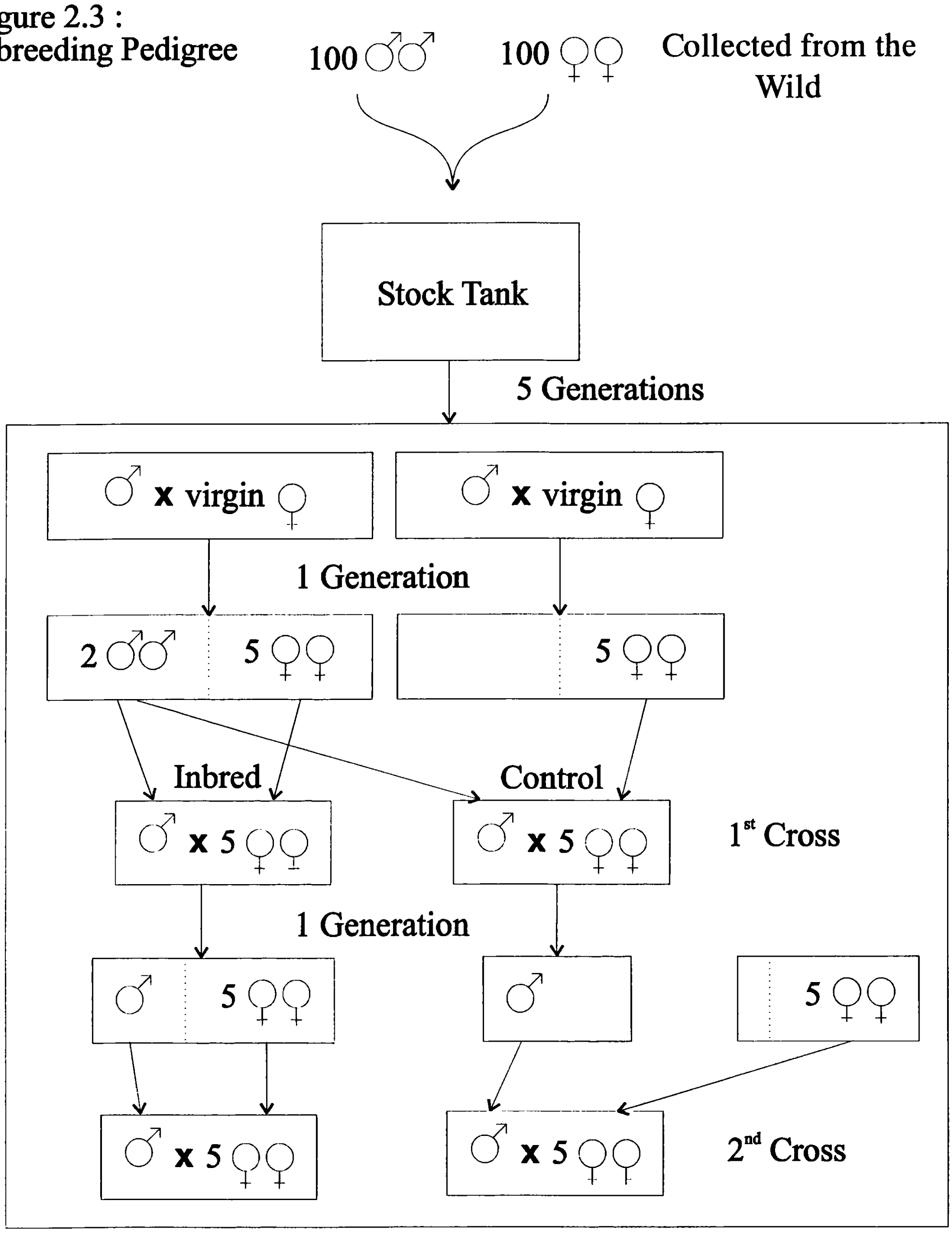
4 Replicates of these paired lineages
(inbred and control) were produced



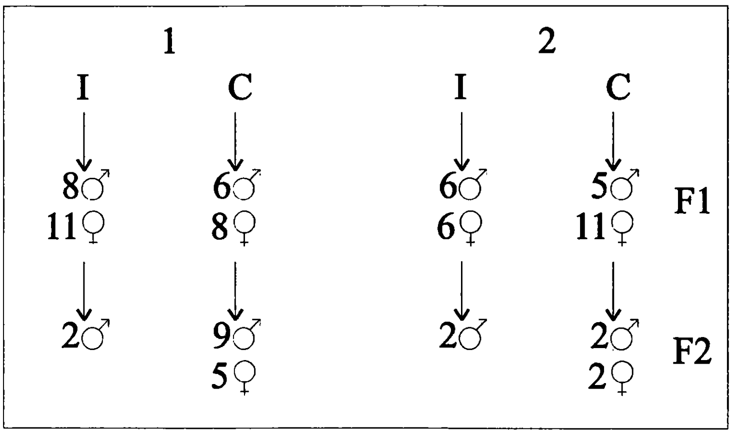
Progeny production of each lineage
(Note second female producing offspring in line 4)
See text in chapter 2

Paria

Figure 2.3 :
Inbreeding Pedigree



2 Replicates of these paired lineages
(inbred and control) were produced



Progeny production of each lineage in
F1 and F2 generations

Chapter 3: Fluctuating asymmetry

3.1 Summary

Fluctuating asymmetry and inbreeding depression in the sexual coloration of male guppy fish.

This chapter tests the hypothesis that fluctuating asymmetry in exaggerated sexual traits is a sensitive indicator of genetic stress. Inbreeding was used to create genetic stress in two populations of male guppies collected from the Aripo and Paria river basins in Trinidad. Fluctuating asymmetry in the size of carotenoid, melanin and iridescent pigments did not differ between inbred and control males. The lack of response was not due to inbreeding having no effect on the male phenotype. Inbred Aripo males had less melanin pigment and Paria males had less carotenoid pigment. These results show that sexual coloration in guppies is sensitive to genetic stress caused by inbreeding. But they provide no support for the hypothesis that genetic stress is reflected particularly well by the degree of fluctuating asymmetry. The different response to inbreeding in the two populations is interesting. There was inbreeding depression in the area of carotenoid pigment in the Paria population where this characteristic is known to influence female mate choice, whereas there was no inbreeding depression in the Aripo population where females show no detectable preference for carotenoid pigments.

3.2 Introduction

It is widely thought that fluctuating asymmetry (FA) is a sensitive indicator of developmental stress (Soulé 1982, Parsons 1990, Møller and Pomiankowski 1993). Both genetic (e.g., inbreeding, hybridisation, spread of new mutants) and environmental (e.g., pH, temperature, pollutants) stresses are thought to lead to increased FA. This has lead to the promotion of FA as a useful measure of well-being at both the individual and population levels and the proposal that FA may be a useful proxy for fitness (Clarke 1995) See further discussion in the Introduction : section 1.4.

The recent revival of interest in fluctuating asymmetry is in large part due to novel applications in sexual selection. In a number of papers Møller and others have argued that exaggerated secondary sexual characters differ from other traits as they i) have much higher mean levels of FA and ii) often show a negative correlation between FA and trait size (Møller 1990, Møller and Höglund 1991). The latter relationship is taken as evidence supporting the 'good genes' hypothesis. It has been suggested that males with larger sexual traits are high quality individuals because they appear able to absorb the costs of producing larger ornaments without loss of developmental control. This hypothesis is particularly interesting as it has been shown that females prefer males with symmetric sexual traits (Møller 1992), a result confirmed in model systems (Swaddle and Cuthill 1994a, 1994b).

This study addressed a more basic consideration. Does genetic stress caused by inbreeding elevate the degree of FA in sexual traits? Inbreeding is expected to cause stress by increasing the frequency of homozygous, deleterious, recessive alleles (Charlesworth and Charlesworth 1987). Evidence that inbreeding increases FA in non-sexual traits is inconsistent. Several studies have reported that population level FA correlates positively with homozygosity in natural populations (Soulé 1979, Leary *et al.* 1984) and is higher under controlled inbreeding (Mather 1953, Reeve 1960). However, the few carefully controlled and well-replicated experiments have failed to show any association between inbreeding and FA (Clarke *et al* 1992, Fowler and Whitlock 1994). None of these experiments have examined genetic stress in sexual traits. Sexual traits are of interest because they are often highly exaggerated and costly to produce (Andersson 1994) and thus might be more sensitive to genetic stress. They are often extravagant traits bearing highly intricate patterns and vivid coloration. Many sexual ornaments are known to be subject to strong directional selection arising from female choice (or male competition) (see section 1.2). In cases where exaggeration has gone to extremes, sexual traits are likely to be very costly to produce and maintain and may be close to the limits of production (Møller & Pomiankowski 1993). Sexual traits in many species(see section 1.3) and particularly in guppies (section 1.5.3) can be extremely costly. For this reason it is expected that sexual traits will be particularly sensitive to stress (such as inbreeding).

The effect of inbreeding was studied in the sexual coloration of male Trinidadian guppies. These fish are well suited for this investigation as males have prominent sexual pigments which females use in mate choice decisions (Endler 1980; see section 1.5.1). Furthermore, male colour patterns and female mate preference vary geographically (Houde and Endler 1990) allowing inbreeding effects to be compared between different populations. The area of carotenoid, melanin and iridescent pigments was calculated for each side of the body (see chapter 2 for inbreeding scheme, methods of photography and analysis). This allowed FA and mean area for each pigment group to be compared between inbred and control males.

Three hypotheses were explicitly addressed. First, did inbreeding lead to an increase in FA in male sexual colour patterns? Second, was FA a more sensitive measure of inbreeding than other phenotypic measures? This question was addressed by measuring whether increases in FA due to inbreeding were more easily detected than reductions in the size of male colour pigment area. Third, were male traits that are preferred by females more sensitive to inbreeding than other sexual traits? This hypothesis was tested by comparing the effects of inbreeding in two guppy populations that have distinct male colour patterns and distinct female mate preferences. If the particular patterns of each population have evolved to provide females with information about male condition, then it would be expected that preferred traits might show increased sensitivity to stress than less important traits. If all pigments are Fisherian traits unconnected with viability then no difference would be expected. In the case of these populations, Paria females show strong preference for carotenoid pigment. Aripo females do not prefer carotenoid, though they do prefer males with more pigment (i.e. melanic and structural iridescent for this population). Therefore the reaction of carotenoid versus other pigments in the two populations might show interesting results.

3.3 Methods

3.3.1 Measuring size

All sexual and morphological traits were examined for evidence of inbreeding depression. This provides a useful comparison with the effects of inbreeding on FA. Mean trait size was compared between inbred and control lines for each population. All of the morphological traits were normally distributed and thus needed no transformation. The colour pigment areas had a skewed distribution, with smaller values being rare. Left and right measures were square-root transformed after the Box-Cox test revealed that this was an appropriate power transformation. Transformed measures were normally distributed. No transformation was required for the left and right dorsal scale measurements. Data from the different lines were pooled and analysed in an ANOVA. This compared the variance within and between inbred and control fish. The ANOVA also compared the variances within and between different lines - if these were different then lines were analysed separately (all showed the same trends as the pooled data from all lines); when there was no significant line effect (in the majority of all tests) it was considered justified to use pooled data. Line effects were tested for in all analyses.

3.3.2 Measuring fluctuating asymmetry

Fluctuating asymmetry of the three colour pigment groups ($\sqrt{}$ transformed) and the single paired morphological trait (10 dorsal scales) were compared between the inbred and control populations for the Paria and Aripo populations. Fluctuating asymmetry was estimated using a two-way, mixed-model ANOVA (sides = random, treatment = fixed), with two measurements of each side (Palmer and Strobeck, 1986). The ANOVA measures population FA as the variance between left and right sides. It also allows directional asymmetry and measurement error to be estimated.

Individual signed asymmetry scores (mean R - mean L) were also examined for the four traits in each population. Individuals were excluded if they lacked any pigment on left or right sides. There was no evidence that the mean level of the remaining signed asymmetry differed from zero. The Kolmogorov-Smirnov test revealed no evidence for antisymmetry, that is broad-peaked or bimodal departures from normality.

The colour pigment data were transformed in two ways to take account of possible confounding effects. To control for correlation with trait area, relative FA was estimated by dividing left and right measures by the mean trait area for each individual. To control for correlation with body area, %FA was estimated by dividing measurements by the body area of each individual and taking a percentage. Transformed data were processed in the same way as before to derive population measures for relative FA and %FA. These transformations are justifiable when scaling exists. So we checked whether the unsigned asymmetry (|mean R - mean L|) in each trait scaled linearly with mean trait area or body area, and whether these relationships intercept at the origin (Cuthill *et al.* 1993). All statistical analyses were carried out using *JMP* (SAS Institute Inc. 1996).

It should be noted that the analysis of male guppy colour patterns was not as straightforward as the estimation of FA above suggests. A high proportion of male guppy pattern elements did not occur in the usual paired manner common to

FA work. Often males showed spots that were in completely different positions on either side, or missing completely on one side. There are no agreed techniques for the analysis of such variable patterns. Individuals which had missing spots were left out from the analysis of FA and size. The amount of fluctuating asymmetry in individuals with missing spots can be several orders of magnitude greater than those with paired pigment spots (similar to the size of many artificial manipulations of FA). If such individuals had been included they would have severely distorted the analysis. These estimates of population FA thus only relate to the small differences between left and right sides, and the measurement of size only relates to those individuals with pigment on both sides. The individuals displaying this different level of asymmetry are dealt with in Chapter 4.

3.4 Results

3.4.1 Morphological characters

There was only weak evidence of inbreeding depression in the morphological traits (Table 3.1). Control fish generally had larger trait values in the Aripo but the reverse was true in the Paria. Any relationship was obscured in part by the large variation between lines. The only significant inbreeding depression was in caudal fin area of the Aripo population. But given the large number of tests, this contrast was not significant after making a sequential-Bonferroni adjustment (Sokal and Rholf 1995). In the Paria population there was no evidence of inbreeding depression.

3.4.2 Directional asymmetry and measurement error

There was no evidence of directional asymmetry for any pigment colour in either population. None of the comparisons of variance due to side compared to non-directional sources of variance were significant (all $P>0.05$; technique in Palmer and Strobeck 1986).

All measurements were carried out twice to allow measurement errors to be estimated. Measurement errors associated with pigment areas were small (mean <1%). *F*-tests comparing the estimate of population FA to measurement error revealed that FA made a significant contribution to the variance observed (melanin, carotenoid and iridescent in both populations $P<0.05$). But measurement errors associated with the 10 dorsal scales were considerably larger (mean ~ 3%). It was much harder to accurately pinpoint where scales began and ended. The estimate of population FA relative to the measurement error was not significant for 10 dorsal scales in the Aripo control ($F_{[22,46]}=1.196$, $P>0.05$) or Paria control ($F_{[18,38]}=1.84$, $P>0.05$) populations, so comparison of FA in dorsal scales between inbred and control populations was not possible.

3.4.3 Fluctuating Asymmetry

For none of the three colour patterns was there evidence that inbreeding increased FA (Table 3.2). This was true for both Paria and Aripo males. The lack of any increase in FA in inbred populations persisted when relative FA and %FA were used (Table 3.2). Controlling for trait area was justified for melanin in Aripo males (positive relationship, $P=0.020$) and controlling for body area was justified for carotenoid in Aripo males (negative relationship, $P=0.035$). All other relationships with trait area and body size were weak and not significant. The only significant difference between inbred and control populations was in the 'wrong' direction, with the control population having greater FA (Aripo population, iridescent ÃFA $F_{[22,19]}=3.44$, $P<0.01$ and iridescent %FA $F_{[22,19]}=3.33$, $P<0.01$).

3.4.4 Pigment areas

Inbreeding depression for pigment area occurred in both populations. Comparisons were made using ANOVA, removing the effect of line. Inbred Aripo males had less melanin pigment than control males (inbred 1.30±0.05, control 1.61±0.06, $P<0.001$) and this difference remained significant when body area was taken into account (Fig. 3.1). There was no effect of inbreeding on iridescent or carotenoid pigments amongst Aripo males (all $P>0.05$). Inbred Paria males had reduced carotenoid pigment area and this relationship was very strong once body area was taken into account (Fig. 3.1). There was no inbreeding depression in melanin or iridescent pigment areas. See Table 3.3.

See Appendix 2 for breakdown of variation in pigment areas and fluctuating asymmetry between families. In the Appendix all offspring of a particular pair of brothers are referred to as a "tribe". In all but one case only one brood per sire was obtained so there was no need to separate broods within tribes.

3.4.5 Brood size

The sizes of inbred versus control broods is summarised in Table 3.4. There seems no reason to expect that (given enough broods) they would not come from a normal distribution; therefore unpaired *t*-tests were used to compare the sizes of inbred and control broods. No significant difference was found (all $P>0.4$; see Table 3.5) though the large variation makes it unlikely that a significant result could be obtained without many more broods to compare; the trend is for inbred broods to be smaller as expected.

3.5 Discussion

3.5.1 Does genetic stress elevate the degree of FA in sexual traits?

The first question addressed in this study was whether the genetic stress caused by inbreeding increases fluctuating asymmetry in sexual traits. FA was measured in three colour pigment areas (melanin, carotenoid and iridescent) from two populations of guppies (from Aripo and Paria river basins). These pigments form part of the male's sexual display and are not present in females. None of the traits in either population showed increased FA under inbreeding (Table 3.2). In addition no inbreeding effect on FA was observed when controlling for mean pigment area or body area. These results follow similar outcomes from experimental studies in *Drosophila* (Fowler and Whitlock 1994) and bees (Clarke *et al* 1992) using non-sexual traits. However this study and that by Fowler and Whitlock (1994) only considered mild degrees of inbreeding (one or two generations of sib-mating) and bees are unusual as they are haplodiploid and so will have been purged of deleterious recessives. It is possible that responses in symmetry would only be found following severe inbreeding.

3.5.2 Is FA a more sensitive measure of inbreeding than other phenotypic measures?

The results of this experiment suggests that the claim that FA is a particularly sensitive measure of genetic stress (Parsons 1990) can be rejected. Whilst there was no effect of inbreeding on FA, there were detectable differences between inbred and control lines in a number of other traits. The size of pigment areas were smaller in inbred males (Fig. 3.1 & Table 3.3). The effects were marked in the Aripo population where inbred individuals had smaller melanin pigment areas (and considerable effects on spot numbers - see Chapter 4). The evidence was more equivocal for the Paria population except that inbreds had smaller carotenoid pigment areas. The difference between the populations is most likely a reflection of the greater sample size and number of inbred lines of the Aripo population; though there is the possibility that it reflects the differences in importance to mate choice. The brood size data did not strongly show inbreeding depression (unlike the results of Fujio and Nakajima (1992) who found high mortality among offspring of full sib matings); a possible explanation is that this study actually examined inbred and *less inbred* guppies (rather than inbred and outbred) due to chance inbreeding under laboratory conditions (in stock tanks). However the detection of inbreeding depression in other characters implies that inbreeding stress was detectable, and that the trend for smaller inbred broods would probably be significant despite the large variation if more lines had been used.

These results serve two purposes. First they show that inbreeding did cause detectable phenotypic differences in some aspects of male sexual coloration. Hence the absence of a response in FA to inbreeding can not be attributed to failure of the inbreeding regime to cause genetic stress. Second they revealed that FA is less sensitive to mild inbreeding than are other measures. It is interesting to note that asymmetry in pigment size (i.e. FA) did not show any response to inbreeding, unlike measurements of pigment area. Standard measurements of size appear to be more sensitive indicators of inbreeding than does asymmetry. This comparison of sensitivity is valid as measurements were made on the same samples using similar statistical procedures. But this does not lead to the conclusion that inbreeding cannot cause an increase in FA. Such a response might well be detectable in studies with larger sample sizes or harsher genetic stress.

3.5.3 Are preferred traits more sensitive to inbreeding than other sexual traits?

The third question raised concerned female choice and whether it was directed at traits that are good at reflecting inbreeding. These findings are in general agreement with the hypothesis. Female preference in the Paria population is strong for carotenoid but non-existent or weak for melanin or iridescent pigments (Houde 1988a). This was paralleled by the distribution of inbreeding depression, which was strongly detected in the area of carotenoid pigment when overall body size was controlled for. There was no inbreeding depression in the other two pigment groups.

Less is known about female mate preferences in the Aripo population. It is clear that females do not have strong preference for carotenoid (like other guppy populations) but evidence on other preferences is conflicting (see section 1.5.2). Some studies have shown strong non-random mating but no information on what colour pattern elements are important (Houde 1988a). Others have shown preferences for the more colourful males (Breden & Hornaday 1994); unless the bright alternative *model*is moving (Stoner & Breden 1988); while another study showed a preference for the duller model (Breden & Stoner1987). Endler (1983) suggested that females prefer greater numbers and area of iridescent and melanic spots.

In the Aripo population inbreeding depression was associated with melanin area but not with carotenoid. There was a strong trend for inbred males to show less iridescent area but this was not significant (though there was an effect on spot numbers - see Chapter 4). There is evidence that Aripo females prefer more colourful males - in this population that applies almost entirely to melanic and iridescent pigments as carotenoids are so insignificant. Though it must be remembered that the very fact that carotenoids are small and pale in Aripo males may be the explanation for the lack of measured effect in this pigment. Overall these findings are suggestive that female preference is directed at male traits that provide information about male genetic quality. But this conclusion can not be strongly held owing to the insufficient knowledge about female preference in the Aripo population.

3.6 References

Andersson, M. B. 1994. Sexual Selection. Princeton University Press, Princeton, New Jersey.

Breden, F. & Hornaday, K. 1994. Test of indirect models of selection in the Trinidad guppy. Heredity 73: 291-297.

Charlesworth, D. and Charlesworth, B. 1987 Inbreeding depression and its evolutionary consequences. Ann. Rev. Ecol. Syst. 18, 237-268.

Clarke, G. M. 1995. Relationships between developmental stability and fitness: applications for conservation biology. Conserv. Biol. 9, 18-24.

Clark, G. M., Oldroyd, B. P. and Hunt, P. 1992. The genetic basis of developmental stability in *Apis mellifera*: heterozygosity versus genic balance. Evolution, 46, 753-762.

Cuthill, I. C., Swaddle, J. P. and Witter, M. S. 1993. Fluctuating asymmetry. Nature, 363, 217-218.

Endler, J. A. 1978. A predator's view of animal colour patterns. Evol. Biol., 11, 319-364.

Endler, J. A. 1980. Natural selection on colour patterns in *Poecilia reticulata* . Evolution, 34, 76-91.

Endler, J.A. 1983. Natural and sexual selection on colour patterns in poeciliid fishes.

Fajen, A. and Breden, F. 1992. Mitochondrial DNA sequence variation among natural populations of the Trinidad guppy, *Poecilia reticulata*. Evolution, 46, 1457-1465.

Fowler, K. and Whitlock, M. C. 1994. Fluctuating asymmetry does not increase with moderate inbreeding in *Drosophila melanogaster*. Heredity, 73, 373-376.

Fujio, Y & Nakajima, M. 1992. Estimation of genetic load in guppy population. Nippon Suisan Gakkaishi - Bulletin of the Japanese Society of Scientific Fisheries 58(9): 1603-1605.

Houde, A. E. 1988a. Genetic difference in female choice between two guppy populations. Anim. Behav., 36, 510-516.

Houde, A. E. 1988b. The effects of female choice and male-male competition on the mating success of male guppies. Anim. Behav., 36, 888-896.

Houde, A. E. and Endler, J. A. 1990. Correlated evolution of female mating preferences and male colour patterns in the guppy *Poecilia reticulata.* Science, 248, 1405-1408.

Houde, A. E. 1992. Sex-linked heritability of a sexually selected character in a natural population of *Poecilia reticulata* (Pisces: Poecilidae) (guppies). Heredity, 69, 229-235.

Leary, R. F., Allendorf, F. W. and Knudsen, L. K. 1984. Superior developmental stability of heterozygotes at enzyme loci in salmonid fishes. Am. Nat., 124, 540-551.

Mather, K. 1953. Genetic control of stability in development. Heredity 7, 297-336.

Møller, A. P. 1990. Fluctuating asymmetry in male sexual ornaments may reliably reveal male quality. Anim. Behav. 40, 1185-1187.

Møller, A. P. 1992. Female swallow preference for symmetrical male sexual ornaments. Nature, 357, 238-240.

Møller, A. P., and Höglund, J. 1991. Patterns of fluctuating asymmetry in avian feather ornaments: implications for models of sexual selection. Proc. R. Soc. Lond. B, 245,1-5.

Møller, A. P. and Pomiankowski, A. 1993. Fluctuating asymmetry and sexual selection. Genetica 89, 267-279.

Nicoletto, P. F. 1991. The relationship between male ornamentation and swimming performance in the guppy, *Poecilia reticulata*.. Behav. Ecol. Sociobiol., 28, 365-370.

Nicoletto, P. F. 1995. Offspring quality and female choice in the guppy, *Poecilia reticulata.* Anim. Behav., 49, 377-387.

Parsons, P. A. 1990. Fluctuating asymmetry: An epigenetic measure of stress. Biol. Rev., 65,131-145.

Palmer, A. R., and Strobeck, C. 1986. Fluctuating asymmetry: Measurement, analysis, patterns. Ann. Rev. Ecol. Syst., 17,391-421.

Reeve, E. C. R. 1960. Some genetic tests on asymmetry of sternopleural chaeta number in *Drosophila*. Genet. Res.Camb. 1,151-172.

Reynolds, J. D., Gross, M. R., and Coombs, M. J. 1993. Environmental conditions and male morphology determine alternative mating behaviour in Trinidadian guppies. Anim. Behav., 45, 145-152.

SAS Institute Inc. 1996. JMP Statistical Discovery Software. Version 3.1.6. SAS Institute Inc., Cary, NC, USA.

Sokal, R. R. and Rholf, F. J. 1995. Biometry. 3rd edition,W. H. Freeman & Co., New York.

Soulé, M. E. 1979. Heterozygosity and developmental asymmetry: another look. Evolution, 33, 396-401.

Soulé, M. E. 1982 Allomeric variation 1. The theory and some consequences. Am. Nat., 120, 751-764.

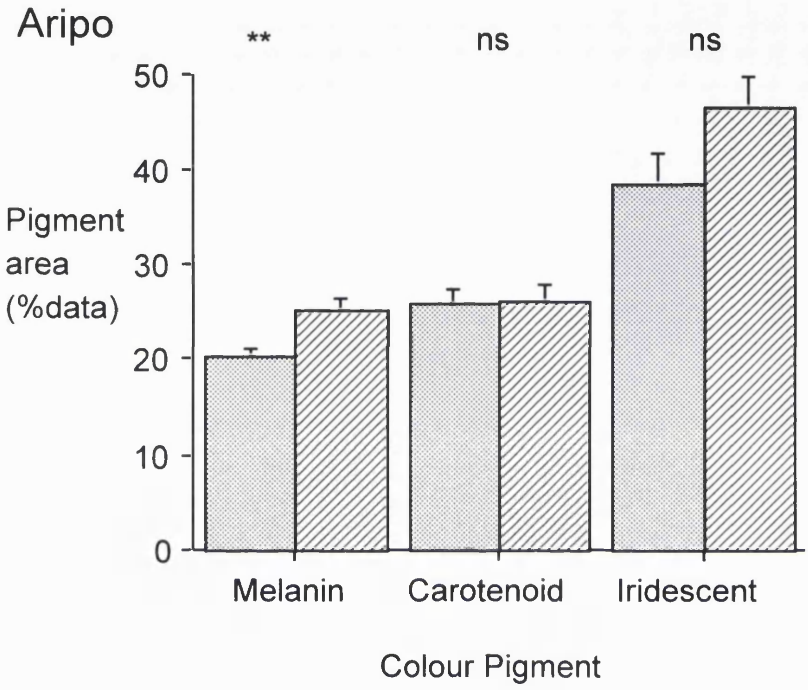
Stoner, G. & Breden, F. 1988. Phenotypic differentiation in female preference related to geographic variation in predation risk in the Trinidad guppy (*Poecilia reticulata)*. Behav. Ecol. Sociobiol. 22: 285-291.

Swaddle, J. P. and Cuthill, I. C. 1994a. Female zebra finches prefer symmetric males. Nature, 367, 165-166.

Swaddle, J. P. and Cuthill, I. C. 1994b. Female zebra finches prefer males with symmetrically manipulated chest plumage. Proc. R. Soc. Lond. B, 258, 267-27

3.7 Tables and figures

Figure 3.1 Mean pigment areas expressed as a percentage of body area for inbred (stipple) and control (cross-line) populations, ** $P<0.01$.



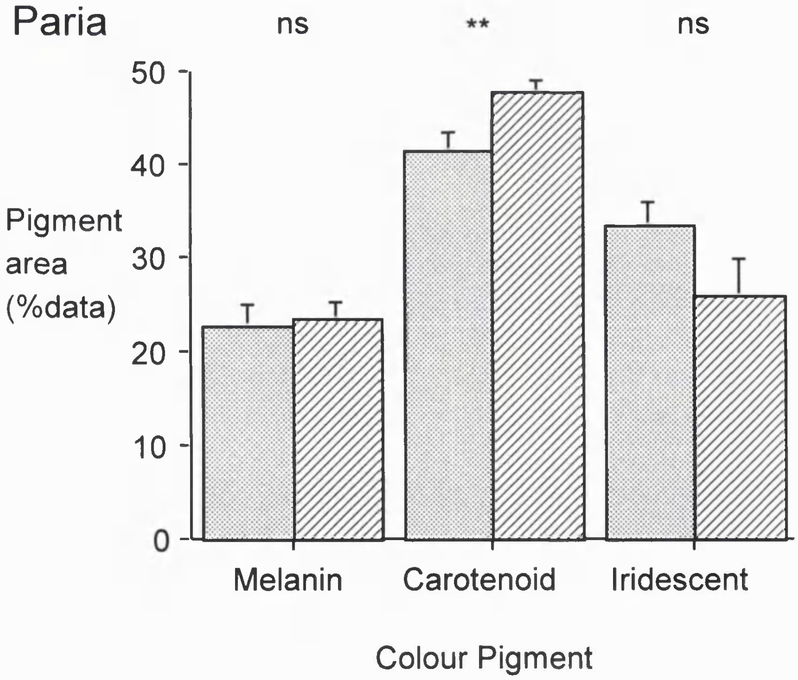


Table 3.1 Means and standard errors for morphological traits (untransformed data - mm & mm^2). All traits were normally distributed and comparison was made controlling for line, * $P<0.05$.

Pop	Measurement	Inbred		Control		P value
		N	mean±se	N	mean±se	
Aripo	Body length	24	14.851±0.240	22	14.996±0.175	0.261
	Total length	24	20.254±0.356	22	20.554±0.236	0.128
	Maximum depth	24	3.553±0.048	22	3.531±0.058	0.993
	Gonopodial length	24	3.523±0.085	22	3.433±0.129	0.506
	Caudal fin area	24	19.188±0.734	22	21.043±0.669	0.044 *
	Dorsal fin area	24	4.779±0.296	22	5.490±0.240	0.142
	Body area	24	41.551±1.211	22	42.019±0.942	0.329
	Total area	24	65.477±1.862	22	68.553±1.590	0.099
	10 dorsal scales	24	4.188±0.077	22	4.211±0.071	0.571
Paria	Body length	14	15.328±0.271	19	14.882±0.309	0.268
	Total length	14	21.786±0.388	19	20.312±0.502	0.025 *
	Maximum depth	14	3.801±0.060	19	3.675±0.087	0.255
	Gonopodial length	14	3.816±0.115	19	3.871±0.124	0.832
	Caudal fin area	14	21.652±0.897	19	19.663±1.047	0.151
	Dorsal fin area	14	6.635±0.389	19	6.536±0.497	0.881
	Body area	14	45.829±1.529	19	43.502±1.709	0.305
	Total area	14	74.116±2.171	19	69.701±3.068	0.252
	10 dorsal scales	14	4.347±0.104	19	4.240±0.109	0.524

Table 3.2 Estimates of population level FA. Inbred and control values are given for √ transformed FA (√mm^{2}x100) for the three colour areas. *F* ratios were calculated to test whether inbreeding increased fluctuating asymmetry for √ FA, relative FA and %FA. No differences were significant at the $P<0.05$ level

Population	Trait	Inbred FA		Control FA		F ratio P value		
		N	FA (√ data)	N	FA (√ data)	√ FA	relative FA	% FA
Aripo	Melanin	24	2.211	23	2.256	0.98 ns	1.30 ns	0.98 ns
	Carotenoid	24	0.527	23	0.615	0.86 ns	0.79 ns	0.82 ns
	Iridescent	20	1.679	23	5.742	0.29 ns	0.88 ns	0.30 ns
Paria	Melanin	14	2.504	18	2.596	0.96 ns	0.90 ns	0.73 ns
	Carotenoid	14	5.359	19	2.708	1.98 ns	2.16 ns	1.99 ns
	Iridescent	9	5.497	8	1.895	3.45 ns	1.82 ns	2.96 ns

Table 3.3 Means and standard errors of pigment areas (√ transformed mm^2) for inbred and control populations. Comparisons of inbred and control figures are given for √ transformed data and pigment area relative to body area (% data). (* $P<0.05$ ** $P<0.01$).

Population	Pigment	Inbred Area		Control Area		*P* value	
		N	mean±se (√ data)	*N*	mean±se (√ data)	(√data)	(% data)
Aripo	Melanin	24	1.304±0.054	23	1.605±0.055	0.0002	0.0018
	Carotenoid	24	1.676±0.0	23	1.674±0.095	0.702	0.746
	Iridescent	20	2.442±0.187	23	2.969±0.174	0.061	0.114
Paria	Melanin	14	1.549±0.150	18	1.523±0.115	0.918	0.795
	Carotenoid	14	2.798±0.124	19	3.138±0.107	0.055	0.0075
	Iridescent	9	2.206±0.194	8	1.708±0.206	0.043	0.063

Table 3.4 Inbred and control brood sizes

	Aripo					Paria		
	Tribe 1	Tribe 2	Tribe 3	Tribe 4	Total Aripo	Tribe 5	Tribe 6	Total Paria
Inbred males	3	3	5	13	24	8	6	14
Control males	4	2	1	8/8 (2 broods)	23	6	5	8
Inbred m+f	8	8	10	23	49	19	12	31
Control m+f	10	8	11	16/17	62	14	16	30
2nd Gen. m Inbred	-	-	-	-	-	2	2	4
2nd Gen. m Control	-	-	-	-	-	9	2	11
2nd Gen Inbred m+f	-	-	-	-	-	2	2	4
2nd Gen. Control m+f	-	-	-	-	-	14	4	18

m= males f= females

Table 3.5 Comparisons of inbred and control fecundity

Group	*t*	*P*	*N*
Aripo males only	-0.59	0.955	8
Aripo total	0.472	0.654	8
Paria males only	0.480	0.648	8
Paria total	0.656	0.536	8

Table 3.5 shows the results of unpaired *t*-tests for differences in brood size between inbred and control guppies, first using males only, then both males and females. In the Paria both generations were used, though there was no difference to the results if either generation were tested separately.

Chapter 4: Spot asymmetry

4.1 Summary

This chapter tests the hypothesis that guppy sexual coloration may respond to inbreeding stress in other ways than the degree of FA or pigment area. Chapter 3 described the analysis of fluctuating asymmetry which showed no response to inbreeding stress. Other facets of colour pattern were also measured: the number of discrete colour spots and whether spots were located in the same or different positions on each side. This chapter describes the separate analysis of colour pattern, comprising data on total spot numbers for each pigment group and also the number of unpaired spots. The latter will be called spot asymmetry.

This allowed the response of FA, size, spot number and spot asymmetry to be compared between inbred and control males. Like FA, spot asymmetry also showed no response to inbreeding. However spot number and iridescent spot colours showed an effect of inbreeding depression. Inbred Aripo males showed fewer melanin spots and fewer iridescent sub-spots and colours; Inbred Paria males did not differ from control males. This difference between the populations may reflect female preferences - Aripo females have been shown to prefer greater numbers of spots. It is interesting that this element of colour pattern shows sensitivity to stress.

4.2 Introduction

Inbreeding depression is expected to affect costly sexually selected traits more severely than non-sexual traits. The reasons to expect this have already been discussed - sexual traits are usually exaggerated, costly and subject to directional selection arising from female choice (Møller & Pomiankowski 1993). Sexual traits which are *not* costly on the other hand, (Fisherian or aesthetic traits) are not predicted to show this relationship; though an increase in variance might be expected. Inbreeding has been shown to be deleterious in guppies, in laboratory populations. Beardmore & Shami (1979) demonstrated that individuals exhibiting low heterozygosity at various isozyme loci had shorter lifespans, exhibited the most extreme numbers of caudal fin rays (an indication of reduced homeostatic abilities) and were less fecund; (but see section 1.4.2 for drawbacks of isozyme data). Another study used inbreeding to estimate genetic load, and found mortality up to ten times higher in offspring of full-sib mating compared to random mating (Fujio & Nakajima 1992). This suggests a large amount of mildly deleterious recessive genes. When successive generations of full-sib mating were produced, offspring mortality decreased rapidly. This suggests that the most marked effects of inbreeding depression can be seen in the first generation of inbreeding. This chapter describes the effects of first generation full-sib mating on colour pattern.

There is evidence that female guppies are not simply concerned with the amount of pigment areas in male colour patterns. The distribution of those pigments into discrete spots can also be important; both certain patterns are preferred (Houde 1988) and greater total spots (in addition to total area) are preferred (Endler 1983). Pattern complexity (numbers of spots and colours) has also been shown to be important (Endler 1980, 1983; Nicoletto 1993)(also see Chapter 5). Symmetrical spot patterns are also preferred (Chapter 5). This implies that the response of spot number and distribution to inbreeding is worthy of investigation.

The pigmentation of sexual displays is markedly different from normal body pigmentation. For example the melanophores found in the pigment calls of typical teleost scales are a stellate shape and respond to chemical stimulation (e.g. epinephrine) to aggregate or disperse melanin granules. These are typically used by fish for camouflage - body ground colour can be considerably lightened or darkened by the movement of these melanin granules to blend in with their surroundings. In contrast, the melanophores from scales in guppy black spots lack any distinct shape and do not respond to chemical stimulation (Dickman 1987). These form the permanent spots of male display (though they are also partially under voluntary control as males may enhance the area of spots for display (personal observation; Brooks & Caithness 1995; Baerends *et al.* 1955)). There is some evidence that pattern complexity is condition dependant - as Nicoletto (1993) found a correlation between overall ornament complexity and prolonged swimming performance. This leads to the prediction that pattern complexity may be expected to show a response to inbreeding stress, in the form of reduced numbers of spots and/or colours. There is also the possibility that this effect could be more marked in Lower Aripo males than Paria males, due to the fact that Aripo males normally have more spots, and females prefer greater numbers of spots (Endler 1983; Chapter 5); whereas Paria females show strong preference for carotenoid area instead. The hypothesis that symmetry at the level of spot pattern may be a sensitive indicator of stress can also be tested by measuring spot locations.

These questions were addressed by measuring whether inbreeding produced reductions in the number of spots or colours, or changes in the distribution of colour spots on left and right sides (i.e., spot asymmetry). This could then be compared to the results on FA, colour area and morphological traits (i.e. different types of inbreeding depression) to see if these different aspects of pattern and symmetry were more sensitive to stress than other measures.

4.3 Methods

4.3.1 Measurement of spot asymmetry

A definition of spot symmetry is needed. A high proportion of male guppy pattern elements did not occur in the usual paired manner common to FA work. I term this “spot asymmetry”. Though there may be continuous variation from paired spots through overlap of varying degrees to "unpaired" spots, the large differences found with missing spots require a separate analysis of symmetry at the level of pattern. This may be simply an extreme example of FA; spot symmetry is considered separately merely to try to see if more information can be found by considering pattern. Fish with missing spots occupy a different distribution of symmetry scores to those with small deviations between paired spots (the usual measures of FA), and therefore it is useful to apply a different analysis to examine the significance of pattern symmetry.

FA refers to small random deviations in paired traits where the normal state is symmetry. Spot asymmetry here refers to unpaired characteristics. Colour pigments were concentrated into a variable numbers of spots. Spot shapes differed and sometimes spots were missing from one side (Fig. 4.1). Around half the males had one or more of their melanin or carotenoid spots missing or in a totally different position on the left and right sides.

There are no agreed techniques for the analysis of such variable patterns. Individuals which had missing spots were left out from the analysis of FA and size. The amount of fluctuating asymmetry in individuals with missing spots can be several orders of magnitude greater than those with paired pigment spots. To bring individuals with missing spots into the analysis, comparison of spot positions by image manipulation was required. To quantify side variation in spot position and number the computer pictures of the left and right sides were manipulated so they were aligned in the same direction, one under the other (Fig. 4.1). This allowed easy assessment of relative spot position by eye. A spot was scored as matched if it overlapped with spots on the other side. Spots that did not overlap were scored as unmatched for each of the three pigments - melanin, carotenoid, and structural. In addition, this analysis was repeated for iridescence at the level of the component iridescent colours (see below). The number of unmatched iridescent sub-spots and the number of unmatched iridescent sub-colours (colours present on one side) were calculated.

4.3.2 Measuring spot number

Total spot number was compared in inbred and control lines. This was done for each pigment colour on each side. Iridescent pigment was broken down into its components, blue, yellow, green, purple and silver. These were termed "sub-colours" and "sub-spots" to make them distinct from the three main pigment groups - melanic, carotenoid and structural iridescent (the term "iridescent spot" refers to the presence of structural colours on one side of a fish; the term "iridescent sub-spot" refers to a particular structural colour spot within that). The total number of iridescent sub-spots and sub-colours was counted (see Chapter 2 for methods and definitions).

As the distribution of spot numbers was not normal and not continuous, measurements were treated as ordinal variables. Data from different lines were pooled and analysed using ordinal logistic regression, taking out the effect of line. Total numbers of spots of all three pigments were compared between both populations using Spearman's rank correlation test. Only control guppies were used to compare differences between populations, to avoid confounding effects of inbreeding depression on pigments. All statistical analyses were carried out using *JMP* (SAS Institute Inc. 1996).

4.4 Results

4.4.1 Spot number

Inbreeding depression was revealed in the number of pigment spots. Inbred Aripo males had significantly fewer spots than control males (Fig. 4.2). This was accounted for by marked reductions in the number of melanin and iridescent sub-spots in inbred males. There was also a less pronounced reduction in the number of iridescent colours. Carotenoid spot number showed no response. There were no differences in spot numbers between inbred and control Paria males (all $P>0.05$).

4.4.2 Spot asymmetry

The amount of spot asymmetry differed between pigments (Table 4.1). The number of melanin spots on each side was highly variable and about one-third of males had at least one unmatched melanin spot (40% Aripo, 30% Paria). In contrast, nearly all males had equal numbers of carotenoid spots on left and right sides and very few spots were unmatched (6% Aripo, 3% Paria). In neither population was there any substantial iridescent spot asymmetry. Only one individual in the Aripo and only four in the Paria had unmatched iridescent spots (i.e. no structural colours measurable on one side). Despite this variation, none of the colour pigments showed any difference in spot asymmetry between inbred and control males in either population (Table 4.1). There was no evidence for any pigment that the number of unmatched spots correlated closely with the total number of spots (all Spearman $\rho<0.34$, $P>0.05$), so controlling for this parameter was not necessary.

4.4.3 Comparison of spot numbers between populations

Differences in spot numbers between the two populations paralleled the differences in pigment area. See Table 4.2. Both populations have similar areas of melanic pigment, while carotenoid area is much larger in the Paria guppies, and iridescent area is much larger in Aripo guppies. The spot distribution is much the same - there is no difference between numbers of melanic spots between populations (Spearman $\rho=-0.246$, $P=0.12$), but Paria guppies have more carotenoid spots (Spearman $\rho=0.546$, $P=0.0002$) and Aripo guppies have more iridescent subspots (Spearman $\rho=0.521$, $P<0.0001$). In terms of total spot number (including iridescent subspots), Aripo males have significantly more spots overall (Spearman ρ=0.47, P=0.001).

4.5 Discussion

This chapter describes the analysis of a different order of symmetry at the level of colour pattern that has not previously been studied. As a result there are no agreed techniques. Previous FA work has only compared the size of paired characters. These experiments concerned unpaired characters. In guppies a form of symmetry involving entirely missing and misplaced colour spots is very common in some pigment groups. This is a different order of magnitude to the small deviations in paired traits that are called FA. Spot asymmetry was analysed separately to determine possible effects of inbreeding. No inbreeding effect was visible in spot symmetry in either population.

However, in Aripo guppies, the numbers of spots and colours decreased in inbred males. Overall ornament complexity (increasing numbers of spots and colours) has been shown to be both preferred by females (particularly in Aripo guppies - see Chapter 5; also Endler 1983) and condition dependent (Nicoletto 1993). This also applies to pigment areas - Paria females prefer large carotenoid area, which is reduced by inbreeding; and Aripo females prefer more colorful males (which in this population refers almost entirely to melanin and iridescence, as carotenoid areas are so insignificant) - and the areas of both melanin and iridescent colours are reduced by inbreeding. It is interesting that inbreeding depression is associated with reductions in preferred traits. The lack of response of spot numbers in Paria guppies may be relevant to the lesser importance of spot numbers to females in this population; or it may simply be an artifact of smaller sample size. It would be unexpected if a reduction in numbers of colours showed up in Paria guppies - as they show very little iridescence normally and it is in these that variation in colour number occurs (all Paria guppies have melanin and carotenoid).

So, to sum up, the effects of inbreeding depression are marked in Aripo males in melanin area and spot number, and number of iridescent spots and colours; Aripo females prefer all these traits to be exaggerated. Inbreeding depression in Paria guppies is only detected in carotenoid area; this is the only reported preference in Paria guppies. Overall these findings are suggestive that female preference is directed at male traits that provide information about male genetic quality. But this conclusion can not be strongly held without further experimentation. More information about whether Paria females prefer increasing pattern complexity or not would be helpful, as would information on correlations between spot numbers and condition (see Chapter 7).

These experiments again provide no support for the hypothesis that symmetry is a better indicator of stress than other traits. Neither asymmetry in pigment size (ie, FA) nor asymmetry in the number and position of pigment spots showed any response to inbreeding, unlike measurements of pigment area and pigment spot number. Standard measurements of size appear to be more sensitive indicators of inbreeding than does asymmetry. The fact that inbreeding depression was detected in other traits suggests that sufficient stress for the experiment to succeed was generated by mild inbreeding. Both aspects of symmetry were less informative measures of stress than measures of size and pattern.

There are reasons to be cautious about interpretation of these results. The analysis is based on a small sample with only a few lines per population. Line affects were controlled for statistically but it would have been better to have had a greater level of replication. This constraint is particularly severe in the Paria population where data were also combined across two generations because of low fecundity. Ideally numerous lines should be used where each may be used as a single data point. However, the hypothesis that symmetry is a more sensitive stress indicator than other traits is still undermined by the finding that inbreeding depression could be detected in other traits but not any measure of symmetry.

4.6 References

Baerends, G.P., Brouwer, R. & Waterbolk, H.T. 1955. Ethological studies on *Lebistes reticulatus* (Peters). Behaviour 8:249-334.

Beardmore, J.A. & Shami, S.A. 1979. Heterozygosity and the optimum phenotype under stabilising selection. Auilo Ser. Zool. 20: 100-110.

Brooks, R. & Caithness, N. 1995. Manipulating a seemingly non-preferred male ornament reveals a role in female choice. Proc. R. Soc. Lond. B., 261, 7-10.

Dickman, M.C. 1987. Effects of age on skin pigmentation of the guppy (*Poecilia reticulata)*. Biological Bulletin 173: 421-422.

Endler, J.A. 1980. Natural selection on colour patterns in *Poecilia reticulata*. Evolution, 34, 76-91.

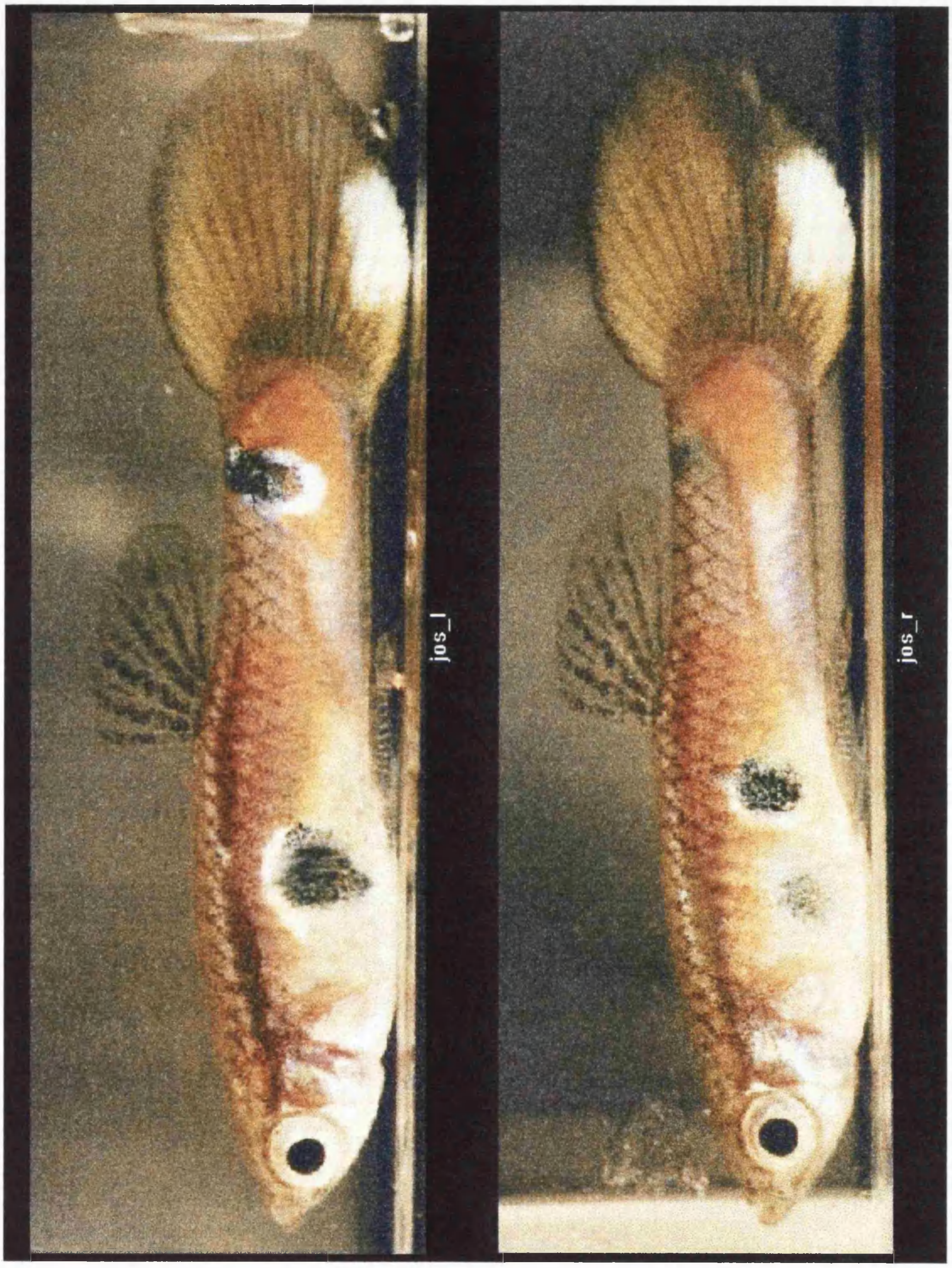
Endler, J.A. 1983. Natural and sexual selection on colour patterns in poeciliid fishes. Environ. Biol. Fish 9: 173-190.

Fujio, Y & Nakajima, M. 1992. Estimation of genetic load in guppy population. Nippon Suisan Gakkaishi - Bulletin of the Japanese Society of Scientific Fisheries 58(9): 1603-1605.

Houde, A. E. 1988. The effects of female choice and male-male competition on the mating success of male guppies. Anim. Behav., 36, 888-896.

Møller, A.P. & Pomiankowski, A. 1993. Fluctuating asymmetry and sexual selection. Genetica 89, 267-279.

Nicoletto, P.F. 1993. Female sexual response to condition-dependent ornaments in the guppy *Poecilia reticulata.* Anim. Behav. 46: 441-450.



4.7 Tables and figures

Fig. 4.1 Spot asymmetry was assessed by comparing right (upper) and left (lower) sides of an individual. This male has a different number of melanin spots on each side (2 left and 1 right) and each spot is unmatched in terms of location.

Fig. 4.2

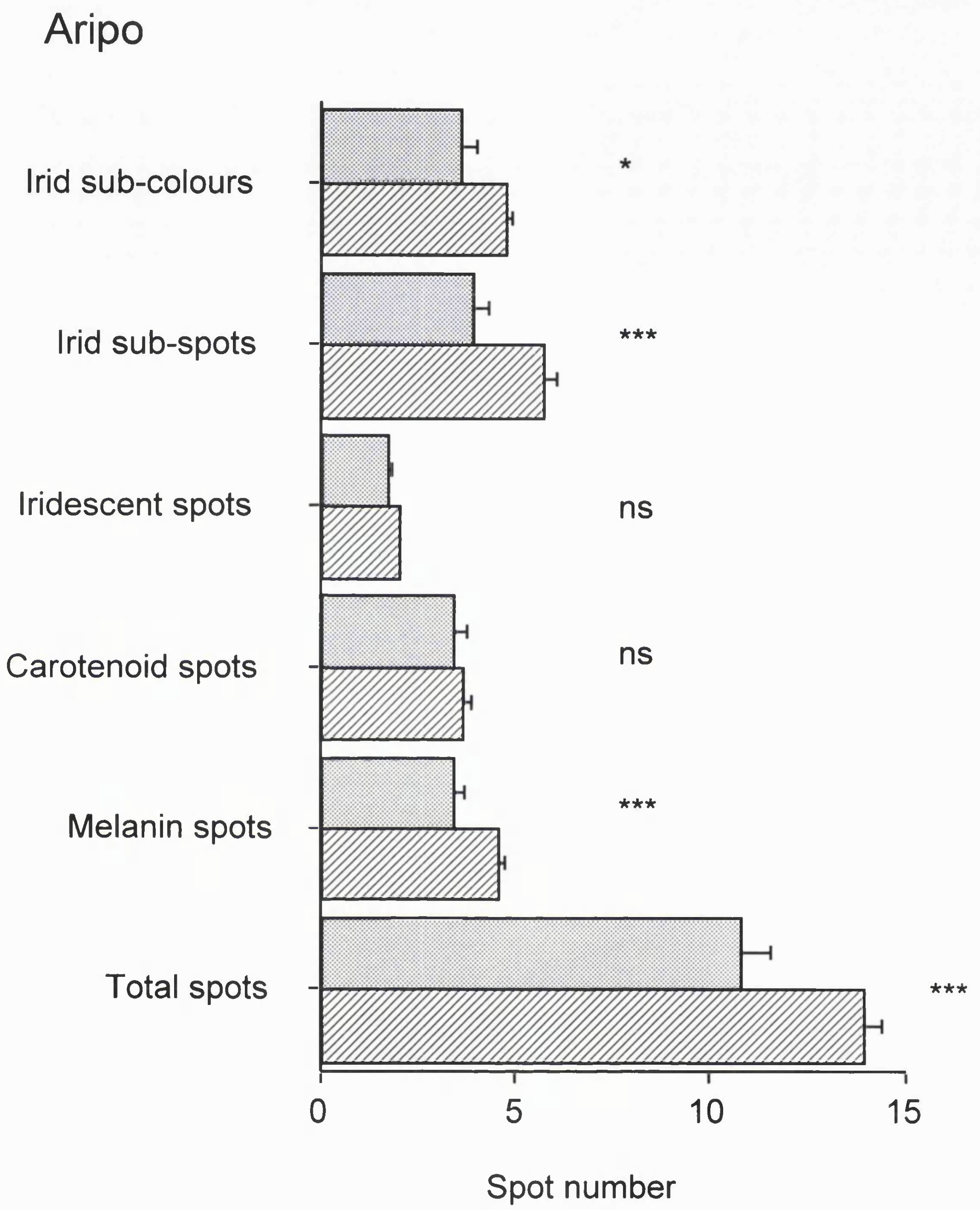


Fig. 4.2 Spot number summed over left and right sides in inbred (stipple) and control (cross-line) Aripo population, * $P<0.05$, *** $P<0.001$.

(Paria population showed no differences in inbred and control spot numbers.)

Table 4.1 Spot asymmetry in inbred and control populations. Comparison controlling for line.

Population	Pigment	Inbred Unmatched Spots		Control Unmatched Spots		P value
		N	mean±se	N	mean±se	
Aripo	Melanin	24	0.542±0.120	22	0.500±0.157	0.546
	Carotenoid	24	0.042±0.042	22	0.0±0.0	0.970
	Irid sub-spots	24	0.583±0.133	22	0.182±0.084	0.057
	Irid sub- colours	24	0.333±0.130	22	0.091±0.063	0.087
Paria	Melanin	14	0.429±0.173	19	0.368±0.113	0.998
	Carotenoid	14	0.214±0.114	19	0.053±0.053	0.213
	Irid sub-spots	14	0.357±0.133	19	0.263±0.104	0.539
	Irid sub-colours	14	0.214±0.114	19	0.105±0.072	0.383

Table 4.2 Spot number summed over left and right sides in inbred and control populations. Comparison controlling for line, * $P<0.05$, *** $P<0.001$.

Population	Pigment	Inbred Spot Number		Control Spot Number		*P* value
		N	mean±se	*N*	mean±se	
Aripo	Total spots	24	10.67±0.76	22	13.91±0.47	<0.001 ***
	Melanin spots	24	3.38±0.31	22	4.55±0.19	<0.001 ***
	Carotenoid spots	24	3.42±0.33	22	3.64±0.25	0.549
	Iridescent spots	24	1.71±0.14	22	2.00±zero	0.959
	Irid sub-spots	24	3.88±0.46	22	5.73±0.36	<0.001 ***
	Irid sub-colours	24	3.58±0.43	22	4.73±0.20	0.024 *
Paria	Total spots	14	11.64±0.76	19	10.11±0.78	0.130
	Melanin spots	14	4.14±0.42	19	3.42±0.49	0.148
	Carotenoid spots	14	5.21±0.24	19	4.63±0.32	0.219
	Iridescent spots	14	1.43±0.23	19	0.95±0.22	0.108
	Irid sub-spots	14	2.29±0.53	19	2.05±0.43	0.919
	Irid sub-colours	14	2.79±0.55	19	1.68±0.38	0.085

Chapter 5: Female choice

5.1 Summary

The effect of spot asymmetry on female mate choice was investigated in the Trinidadian guppy (*Poecilia reticulata*). Spot asymmetry arises because whole pattern elements are missing from one side or are located in a different position in either side. Pairs of males were selected that differed in melanic spot asymmetry. In other respects the pair of males were matched for body size, display rate and other aspects of colour pattern. Two experiments were carried out, one in which asymmetry was caused by missing spot elements, the other in which asymmetry was caused by misaligned spots. The latter experiment does not confound differences of pigment area with differences in asymmetry. In both cases females showed strong preference for the symmetric male. This was not sensitivity to fluctuating asymmetry (small differences in the size of spot areas) but to asymmetry at the gross level of spot pattern. Preliminary experiments checked the preferences of the study populations; Paria females showed the expected preference for orange pigment, though not when given choices between larger males; Aripo females showed a preference for males with greater numbers of spots.

5.2 Introduction

Asymmetry in male ornaments has been hypothesised to be widely used in sexual selection (Møller & Pomiankowski 1993). Work on the role of symmetry in mate choice has concentrated on fluctuating asymmetry (FA), a particular kind of asymmetry supposedly generated by small random deviations in the development of paired traits. One of the main reasons for the concentration on FA is the hypothesis that individual level FA reflects individual condition and thus may signal useful information to others (Møller & Pomiankowski 1993)

Several studies have shown that mate choice is sensitive to the degree of FA (Møller and Swaddle 1997). The best known example of this is Møller's work on tail feather asymmetry in the barn swallow which showed that asymmetry as well as trait size had independent effects on mate choice (Møller 1992). A few studies of model systems have confirmed that symmetry *per se* is used by females in their mate choice (Swaddle and Cuthill 1994a; Bennett et al. 1996) and natural levels of asymmetry can be detected by females (Swaddle & Cuthill 1994b). However, the amount of individual FA, even in sexual traits, is usually extremely small and it is hard to imagine that such differences can often be used directly in mate choice decisions.

This chapter investigates a further, more gross, form of asymmetry which I term spot asymmetry. Male guppies show variation in colour pattern on each side. This variation can be measured in terms of the total area differences of each colour (i.e. FA - Chapter 3) or at the level of spots into which colours are concentrated (Chapter 4)(Sheridan & Pomiankowski 1997). Whole spots are frequently missing from one side of a male. Asymmetry at the level of the spot is very obvious to the human observer and is in this respect similar to the order of asymmetry created in most studies of artificially manipulated FA (e.g. Møller 1992). This is a first attempt to show whether there is female preference for gross asymmetry in spot pattern, as opposed to FA in the area of colour on each side. It seems intrinsically far more likely that females are able to detect large pattern differences between left and right sides than FA, especially in the guppy which has complex colour patterns. This intuition is supported by the only previous study of symmetry in guppies which reported that there was no female preference for males with less FA in orange or melanin pigments (Brooks and Caithness 1995b).

5.3 Methods

5.3.1 Female choice trial protocol

Choice tests were carried out to uncover female mate preferences. Standard techniques were followed (Kodric-Brown 1985; Houde 1987, 1988a). Females were placed singly in the centre compartment of a aquarium (60 cm x 30 cm x 35 cm) divided into three compartments. Each end compartment was 15 cm wide, with the central female compartment being 30 cm wide to give a large neutral space (20 cm) relative to the "preference" zone within 5 cm of each barrier. Test males were put in each of the two end compartments. Test females were introduced to the middle compartment and allowed to settle for 10 minutes, followed by a 10 minute trial period. Test males were swapped to control for end effects every other trial.

One possible criticism of this female choice method is that two-way choice tests do not directly measure female mating preferences. Males are not allowed to interact with females in a natural way, in order to facilitate observation of female responses. However, females in the partitioned aquaria respond to courting males with the same type of orientation and other behaviours indicating female sexual receptivity as are observed in open field tests. Females respond to males in a sequence of well-defined behaviour patterns 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 towards him (often termed the orient response). Following this, the female may break off the interaction or swim towards the male with a smooth gliding motion using her pectoral fins, often with her tail curved up and to one side exposing her genital pore (the glide response). This smooth gliding motion is very different from the normal jerky swimming motion (Liley 1966). Lastly, in very few cases, the interaction may continue until the female swims in a tight circle exposing her cloaca and accepts copulation (Liley 1966). Data from studies directly measuring mating success have corroborated results from tests measuring only female responses independent of copulation (Bischoff *et al.* 1985; Houde 1987; Kodric-Brown 1993). All data indicates that female responses in partitioned aquaria trials are a good predictor of subsequent mate choice.

Preference was measured by the amount of time a female spent near a particular male. This was defined as the female being within 5 cm of the partition, and oriented towards the male. Females could spend time in the 20 cm neutral which was not scored. It has been shown that close viewing of males in adjoining tanks is sufficient for females to express their mate preferences and is a good predictor of subsequent male mating success (Bischoff 1985, see above). The proportion of displays that elicit at least an orient response reliably indicates male reproductive success (Brooks & Caithness, unpublished data). Therefore measuring the amount of orient response while controlling display rate (see below) gives a reliable measure of male attractiveness. Eight different females were tested with each pair of males to give a reasonable sample of preference levels across populations. 6 pairs of Paria males and 6 pairs of Aripo males were tested. During testing females were kept in an aquarium where they were not able to view the choices of other females.

One of the main determinants of female preference is male display (Farr 1980). To control for differences in display rate any trials in which males differed by more than 3 displays per 10 minutes were excluded from the analysis (after Kodric-Brown 1985). Other studies have already shown that when display rates differ by more this, females will always choose the male with the higher display rate (Farr 1980; though others have not found this relationship, Houde 1987). If a trial had to be discarded due to different display rates between males, then trials with that pair were continued with the same female until controlled trials were obtained for all males with the same set of females.

5.3.2 Preliminary trials

Preliminary trials were carried out to check the preferences of the study populations.

Paria females were tested with pairs of males differing in amount of orange pigment. Three mature test females from mixed sex tanks were chosen at random and presented choices between pairs of males within 1mm of the same body length, differing in orange area by >10% (with similar areas of other pigments). Two size classes were used - 6 pairs of males both <13.5mm and 7 pairs both>16.5 mm standard length. This test was simply to check that the study populations showed the reported orange area preferences of typical Paria guppies.

Another three Paria females chosen at random were presented with 6 pairs of males differing in body (standard) length by >3mm - the smaller showing more orange (10%) as a proportion of body area. The point of this test was to find out if preference for orange area was more important than the size of males. Finally another three females were presented with 6 large pairs of males (>16mm standard length) within 1mm of the same body length, with orange area within 5%, differing in amount of melanic and iridescent colours by>10%. This last test was intended to find any preference for other pigments when orange area was controlled.

Three Aripo females (chosen in the same way) were given choices between males of differing spot numbers. They were presented with 8 pairs of males differing in total spot numbers by >2 spots per side, and within 1.5mm body length. Three other Aripo females chosen at random were presented with choices between males with large differences in body size but similar proportional colour patterns, to try to uncover any effect of male size. 8 pairs of males were used, differing in standard length by >3 mm, with similar proportions of colours..

Cross-populational preferences were then tested. Aripo and Paria females were given choices between males from their own population and males from the other population. 8 pairs of males were matched to within 2.5mm body length, and large differences in total colour areas were avoided (but not colour differences themselves- this was unavoidable as the typical patterns contain very different elements). Three Aripo females and three Paria females were tested with these pairs.

For all these preliminary trials, males were selected from photographs by eye and colour pattern was analysed using a more primitive method than the computer analysis later used. Fish body length was measured by hand (from the photographs) with reference to the standard cuvette size, and colour areas were measured using the numbers of whole squares in a grid of 1mm square units and divided by body length..

5.3.3 Experiment 1: Missing spots

The females used in this experiment were six months old and had previous experience of males. They were taken from mixed sex aquaria at the time of the trials. Virgin females were not used as they are well known to mate indiscriminately (Houde 1988a) and this was confirmed in preliminary trials.

Suitable males were selected by using their photographs (see Chapter 2 for methods) and the computer programme Image. Guppy coloration can be broken down into carotenoid (orange), melanin (black) and structural iridescent (green, blue, purple, yellow, silver) colours. Carotenoid spots were almost never asymmetric. Iridescent spots were sometimes asymmetric, but the boundaries of these spots were very difficult to measure reproducibly and therefore it was difficult to define fish symmetry for iridescence. However melanin spots were both variable in number per side and formed very distinct spots and so allowed an easy assignment of fish to symmetric or asymmetric categories.

A guppy was scored as asymmetric if one side lacked a melanin spot or had a melanin spot in a different, non-overlapping position. Fish were classified as either symmetric or asymmetric. Fish that had spots that only overlapped a little or had fractured spots or some other ambiguity were not selected. Fish without structural colours present were excluded. Fish body lengths and areas were measured using Image.

One symmetric and one asymmetric male were picked for each pair. Pairs of males were matched as far as possible for body length (±1 mm), age (6 months) and general colour pattern. 6 Paria pairs and 6 Aripo pairs were selected. Each pair of males was tested sequentially with the same 8 females in random order (each population had 8 test females). Each pair of males was tested on a different day.

5.3.4 Experiment 2: Unpaired spots

A second experiment was carried out in the same way as the first. This proved to be necessary because overall pigment area was not controlled for in the first experiment. In the first experiment symmetric males tended to have greater melanin pigment areas (summed over both sides) as asymmetric males had missing spots. In effect the first experiment looked at whether missing spots affect mate choice which confounds asymmetry and overall size. The second experiment avoided this problem by using males without missing elements but with spots in different positions.

Twelve pairs of Aripo males were selected. Eight of these pairs satisfied the definition that the asymmetric male's spots were non-overlapping. In addition, four further pairs were included in which the asymmetric male had melanic spots in clearly different positions but slightly overlapping (thus not conforming to the previous definition). These fish were clearly more asymmetric than their matched pairs.

Again males were matched for age and body length (±1 mm, apart from 2 pairs in which the symmetric males were 1.7 mm and 3 mm larger). Only trials in which the males were matched for display rate were used for analysis (as in experiment 1). Females were offered a choice between symmetric and asymmetric males in the same way as in experiment 1. In experiment 2, each pair of males was tested with 3 females in random order. Each pair of males was tested on a different day.

A more extensive set of measurements of male coloration were made to make sure that symmetric and asymmetric males differed only in the amount of melanic spot asymmetry. Total pigment area on each side was measured for carotenoid, melanin and structural iridescent colours. In addition the number of different coloured iridescent spots and number of different iridescent colours was calculated. These were termed "sub-spots" and "sub-colours" respectively (as in Chapter 4) - their numbers were recorded but their individual areas were not measured due to the difficulty of reproducibly determining their boundaries. The amount of individual FA for each colour was calculated as left minus right.

5.3.5 Statistical analysis

Statistical analyses were performed using JMP for Macintosh and JMPIN for Windows software. For all choice experiments ANOVAs were carried out on the difference between the time spent with the symmetric and the asymmetric male, with male pair and trial order as effects. In no case was there any significant difference between pairs; there was a trial order effect in Experiment 2 only (see Section 5.4.3). Preferences were measured by t-tests - females were pooled so they contributed a single data point for each pair of males (mean time difference).

In experiment 2 pigment area, fluctuating asymmetry and morphology were compared using a *t*-test as these traits are normally distributed. The spot traits were not normally distributed and were compared using a non-parametric Wilcoxon matched pairs test.

5.4 Results

5.4.1 Preliminary trials

5.4.1a Paria preferences

As expected, Paria females showed a preference for increased orange area in males of similar body sizes. However, this effect was much stronger when males were small - unexpectedly the preference was much weaker (though still significant) when large males were used (with the same females). The mean time difference (more orange minus less orange) for small males was 178±12.578 (s.e.)($P < 0.0001$, $t=6.66$, $N=6$) and for large males was 26.357±13.830 ($P=0.0243$,$t=2.40$,$N=7$). When given choices between larger, less orange males and smaller, more orange males, females showed no significant difference in the time they spent with either(mean 105.667±16.591, $P=0.1674$,$t=1.45$,$N=6$). When presented with choices between large males of equal orange area but differing in other pigments (i.e. secondary preference for iridescent or melanic pigments), females preferred the males with more iridescent and melanic colours (mean time difference 11.278±30.893, $P=0.0385$,$t=2.27$,$N=6$).

5.4.1b Aripo preferences

Aripo females showed a significant preference for males with more spots when given choices between males of similar size but differing pattern complexity (mean difference 83.708±21.666, *P*=0.0206,*t*=2.50,*N*=8). Spot number was unavoidably correlated with overall pigment area, so these trials could not distinguish between these two effects. Aripo females also showed a preference for larger males (mean difference 134.125±61.923, *P* =0.030, *t*=2.32, *N*=8).

5.4.1c Cross-population preferences

Aripo females significantly preferred males from their own population, but Paria females showed no discrimination between Paria males and alien males. Aripo preference for Aripo males: mean 123.625±20.904 (*P* =0.0189, *t*=2.54,*N*=8); Paria preference for Paria males: mean 79.000±20.621 (*P*=0.630,t=-0.49,*N*=8).

5.4.2 Experiment 1.

In both populations females spent more time on average with the symmetric male (mean±se time difference Aripo 88.54±18.47 seconds, Paria 50.0±9.33 seconds). In all pairs in both populations (6/6 Aripo and 6/6 Paria) females spent more time with symmetrical males (Fig. 5.1). Each population was analysed separately in an ANOVA with male pair and the order in which females were presented as effects. In neither case was there a trial order effect (*P* >0.6) or evidence of heterogeneity between pairs of males (*P* >0.3). Preference was tested with *t*-tests. Both populations showed strong preference for the symmetric male (Aripo *N*=6, *t*=4.39 *P* =0.0001; Paria *N*=6, *t*=4.02, *P* =0.0002).

5.4.3 Experiment 2.

In the repeat experiment controlling for pigment area, females still spent more time with the symmetric males (time difference Aripo 167.36±24.25 seconds). In 11/12 pairs females spent more time with the symmetric male, the one exception was only marginally in favour of the asymmetric male (Figure 5.2). Overall there was strong evidence for female preference for the symmetric male (N=12, t = 5.73, P <0.0001). There was again no evidence for heterogeneity between pairs of males (P =0.735), but there was a trial order effect (P = 0.006). The response in favour of the symmetric male was reduced in the second trial compared to the first and last. It is hard to see this as anything more than noise because females were presented in random order to males and there seems no reason why the female response should have been lower in the middle trial.

An extensive comparison was carried out to make certain that symmetric and asymmetric males differed only in the degree of melanin spot asymmetry (Table 5.1). Pigment area, fluctuating asymmetry and morphology were compared using a t-test as these traits are normally distributed. The spot traits were not normally distributed and were compared using a non-parametric Wilcoxon matched pairs test. There were no phenotypic differences between symmetric and asymmetric males in body size, nor in the amount of any colour group (carotenoid, melanin and structural iridescent) or relative area of colour (compared to body area), nor any measure of fluctuating asymmetry in colour (FA, relative FA or FA as a percentage of body area), nor spot numbers or iridescent sub-spot number.

There were three detectable difference between symmetric and asymmetric pairs. First asymmetric males had a greater number of asymmetric melanic spots (Wilcoxon rank sums test, *P* =0.020, *N*=12). But in addition, the analysis revealed that there was a correlated increase in the number of asymmetric iridescent sub-spots in the asymmetric groups (Wilcoxon rank sums test, *P*=0.016, *N*=12). So, my selection procedure unwittingly distinguished two aspects of male pattern asymmetry. There was also a difference in the number of iridescent structural colours; symmetrical males had more colours (Wilcoxon ranks, *P* =0.047, *N*=12). This was the only difference found in size and colour patterns between the pairs of males apart from the differences in spot symmetry. All other measures of colour area and FA were the same. Any of these differences could potentially explain female preference for the symmetric male. In addition, some other unmeasured variable that differed between the two groups could be causally involved.

5.5 Discussion

Female guppies given choices between pairs of males differing in the symmetry of their melanin spots consistently preferred the more symmetric male. In the first experiment this was demonstrated in two populations of guppies, from the Paria and Aripo rivers, that have very different colour patterns. In this experiment, asymmetric males had a melanic spot missing from one side. However, the experiment was compromised because asymmetric males tended to have less melanin summed over both sides. Melanin pigment area has been shown to be of secondary importance in mate choice in one population (Brooks

& Caithness 1995b). Thus it is possible that the only reason females preferred symmetric males was because they had more pigment area.

To avoid this confounding variable a second experiment was undertaken that standardised overall melanin area. Pairs of males had very similar areas of melanin pigments but asymmetric males had spots in different positions. Under these conditions females still showed a strong preference for the more symmetric male. Tests revealed no significant phenotypic differences between symmetric and asymmetric males apart from spot asymmetry. Both iridescent and melanic spots showed more asymmetry in the less preferred males. In addition the less preferred males had fewer iridescent colours. As this is not a manipulation experiment it is impossible to be certain whether these differences in colour pattern were used by females in mate choice. Females could have used an unmeasured correlated variable. Most of the obvious confounding variables, like age, display rate, body size and colour pattern were controlled for. However the result still needs to be confirmed by manipulation experiments (such as the freeze branding technique used by Brooks & Caithness (1995b)).

There is another caveat that must be drawn. Male guppies were sorted by human observers with extremely different vision to female guppies, the responders to male signals. In particular, guppies can see in the ultraviolet and this UV information is liable to be important in natural situations (Endler 1980, 1987). On a more positive note, as these experiments were only concerned with presence or absence of melanin patches and their size, human perception may be sufficient because the structural changes (presence or absence of melanin deposits) alter the reflectance spectra in ways that both guppies and humans are sensitive to. We can be confident that melanin differences are perceived by guppies (because melanin absorbs a wide portion of the spectrum including the UV) and this was the major obvious difference between males presented to females. Other factors were controlled as far as possible.

The results of the preliminary trials served two purposes. Firstly, they confirm that the study populations showed typical preferences that have been previously demonstrated (e.g. Houde 1988a; Endler 1983; Breden & Hornaday 1994) and thus were representative samples of the wild populations. Paria females showed the expected preference for orange area (though unexpectedly less strongly when given choices between very large males). It is possible that this indicates an influence of male size on mate choice, with large size taking precedence over carotenoid area. It may be more difficult for smaller (often younger) males to obtain large amounts of dietary carotenoids. However when given choices between large and small males differing in orange area, no difference in preference was found. Further experimentation would be needed to demonstrate whether this indicated some influence of male size.

Aripo females strongly preferred males with larger numbers of spots. Unfortunately this cannot be distinguished from the effect of total pigment area, as males with larger spot numbers tended to have greater areas of pigment. Also the possibility that females were making choices based upon other, unmeasured cues cannot be excluded with certainty (and the primitive method of colour pattern analysis in the preliminary experiments must be taken into account). Male size was controlled, as well as age and differences in display rates (also no effect of male size has been demonstrated previously with Aripo females). Also previous findings have shown Aripo preferences for pattern complexity and the more colourful male (Endler 1983; Breden & Hornaday 1994), which implies that this result may be accepted with more confidence.

The Aripo preference for larger males with similar relative amounts of pigment conflicts with Houde 1988b, when no effect of body size could be found. However in that case display rate was not controlled - and as can be seen in chapter 6, this can override other considerations. A female preference for large male size has been shown to provide considerable genetic benefits in other populations (e.g. Reynolds & Gross 1992), due to large fathers siring both sons and daughters with higher growth rates. Female size and fecundity are strongly correlated within populations (Reznick 1983). This all indicates a preference for large male size may be advantageous, especially in populations without strong preferences for particular pigments like the Aripo.

The strong preference of Aripo females for Aripo males over Paria males may reflect the strength of counter-selection in their natural environment against preferences for males that are too bright. Paria females did not strongly prefer their own population males, despite their brightness and orange area. It is possible that this reflects a preference for rare males (c.f. Farr 1980) which would be adaptive for outbreeding; also Paria females are less constrained by predation pressure in the wild to prefer their own male pattern type. There is evidence that female mate preference can be strongly influenced by the brightness of males they grew up with (Breden *et al.*1995) which can be of more influence than their genetic background (Rodd & Sokolowski 1995). These females were raised in the same conditions with conspecifics from their original population, so this in addition to genetic influences would imply that females should prefer familiar to alien males. The difference in strength of preference therefore probably reflects genetic variation in mate preference.

The within-population results serve a second purpose - they are interesting when considered in the context of the results of Chapters 3 and 4. Inbreeding depression was shown in different sexual traits - carotenoid area in the Paria males, and melanic and iridescent area and spot number in the Aripo males. Paria females prefer larger carotenoid area and this is the pigment that showed sensitivity to inbreeding depression. Aripo females prefer greater pattern complexity and/or larger areas of melanic and iridescent, and these pigments showed reduced area and spot numbers. In both populations, preferred traits were the ones that showed sensitivity to stress caused by inbreeding. This is further support for the hypothesis that these traits preferred by females are good indicators of male quality.

Spot asymmetry on the other hand shows no sensitivity to inbreeding depression. Similarly fluctuating asymmetry was not a more sensitive indicator than other traits, as theory predicted, but showed no effect. The female choice experiments demonstrated a preference for males with symmetrical spot patterns. This is a preference for a trait which does not show sensitivity to stress. This is further support for the hypothesis that female mate preference is a subtle process based on a complex set of traits (Kodric-Brown 1993), some of which are condition dependent (e.g. display rate, pigment areas) and some of which are relatively condition-independent (such as spot pattern symmetry). Some cues are more important than others (such as display rate - see Chapter 6) and it is interesting that the more important traits in mate choice are also the traits with the strongest evidence for condition-dependence.

This is the first demonstration that female mate choice is sensitive to asymmetry in the guppy. It should be stressed that this did not show sensitivity to fluctuating asymmetry, the small differences observed in the size of colour spot areas on left and right sides of males. A previous study had already established that there was no female preference for FA in carotenoid or melanin pigments (Brooks & Caithness 1995a). The experiments here compared asymmetry at the level of spots. Spot asymmetry arises because whole pattern elements are missing from one side (experiment 1) or are located in different position (experiment 2). This form of asymmetry is much more obvious than differences in FA (at least to a human observer). The males of experiments 1 and 2 did not differ significantly in FA, only in spot symmetry.

These results confirm what seems obvious - symmetry at the level of pattern should be intrinsically more likely to be detectable than the tiny deviations of FA. Pigments are concentrated into a number of spots, that vary in shape and have non-regular edges. In addition, only one side of a male is displayed to the female at any one time (unlike the situation in many bird sexual displays when both sides of bilateral traits can be viewed simultaneously). It seems unlikely that the tiny deviations from symmetry in pigment area are easily detectable or utilised by female guppies. However the kind of gross asymmetries that are extremely common in male guppies, involving entirely missing pattern elements, would appear to be far easier to detect even when sides are displayed sequentially.

There are a number of perplexing outcomes from this study. First, melanic and iridescent elements of male colour patterns showed high levels of spot asymmetry, whereas carotenoid colours were nearly always symmetrically positioned and did not differ in number per side. It is not clear why this should be the case or whether it reflects something interesting about the development of coloration. Second, the amount of spot asymmetry found in male colour patterns was great. Given that this study shows that females use this as a cue in mate choice, why has not this selection led to a reduction in asymmetry? Third, why do females prefer males with symmetric spot patterns? It has been hypothesised that females gain information about male condition using FA (Møller and Pomiankowski 1993). However the previous experiment did not show that FA or spot asymmetry in male guppies were particularly sensitive to genetic stress induced by inbreeding (Sheridan and Pomiankowski 1997; see Chapters 3 and 4). As yet the function of mate choice for spot asymmetry in the guppy remains unclear.

5.6 References

Baerends, G.P., Brouwer, R. & Waterbolk, H.T. 1955. Ethological studies on *Lebistes reticulatus* (Peters). Behaviour 8:249-334.

Bennett, A.T.D., Cuthill, I.C., Partridge, J.C. and Maier, E.J. 1996 Ultraviolet vision and mate choice in zebra finches. Nature 380, 433-435.

Bischoff, R. J., Gould, J. L. & Rubenstein, D. I. 1985. Tail size and female choice in the guppy *(Poecilia reticulata).* Behav. Ecol. Sociobiol., 17, 253-255.

Breden, F., Novinger, D. & Schubert, A. The effect of experience on mate choice in the Trinidad guppy, *Poecilia reticulata.* Environ. Biol. Fish. 42: 323-328

Brooks, R. & Caithness, N. 1995a. Female choice in a feral guppy population: are there multiple cues? Anim. Behav., 50, 301-307.

Brooks, R. & Caithness, N. 1995b. Manipulating a seemingly non-preferred male ornament reveals a role in female choice. Proc. R. Soc. Lond. B., 261, 7-10.

Endler, J. A. 1987. Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces:Poecilidae). Anim. Behav., 35, 1376-1385.

Endler, J. A. 1980. Natural selection on colour patterns in *Poecilia reticulata*. Evolution, 34, 76-91.

Farr, J. A. 1980. Social behaviour patterns as determinants of reproductive success in the guppy, *Poecilia reticulata* Peters (Pisces: Poecilidae). Behaviour. 74, 38-91.

Houde, A. E. 1987. Mate choice based upon naturally occurring colour-pattern variation in a guppy population. Evolution, 41,1-10.

Houde, A. E. 1988a. Genetic difference in female choice between two guppy populations. Anim. Behav., 36, 510-516.

Houde, A. E. 1988b The effects of female choice and male-male competition on the mating success of male guppies. Anim. Behav., 36, 888-896.

Houde, A. E. and Endler, J. A. 1990. Correlated evolution of female mating preferences and male colour patterns in the guppy *Poecilia reticulata.* Science, 248, 1405-1408.

Kodric-Brown, A. 1985. Female preference and sexual selection for male coloration in the guppy (*Poecilia reticulata*). Behav. Ecol. Sociobiol., 17, 199-205.

Kodric-Brown, A. 1993. Female choice of multiple male criteria in guppies: interacting effects of dominance, coloration and courtship. Behav. Ecol. Sociobiol. 32: 415-420.

Liley, N.R. 1966. Ethological isolating mechanisms in four sympatric species of poeciliid fishes. Behav. Suppl. 13: 1-197.

Møller, A. & Pomiankowski, A. 1993. Fluctuating asymmetry and sexual selection. Genetica, 89, 267-279.

Møller, A. P. & Swaddle, J. P. 1997 Asymmetry, developmental stability and evolution. Oxford: Oxford University Press (in press).

Møller, A. P. 1992. Female swallow preference for symmetrical male sexual ornaments. Nature, 357, 238-240.

Reynolds, J.D. & Gross, M.R. 1992. Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata.* Proc. R. Soc. Lond. B. 250: 57-62.

Reznick, D. 1983. The structure of guppy life histories: the trade-off between growth and reproduction. Ecology 64: 862-873.

Rodd, F.H. & Sokolowski, M.B. 1995. Complex origins of variation in the sexual behaviour of male Trinidadian guppies, *Poecilia reticulata* - interactions between social environment, heredity, body-size and age. Anim. Behav. 49: 1139-1159.

Rosen, D.E. & Bailey, R.M. 1963. The poeciliid fishes (Cyprinodontiformes), their structure, zoogeography, and systematics. Bull. Amer. Mus. Nat. Hist. 126, 5-176.

Sheridan, L. & Pomiankowski, A. 1997. Fluctuating asymmetry, spot asymmetry and inbreeding depression in the sexual coloration of male guppy fish. Heredity, *in press.*.

Swaddle, J. P. and Cuthill, I. C. 1994a . Preference for symmetrical males by zebra finches. Nature 367, 165-166.

Swaddle, J. P. and Cuthill, I. C. 1994b. Female zebra finches prefer males with symmetrically manipulated chest plumage. Proc. R. Soc. Lond. B, 258, 267-271.

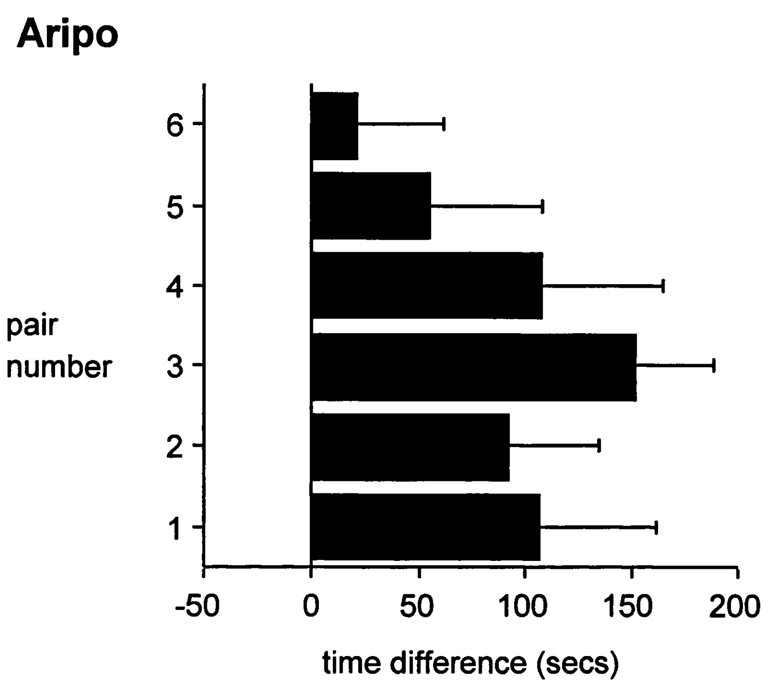
5.7 Tables & Figures

Table 5.1 Phenotypic differences between symmetric and asymmetric males used in experiment 2

Trait	mean±se (symmetric minus asymmetric)	P value
Body length	0.400±0.309	0.222
Melanin area	0.082±0.451	0.859
Carotenoid area	-0.814±0.995	0.431
Iridescent area	1.849±1.766	0.317
% melanic area	-0.007±0.019	0.721
% carotenoid area	-0.031±0.028	0.287
% iridescent area	0.0238±0.034	0.499
Melanin FA	-0.229±0.250	0.380
Carotenoid FA	-0.070±0.212	0.747
Iridescent FA	-0.605±0.913	0.521
Melanin relative FA	-0.053±0.039	0.196
Carotenoid relative FA	-0.005±0.023	0.826
Iridescent relative FA	-0.034±0.042	0.438
Melanin %FA	-0.013±0.009	0.196
Carotenoid %FA	-0.003±0.006	0.689
Iridescent %FA	-0.018±0.019	0.348
Melanin spot number	-1.000±0.537	0.086
Carotenoid spot number	0.333±0.644	0.813
Iridescent spot number	0±0	1.000
Iridescent subspot number	0.667±0.376	0.188
Iridescent subcolour number	1.000±0.408	0.047*
Melanin asymmetric spots	-1.000±0.302	0.020*
Carotenoid asymmetric spots	0±0	1.000
Iridescent asymmetric subspots	-0.583±0.149	0.016*
Iridescent asymmetric subcolours	-0.167±0.112	0.500

Footnote Table 5.1: Pigment area, fluctuating asymmetry and morphology were compared using a *t*-test as these traits are normally distributed. The spot traits were not normally distributed and were compared using a non-parametric Wilcoxon matched pairs test. "%" refers to areas as a percentage of the total body area of each fish. FA is the fluctuating asymmetry calculated as the absolute value of | L-R |; "Relative FA" is calculated as | L-R | divided by | L+R |; and "%FA" is the fluctuating asymmetry expressed as a percentage of total body area. Asymmetric spots are those that do not overlap with spots of the same pigment group on the other side of the animal.

Figure 5.1



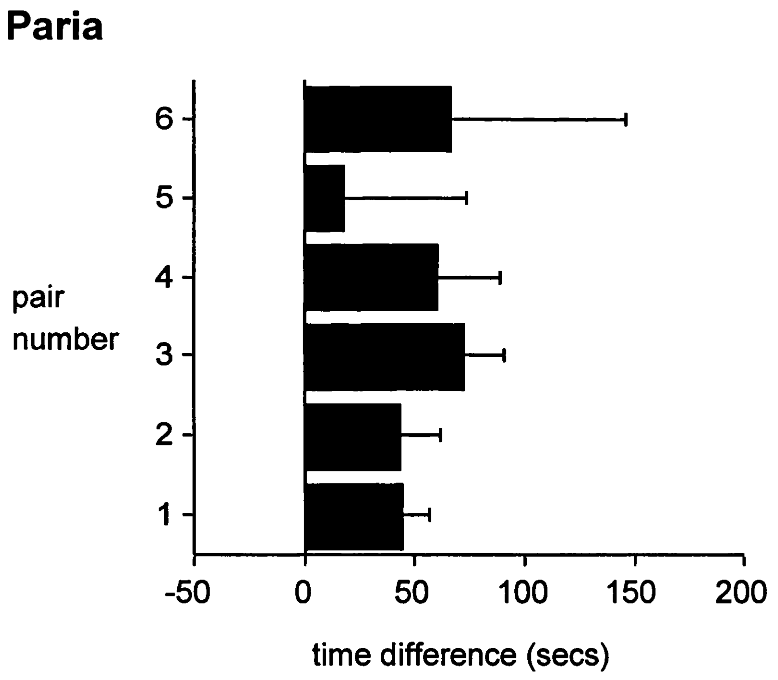


Figure 5.1 Female choice tests in experiment 1 expressed as the mean plus standard error time difference (time with symmetric minus time with asymmetric) that six trial females spent with each pair of males. Aripo and Paria experiments are shown separately.

Figure 5.2

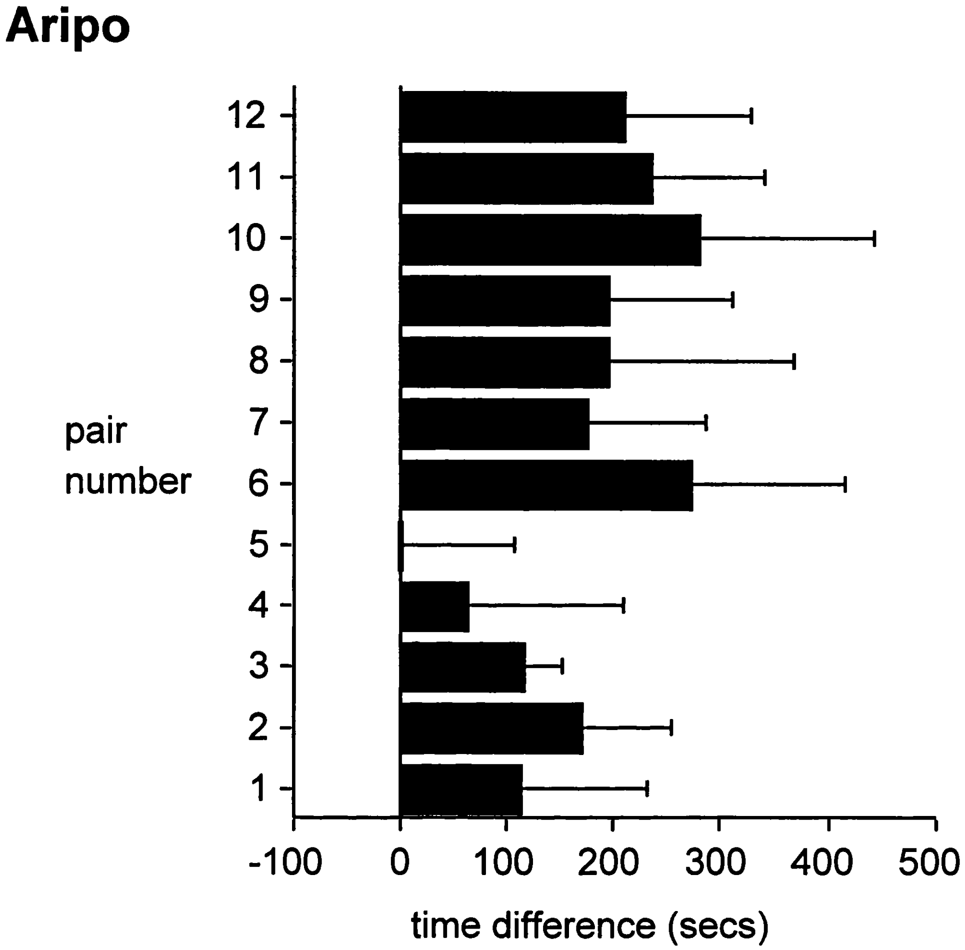


Figure 5.2 Female choice tests in experiment 2 expressed as the mean time difference (time with symmetric minus time with asymmetric) that three trial females spent with each pair of males. The experiment was carried out only with pairs of Aripo males

Chapter 6: Display

6.1 Summary

Courtship displays in guppies are known to be costly. This experiment investigated the effect of inbreeding and symmetry on display rate and attractiveness of male guppies. It was found that genetic stress adversely affected the rate at which male guppies are capable of displaying. Females showed no strong preference for control over inbred males, but showed an overwhelming preference for the most frequently displaying male regardless of all other measured characteristics. Fluctuating asymmetry (FA) had no measurable relationship with display rate. The relationship of pattern asymmetry (NOT conventional FA) and display rate unexpectedly revealed a positive correlation - in experienced males the more asymmetrical individuals displayed more. This effect was not seen in younger males or males of the same age who had not been exposed to females since shortly after maturity. It is suggested that this reveals a compensation behaviour as a result of experience of female responses, as asymmetrical males have been shown to be less attractive to females.

6.2 Introduction

The courtship displays of guppies have been shown to be costly in several ways - they attract predators (Endler 1980, 1983), reduce foraging time, and most of all the sigmoid display is so energetically costly that weakened males cannot display at normal rates (Kennedy *et al.* 1987, McMinn 1990). Thus it would appear that display rate would be an ideal trait to examine to reveal male quality. Female guppies have been shown be strongly influenced by male display rate (Farr 1980a), but on the other hand display rate has also been shown to correlate negatively with male mating success in another case (Houde 1988b). This has been suggested to be due to males detecting the receptiveness of females and, seemingly paradoxically, reducing their display rate when the chance of success is higher (Houde 1988b). This may be an energy saving change in behaviour.

The experiments in this chapter attempt to determine if any correlation exists between symmetry and display rate. Theory predicts that very asymmetrical individuals should be less able to pay the costs of condition-dependent traits. It is possible that male guppies are aware of the degree of their attractiveness to females (e.g. it has been found that male guppies display their most orange side preferentially - pers. comm. J. Woods, Toronto; males assess other males when making choices between associates - Dugatkin & Sargent 1994). These experiments were designed to uncover differences between naïve males and experienced males in their display strategies. The condition dependence of display rate has been demonstrated (McMin 1990). If spot asymmetry is an indicator of condition, then it is expected that display rate should correlate positively with increasing symmetry. However if experienced guppies are aware of their relative attractiveness, more asymmetric males are expected to compensate with higher display rates. The display rates of several groups of male guppies were measured, both young and old, with and without previous exposure to females, to attempt to test these predictions.

Previous work on symmetry in guppies has concentrated on phenotypic characteristics (e.g. colour patterns) but not behavioural characters. The existing work on condition-dependent or "revealing" male sexual traits predicts a negative correlation between asymmetry and the size of the trait. However previous work on asymmetry in male guppy colour patterns found a positive correlation (Brooks & Caithness 1995) between orange pigment area and asymmetry. Orange intensity is known to be condition dependent (Kodric-Brown 1989, Houde & Torio 1992) but there is uncertainty about orange area. The experiments reported here concern another male sexually selected trait, display rate, which has been shown to be condition dependent (Kennedy *et al.* 1987). However this trait, being behavioural rather than physiological, is harder to examine without the possibility of confounding effects such as male behavioural adjustment due to their perception of other factors (such as female receptivity, perceived predation risk etc).

Guppies are ideal for studying sexual selection being highly promiscuous and female choice is the most important factor in their mating system. The most successful type of mating requires female co-operation, which only occurs when females respond to a displaying male. Mature males have several secondary sexual characteristics (colour patterns involving different pigment groups on body and fins, several types of display and attempts to circumvent female choice) and females receive no direct benefits from her choice of mate. Fertilisation is internal and females are ovoviviparous.

The colour patterns of males show considerable variation both between males and on either side of individuals. Colour pattern elements may be paired, in which case conventional analysis of fluctuating asymmetry (FA) may be done, and they may also be missing or in a completely different position on one side. I term this spot asymmetry, as it is much larger than conventional FA and can be orders of magnitude greater.

6.3 Methods

6.3.1 Display trial protocol

Groups of 4 males (in 2 pairs) were tested for display rates and female preferences. A pair of males was placed in *both* end compartments of a three compartment aquarium (60x30x35cm). Males usually display at maximum rate under these conditions (Farr 1980a). Four females were placed in the centre compartment, as it was found in preliminary trials that when only 2 were present it was common to find both females near one end, with the result that the males in the neglected end reduced their display rate and had fewer opportunities to gain positive female responses. This problem very rarely occurred when four females were used in each trial. This is in accordance with many other findings that an equal or female-biased sex ratio reduces the effects of inter-male aggression (as opposed to display) in artificial conditions as more females are available to court (after Houde 1987). The groups of guppies in each trial were allowed to settle for twenty minutes before any scoring. Each male was watched (in random order) for ten minute periods and the numbers of display behaviours exhibited in that time were recorded. Types of display recorded were left or right sigmoid displays (vibrating the body and fins in a stylised s-shaped position) and gonopodial swings. The body side of each male sigmoid display was recorded to check for side preferences, and the number and sequence of all gonopodial swings and sigmoid displays for each male was recorded in two ten minute sessions of watching a single male

6.3.2 Round 1:

Males in each group of four were matched only for age - all males were at least 6 months old, Aripo males only, and had been housed in mixed sex aquaria. Males were photographed under standard conditions of lighting on both sides. These photographs were scored by eye to give each fish a symmetry score for the three pigment groups (carotenoid - orange, melanic - black, and structural iridescent - yellow, blue, green, silver). A maximum of 5 was scored for each pigment group (e.g. a fish with matched spots of very similar shapes), with a minimum of 1 being scored for the most asymmetrical (e.g. if a fish had 2 or more spots completely unpaired), giving a possible maximum symmetry score of 15 for each male. During trials the number and side of sigmoid displays and number of gonopodial swings were recorded. Each male was watched for 3 ten minute periods.

6.3.3 Round 2:

Males in each group were matched for age and large body size differences were avoided as much as possible. A group of older males were used (6 months) which had been housed in male only aquaria and unable to view females in adjoining aquaria since the age of 14 weeks (naïve old males). Also a group of young males (15-17 weeks) were tested. The group of younger males comprised both inbred and control males (see inbreeding scheme, Chapter 2) which had been housed in mixed aquaria. The inbred males, being genetically stressed, were predicted to show lower display rates and correspondingly low female response rates. The younger males were tested in pairs, with one pair each of inbred males and control males. Each male was watched (in random order) for two ten minute periods and their behaviour recorded. The number, sequence and side of sigmoid displays were recorded, the number and sequence of gonopodial swings and the number of female responses were also recorded. A female response to a display is an unambiguous orient towards the male usually followed by a glide towards him. These responses sometimes occur when a male is not displaying.

All males were photographed at 14 weeks (for young inbred and control males) or 6 months ("old" group), and the images were input into a Macintosh computer for analysis using the software Image. Detailed analysis of their morphology and colour patterns is described in Chapter 2. All colour pigment areas, spot numbers and body dimensions were measured, allowing fine scale determination of asymmetries. For each pigment group, paired spot FA was analysed for absolute values (L-R), relative to trait size and relative to body area.

All spot numbers and positions were recorded to give total spot asymmetry scores.

A total of 7 pairs of inbred Aripo males were tested against 7 pairs of control Aripo males, with a further 4 control males tested in an unmixed group, and 6 groups of 4 Aripo males in the older, outbred category.

5 pairs of inbred Paria guppies were tested against 5 pairs of control guppies, with one group of 4 controls tested alone and 2 groups of 4 older Paria males. (Total numbers tested: 14 inbred and 18 control Aripo males, 24 old Aripos, 10 inbred Parias, 14 control Parias, 8 old Parias).

6.3.4 Statistical analysis

Statistical analyses were performed using JMP for Macintosh and JMPIN for Windows software. It was predicted that there would be a negative correlation between asymmetry and display rate. For round 2 it was predicted that inbred males would have lower display rates than control males, and that females might prefer control males as a result. For both rounds the realistic assumption that male behaviour is influenced by their companions was taken into account. Each male was given a rank of 1 to 4 within its group determined by the number of female responses received. Then Spearman's rank order correlation could be used to analyse relationships between attractiveness and male variation. Mann-Whitney's U test was used to test for differences in display and female response between inbred and control males. There were many variables available - body dimensions, numbers of displays, colour areas, pigment symmetry (absolute and relative to both trait size and body size) and spot symmetry - but rather than examining them all certain predictions were tested: inbred males were expected to display less than control males, and females were expected to prefer control males with higher display rates. Symmetrical males were expected to display more.

6.4 Results

6.4.1 Round 1:

Rather than the expected negative correlation between increasing display rate and asymmetry, a significant positive correlation was found (see Figure 6.1). Asymmetrical males displayed *more* than symmetrical males. This relationship was especially strong when considering sigmoid display rate (Spearman, *P* =0.03, *N*=22) but also true for total displays (including gonopodial swings)(Spearman *P* =0.044, *N*=22). Males with higher symmetry scores displayed significantly less than more asymmetrical individuals.

6.4.2 Round 2:

The sigmoid display rate of control males was on average much higher than that of inbred males in Aripo only (Mann Whitney U statistic = -2.626, *P* =0.009, *N*=32. See Table 6.1). Total displays (sigmoid displays + gonopodial swings) were also higher in control males (Z=-2.232, p=0.026, *N*=32). Control males did not receive significantly more responses from females than did inbred males.

Males were ranked from 1 to 4 within each group (4 being most preferred) according to how many positive female responses they receive. Using these rankings Spearman's rank order correlation could be used to determine predictors of attractiveness. Overall females overwhelmingly prefer the most displaying male in each group above all other characteristics (body size, pigment areas, pigment Fas, spot Asymm). (Aripo: Spearman rho=0.592, $P<0.001$ for total number of sigmoid displays only, and Spearman rho=0.522, $P=0.004$ for total number of displays, $N=28$; Aripo old group Spearman rho=0.562, $P=0.004$ (total sigmoid displays), and rho=0.630, p=0.001 (total displays), $N=24$) (Paria Spearman rho=0.680, $P=0.0003$ for total sigmoids, rho=0.701, $P=0.0001$ for total displays $N=24$; Paria old group Spearman rho=0.932, $P=0.0008$ (total sigmoid displays);rho=0.926, $P=0.001$(total displays), $N=8$ (very strong effect even in tiny sample) - See Table 6.2.

There was no difference in the display rates of symmetrical and asymmetrical males, whether scored by conventional FA or spot asymmetry.

6.5 Discussion

Theory predicts that very asymmetrical individuals should be less able to pay the costs of condition-dependent traits. The level of spot asymmetry in guppy colour patterns is very high, and very asymmetric individuals are predicted to perform badly at a very costly behaviour such as display rate. Unexpectedly exactly the opposite result is seen in the first experiment: asymmetric guppies display significantly more than symmetrical ones.

If this effect is real it has interesting implications. Display rate has been shown to be a condition dependent trait (Kennedy *et al.* 1987; Nicoletto 1993);

and has been shown to be more sensitive to stress such as parasitism than other non-sexually-selected traits such as swimming and foraging (McMinn 1990), this suggests that display is particularly revealing of male quality. The second experiment indicates that females use display rate as the most important cue for mate choice, above all other measured factors. This is in agreement with Farr (1980a).

The genetic stress of inbreeding was also shown to reduce display rate, which is further evidence for condition-dependence. This is in agreement with Farr 1983 (inbred guppies showed reduced display rates); though Farr & Peters (1984) did not find that inbreeding suppressed display. There is much other evidence that display is condition dependent, and this finding that display rate is sensitive to inbreeding depression supports this. It also indicates that females may gain information about male quality by using it as the most important cue.

All this evidence for the condition-dependence of display rate has implications for the findings of the first experiment . Females prefer symmetrical males (based on spot asymmetry only *not* FA, see Chapter 5) when tested with choices of two males differing only in spot symmetry - but most importantly display rate was controlled in these experiments. When display rate is not controlled, asymmetrical males display more - this could mean they are better quality, though this conflicts with every prediction of theory. However it could mean the more asymmetrical males are aware they are less attractive to females and are compensating with a higher rate of display. If this is the case they must be paying a cost, as the inbreeding experiment clearly shows that display rate suffers under genetic stress. Perhaps these asymmetric males are trading reduced lifespan against the chance of increased reproductive success. (This would be difficult to measure directly and so an experiment to determine swimming endurance was devised as a measure of male quality independent of the confounding effects of females - see Chapter 7).

It is important to note that the males used in the first experiment were all “old” males (6 months) who therefore had far more experience of female responses than the younger males used in the second experiment. This could be why no effect is seen in the second experiment. It is possible that these young males had too little experience to learn to compensate for unattractiveness by increasing their display rate. Conversely there were also a group of older but naïve males used in experiment 2 and these show no difference in display rate with differing spot symmetry - they do not reproduce the strong relationship seen in experiment 1. These males were also 6 months old and they do not back up the strong-compensation-with-increased-experience hypothesis. However these older males did *not* have increased experience. They had been kept in all male groups since the age of 14 weeks (the same age as the “young” cohort which showed no experience effect), which could have removed any learning-effect of prolonged interaction with females and influenced their behaviour.

The finding that "ugly males try harder" fits neatly with the "differential benefit" theory of courtship strategy proposed by Reynolds (1993). This hypothesis emphasises differences in mating gains with courtship. In this case, symmetric males may enjoy a relatively greater gain in mating success than asymmetric males with increasing display. This means that the rate of improvement in mating success may level out sooner for symmetric males with increasing courtship. Beyond a certain point, it does not pay for attractive males to increase their display rate as the success rate levels out. For "ugly" (asymmetric) males however, benefits (in terms of mating opportunities) carry on increasing with increasing display (even though their overall success remains lower than for attractive males). So ugly males can gain by increasing their level of courtship, while attractive males do not. This could result in the strategy seen in these results: ugly (asymmetric) males compensate with increased courtship rates compared to attractive (symmetric) males. Young males and naive old males do not show this strategy - they have not had prolonged interaction with females and had no opportunity to learn whether they were ugly or attractive. The theory that the optimum display rate may be higher for unattractive males has also been proposed by Houde (1988), as long as courtship does not emphasise other features that females do not prefer. This stemmed from her results showing that male guppies displaying at higher rates had less success. An interesting parallel has been found in bowerbirds (Ptilonorhynchidae) - the less ornate a male's plumage, the more complex his bower (Gilliard 1969). This is at a species level (more ornate species build less complex bowers) rather than an individual level - and it is assumed that trade-offs acting within species can also be seen among species (Gilliard's "transference" effect (1969)). It may be due to costliness (species cannot afford to invest in both traits at once) or "saturation" of benefits (little advantage to be gained from investing in both). This is more evidence that the costs and benefits of courtship are complex trade-offs and predictions based on traditional condition-dependent theory are not always reliable. There is evidence that animals may adjust their courtship according to their own and other's attractiveness (Dugatkin & Sargent 1994) environmental conditions (Reynolds *et al.* 1993) and particularly according to the receptivity of the opposite sex (Houde 1988; Farr 1980b). It is common that only the changes in receptivity according to the amount of courtship are considered - though the reverse is clearly important. The experienced guppies in these experiments were clearly adjusting their courtship according to previous female responses - and this confounds expectations that the most attractive males might display the most.

In classic condition-dependent theory, individuals that invest most heavily in sexually-selected displays should be the ones most able to pay those costs (Kodric-Brown and Brown 1984; Grafen 1990; Andersson 1982; Zahavi 1977). The density of carotenoid pigmentation in male guppies may be an example of this (Kodric-Brown 1989; Endler 1980) especially as this trait correlates positively with swimming performance (Nicoletto 1991). There are other examples: black grouse (*Tetrao tetrix*) survive better if they are more competitive; (Alatalo *et al.* 1991); house finches (*Carpodacus mexicanus*) have greater survival if they are more colourful (Hill 1991); across dimorphic wood warbler species more adorned males have greater song rates and repertoires(Shutler & Weatherhead 1990); male scorpionflies (*Panorpa vulgaris*) investing most heavily in secreting salivary nuptial gifts produced more competitive sons and daughters (Thornhill & Sauer 1992); preferred male blue tits have greater survival (Kempenaers *et al.* 1992). The finding here that inbreeding stress decreases the ability of male guppies to display is more evidence that display *can* be a classic condition dependent trait; however the finding that experience causes males to modify their behaviour on the basis of attractiveness makes it clear that caution must be applied when interpreting results of condition-dependence experiments; a complex suite of conditions and experience must be taken into account before it can be claimed with confidence that a trait conforms to traditional condition-dependent sexual selection theory.

The overwhelming female preference for the most displaying male in each group (for both populations and age groups) shows that display rate is very important in mate choice. This is in agreement with previous work (eg Farr 1980a).

The inbreeding effect shows that males in worse condition have reduced display rates. This backs up other work showing display to be a condition dependent trait (eg McMinn 1990; Nicoletto 1993). This result was very clear for sigmoid displays, and less strong when gonopodial displays are also considered. This is not surprising considering the evidence that the sigmoid display is very costly and the rate of display is particularly sensitive to stress (e.g. Kennedy *et al.* 1987), whereas the gonopodial swing requires far less effort and is much less conspicuous to both predators and females. The finding that sigmoid display rate is sensitive to inbreeding depression agrees well with the evidence that it is more sensitive to parasitism than non-sexual traits (McMinn 1990); this coupled with the finding that females use display rate above all other cues in mate choice indicates that they may gain information about male quality by using this strategy.

However the unexpected relationships between spot asymmetry and display rate imply that caution should be exercised when interpreting data on male courtship in terms of male quality. It seems likely that males may be aware of their own attractiveness and may alter their behaviour accordingly (c.f. the finding that males display their most orange side more, pers. comm. J. Woods, Toronto). There is other evidence that males are aware of relative attractiveness - Dugatkin & Sargent (1994) showed that they assess other males and categorize them on their proximity to females (and female responses). Males use this information when choosing between other males as associates. This strategy may increase a male's chances of being the individual chosen by a female assessing nearby males. This is another example that indicates that experiments may be confounded by variables that experimenters cannot detect (c.f. Houde 1988b - male guppies unexpectedly reduce their display rate when they detect receptive females); males have also been shown to prefer to court females from their own population, and the basis for this male preference is not known (Ballin 1973; Crow 1981). There is another piece of evidence for compensation behaviour in males - Kodric-Brown (1989) found that males deprived of carotenoids (and thus unattractive to females) resorted to continuous following behaviour and more attempts at sneak copulations.

Mating behaviour is complex; certainly female choice has been shown to be subtle and based on a complex set of male behavioural and morphological variables (Kodric-Brown 1993); and there is no reason to believe that male strategy is any less complex (e.g. males change their courtship behaviour depending on female receptivity (Houde 1988b), population of female (Crow 1981), presence of predators (Magurran & Seghers 1990), and clarity of water (Luyten & Liley 1991)). A method of determining male fitness independently of the confounding effects of females (such as swimming or foraging performance) would be more useful in order to obtain insight into the issue of any possible relationship between symmetry and quality. This conclusion led to the design of Chapter 7.

6.6 References

Alatalo, R.V., Höglund, J. & Lundberg, A. 1991. Lekking in the black grouse - a test of male viability. Nature (Lond.) 352: 155-156.

Andersson, M. 1982. Sexual selection, natural selection and quality advertisement. Biol. J. Linn. Soc. 17: 375-393.

Ballin, P.J. 1973. Geographic variation in courtship behaviour of the guppy; *Poecilia reticulata.* M.Sc. thesis, University of British Columbia, Vancouver.

Brooks, R. & Caithness, N. 1995. Female choice in a feral guppy population: are there multiple cues? Anim. Behav., 50, 301-307.

Crow, R.T. 1981. Behavioural adaptations to stream velocity in the guppy, *Poecilia reticulata.* M.Sc. Thesis, University of British Columbia, Vancouver. Pp 1-106.

Dugatkin, L.A. & Sargent, R.C. 1994. Male-male association patterns and female proximity in the guppy, *Poecilia reticulata.* Behav. Ecol. Sociobiol. 35: 141-145.

Endler, J.A. 1980. Natural selection on colour patterns in *Poecilia reticulata.* Evolution, 34, 76-91.

Endler, J.A. 1983. Natural and sexual selection on colour patterns in poeciliid fishes. Environ. Biol. Fish 9: 173-190.

Farr, J. A. 1980a. Social behaviour patterns as determinants of reproductive success in the guppy, *Poecilia reticulata* Peters (Pisces: Poecilidae). Behaviour. 74, 38-91.

Farr, J.A. 1980b. The effects of social experience and female receptivity on courtship-rape decisions in male guppies, *Poecilia reticulata* (Pisces: Poeciliidae). Anim. Behav. 28: 1195-1201.

Gilliard, T.E. 1969. Birds of paradise and bowerbirds. Wiedenfeld & Nicolson, London.

Grafen, A. 1990. Sexual selection unhandicapped by the Fisher process. J. Theor. Biol. 144: 475-516.

Hill, G.E. 1991. Plumage coloration is a sexually selected indicator of male quality. Nature (Lond.) 350: 337-339.

Houde, A. E & Torio, A. J. 1992. Effect of parasite infection on male colour patterns and female choice in guppies. Behavioural Ecology, 3, 346-351.

Houde, A. E. 1987. Mate choice based upon naturally occurring colour-pattern variation in a guppy population. Evolution, 41,1-10.

Houde, A. E. 1988. The effects of female choice and male-male competition on the mating success of male guppies. Anim. Behav., 36, 888-896.

Kempenaers, B., Verheyen, G.R., Van den Broeck, C., Berk, T., Van Broeckhoven, C.V., and Dhondt, A.A. 1992. Extra-pair paternity results from female preference for high quality males in the blue tit. Nature 357: 496-497.

Kennedy, C. E. J., Endler, J. A., Poynton, S. L., & McMinn, H. 1987. Parasite load predicts mate choice in guppies. Behav. Ecol. Sociobiol., 21, 291-295.

Kodric-Brown, A., and Brown, J.H. 1984. Truth in advertising: the kinds of traits favored by sexual selection. Am. Nat. 124: 309-323.

Kodric-Brown, A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. Behav. Ecol. Sociobiol., 25, 393-401.

Kodric-Brown, A. 1993. Female choice of multiple male criteria in guppies: interacting effects of dominance, coloration and courtship. Behav. Ecol. Sociobiol. 32: 415-420.

Luyten, P.H. & Liley, N.R. 1991. Sexual selection and competitive mating success of male guppies *(Poecilia reticulata)* from four Trinidad populations. Behav. Ecol. Sociobiol. 28: 329-336.

Magurran, A.E. & Seghers, B.H. 1990. Risk sensitive courtship in the guppy *(Poecilia reticulata).* Behaviour 112: 194-201.

McMinn, H. 1990. Effects of the nematode *Camallanus coti* on sexual and non-sexual behaviour in the guppy (*Poecilia reticulata)*. Am. Zool., 30, 245-249.

Nicoletto, P. F. 1991. The relationship between male ornamentation and swimming performance in the guppy, *Poecilia reticulata*. Behav. Ecol. Sociobiol., 28, 365-370.

Nicoletto, P.F. 1993. Female sexual response to condition-dependent ornaments in the guppy *Poecilia reticulata.* Anim. Behav. 46: 441-450.

Reynolds, J. D. 1993. Should attractive individuals court more? Theory and a test. Am. Nat. 141:914-927.

Reynolds, J. D., Gross, M. R., and Coombs, M. J. 1993. Environmental conditions and male morphology determine alternative mating behaviour in Trinidadian guppies. Anim. Behav., 45, 145-152.

Shutler, D., and Weatherhead, P.J. 1990. Targets of sexual selection: song and plumage of wood warblers. Evolution 44: 1967-1977.

Thornhill, R. & Sauer, P. 1992. Genetic sire effects on the fighting ability of sons and daughters and mating success of sons in a scorpionfly. Anim. Behav. 43: 255-264.

Zahavi, A. 1977. The cost of honesty (further remarks on the handicap principle). J. Theor, Biol. 67:603-605.

6.7 Tables and figures

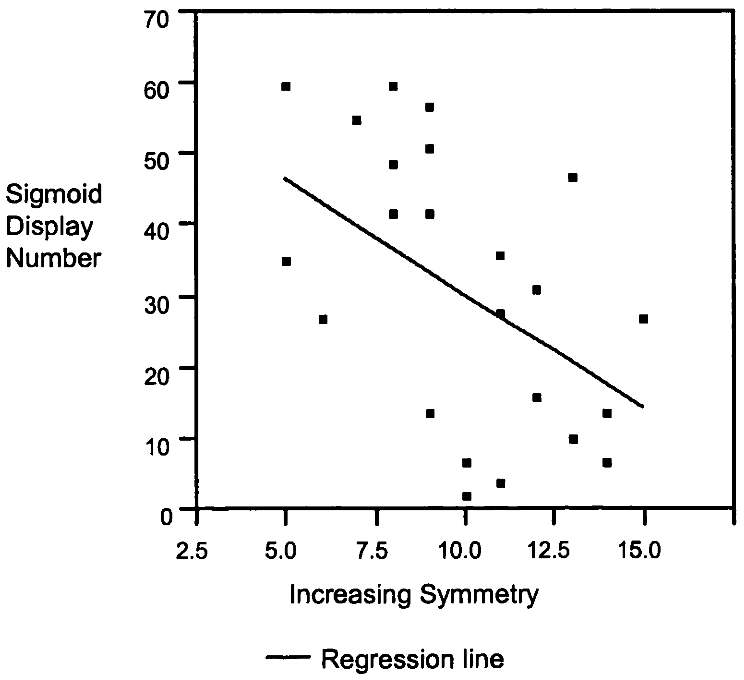
Group	Variables	Mann-Whitney U statistic	p value	n	
Aripo	Sigmoid display + inbreeding	-2.626	0.009	32	**
	Total display + inbreeding	-2.232	0.026	32	*
	Female ranking + inbreeding	-0.286	0.775	32	ns
Paria	Sigmoid display + inbreeding	-1.053	0.292	24	ns
	Total display + inbreeding	-0.705	0.481	24	ns
	Female ranking + inbreeding	0.893	0.372	24	ns

Table 6.1. Correlations between inbreeding , display and female response using the Mann-Whitney U test. (* = $p<0.05$; **=$p<0.01$)

Group	Variables	Spearman rho	P value	n
Aripo young	Sigmoid displays + female ranking	0.592	0.0009	28
	Total displays + female ranking	0.522	0.004	28
Aripo old	Sigmoid displays + female ranking	0.562	0.004	24
	Total displays + female ranking	0.630	0.001	24
Paria young	Sigmoid displays + female ranking	0.680	0.0003	24
	Total displays + female ranking	0.701	0.0001	24
Paria old	Sigmoid displays + female ranking	0.932	0.0008	8
	Total displays + female ranking	0.926	0.001	8

Table 6.2. Correlations between two types of display and ranking in order of female responsiveness using Spearman's rank order test. (all $p < 0.01$)

Figure 6.1. Plot of symmetry score against sigmoid displays:



Chapter 7: Swimming performance

7.1 Summary

This chapter describes an attempt to find a measure of male quality independent of the confounding effects of courtship effort and response to females. Swimming performance was chosen as a suitable independent variable to measure male vigour. Male swimming endurance was measured and compared with morphological and sexual traits. The technique was a poor discriminator between males, though an effect of inbreeding stress could be detected. Inbreeding depression affected swimming performance - only some outbred males were capable of strong sustained swimming. There were no strong correlations with pigment areas or body size. There was a strong age effect - older males being far better swimmers. No correlation with fluctuating asymmetry or spot symmetry was found.

7.2 Introduction

It transpires that display rate is not a good variable to measure for indications of male quality. There was evidence of compensation behaviour in unattractive males in Chapter 6, plus other evidence exists of complex adjustment of courtship strategies by males due to various factors - relative attractiveness of other males (Dugatkin & Sargent 1994); female receptivity (Houde 1988); their own attractiveness (Kodric-Brown 1989) and the origins of the female (Ballin 1973; Crow 1981). There is also a wealth of evidence that males adjust their courtship depending on both the perceived predation risk and the predation regime of their population of origin (Magurran & Seghers 1990).

These findings made it obvious that measurement of display rate was an inadequate measure of male vigour, and a new method was required to attempt to determine male quality. Male fish from all populations, regardless of predation regime, require good swimming ability for foraging as well as searching for females. Swimming is a major survival component in fish and so should correlate with any traits used in mate choice that indicate long term male vigour (though not with condition-independent traits).

It could be argued that populations from high predation environments experienced a higher level of attacks from predators and thus were selected for greater swimming ability for fleeing. However there is evidence that guppy swimming performance is strongly influenced by the water velocity during ontogeny (Nicoletto 1996) (frequent fleeing from predators would be expected to show a similar effect). Guppies raised in different conditions of water velocity show pronounced differences in swimming performance, with correlated increases in display duration (but not display rate). These differences would only be expected between populations. However all the guppies used in this study were laboratory adapted for more than one generation, so all experienced the same swimming conditions and no predatory attacks, so conditions during ontogeny were standardised.

The existence of a female preference for symmetry prompted this study of the relationship between symmetry and swimming ability. If symmetrical males were fitter, then this was expected to be reflected by higher display rate. This was not the case, and evidence of compensation behaviour emphasised that the measurement of display had too many confounding variables. It was hoped that measurement of swimming performance would be a more accurate predictor of male condition, thus giving information about the quality of symmetrical versus asymmetrical males. Also, it would be useful to demonstrate an effect of inbreeding depression on swimming ability as further proof that the inbreeding scheme had succeeded in inducing stress; this confers greater confidence in the findings on symmetry and inbreeding.

7.3 Methods

The swimming endurance of male guppies was tested using an adaptation of Nicoletto's swim test method (1991). Guppies were placed individually in a transparent PVC pipe (24mm internal diameter, 1.25m in length) with a flowmeter and valves to a pump at one end (see diagram, Figure 7.1). Water velocity was adjusted using the valves to increase or decrease the proportion of water flowing through the two routes - either directly back to the reservoir or through the test chamber. The outflow of the test chamber drained directly back into the reservoir feeding the pump. The fish were introduced to the test chamber at an initial flow speed 7.8 cm/s (guppies can swim at this rate for several hours). This flow speed was maintained for three minutes for each trial, to give the fish time to acclimatise and to ensure that all fish reached the horizontal testing zone of the apparatus before the water velocity was increased.

Then the flow speed was increased to 16.5 cm/sec (2.5 litre/minute) and kept constant until the fish was washed out of the chamber to determine critical swimming time (in seconds). Each male was tested three times as times showed considerable variation (unlike Nicoletto's (1991) method which tested each fish only once). There are other differences - Nicoletto's apparatus used a submerged pump and needle valves, in contrast to the enclosed pump (common central heating pump, capable of easily producing 7 litres/minute through this apparatus) and simple compression valves for adjustment used here. Also a collimator made of 6cm plastic straws was used in Nicoletto's apparatus to facilitate rectilinear flow. This was not used in my apparatus. Calculation of the appropriate Reynolds numbers ((constant x density x velocity x diameter) / viscosity) revealed that for the initial flow rate the flow was laminar (R< 2300) and for the high flow rate the flow was definitely turbulent (R>2300). These conditions were the same for every fish trial, so no attempt was made to rectify this.

Methods of trial were also different - the 1991 study increased the flow rate by 2.9 cm/s every three minutes until the fish was washed out of the test chamber. The experiment described here measured the total time spent at 2.5 litres/minute; this avoids difficulties caused by transition between laminar and turbulent flow across the different rates and makes comparisons between fish of widely differing abilities easier. Corrected swimming speed (ratio between time at top rate (critical swimming speed) and standard length of the fish) (Stahlberg & Peckman 1987) was also not used. This was firstly because "critical swimming speed" was discarded after preliminary trials showed its difficulties and a constant top rate was used to compare all fish; and secondly because analysis showed no relationship between male body length and swimming times. Also Nicoletto (1996) showed no difference in standard length and body width between strong and poor swimmers (though they had wider caudal peduncles - the base of the tail). The use of endurance rather than "critical swimming speed" is not a direct measure of how fast the fish can swim, but it is positively correlated with it, and also with active metabolism (Beamish 1978; Brett 1964; Brett & Glass 1973; Jones *et al.* 1974; Smit 1965).

All available inbred and control males were swim tested, and also a further group of older males (~6 months) from each population were tested concurrently. Each fish was tested three times on different days. Results were not normally distributed nor transformable to normality, so a non-parametric test was used (Spearman's rank correlation) to compare swimming times with inbreeding status, morphological variables and colour pattern data. Body area, standard length, display rates, relative FA (absolute value of left minus right colour areas as a proportion of body length) and colour areas were all normal or transformable to normality (by taking the square root after consulting the Box-Cox transformation, see Chapter 3) so ANOVAs could be used to compare these variables between young and old groups.

7.4 Results

There was good agreement between the three trials for each fish (Spearman, all P >0.001) - times were variable but quite consistent. This justified the use of the mean of three trials in all further analysis.

No statistically significant difference was found between the swimming performance of inbred and control males in either population (see Table 7.1), though mean swimming times were much higher in controls. The distribution was odd - most males swam for a very short time (<100 seconds) but some swam for 10 minutes. For this reason the top and bottom 50% were analysed separately - in the bottom 50% there was no difference, and in the top 50% the difference in means became very pronounced between inbred and control males. However the variance was high so this trend was still not significant.

There were no correlations found between swimming performance and any morphological variable in either population (Aripo all $P > 0.16$; Paria all $P > 0.14$). The morphological variables tested were: standard length, total length, maximum depth, caudal area, dorsal area, body area and gonopodial length (see Chapter 2). No elements of colour pattern correlated with swimming performance in Aripo guppies (all $P > 0.12$; except √total carotenoid area ($P = 0.023$) (but this was a *negative* correlation and due to the influence of one individual); also total melanic area ($P = 0.052$) and √total melanic area ($P = 0.063$) - these were also negative correlations produced by the influence of one fish and therefore spurious). Similarly no significant correlations were found with colour pattern in Paria guppies (all $P > 0.118$ - though interestingly the strongest trend in this population was a positive correlation between carotenoid area and swimming performance (c.f. negative trend for all pigments in Aripo) though probably due to smaller sample size this was not significant ($P = 0.118$)).

No relationship with fluctuating asymmetry was found in Aripo guppies (for FA, relative FA, √FA or %FA) - all $P > 0.09$; Similarly there was no effect of FA in Paria guppies (all $P > 0.09$). There was no correlation between swimming and total spot numbers or spot asymmetry in either population (Aripo all $P > 0.27$; Paria all $P > 0.13$).

The only strong result was an age effect on swimming. There were no real differences between inbred and control guppies, so the whole cohort was used as the "young" group when comparing with the old group (though there was no difference in the results when they were divided into inbred and control). The older group were significantly stronger swimmers (*P* <0.0001; see Table 7.2). Similarly to the young group, within the older guppies of both populations there was no correlation between swimming performance and any colour variable, symmetry (FA or spot symmetry), or any morphological variable (except in the Aripo group - body depth (*P* =0.047)).

This large difference in swimming performance warranted a more detailed comparison of old males with young inbred and control guppies (see Tables 11 & 12). Older males were significantly larger than both inbred and control males for both area and length. Due to this difference, colour areas as a proportion of body area (referred to as "%" melanin or carotenoid etc.) were used for comparisons; also due to the known inbreeding effect of reduced pigment areas (Chapter 3) inbred and control guppies were considered separately.

Old fish perform better, therefore information on which traits correlate with age would be useful. Colour patterns in young and old fish were compared. Aripo control guppies (see Table 7.3) showed no significant difference in % colour areas compared to the old males for all three pigment groups (all p>0.19). Inbred guppies however showed significantly less relative area of melanic and iridescent colours than the older males (% melanin p=0.010; % iridescent *P* =0.006). Inbred guppies showed no difference in FA as a proportion of body area compared to the old males apart from a trend in %FA carotenoid (*P*=0.067) though this was for higher FA in the old males. This continued in the control males, which had significantly *less* %FA carotenoid than the old males (*P* =0.015). FA in iridescence showed the opposite correlation - old males had less %FA iridescent than young control males (*P* =0.042).

Paria inbred and control guppies (see Table 7.4) were significantly smaller than older males (all *P* <0.04). Both groups showed significantly less iridescent pigment as a proportion of body area (*P*<0.0005). The only other difference involved carotenoid area - control guppies had proportionally more orange area than older males (*P* =0.006). There were no other differences in colour areas or relative FAs (all *P* >0.08).

There were no correlations in either population (young or old, inbred or control) between swimming performance and any aspect of display (total amounts of sigmoid displays, gonopodial swings, female responses or female ranking) (all *P* >0.1); though old males showed significantly more displays (*P* <0.0001 in both populations).

7.5 Discussion

This experiment was a disappointingly poor discriminator between males. Without the large difference seen between age groups, the most likely conclusion would be that the apparatus was simply unsuitable for this test. However, the major differences seen between the age groups in both populations shows that the technique can discriminate differences in condition between males; though it seems that the inbred and control males of the same age were too similar for this test to distinguish clearly.

Having said that, there were large differences in the mean swimming times of inbred and control males. Control males had consistently higher times and of the top males who swam up to 10 times longer than the majority, almost all were controls. This implies that an effect of inbreeding depression on swimming performance was visible, and perhaps a greater sample size would be all that was necessary to distinguish significantly between the two groups rather than a major re-design of the experiment.

Analysis of the repeatability of trials suggested that three trials per fish were adequate - though if Nicoletto's one trial per fish method had been used that could have given a very different result for some fish (particularly the strong swimmers, which were far more variable than weak males). This perhaps illustrates the problem with this test - there seems no way to standardise motivation to swim. All guppies will orient into a current, and will resist being swept over an edge to an unknown destination (personal observation; also Stahlberg & Peckman 1987) - this is why swim testing can work. This correlates closely with the common situation of guppies in the wild, often living between waterfalls. Guppies do spread downstream via waterfalls (e.g. Shaw *et al.* 1992) and this must be important for colonisation of new areas; but for the majority of their lives avoidance of being swept away should be advantageous. The problem with the test appears to be that some fish "give up" more easily than others. The large numbers of fish swimming for less than a minute, coupled with the few fish swimming for ten minutes or more suggests that this must be the case. It seems unlikely that the differences in condition between fish are that large. In preliminary trials, it did appear that "frightening" the fish (by tapping the swim tube behind it) caused them to try harder - though it seemed impossible to standardise this for all fish and so could not be used. Other options such as placing a predator in view behind the fish were considered but abandoned as impractical.

However, the fact that the older groups swam consistently longer (a mean of almost 500 seconds in the Aripo guppies versus a mean of only 60 seconds in younger fish, see Table 7.2), does suggest that after all there may be differences in condition causing the wide range of swimming performance. So perhaps more confidence in the apparatus is justified.

This finding caused a focus on the differences between the old and young groups. *Within* all groups, no correlations between body size and swimming were found. This is in agreement with Nicoletto 1996; apart from a correlation in the old Aripo group between maximum body depth and swimming. In Nicoletto's (1996) study the only aspect of body size to correlate was the width of the tail attachment (not body width). However, *between* groups, a major difference in body size was found. The larger, older males swam consistently longer than the younger, smaller males in both populations. These two variables unfortunately could not be disentangled, as there were no effects of body size within groups. The lack of effect of body size within any group, plus the findings of Nicoletto (1996) could imply that the important factor causing the difference between the groups was age rather than sheer size. Male size is controlled by both genetic (Reynolds & Gross 1992) and environmental (Reznick 1990) components and is nearly fixed at maturity (Reynolds *et al.* 1993) (around 14 weeks).

Within groups, there were no real correlations between any measure of symmetry or pigment group and swimming. The exceptions were higher %FA in carotenoids in old males and lower iridescent %FA in old males, both compared to control Aripo males. These two were the only correlations, not overwhelmingly strong and in conflicting directions - so probably not something to get excited about. However, there were very strong differences in proportional pigment area between young and old guppies. Aripo guppies showed significantly less iridescent and melanic pigment as a proportion of body area than older males. This effect was only present in inbred guppies - control guppies showed no difference in relative pigment areas. Male guppies do occasionally gain colour spots after maturity, which could explain the difference. Paria guppies (both inbred and control) also showed less iridescent area than old males. No effect of carotenoid area could be seen in either population (unlike Nicoletto 1991, in which carotenoid area correlated positively with swimming). This is perhaps unsurprising in these circumstances - carotenoid is most important in Paria guppies which had a small sample size; whereas carotenoid is unimportant in mate choice in Aripo guppies, and correspondingly small and dull.

These findings may indicate that females choosing males on the basis of relative area of pigment (melanic or iridescent) might be preferring older males. It has already been demonstrated that these pigments decrease in area due to inbreeding. The results discussed here indicate that larger males with more relative pigment area are better swimmers - Aripo females have been shown to prefer both these traits. These preferences may result in females choosing older males. A female preference for large male size has been shown to provide considerable genetic benefits in other populations (Reynolds & Gross 1992), due to large fathers siring both sons and daughters with higher growth rates. Other data also shows offspring size increases with increasing parental age (Rocchetta *et al.* 1993) Relative pigment area has been shown to be sensitive to inbreeding, and now also to male age. This may be evidence of truthful advertisement. Why this effect in the Paria males only shows in iridescence (an unimportant pigment in mate choice) rather than the more important carotenoid pigment is unclear, and may be an artefact of sample size.

Display rate is known to be condition-dependent, but was a poor predictor of swimming performance. This may demonstrate that swimming performance is a poor test to distinguish condition dependent traits. Aside from this, age was a good predictor of display rate - which both populations prefer above other cues. As age correlates with both display rate and swimming performance, females choosing on the basis of display rate may be choosing older, fitter males.

Female preference for older (and hence fitter) males has been found in other species (e.g. Yellow-knobbed Curassow (*Crax daubentoni*) - Buchholz 1991) and large size (often correlated with age) has also been documented as a preferred trait in other species (e.g. swordtails (*Xiphophorus nigrensis*) - Ryan *et al.* 1990); high courtship intensity has also been shown to be both preferred and an indicator of outbreeding (in *Drosophila subobscura* - Maynard-Smith 1956). All these characters (large size, age and courtship intensity) have been shown to be directly or indirectly important to female guppies in the previous experiments and also affected by inbreeding depression. The experiments of this chapter show that a marked inbreeding effect showed in a basic survival trait (swimming); and that swimming performance showed marked correlations with age and size. The implication is that females prefer traits correlated with vigour because these traits are less developed in inbred males.

Once again symmetry was a poor indicator of male quality, this time in terms of swimming performance. Other traits were far better predictors of male quality. Age and body size were the best predictors of performance, and correlated with relative pigment areas. These traits have been shown to be both important in female mate choice and sensitive to inbreeding depression. These preferences may therefore be useful in discriminating differences in male condition.

7.6 References

Ballin, P.J. 1973. Geographic variation in courtship behaviour of the guppy; *Poecilia reticulata.* M.Sc. thesis, University of British Columbia, Vancouver.

Beamish, F.W.H. 1978. Swimming capacity. In: Fish Physiology, vol 7. Pp 101-187. Eds Hoar & Randall. New York : Academic Press.

Brett, J.R. & Glass, N.R. 1973. Metabolic rates and critical swimming speeds of sockeye salmon (*Oncorhynchus nerka*) in relation to size and temperature. J. Fish Res. Board Can. 30: 379-387.

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21: 253-255.

Buchholz, R. 1991. Older males have bigger knobs: correlates of ornamentation in two species of Curassow. The Auk 108: 153-160.

Crow, R.T. 1981. Behavioural adaptations to stream velocity in the guppy, *Poecilia reticulata.* M.Sc. Thesis, University of British Columbia, Vancouver. Pp 1-106.

Dugatkin, L.A. & Sargent, R.C. 1994. Male-male association patterns and female proximity in the guppy, *Poecilia reticulata.* Behav. Ecol. Sociobiol. 35: 141-145.

Houde, A. E. 1988. The effects of female choice and male-male competition on the mating success of male guppies. Anim. Behav., 36, 888-896.

Jones, D.R., Kiceniuk, J.W. & Bamford, O.S. 1974. Evaluation of swimming performance of several fish species from the Mackenzie River. J. Fish Res. Board Can. 31: 1641-1647.

Kodric-Brown, A. 1989. Dietary carotenoids and male mating success in the guppy: an environmental component to female choice. Behav. Ecol. Sociobiol., 25, 393-401.

Magurran, A.E. & Seghers, B.H. 1990. Risk sensitive courtship in the guppy (*Poecilia reticulata).* Behaviour 112: 194-201.

Maynard-Smith, J. 1956. Fertility, mating behaviour, and sexual selection in *Drosophila subobscura.* J. Genet. 54: 261-279.

Nicoletto, P. F. 1991. The relationship between male ornamentation and swimming performance in the guppy, *Poecilia reticulata..* Behav. Ecol. Sociobiol., 28, 365-370.

Nicoletto, P.F. 1996. The influence of water velocity on the display behaviour of male guppies, *Poecilia reticulata.* Behav. Ecol. 7: 272-278.

Reynolds, J.D. & Gross, M.R. 1992. Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata.* Proc. R. Soc. Lond. B. 250: 57-62.

Reynolds, J. D., Gross, M. R., and Coombs, M. J. 1993. Environmental conditions and male morphology determine alternative mating behaviour in Trinidadian guppies. Anim. Behav., 45, 145-152.

Reznick, D.N. 1990. Plasticity in age and size at maturity in male guppies (*Poecilia reticulata)*: an experimental evaluation of alternative models of development. J. Evol. Biol. 3: 185-203.

Rocchetta, G., Vanelli, M.L. & Pancaldi, C. 1993. Parental age effects on body size and growth rate in guppy-fish. Growth, Development and Aging. 57: 3-11.

Ryan, M.J., Hews, D.K., and Wagner W.E. 1990 Sexual selection on alleles that determine body size in the swordtail *Xiphophorus nigrensis*. Behav. Ecol. Sociobiol. 26: 231-237.

Shaw, P.W., Carvalho, G.R., Seghers, B.H. & Magurran, A.E. 1992. Genetic consequences of an artificial introduction of guppies (*Poecilia reticulata*) in North Trinidad. Proc. R. Soc. Lond. B. 248: 111-116.

Smit, H. 1965. Some experiments on the oxygen consumption of goldfish (*Carassus auratus* L.) in relation to swimming speed. Can. J. Zool. 43: 623-633.

Stahlberg, S. & Peckman, P. 1987. The critical swimming speed of small Teleost fish species in a flume. Arch. Hydrobiol. 110: 179-193.

7.7 Tables and figures

Figure 7.1: Diagram of swim test apparatus

c

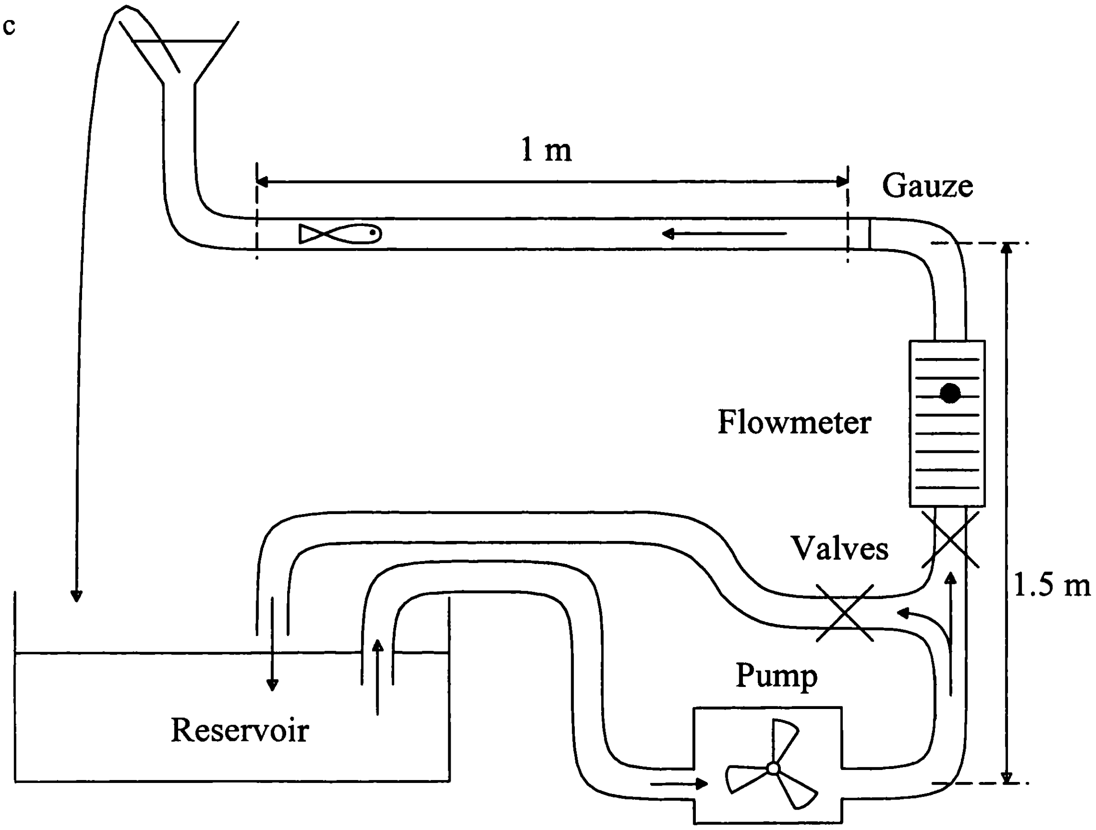


Table 7.1 Table of mean swimming times for inbred and control males.

Group	N	**Inbred mean±s.e.**	N	**Control mean±s.e.**	**p value**
Aripo all	24	42.208±26.718	23	82.278±27.293	0.482
Aripo bottom 50%	11	20.546±3.220	12	19.750±3.083	0.905
Aripo top 50%	13	60.538±48.080	11	150.364±52.268	0.429
Paria all	14	69.357±35.050	19	143.211±30.087	0.486
Paria bottom 50%	8	38.375±4.484	8	26.250±4.484	0.130
Paria top 50%	6	110.667±60.588	11	226.818±44.747	0.166

Table 7.2 Table of mean swimming times for old and young males.

Population	N	Young age group mean ± s.e.	N	Old age group mean ± s.e.	p value
Aripo	46	61.787±36.512	27	497.074±48.172	<0.0001
Paria	33	111.879±27.343	17	170.941±38.096	0.062

Table 7.3 Comparison of young and old Aripo males.

Comparison	**N**	**Inbred group mean ± s.e.**	**N**	**Old group mean ± s.e.**	**p value**
Body area	24	41.826±1.432	31	52.567±1.260	<0.0001
Body length	24	14.701±0.250	31	16.483±0.220	<0.0001
% Melanic area	24	0.199±0.010	27	0.235±0.010	0.010
% Carotenoid area	24	0.255±0.017	27	0.291±0.016	0.127
% Iridescent area	20	0.383±0.028	27	0.489±0.028	0.006
% FA Melanin	24	0.023±0.004	27	0.018±0.004	0.294
% FA Carotenoid	24	0.018±0.004	27	0.029±0.004	0.067
% FA Iridescent	20	0.036±0.008	27	0.042±0.007	0.567
Comparison	**N**	**Control group mean ± s.e.**	**N**	**Old group mean ± s.e.**	**p value**
Body area	23	41.862±1.623	31	52.567±1.398	<0.0001
Body length	23	14.886±0.240	31	16.483±0.206	<0.0001
% Melanic area	23	0.245±0.012	27	0.235±0.011	0.559
% Carotenoid area	23	0.259±0.018	27	0.291±0.016	0.191
% Iridescent area	23	0.463±0.026	27	0.489±0.024	0.461
% FA Melanin	23	0.043±0.007	27	0.018±0.007	0.910
% FA Carotenoid	23	0.015±0.004	27	0.029±0.004	0.015
% FA Iridescent	23	0.028±0.003	27	0.042±0.003	0.042

Table 7.4 Comparison of old and young Paria males

Comparison	**N**	**Inbred group mean ± s.e.**	**N**	**Old group mean ± s.e.**	**p value**
Body area	14	45.829±2.404	17	52.962±2.181	0.036
Body length	14	15.328±0.347	17	16.425±0.315	0.026
% Melanic area	14	0.228±0.017	17	0.232±0.016	0.861
% Carotenoid area	14	0.415±0.020	17	0.414±0.013	0.964
% Iridescent area	9	0.333±0.026	17	0.463±0.019	0.0004
% FA Melanin	14	0.027±0.005	17	0.016±0.005	0.106
% FA Carotenoid	14	0.037±0.007	17	0.021±0.006	0.086
% FA Iridescent	9	0.040±0.009	17	0.030±0.007	0.418
Comparison	**N**	**Control group mean ± s.e.**	**N**	**Old group mean ± s.e.**	**p value**
Body area	19	43.502±2.126	17	52.962±2.248	0.004
Body length	19	14.882±0.325	17	16.425±0.343	0.002
% Melanic area	19	0.221±0.017	17	0.232±0.018	0.667
% Carotenoid area	19	0.427±0.015	17	0.414±0.015	0.006
% Iridescent area	8	0.259±0.032	17	0.463±0.022	<0.0001
% FA Melanin	19	0.028±0.005	17	0.016±0.005	0.092
% FA Carotenoid	19	0.026±0.005	17	0.021±0.005	0.445
% FA Iridescent	8	0.024±0.010	17	0.030±0.007	0.608

Chapter 8: Summary

The objective of this thesis was to investigate the role of symmetry in sexual selection in guppies. Theory predicts that fluctuating asymmetry should be a sensitive indicator of genetic stress. Genetic stress was induced by mild inbreeding in order to measure differences in the responses of symmetry and other variables of male morphology, behaviour and sexual display.

The results show that stress was successfully induced by the inbreeding experiment because several traits showed inbreeding depression. This took the form of reduced pigment areas, of carotenoid in Paria guppies, and melanic (and to a lesser extent iridescent) pigments in Aripo guppies. There was some weak evidence of inbreeding depression in morphological traits, but no significant differences between inbred and control males (Chapter 3; Sheridan & Pomiankowski 1997a).

In contrast, there was no evidence of sensitivity to inbreeding in any measure of fluctuating asymmetry. This does not agree with the prediction that FA should be a more sensitive indicator of stress than other measures. Similarly, there was no evidence of inbreeding depression in another measure of guppy symmetry at the level of spot pattern. Guppy spots may be unpaired, and this far larger level of asymmetry can be analysed separately. Colour pattern overall *did* show sensitivity to inbreeding stress, in the form of reduced numbers of spots and colours in inbred Aripo males. Asymmetric spots however showed no relation to inbreeding, and were a less sensitive indicator of stress than other measures such as numbers of spots and colours (Chapter 4; Sheridan & Pomiankowski 1997a).

Females did not directly discriminate between inbred and control males when given choices. However, male display rate was significantly reduced by inbreeding, and females of both populations always preferred the most frequently displaying male (Chapter 6). Also, females given choices between males differing in the number of unmatched spots preferred the more symmetrical male in both populations (Chapter 5; Sheridan & Pomiankowski 1997b).

There was some evidence of compensation behaviour - unattractive males (with unmatched spots) displayed more frequently, but only if they had considerable experience of females (Chapter 6).

Swimming tests showed weak evidence of inbreeding depression, but strong evidence of an age effect. Older males were far better swimmers, and this correlated with larger body size, higher display rate and greater area of the body covered with sexual pigments (Chapter 7).

These results may be considered in the light of the hypothesis that preferred traits, *if* they are condition dependent, should be the traits most sensitive to inbreeding depression. These results agree fairly well with this hypothesis.

Aripo females prefer more spots, more colours, and larger melanic and iridescent areas. Inbreeding depression reduces numbers of spots and colours, and reduces melanic and iridescent areas. Aripo females also prefer larger males (Chapter 5). Body size showed only weak evidence of inbreeding depression (Chapter 3) - though swimming performance was highly correlated with larger body size in old males (Chapter 7). Larger, older males have been shown to produce considerable genetic benefits in their offspring in other populations (Reynolds & Gross 1992; Rocchetta *et al* 1993).

Paria females strongly prefer carotenoid area - which was the only pigment to respond to inbreeding depression in this population, showing reduced area in inbred males. There is no information on whether Paria females prefer larger males (the tests in Chapter 5 compared larger, less orange males with smaller, more orange males - so any size preference may have been confounded by the strength of orange preference in this population).

In addition, females of both populations strongly preferred the most frequently displaying male of any group, above other variables such as pigment areas and body size. Display rate was significantly reduced by inbreeding depression. Larger, older males also showed much higher display rates. By the use of display rate as a cue, females of both populations may indirectly be selecting older, fitter males (which have been shown to produce larger offspring).

Overall these findings are suggestive that most female preference in guppies is directed at male traits that provide information about male genetic quality. However, females from both populations showed a preference for the more symmetric of pairs of males differing only in spot symmetry. Spot symmetry showed no response to stress in the form of inbreeding, and showed no correlations with any other variables known to be condition dependent. This preferred trait showed no evidence of sensitivity to genetic stress (unlike the other preferred traits). The over-riding importance of display rate to females, plus the finding that other factors become important when display rate is controlled (such as size, pattern complexity, and spot symmetry) add support to the assertions of other guppy workers (e.g. Kodric-Brown

1993)- that female mate choice is a subtle process, involving a complex suite of morphological and behavioural traits in males.

The mixed evidence of condition dependent sexual traits (pigment areas, numbers of spots and colours, display rate, possibly swimming endurance and body size), condition independent sexual traits (spot symmetry), and the lack of response in FA can suggest two conclusions. Firstly, mild inbreeding is not a severe enough stress to induce responses in symmetry (either FA or pattern); or sample size was too small to detect the effects. The analysis is based on a small sample with only a few lines per population. Line affects were controlled for statistically but it would have been better to have had a greater level of replication. This constraint is particularly severe in the Paria population where data were also combined across two generations because of low fecundity. The ideal situation would be to have more lines where each could be used as a single data point. However, there is the point that inbreeding depression *was* detected in other traits, so lack of response in symmetry cannot be attributed to failure of the inbreeding to produce genetic stress. Also, the predictions of theory led to the expectation that symmetry (in particular FA) should be *more* sensitive to stress than other measures. This has proved false in this case.

The second possible conclusion, if the findings are real, is that most sexual traits measured in this study are condition dependent traits (pigment areas, pattern complexity, display rate) and females using these cues are gaining information about male quality. Body size correlates well with age and swimming endurance, so females using this cue are also gaining truthful information about male vigour. In addition, females also prefer traits that are not condition dependent (spot symmetry) and gain no information about male quality. The evidence on condition-dependent sexual traits is consistent with predictions from "good genes" models of mate choice

(Pomiankowski 1988; Grafen 1990; Iwasa *et al.* 1991) though this does not preclude the action of other models. Unfortunately most sexual selection models make few mutually exclusive predictions (Balmford & Read 1991). For example, preferences for large pigment areas, complex spots, large size and frequent display could all have arisen through "sensory bias" (Ryan 1990) as these traits all confer increased conspicuousness. The fact that these traits all correlate with male quality (in this case illustrated by outbreeding) may be a secondary advantage. These traits could also indicate parasite load (in one case, display rate, this has been shown - Kennedy *et al.* 1987). Preferences could coevolve with the mating advantages conferred by these conspicuous traits (Fisherian sexual selection) in addition to the potential benefits in terms of offspring fitness (good genes).

The existence of a female preference for spot symmetry appears to support the action of "aesthetic choice" (Fisherian sexual selection) as this trait shows no sensitivity to stress or correlations with other variables known to be condition dependent. This would suggest that both good genes and Fisherian processes are both acting on guppy mate choice, without excluding the possibility of other mechanisms. There is also the possibility that spot asymmetry *is* sensitive to stress but the methods used did not detect it. Aside from this, there are reasons to believe that spot symmetry is unlikely to conform to predictions of theory regarding fluctuating asymmetry. Spot asymmetry is extremely common in guppies, and this extreme form of asymmetry has not been properly investigated. If female preference for symmetry is important, surely selection should have reduced the high level of asymmetry in males. The evidence that female preference for display rate is independent of spot symmetry suggests that under natural conditions (when display rate is not controlled) this preference for spot symmetry is relatively unimportant.

Conversely, there is still the finding that condition dependence was detected in other preferred traits, but not any measure of symmetry. Overall, these findings are suggestive that most female preference is directed at traits which may give information about quality (but some preference is directed at traits that only confer a mating advantage). There is no support for the hypothesis that any form of symmetry in sexual traits (whether fluctuating asymmetry or pattern asymmetry) is a better indicator of stress than other measures.

References

Balmford, A. & Read, A.F. 1991. Testing alternative models of sexual selection through female choice. Trends Ecol. Evol. 6: 274-276.

Grafen, A. 1990. Sexual selection unhandicapped by the Fisher process. J. Theor. Biol. 144: 475-516.

Iwasa, Y, Pomiankowski, A. & Nee, S. 1991. The evolution of costly mate preferences 2: The "handicap" principle. Evolution 45: 1431-1442.

Kennedy, C. E. J., Endler, J. A., Poynton, S. L., & McMinn, H. 1987. Parasite load predicts mate choice in guppies. Behav. Ecol. Sociobiol., 21, 291-295.

Kodric-Brown, A. 1993. Female choice of multiple male criteria in guppies: interacting effects of dominance, colouration and courtship. Behav. Ecol. Sociobiol. 32: 415-420.

Pomiankowski, A.N. 1988. The evolution of female mate preferences for male genetic quality. Oxford Surveys in Evolutionary Biology 5: 136-184

Reynolds, J.D. & Gross, M.R. 1992. Female mate preference enhances offspring growth and reproduction in a fish, *Poecilia reticulata.* Proc. R. Soc. Lond. B. 250: 57-62.

Rocchetta, G., Vanelli, M.L. & Pancaldi, C. 1993. Parental age effects on body size and growth rate in guppy-fish. Growth, Development and Aging. 57: 3-11.

Ryan, M.J. 1990. Sexual selection, sensory systems, and sensory exploitation. Oxf. Surv. Evol. Biol. 7: 157-195.

Sheridan, L. & Pomiankowski, A. 1997a. Fluctuating asymmetry, spot asymmetry and inbreeding depression in the sexual coloration of male guppy fish. Heredity, *in press.*

Sheridan, L. & Pomiankowski, A. 1997b. Female choice for spot asymmetry in the Trinidadian guppy. Anim. Behav., *in press.*

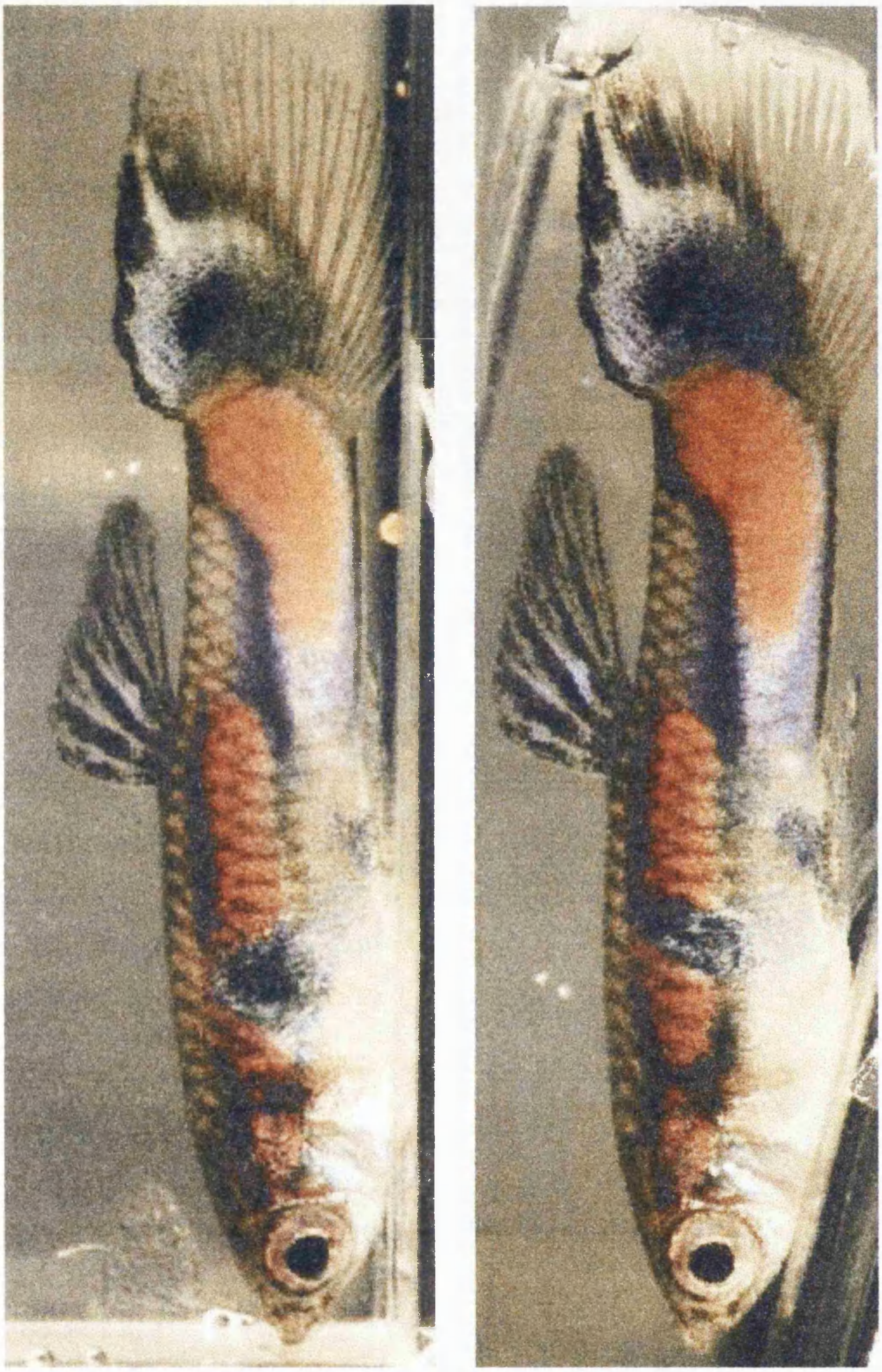
Acknowledgements

I would like to thank Andrew Pomiankowski for unique help, input and supervision.

Thanks also go to Professor D.A. Hopkinson for help and advice in the absence of Dr. Pomiankowski;

- to Ricardo Azevedo and Kevin Fowler for analysis assistance and advice;
- John Reynolds for advice on transport (and his map of Trinidad!)
- David Reznick for use of equipment at the Asa Wright Nature Centre - William Beebe Tropical Research Station, Simla, Trinidad
- Trinidad Ministry of Agriculture, Lands and Fisheries for permission to export fish
- Duncan Franklin for experimental help
- Home Office for the import license
- Jos Martin and Lenham Computing Services for computing assistance.

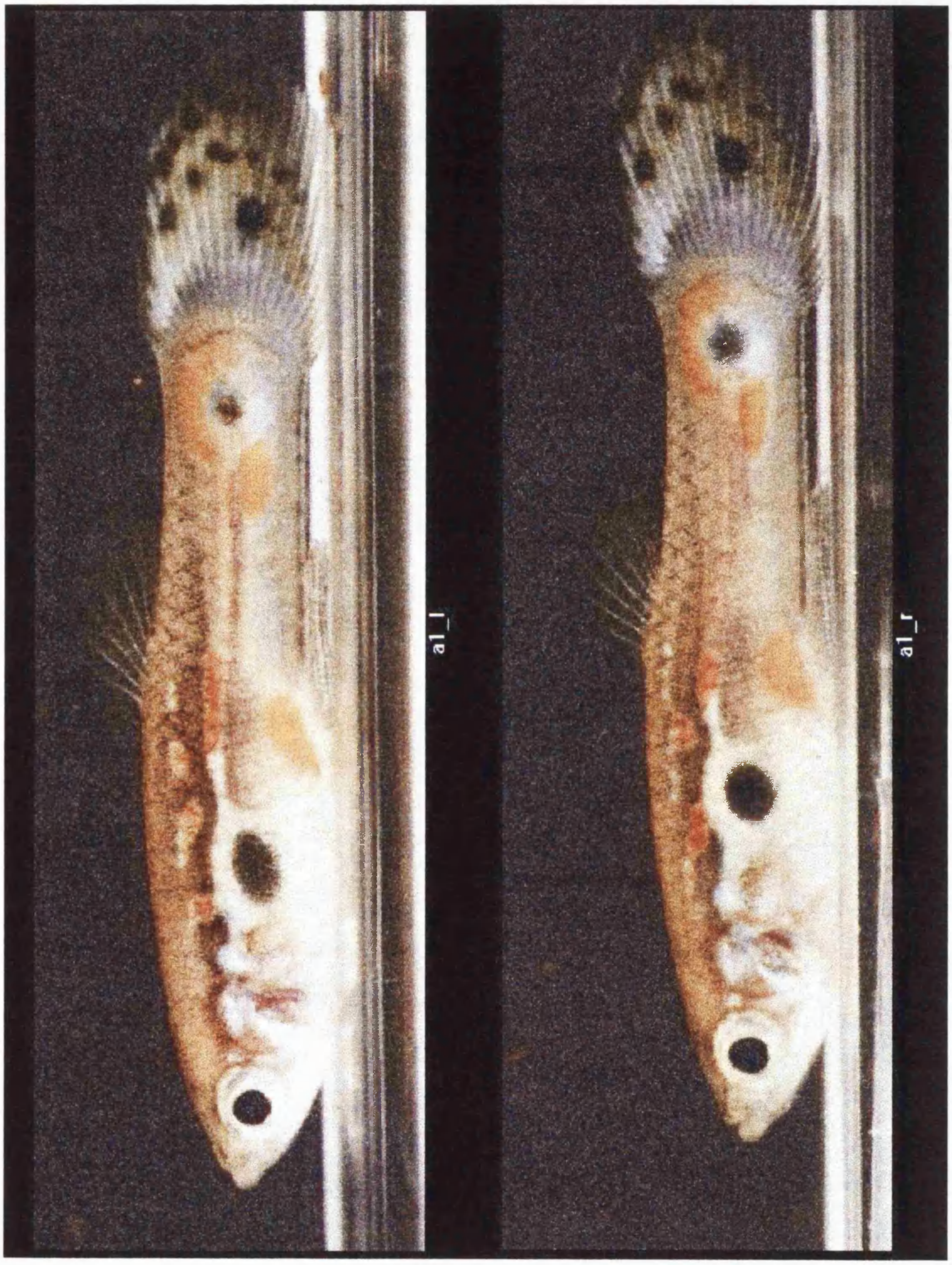
This work was supported by grants from the Leverhulme Trust



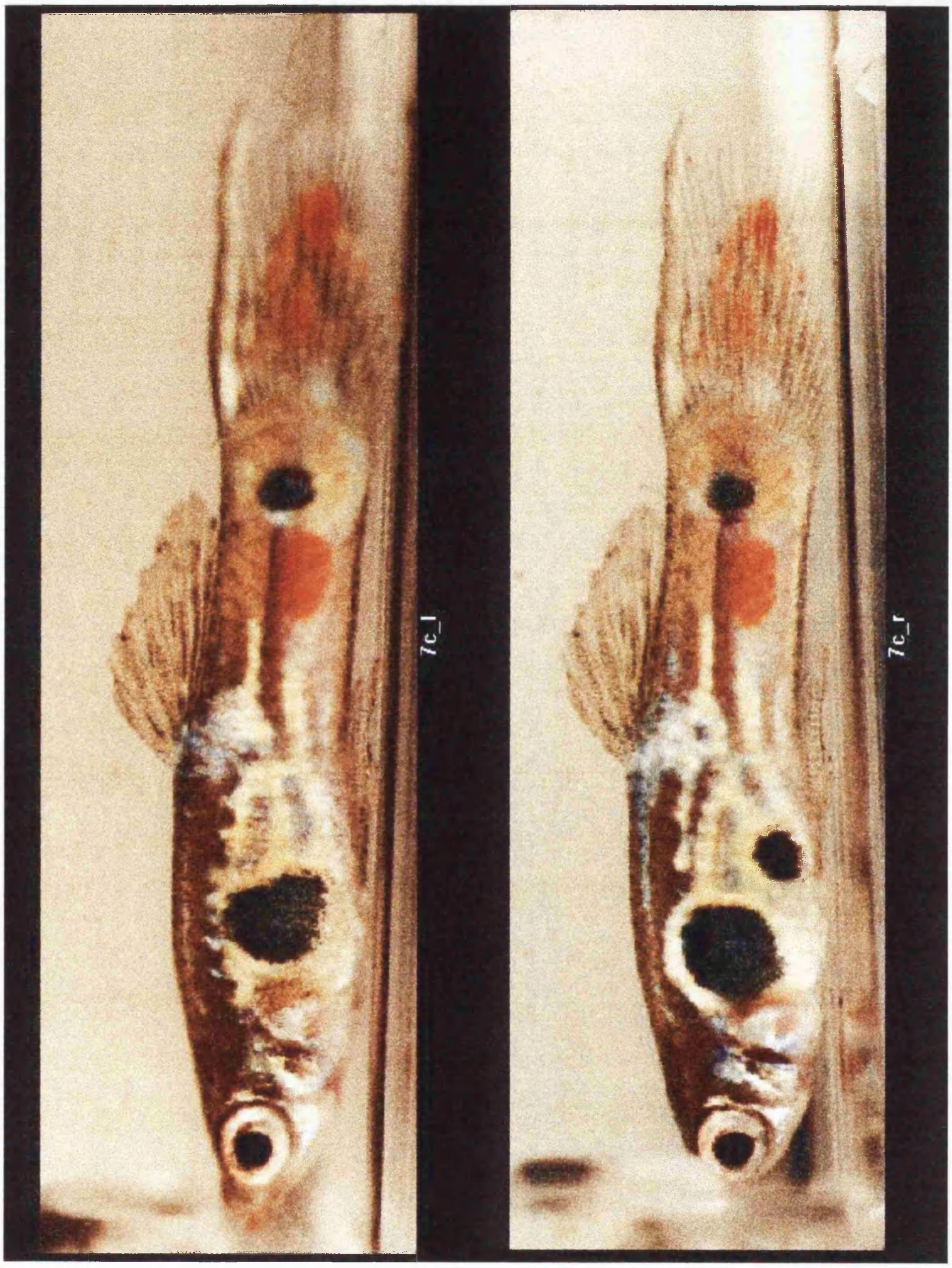
Appendix 1

Symmetrical Paria Fish

Asymmetrical Paria Fish



Symmetrical Aripo Fish



Asymmetrical Aripo Fish

Appendix 2

Breakdown of colour pigment areas by families (tribe = offspring of one sire).

"faM" refers to the absolute value of left minus right melanin,

"fa√M" is the square root of this; "faRelM" referes to relative FA - absolute value of left minus right

divided by (left plus right /2); and "fa%m" refers to FA divided by body area and so on .....

ARIPO

MELANIN							
	N	faM	SE	fa√M	SE	faRelM	SE
Aripo controls	23	0.411	0.092	0.176	0.028	0.115	0.019
Aripo inbreds	24	0.562	0.090	0.149	0.027	0.111	0.019
tribe 5:controls	13	0.437	0.123	0.162	0.036	0.125	0.025
tribe 5:inbreds	16	0.573	0.111	0.167	0.032	0.102	0.022
tribe 3:controls	5	0.652	0.295	0.216	0.091	0.144	0.055
tribe 3:inbreds	1	0.960	0.659	0.326	0.202	0.222	0.123
tribe 2:controls	3	0.233	0.184	0.098	0.065	0.084	0.045
tribe 2:inbreds	4	0.594	0.159	0.205	0.056	0.141	0.040
tribe 6:inbreds	3	0.077	0.098	0.030	0.046	0.024	0.045
tribe 6:controls	2	0.218	0.120	0.110	0.056	0.112	0.055
CAROTENOID							
	N	faC	SE	fa√C	SE	faRelC	SE
Aripo controls	24	0.295	0.052	0.096	0.019	0.067	0.017
Aripo inbreds	23	0.368	0.051	0.115	0.019	0.078	0.016
tribe 5:controls	13	0.436	0.050	0.012	0.015	0.069	0.010
tribe 5:inbreds	16	0.260	0.045	0.071	0.013	0.040	0.009
tribe 3:controls	1	0.251	0.108	0.080	0.034	0.052	0.022
tribe 3:inbreds	3	0.790	0.242	0.221	0.077	0.124	0.050
tribe 2:controls	4	0.183	0.156	0.053	0.061	0.031	0.048
tribe 2:inbreds	3	0.406	0.135	0.174	0.053	0.149	0.042
tribe 6:inbreds	3	0.453	0.281	0.215	0.122	0.208	0.109
tribe 6:controls	2	0.105	0.344	0.070	0.150	0.093	0.133
IRIDESCENT							
	N	faI	SE	fa√I	SE	faRelI	SE
Aripo controls	24	1.781	0.284	0.276	0.046	0.090	0.019
Aripo inbreds	23	1.053	0.304	0.227	0.050	0.111	0.020
tribe 5:controls	13	0.640	0.427	0.136	0.054	0.078	0.020
tribe 5:inbreds	16	1.919	0.320	0.276	0.041	0.083	0.015
tribe 3:controls	1	0.815	0.174	0.170	0.040	0.077	0.025
tribe 3:inbreds	3	5.110	0.388	0.832	0.089	0.271	0.055
tribe 2:controls	4	0.562	0.302	0.153	0.089	0.092	0.055
tribe 2:inbreds	3	0.981	0.262	0.207	0.077	0.089	0.048
tribe 6:inbreds	3	3.18	0.615	0.667	0.152	0.283	0.074
tribe 6:controls	2	0.618	0.753	0.667	0.187	0.255	0.090

MELANIN			
	N	Mfa%M	SE
Aripo controls	23	0.028	0.004
Aripo inbreds	24	0.023	0.004
tribe 5:controls	13	0.025	0.006
tribe 5:inbreds	16	0.026	0.005
tribe 3:controls	5	0.033	0.014
tribe 3:inbreds	1	0.047	0.032
tribe 2:controls	3	0.016	0.011
tribe 2:inbreds	4	0.033	0.010
tribe 6:inbreds	3	0.005	0.007
tribe 6:controls	2	0.017	0.008
CAROTENOID			
	N	Cfa%C	SE
Aripo controls	23	0.015	0.003
Aripo inbreds	24	0.018	0.003
tribe 5:controls	13	0.019	0.002
tribe 5:inbreds	16	0.011	0.002
tribe 3:controls	5	0.012	0.005
tribe 3:inbreds	1	0.032	0.011
tribe 2:controls	3	0.008	0.011
tribe 2:inbreds	4	0.028	0.009
tribe 6:inbreds	3	0.035	0.021
tribe 6:controls	2	0.010	0.025
IRIDESCENT			
	N	Ifa%I	SE
Aripo controls	24	0.043	0.007
Aripo inbreds	23	0.036	0.008
tribe 5:controls	13	0.021	0.009
tribe 5:inbreds	16	0.044	0.007
tribe 3:controls	1	0.026	0.006
tribe 3:inbreds	3	0.119	0.013
tribe 2:controls	4	0.024	0.014
tribe 2:inbreds	3	0.032	0.012
tribe 6:inbreds	3	0.105	0.025
tribe 6:controls	2	0.019	0.030

MELANIN							
	N	L+R M	SE	L+R √M	SE	L+R %M	SE
Aripo controls	23	2.563	0.186	1.561	0.062	0.245	0.011
Aripo inbreds	24	1.715	0.182	1.284	0.060	0.199	0.011
tribe 5:controls	13	1.765	0.260	1.302	0.082	0.202	0.015
tribe 5:inbreds	16	2.901	0.234	1.670	0.074	0.265	0.014
tribe 3:controls	5	2.112	0.203	1.440	0.068	0.222	0.011
tribe 3:inbreds	1	2.190	0.454	1.471	0.151	0.210	0.024
tribe 2:controls	3	1.333	0.153	1.149	0.059	0.183	0.018
tribe 2:inbreds	4	2.119	0.132	1.449	0.051	0.230	0.015
tribe 6:inbreds	3	1.215	0.294	1.080	0.128	0.168	0.017
tribe 6:controls	2	0.926	0.361	0.959	0.156	0.141	0.021
CAROTENOID							
	N	L+R C	SE	L+R √C	SE	L+R %C	SE
Aripo controls	23	2.961	0.355	1.597	0.072	0.259	0.017
Aripo inbreds	24	2.936	0.347	1.488	0.070	0.255	0.016
tribe 5:controls	13	3.497	0.473	1.568	0.082	0.279	0.020
tribe 5:inbreds	16	3.654	0.426	1.778	0.074	0.297	0.018
tribe 3:controls	5	2.628	0.494	1.554	0.063	0.246	0.023
tribe 3:inbreds	1	3.195	1.105	1.490	0.142	0.255	0.052
tribe 2:controls	3	3.020	0.121	1.466	0.054	0.276	0.010
tribe 2:inbreds	4	1.298	0.105	1.266	0.047	0.179	0.009
tribe 6:inbreds	3	0.932	0.181	1.055	0.104	0.148	0.017
tribe 6:controls	2	0.623	0.222	0.868	0.128	0.115	0.021
IRIDESCENT							
	N	L+R I	SE	L+R √I	SE	L+R %I	SE
Aripo controls	24	9.302	0.981	2.945	0.174	0.463	0.030
Aripo inbreds	23	6.697	1.052	2.437	0.186	0.383	0.033
tribe 5:controls	13	6.280	1.779	2.291	0.311	0.363	0.055
tribe 5:inbreds	16	10.662	1.334	3.156	0.233	0.501	0.041
tribe 3:controls	1	7.098	1.640	2.576	0.336	0.399	0.059
tribe 3:inbreds	3	9.605	3.668	3.071	0.751	0.439	0.131
tribe 2:controls	4	7.526	2.196	2.586	0.419	0.411	0.069
tribe 2:inbreds	3	5.917	1.902	2.422	0.363	0.383	0.060
tribe 6:inbreds	3	6.442	1.003	2.494	0.209	0.391	0.033
tribe 6:controls	2	5.039	1.228	2.231	0.256	0.327	0.040

PARIA

MELANIN							
	N	faM	SE	fa√M	SE	faRelM	SE
paria controls	18	0.55868	.09287	0.18739	0.03371	0.13643	0.02628
paria inbreds	14	0.56143	0.1036	0.18712	0.03494	0.13752	0.02953
tribe 1:controls	14	0.60286	0.11421	0.19288	0.03833	0.13413	0.03075
tribe 1:inbreds	10	0.548	0.13441	0.20451	0.04718	0.16419	0.03836
tribe 4:controls	4	0.40125	0.107517	0.168179	0.053683	0.144506	0.056968
tribe 4:inbreds	4	0.595	0.163529	0.143624	0.030607	0.070831	0.010744
CAROTENOID							
	N	faC	SE	fa√C	SE	faRelC	SE
paria controls	19	1.07237	0.2051	0.17375	0.03467	0.05783	0.0129
paria inbreds	14	1.40321	0.33073	0.24506	0.05568	0.08901	0.02029
tribe 1:controls	15	0.99	0.2401	0.14762	0.03562	0.0445	0.01069
tribe 1:inbreds	10	1.664	0.42444	0.29029	0.07145	0.10589	0.02617
tribe 4:controls	4	1.38125	0.386967	0.271747	0.089404	0.107825	0.041069
tribe 4:inbreds	4	0.75125	0.33086	0.131976	0.052604	0.046816	0.016646
IRIDESCENT							
	N	faI	SE	fa√I	SE	faRelI	SE
paria controls	19	0.41237	0.20762	0.20383	0.10776	0.135788	0.077722
paria inbreds	14	1.03286	0.27551	0.36182	0.1321	0.127633	0.035544
tribe 1:controls	15	0.52233	0.2571	0.25818	0.13383	0.135788	0.077722
tribe 1:inbreds	10	0.785	0.26587	0.22971	0.08593	0.101911	0.027777
tribe 4:controls	4	0	0	0	0	0	0
tribe 4:inbreds	4	1.6525	0.664798	0.692101	0.397992	0.179078	0.09653

MELANIN			
	N	Mfa%M	SE
paria controls	18	0.02966	0.00521
paria inbreds	14	0.02729	0.00488
tribe 1:controls	14	0.03027	0.00628
tribe 1:inbreds	10	0.0298	0.00658
tribe 4:controls	4	0.027511	0.00953
tribe 4:inbreds	4	0.021026	0.004254
CAROTENOID			
	N	Cfa%C	SE
paria controls	19	0.02643	0.00541
paria inbreds	14	0.03706	0.00881
tribe 1:controls	15	0.02171	0.0051
tribe 1:inbreds	10	0.0442	0.0114
tribe 4:controls	4	0.044161	0.015746
tribe 4:inbreds	4	0.019227	0.007315
IRIDESCENT			
	N	Ifa%I	SE
paria controls	19	0.023821	0.009631
paria inbreds	14	0.039605	0.009097
tribe 1:controls	15	0.023821	0.009631
tribe 1:inbreds	10	0.035531	0.009283
tribe 4:controls	4	0	0
tribe 4:inbreds	4	0.047751	0.022637

MELANIN							
	N	L+R M	SE	L+R √M	SE	L+R %M	SE
paria controls	18	2.55764	0.41973	1.52262	0.11536	0.23372	0.01676
paria inbreds	14	2.70571	0.51103	1.54889	0.15035	0.22794	0.02065
tribe 1:controls	14	2.81571	0.519	1.59458	0.14112	0.2432	0.02075
tribe 1:inbreds	10	2.2125	0.60332	1.38582	0.17525	0.20309	0.02337
tribe 4:controls	4	1.654375	0.239436	1.270759	0.100499	0.200515	0.01271
tribe 4:inbreds	4	3.93875	0.722029	1.956545	0.186884	0.290052	0.023856
CAROTENOID							
	N	L+R C	SE	L+R √C	SE	L+R %C	SE
paria controls	19	10.05329	0.61869	3.13830	0.10663	0.47677	0.01137
paria inbreds	14	8.07089	0.6624	2.79771	0.12422	0.4148	0.01939
tribe 1:controls	15	10.818	0.63311	3.26405	0.12057	0.49151	0.01128
tribe 1:inbreds	10	8.3235	0.87273	2.83206	0.14766	0.41997	0.02716
tribe 4:controls	4	7.185625	0.650074	2.66672	0.14637	0.421511	0.012918
tribe 4:inbreds	4	7.43938	0.86959	2.71185	0.14637	0.401884	0.009546
IRIDESCENT							
	N	L+R I	SE	L+R √I	SE	L+R %I	SE
paria controls	19	3.34531	0.82258	1.70754	0.20626	0.25922	0.038722
paria inbreds	14	5.08833	0.77553	2.20574	0.19447	0.332737	0.02428
tribe 1:controls	15	3.34531	0.84263	1.70754	0.21464	0.25922	0.038722
tribe 1:inbreds	10	5.91	0.97299	2.38884	0.24785	0.357952	0.030456
tribe 4:controls	4	0	0	0	0	0	0
tribe 4:inbreds	4	3.445	0.38311	1.83955	0.11473	0.282308	0.022998