Condition-dependent mate preference in the stalk-eyed fly *Cyrtodiopsis dalmanni*.

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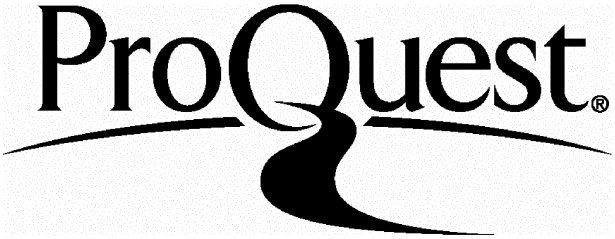
Ph.D. Thesis



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Abstract

This thesis investigates whether female mate preference is dependent upon female morphology or condition using the stalk-eyed fly, *Cyrtodiopsis dalmanni,* as a model. Workers in the field of sexual selection are interested in evidence for this type of variation in mate preference because it is believed it may affect the rate of evolution of male trait and female preference and increase our understanding of the mechanistic nature of mate choice.

Little is known or understood about variation in female mating preferences despite the great attention paid to mating systems. There is some evidence for genetic variation in female preference. There is more evidence for environmental factors affecting preference, with sources as diverse as morphological phenotype, predation risk, parasitism, copying, age and experience having been identified.

The phenotype of females was manipulated in two ways. First by varying larval food density to create females that varied in eyespan. Second, adult females were fed a sucrose diet to create a transient reduction in nutrition. Both manipulations are likely to have depressed female condition. Female eyespan correlates with body size and thus with fecundity. The sucrose diet also reduced female fecundity by causing a reduction of egg production. Under both manipulations, females in lower condition (small eyespan or sucrose diet) were

less choosy. Female *C. dalmanni* normally prefer large eyespan males. Eyespan-dependent mate choice is probably due to large eyespan females having better vision and so being better able to distinguish between male phenotypes. Diet-dependent mate choice is caused by females with reduced egg production having weaker mate choice.

Further investigation of eyespan-dependent mate preference was made by mixing large and small eyespan females in the same cage to see whether there was competition between the two female phenotypes for mates. The results did not provide evidence that females competed for mates.

In addition, the use of fluctuating asymmetry (FA) as a tool for conservation biologists monitoring endangered populations was evaluated. Fluctuating asymmetry in exaggerated sexual ornaments has been proposed to show a strong positive relationship with environmental stress. No evidence was found to support this theory.

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Chapter One

Introduction and literature review

Sexual selection theory

While Darwin was collecting evidence for his theory of evolution by natural selection, he observed that some traits, nearly always possessed by males, did not fit with his hypothesis that all traits should be adaptive to the organism's environment. These traits, such as bright plumage in birds and horns of beetles, could not give the possessor a survival advantage and therefore seemed to oppose the theory of natural selection. Darwin (1859) proposed that these traits were adaptive if they were used to gain access to females and therefore increase the possessor's reproductive success.

Differential reproductive success in males is common and is especially prominent in polygynous species where a few males within a population sire a disproportionate number of offspring (Cox and LeBouef 1977; Clutton-Brock *et al.* 1982; Alatalo *et al.* 1992). Such extreme variation in male reproductive success can arise by two mechanisms, which are not mutually exclusive. Males can compete amongst each other for mates or females may choose between

potential mates. Both male competition and female choice can produce strong selective pressure on males for large size or weapons and traits that are attractive to females (Andersson 1994). These characters often decrease the survivorship of the possessor by making them more conspicuous or more cumbersome when trying to flee a predator (Ryan 1985; Houde 1997).

The evolution of male weapons and greater male size is not controversial. Any trait that increases a males fighting ability and hence his access to females will give that male a reproductive advantage over his competitors. Female choice for direct benefits (e.g. choosing males with large nuptial food gifts, a good breeding territory, or males that will provide better parental care) is also not controversial. By choosing such males, females are likely to increase their reproductive success over females that don't discriminate between potential mates. What is controversial however, is how female choice evolved in mating systems where females seem to gain nothing from their choice other than sperm (Kirkpatrick and Ryan 1991). This behaviour is most pronounced and obvious in polygynous leking species where males often possess exaggerated ornaments and elaborately coloured traits that must be the result of strong sexual selection (Höglund and Alatalo 1995). Why do females choose in these species when there appears to be no direct benefit?

Two central mechanisms for the evolution of female mate choice by indirect benefits exist. These two mechanisms differ in the central issue of

whether ornaments represent males of good genetic quality or just male attractiveness. Good genes theories stipulate that females gain survivorship advantages for their offspring by mating good genetic quality males, while Fisherian theories assume females choose attractive males so that their sons will be attractive too.

Fisher and runaway sexual selection

Fisherian models of sexual selection suggest that male exaggerated traits evolved as a result of the co-evolution between genes for female mate choice and genes for the male trait. Male traits are just attractive to choosy females and serve no other signalling purpose. Such male Fisherian traits may be costly but give the possessor a reproductive advantage over males who do not possess the trait. Fisher's original hypothesis was only verbal (Fisher 1930), but recently population and quantitative genetic models have shown that mate choice may evolve in this way (Lande 1981, Kirkpatrick 1982, Pomiankowski *et al.* 1991). All such models work in the same basic way.

An initial preference arises in a population. This gene for preference rises in frequency in the population because it makes females prefer a male trait which increases male fitness (or fitness in both sexes). As a result, male offspring have greater fitness. An alternative model suggests that the initial spread of the female

preference is due to random genetic drift. Once the female preference gene reaches a threshold frequency in the population a positive feedback process begins which leads to the rapid increase in frequency of both male trait and female preference gene. This process is termed runaway.

Runaway occurs because trait and preference genes are inherited together, as preference genes are in linkage disequillibrium with trait genes within the population. Offspring of a female, who mated with a male with the preferred trait, will inherit both preference and trait genes. The female preference gene increases the reproductive success of males with the preferred trait, driving the trait to higher frequency in the population. Because the preference and trait genes tend to be found in linkage disequillibrium (i.e. associated together within individuals more frequently than expected under random mating) the female preference is also dragged to a higher frequency in the population. This causes an increase in the strength of female preference in the population and so further increases the frequency of the male trait. In this way both female preference and the preferred male trait co-evolve together.

The male trait may be selected to a point where it becomes maladaptive in ways other than reproductive success. Males will then evolve to a compromise state between natural selection against the trait which reduces survivorship and sexual selection in favour a trait which increases reproductive success. In simple

terms males must survive to mate but also have a large enough trait to out compete other males for matings.

Good genes sexual selection

Unlike Fisherian male traits, good genes male traits are correlated with overall viability of the possessor. Fisher first proposed good genes sexual selection but failed to present a formal description (Fisher 1915). Zahavi is accepted as one of the main proponents of the most widely studied mechanisms of good genes sexual selection, which he termed Handicap theory (Zahavi 1975, 1977). Zahavi suggested that large ornamented males suffered increased predation and that only the highest quality males could produce the exaggerated male ornament and survive to sexual maturity. The trait itself must be costly, or poor quality males could cheat and produce a male ornament without a survival cost. The trait would then confer no information about male viability. The cost of expression makes male exaggerated secondary sexual traits an uncheatable signal of male quality.

In good genes sexual selection females prefer to mate with large ornamented males. These males also tend to have genes for greater viability which are then passed on to the offspring. Integral to good genes sexual selection is the relationship between male quality and the expression of the male trait. Unlike

Fisherian models, the expression of the male trait gene is assumed to be an increasing function of quality. This relationship is born of the assumption that the cost of producing a large ornament is greater for males in poor condition (those lacking high viability genes). In good genes models, choosy females gain the advantages of runaway by having sexy sons, but also from the increased viability of their offspring.

Early models of good genes sexual selection showed that the female preference and the male trait failed to evolve beyond low frequencies (Maynard Smith 1976, Tomlinson 1988). A problem in these early models was the way investigators modelled the link between ornament expression and male quality using epistatic expression of the trait (i.e. males developed the trait to the same degree if they had the trait gene but few of these survived to sexual maturity if they did not also have the viability gene). Taking two more realistic approaches to ornament expression solved this problem. These were condition-dependent and 'revealing' expression of the ornament. Condition-dependent models assume that ornaments develop in proportion to the phenotypic quality of the male. Revealing ornaments reflect the resistance of a male to environmental stress, in particular, parasites (Hamilton and Zuk 1982). Investigators also found that genetic variation for male viability soon disappeared as viability genes were pushed to fixation by selection. Genetic variation in male viability was maintained in later models by assuming mutation-selection balance, migration, or spatially and temporally

varying selection pressure. Later models, using condition-dependent and revealing ornaments showed that good genes sexual selection could work (Kirkpatrick 1986; Pomiankowski 1987a, 1987b).

Evidence for indirect benefit mate choice

Female mate choice is now a widely accepted phenomenon, but it has not always been assumed as such by evolutionary biologists (Trivers 1985). As recently as thirty years ago females were assumed to be passive in selection of mates (Trivers 1985).

In mating systems with no parental care or nuptial food gifts, males rarely contribute more to the production of offspring than their genes. Sperm are usually unlimited in number and cost little to produce. In contrast, females invest heavily in the production of large costly gametes that are limited in number. Males and females therefore have conflicting mating strategies (Parker 1983). Males will maximize their reproductive success if they mate as many females as possible. In contrast, females will maximise their reproductive success by carefully choosing good quality mates.

Numerous studies have now shown that female choice of partner is influenced by variation in male traits (Andersson 1994). Females have also been shown to have highly repeatable mate preferences when presented with a choice

between males or male signals in such diverse species as jungle fowl (Johnsen and Zuk 1996), stalk-eyed flies (Wilkinson 1998), guppies (Godin and Dugatkin 1995), fruit flies (Isoherranen *et al.* 1999), field crickets (Wagner *et al.* 1995) and African painted reed frogs (Jennions *et al.* 1995). Even in the wild where observations of mating attempts may be confounded by many other factors, female choice has been directly demonstrated (Andersson 1982; Wilkinson and Reillo 1994).

For females to exert a choice, they must be able to reject unsolicited copulations with undesirable males. Numerous studies have found that females have the ability to reject undesired copulations (Merrell 1949, Beach and LeBoeuf 1967, Trivers 1985; Day *et al.* 1990).

Mate choice in the stalk-eyed fly

Studies of sexual selection in stalk-eyed flies are limited to the investigation of two closely related species, *Cyrtodiopsis whitei* and *Cyrtodiopsis dalmanni* (Wilkinson *et al.* 1998). These species are found throughout South East Asia. Most laboratory populations used in studies of sexual selection originate from peninsular Malaysia. Both species are sexually dimorphic for eyespan (Schillito 1971; de la Motte and Burkhardt 1983), males typically having eyespans much larger than their body length, whereas females, whilst still having

stalked-eyes, have eyespans less than their body length. Sexual selection in *C. dalmanni* and *C. whitei* is mediated by male-male competition and female mate choice (Burkhardt and de la Motte 1988; Panhuis and Wilkinson 1999).

Competition between males takes place in the form of highly ritualised fights (Burkhardt and de la Motte 1987). Males face each other approximately one eyespan apart and stand on their mid and hind legs. Males will then display their large forelegs parallel to their eyestalks. If one of the males does not retreat, the males will rear up on their hind legs and strike at each other with their forelegs. The loser is forced into retreat and chased away. Most ritualised fights occur between males of similar eyespan as small males nearly always back down in confrontations. When fights do occur, large eyespan males nearly always win and this effect has been shown to be independent of male body size (Panhuis and Wilkinson 1999).

During the day, stalk-eyed flies forage on the forest floor or the banks of streams (Burkhardt and de al Motte 1987). In the early evening, flies congregate on plant root hairs on the edge of riverbanks. Large eyespan males arrive first, each occupying a different root hair. As dusk falls small males and females arrive and collect in aggregations. Large eyespan males will drive other males off their root hair, creating a harem of females. Male eyespan shows a strong relationship with the number of females on its root hair (Burkhardt and de la Motte 1988; Wilkinson and Reillo 1994). At dawn a male will attempt to mate with the

females in its harem. About 95% of copulations occur during this dawn mating period (Wilkinson and Reillo 1994). Females appear to gain nothing from their mates other than a spermatophore. The spermatophores of *C. dalmanni* and *C. whitei* are small and are unlikely to provide any nutrition for females (Kotrba 1996).

In laboratory studies females have been shown to prefer roosting with large eyespan males. Their choice is not chemically based as washing dummy males to remove pheromones had no effect on female choice (Burkhardt and de la Motte 1988). In addition females no longer showed any roosting preference when dummy males were covered with a perforated paper bag, suggesting that choices between males are visually based (Burkhardt and de al Motte 1988). Additionally, significant female preference for large eyespan males has been demonstrated in two studies using live males (Wilkinson and Reillo 1994, Wilkinson *et al.* 1998).

Condition-dependence is one of the assumptions of some handicap models of the evolution of female preference and male trait. Male eyespan in *C. dalmanni* has been shown to have greater condition-dependence than standard morphological traits and the homologous trait in females (David *et al.* 1998, Wilkinson and Taper 1999; chapter five). In addition male eyespan has been shown to be heritable (Wilkinson 1993), another basic assumption of indirect benefit models. Further to this David *et al.* (2000) found that the condition-

dependence of the male trait was mediated by underlying genetic mechanisms which were heritable.

Variation in female mate preference

In the last ten years the investigation of variation in female mate preference has escalated. Before this period of intensive research, studies of variation in female mate choice were limited to estimating the heritability of female mate preferences. Rosenqvist and Berglund (1992) and Ahnesjö *et al.* (1993) suggested that more wide ranging research was required as variation in female mating behaviour may be more important in sexual selection than had previously been thought. In a recent review of variation in mate choice, Jennions and Petrie (1997) identified five main areas of interest in variation of female mating preferences. Most importantly, the mean strength and variation of female preference in a population determine the direction and rate of sexual selection by female mate choice. In addition, variation in female mate preference can tell us something about the costs and benefits of mate choice and also help us understand the underlying mechanistic nature of female choice. For example, if a slight increase in the cost of mating makes previously choosy females mate randomly, we might suppose the benefit of mating a desirable male is small. In a similar

way, if we find that variation in a female sensory organ affects a female's ability to choose between males, we can infer something about the mechanism of mate choice.

Before this explosive increase in research into variation in female mate preferences, the prevailing view tended to be that females in a species prefer to mate males of a certain phenotype. Now, female mate preferences are seen to be plastic in their expression and dependent on many genetic and environmental factors.

Genetic variation in female mate choice

Theoretical models of sexual selection assume that female mate preferences are heritable and passed on to the offspring. These models also assume that preference and male trait genes will be in linkage disequillibrium. The evidence for a heritable genetic basis to female mate preference is overwhelming (Bakker and Pomiankowski 1995; see also Bakker 1999 for a more recent review). In their review Bakker and Pomiankowski (1995) found heritable variation in female mate preference within populations from a wide range of taxa, with estimates of heritability often exceeding 0.5. In addition they found some evidence for a positive genetic correlation between female preference and male trait genes even though some of the experimental designs used were likely to

weaken the chances of finding linkage disequillibrium (Pomiankowski and Sheridan 1994).

Investigations into the repeatability of female mate choice can give us an upper estimate of the heritability of female mate choice whilst also giving us a measure of how consistent females are in their choice. The repeatability measures the amount of variation in female preference that is due to variation between females. Jennions and Petrie (1997) reviewed this literature and found 11 examples of repeatable female mate choice in contrast to 3 studies where no repeatability could be found. More recently Howard and Young (1998) found evidence for repeatable female preference for male call frequency in the American toad (*Bufo americanus*). The other three call parameters measured had repeatabilities close to or equal to zero. This was because all females were consistent in their choice for one type of call. Wilkinson *et al.* (1998) also found repeatable female mate choice in the stalk-eyed fly, *C. dalmanni.*

From the accumulated evidence it seems clear that a proportion of the variation in female mate preferences can be explained by genetic differences between females. Repeatability estimates do tell us how consistent a female's choice is likely to be and how much females vary between individuals. However, no one has yet has mapped any genes for mate preference.

Environmental and social variation in mate choice

Many sources of environmental and social variation in mate choice have been found. These are wide ranging and include environmental factors such as predation pressure and search costs, and social factors such as age, experience, mate choice copying, and the operational sex ratio.

Experimental studies have shown that females tend to mate randomly, if at all, when predators are in close proximity. Females in populations under high predation pressure are likely to visit fewer male territories or leks, so they can reduce their risk of exposure to a predator to a minimum. The first evidence for this phenomenon came from the goby, which has the cod as a predator (Forsgren 1992). Females spent more time observing males in a choice chamber when a cod was not within visual range and preferred large colourful males. When a fish tank containing a cod was placed adjacent to the choice chamber, female preference for the previously desirable male disappeared. Similar evidence for reduced female preference when there is a perceived risk of predation have been found in guppies (Magurran and Seghers 1994; Gong and Gibson 1996), fiddler crabs (Koga *et al.* 1998), crickets (Hedrick and Dill 1993) and the sex role-reversed pipe fish (Bergland 1993). The presence of a predator was also found to reduce mating activity in water striders (Sih *et al.* 1990), further indicating the caution females take when under threat of predation. It appears clear that predation risk reduces

female mating preferences and therefore the strength of sexual selection by female mate choice.

Milinski and Bakker (1992) showed that female mate preference in the stickleback (*Gasterosteus aculeatus*) was dependent upon the cost of sequential mate searching. Stickleback females were exposed to a bright red-throated male. Previous investigation had shown that bright red males are preferred by females (Milinski and Bakker 1990). The strength of preference of females for the bright male was measured and females were then exposed to one of two treatments, either being left in a holding tank for ten minutes or being left in a similar holding tank with an artificial current for the same period. All females initially showed strong preferences for the first male. Females exposed to a current swam against it to maintain their position and stability in the water. Females were then introduced into a new tank containing a dull red-throated male. If females had been in the holding tank with the current, they accepted the dull male. If females had been in the normal holding tank, they rejected the dull male. Females were also more likely to accept the dull male if they spent longer in the holding tank. This suggests that females assess the costs of returning to desirable males compared to the benefits they will gain from mating a desirable male. Milinski and Bakker (1992) commented that the strength of female mate choice might be dependent upon the costs of mate searching or the habitat structure.

Interestingly, predatory soil mites with a polymorphism for prey preference have been shown to mate males depending on the current prey mixture, thereby maximising their offsprings' fitness (Lesna and Sabelis 1999). Predatory mites either preferred mite A or B as prey. Selection experiments showed prey preference was heritable and likely to be due to the action of a single locus containing one gene or a few tightly linked genes. Hybrids between A and B preferring selection lines had greater population growth than either isogenic line when prey was mixed (A and B). With only A prey, the A-preferring line was fittest, but there was no difference between lines on B prey. Mate preference tests showed that when selection-line females were fed mixed prey they mated disassortatively, yielding hybrid offspring which were the fittest on mixed prey. When reared on each prey type alone, A-line females always mated A-line males and B-line females also mated A-line males. From this mating system it is clear females are choosing males with the best genes to cope with the current environmental conditions. Frequency-dependent hybrid vigour is more likely to be maintaining the male trait polymorphism in the population than mutation-selection balance, which would not maintain variation in a trait controlled by so few genes.

Female age, and specifically experience, is likely to affect female preference. A female's threshold for accepting an attractive male is likely to be greater for females who have been exposed to large ornamented males compared

to females that have previously only been exposed to small unornamented males. Collins (1995) found such an effect of variation in female exposure to males. Female zebra finches were given a choice between two males. Females were then exposed to one of two treatments; the preference of females was then re-tested using the same two males as in the previous choice test. In all pre-treatment choice tests females chose the most rapidly displaying male. Treatments consisted of either exposure to rapidly displaying males or exposure to slowly displaying males. Females exposed to rapidly displaying males had weaker preferences for the rapidly displaying male in post-treatment choice tests. The strength of preference for rapidly displaying males increased in females exposed to slowly displaying males. Control females did not change in their preference. This effect of female experience was thought to be temporary.

Rosenqvist and Houde (1997) also found similar effects in guppies. Virgin females were reared in visual range of either bright orange males, dull orange males or a mixture of both. Females exposed to only one type of male mated randomly in choice tests using the two types of male. Females, which had been exposed to both types of male, preferred the bright orange male in choice tests and had stronger preferences than either of the other two female groups.

Some mate preferences have also been shown to be culturally based (Oeting and Bischof 1996; Freeburg *et al.* 1999). Culturally based preferences are those which are inherited from observations of individuals from the population

during sexual maturation. Freeburg *et al.* (1999) found that in cow birds (*Molothrus ater*) female preference was dependent upon the population in which the chicks had been reared. Females preferred males from their rearing population when sexually mature. Oeting and Bischof (1996) showed that female Zebra fiches reared by Bengalese finches preferred Bengalese males to Zebra males. This effect was much reduced if Zebra females were exposed to Zebra males shortly after fledging. Such culturally based preferences may be selected for to prevent sexually mature adults mating outside their species. How culturally based preferences affect the offspring of brood parasites will be particularly interesting as these species are often reared in by hosts that bear little resemblance to them.

Some theoretical studies of mate choice have shown that females will benefit from copying the mate choice of others when the costs of assessing males is high or when a female cannot discriminate between males herself (Losey *et al.* 1986; Wade and Pruett-Jones 1990). Copying has the effect of increasing the variation in male mating success (females are more consistent in their choice) and increases the rate of sexual selection. Evidence for mate choice copying is difficult to demonstrate conclusively as many explanations for such observations must first be excluded (Houde 1997). Observations of females mating with males which they had previously observed mating could be explained by anti-predator flocking, schooling behaviour, differences between males, female preference to mate where copulations have previously taken place or female preference for

recently sexually active males. This makes observation of female mate choice copying only possible in controlled laboratory experiments where these other possibilities can be excluded.

Dugatkin's experiments on guppies are the most comprehensive and convincing evidence for the existence of mate choice copying. Dugatkin (1992) initially showed that females preferred males that had previously been observed courting another female. Other possible alternatives for these observations were excluded by numerous control experiments. Dugatkin and Godin (1992) showed that females reversed their own mate preference when copying the choice of other females. Further analysis showed that females reversed their preference if they had the chance to copy the choice of another female whose males differed in colouration by less than 25% (Dugatkin 1996a). If males differed by more than this, females maintained their original preferences. Further to this females were shown to always reverse their choice if two females were seen to court a male or if one female was seen to court a male for a long period of time (Dugatkin 1996b). Many other authors have tried to demonstrate mate choice copying; some have failed (Brookes 1996; Lafleur *et al.* 1997; Partriquin-Meldrum and Godin 1998) while others have succeeded (McComb and Clutton-Brock 1994; Grant and Green 1995; Witte and Ryan 1998). It is unclear how common copying effects are under natural conditions.

The evidence for mate choice copying is far from conclusive. However, the process itself does make some evolutionary sense. If the Fisher process were operating, a female's inclusive fitness would be dependent upon having sons that bare the desirable trait. It therefore makes sense for females to monitor the preferences of other females in a population lest they choose a male which no other females find attractive.

The operational sex ratio and level of harassment by males has also been shown to effect female mate preference. Females have been shown to mate more frequently when in male biased populations (Rowe 1992) and this may be to reduce male harassment for copulations (Arnqvist 1992). Mating in order to reduce male harassment is likely to reduce a female's ability to select mates.

Female biased sex-ratios can also increase the competition between females for mates. In the facultatively polygynous blue tit, females in female biased populations have reduced choice of males (Kempenaers 1994). Often the dominant monogamous female in a male territory will fight off intruding females. Gottard *et al.* (1999) showed that female propensity to mate (measured as the inverse time from emergence to mating) was greater in a female biased populations of speckled wood butterfly than in male biased populations. This may be linked to the cost of mate searching in the female biased population being greater. The cost of searching for males may reduce female mate preference in this species. Palokangas *et al.* (1992) showed that female kestrels become non-

choosy when the sex ratio is equal compared to having strong preferences for long tailed males when sex-ratios during the breeding season were male biased.

The relative importance of environmental and social factors versus genetic factors in determining the variation in female mate choice is unclear. Not all environmental factors will be independent of genetic influence however. The degree to which social and environmental fluctuations affect female mate choice may be dependent on the phenotype and consequently genotype of females.

Phenotype and Condition-dependence

First I describe what is meant by condition and whether it is a heritable characteristic and show that many phenotypes are used to measure condition. I then move on to describe some male secondary as examples of condition-dependent traits, concentrating on the stalk-eyed fly. I will conclude this section by describing why we might expect female preference to be condition-dependent.

What are condition and condition dependence?

Condition and measures of condition (condition indices) have been used for decades in the fisheries and agricultural industries (LeCren 1951; Wildman *et al.* 1982; Bolger and Connolly 1989). Condition in these trades has been

traditionally thought of as the nutritional status of an individual and all the condition indices they use give a general measure of fatness (Le Cren 1951; Bolger and Connelly 1989; Gerhart *et al.* 1996). Indices of condition in fish are generally based on phenotypic measures such as the body mass to body length ratio and similar phenotypic measures are used in cattle with body size also being used. In addition, in cattle, condition can be accurately assessed by palpation; the prodding of the rump to determine the depth of sub-cutaneous fat (Gerhart *et al.* 1996). In fisheries, condition indices have been used to predict growth rate and timing of maturation (reviewed in Bolger and Conolly 1989). In livestock, condition indices are widely used to measure nutrition, reproductive potential and herd health or productivity (Wildman *et al.* 1982; Cameron *et al.* 1993).

Ecologists have recently imported this general method of determining the health of an individual as it is less invasive than other methods (Jakob *et al.* 1996). Some of the most commonly used indices in ecology are the ratio of body mass to body size and the residuals of this regression, which eliminates any body size dependence of this measure (Jakob *et al.* 1996). Studies of ecology and animal behaviour have found individual condition indices to affect predator inspection (Külling and Milinski 1992) in fish, as well as fecundity in spiders (Morse 1988) and courtship activity in Diptera (Droney 1998).

Individuals in poor condition are those which are lacking key nutrients in their diet or suffering from general malnutrition. Condition also appears to be

linked to a number of fitness traits. Rowe and Houle (1996) developed a generalised model of condition as a pool of resources (be it fat or some other kind of nutritional reserve). This pool of resources (condition) is attributed to certain characters for growth or behaviours. Some behaviours, foraging for example, are likely to increase the pool of resources. The pool of resources can also be reduced in size by unavoidable costs such as fighting infection or healing wounds (Poulin 1994; Lopez 1999). As at any time the pool of resources is limited, costly traits and behaviours will be dependent upon condition (Rowe and Houle 1996). From this word model it is clear that a number of phenotypes are expected to be representative of condition (Jennions and Petrie 1997).

Is condition heritable?

As mentioned earlier, condition has been linked to a number of fitness components in the fisheries and agricultural industries and is likely to be a life history correlate (Houle 1991). Because condition is linked to fitness, the heritability of this trait is expected to be low (Falconer and Mackay 1996). Fisher's fundamental theorem states that any character that is subject to strong directional selection (as we might expect of mutations that increase condition or fitness) will have low additive genetic variation in an equillibrium population. This is because any variation will soon be exhausted due to directional selection

which will push alleles for greater condition or fitness to fixation. However, Houle (1992) showed that many fitness components do have large additive genetic components. Low narrow sense heritability may be due to either low additive genetic variation for the trait or a large residual variance component (consisting of other genetic and environmental components). Houle (1992) showed that fitness components have low heritability not because they lack additive genetic variation, but because they have high residual variation. Fitness components also tend to integrate variability over a lifetime and are subject to selection on other characters. Therefore these traits are subject to variation in a large number of loci (Houle 1991) and environmental conditions (Price and Schulter 1991).

Genetic variation in condition may be maintained under a number of circumstances (Andersson 1994). A balance between selection and mutation can maintain additive genetic variation in condition. For any complex trait, the number of genes contributing to its expression will be a large proportion of the total for the individual. The mutation rate of these genes is likely to counter the exhaustion of variation through selection. Most of these mutations will be deleterious as random mutation in a complex trait is unlikely to improve upon it in much the same way as randomly tightening or loosening bolts on a car is unlikely to improve its performance. Houle *et al.* (1994) showed that mutation accumulation was strong enough to counteract the effects of selection when

comparing 200 lines of *D. melanogaster* containing the same high fitness second chromosome. After 44 generations six out of eight life history components showed a significant increase in genetic variation between lines. The rate of mutation accumulation was close to that expected for the maintenance of additive genetic variation in the face of selection (Houle *et al.* 1994). Houle *et al.* (1996) reviewed the literature on variation in mutation rate (variation in a trait caused by mutation each generation). The mutation rate component to life history traits was found to be six times greater than that for morphological traits, consistent with mutation-selection balance theory. As condition-dependent traits are dependent on a large number of loci it is likely they will be under the same type of mutation-selection balance as is predicted for life history traits (Rowe and Houle 1996).

In addition, components of condition may maintain high genetic variance through fluctuation in the environment through time. Fluctuations in host-parasite cycles are a well documented example of this and as we have seen are intimately involved in female mate choice sexual selection. Spatially varying selection may also contribute to the maintenance of additive genetic variation in condition. Given that individuals will be adapted to their local environment, migration between habitats may provide an important source of genetic variation.

Condition dependence of male traits.

The focus of condition-dependent traits to date in sexual selection has been on male secondary sexual characters and displays (reviewed in Maynard Smith 1985). Condition-dependence of male traits was first proposed by Fisher (1915) and was later taken up by Zahavi (1975 and 1977). If male ornaments and displays are condition-dependent, males in poor condition will have smaller ornaments than males in high condition. This means that females choosing between males on the basis of a condition-dependent trait will be assessing an honest signal of male quality. The honesty in the male signal is effectively enforced by its cost of production and maintenance (Grafen 1990).

Examples of condition-dependent male traits are now numerous (Andersson 1994). In his review of honest advertisement of male quality through sexually selected traits Johnstone (1995) found 11 examples in the published literature of condition-dependent male secondary sexual traits. Johnstone's definition of condition is less expansive than that stated above, being limited to measurements of body size versus bodyweight. If we expand acceptable evidence for condition-dependence beyond Johnstone's definition to include trait correlations with parasite load, nutritional status and weight, we find a further 34 examples out of 132 observed traits in 109 species. Many of these studies are not experimental and do not compare the male ornament with non-sexual morphological male traits. To establish if the male trait is indeed a signal of male

quality, then it must be more representative than any other morphological traits of condition.

More recently, experimental studies in *C. dalmanni* have found evidence for condition-dependent expression of the male trait, relative eyespan. David *et al.* (1998) artificially manipulated condition in the stalk-eyed fly *C. dalmanni* by varying the larval food density of flies during their development. Stalk-eyed flies have eyes on the end of stalks that grow distally from the head capsule. David *et al.* (1998) found that male eyespan showed a disproportionately greater sensitivity to condition than female eyespan and two non-sexual male wing traits. This study was the first to compare explicitly the degree of condition-dependence of the male ornament to that of the female homologous trait and to other male morphological traits.

Further to the above study, Wilkinson and Taper (1999) studied condition-dependence by assuming that body length could be taken to be representative of individual condition. The use of body size as a measure of condition is a reasonable assumption as growth is only possible during the larval stages and is set once an adults cuticle has hardened a few hours after eclosion. The additive genetic variance for eyespan, thorax width and body length were estimated from a half sib breeding design. Wilkinson and Taper (1999) found that 97% of eyespan genetic variance was explained by its dependency on condition, whereas only 7%

of genetic variation in thorax width, a character under net stabilising selection, could be attributed to condition-dependence.

Finally, David *et al.* (2000) found that male eyespan became more variable with increasing nutritional stress. When food quality was high (normal corn media) nearly all males had large eyespan. When food quality was worse (spinach) the variation in male eyespan increased with fewer males having large eyespans. When food media was very poor (moist cotton wool and mould) few males produced large eyespans and the variance in male eyespan was greater than in the other two treatments. By using a mid-parent heritability analysis it was shown that resistance to nutritional stress was revealed by male eyespan, and that this was heritable.

Although many studies showing condition-dependence of male ornaments do not show they are better signals of male condition than morphological traits, it is now generally accepted that condition-dependent exaggeration of male ornaments and displays is common and the rule rather than the exception. These traits are particularly good examples of condition-dependent traits because they have been selected to become exactly that–signals of condition (Grafen 1990). Because of this, sexual ornaments are also the most extreme of a wide range of condition-dependent traits.

Cost of mate choice

Additive genetic variation in female mating preferences may be maintained in the same way as variation is maintained in male ornaments-via condition-dependence (Bakker 1999). As mentioned above, there is evidence of additive genetic variation for female preference. The assumptions that have to be met for condition-dependence are simple-the trait must be costly and individuals in poor condition must suffer higher costs than those in high condition. So is choosing between males costly?

Expressing female preferences can be costly for a number of reasons. Searching for males is likely to consume energy reserves, increase female exposure to predators and perhaps parasites, and reduce foraging time. The cost of female choice has been previously investigated in a small number of studies due to interest in its effects on indirect benefit models of sexual selection (Pomiankowski 1987b, Bulmer 1989 and Pomiankowski *et al.* 1991). Here I will only give a short review of the evidence for costly mate choice as it has been reviewed extensively elsewhere (Reynolds and Gross 1990). The costs of choice can be divided into the costs of searching for desirable males and the costs of being in close proximity to large groups of individuals and in particular males.

The costs of searching for a mate are obvious. Such behaviour consumes energy reserves, will increase the likelihood of encountering a predator and reduce

the time available for foraging. In the garibaldi damselfish, females defend feeding and shelter territories but have to search for and spawn with nesting males (Sikkel 1998). Sikkel (1998) found that intruders harvested food from the territory and that the duration of female searching and spawning was negatively related to the territorial intruder pressure. Delayed breeding also imposes greater reproductive costs in some species, therefore reducing the time available for assessment of males. In the pied flycatcher, brood success and brood size decrease rapidly as the breeding season progresses and so females settle as early as possible and before they can assess many males (Slagsvold *et al.* 1988).

Increased searching activity may increase the risk of encounters with predators, while assessing males may divert attention from possible attack (Magnhagen 1991). The conspicuousness of ornamented or displaying males may also attract the attention of predators on mating pairs or leks. In guppies, one type of natural predator (the pike cichlid) has been shown to prefer attacking females when given a choice between a male and a female (Pocklington and Dill 1995). Success rate per attack was the same for both males and females, but the predator more often attacked the female which is larger and nutritionally more profitable. Under such conditions, females may find it more profitable to court duller males in the presence of predators, as they are less likely to attract attention.

When females do not have to search for mates because they are either social species mating occurs on leks, costs of choice can still occur. In many

species males aggressively harass females for copulations. Females can either rebuff males or accept matings to reduce harassment. Waage (1979) studied such a system in the damselfly. In this species females may mate with undesirable males to reduce harassment. A female can exert her choice by then mating a desired male, as 88-100% of male sperm is displaced by the current male during copulation (Waage 1979). In this way females can reduce harassment but still assure paternity by a desired male, but at a cost of wasted time. Females of many species have been found to actively discourage males from engaging in mating through aggressive and energetically costly behaviour such as biting and struggling free of males (Hölldobler 1976).

Borgia and Collis (1989) found that female bower birds avoid mating with males parasitised by the louse *Myrsidea ptilonorhynchi*. Although they presented no direct evidence for increased risk of parasite transmission with greater search range, they did report the presence of an effective vector (two species of hippoboscid flies) that can transmit the parasite without direct contact between birds. It is therefore reasonable to assume that the more bowers visited the greater the risk or severity of infection. When visiting leks, increased opportunity for parasites to spread between hosts is likely.

It is clear that there are many types of cost associated with mate choice and that these are not solely dependent upon searching for desirable males. Females in poorer condition may be expected to reduce their efforts at

discriminating between males, as this will reduce the resources put into mate choice that would otherwise be invested in other traits and behaviours. We therefore expect mate choice to be condition-dependent.

Evidence for condition-dependent mate preference

Evidence for condition-dependent mate preference has begun to appear in the literature over the last two years, although it is still neither extensive nor conclusive. I will begin by describing examples of parasitic infection reducing female condition and strength of mate choice and then move onto other examples in direct benefit mating systems and an example where genetic variation in condition affects female mate preference. I will conclude the section by describing a potentially interesting area of investigation where other examples of condition-dependent mate preference may be found.

Parasites often cause phenotypic changes in individuals, especially in behaviour, and some parasites have been shown to bring about a reduction in an individuals condition (Poulin 1993; Zuk 1998; Holmes and Zohar 1990). A reduction in condition is likely to occur because of the cost of immune response and the drain on physiological resources directly by the parasite. López (1999) showed that unparasitised female guppies preferred to mate bright actively

displaying males when given a binary choice between a bright displaying male and a dull less actively displaying male. A second group of females were infected with a fluke parasite that attaches to the fins of guppies. These infected females chose randomly between the males and had significantly weaker preferences than uninfected females. The number of parasites on a female was negatively correlated with female activity and strength of preference. Infected females also swam between males less frequently, indicating that parasite infection reduced female condition.

Poulin (1994) found a similar reduction in female mate preference in parasitised upland bullies, *Gobiomorphus breviceps*. Wild caught females were given a choice between a large and a small male. After mate choice decisions were made females were dissected and the number of trematode parasite cysts in the body of the fish were counted. Poulin (1993) had previously shown that there is a negative correlation between the physical condition of a fish and parasite load. In surprising concurrence with López (1999), more parasitised females were found to make fewer mate inspections and chose the smaller male more often than lightly parasitised females.

In contrast to these studies, Zuk *et al.* (1998) found that a parasitic nematode infection that reduced condition in females did not effect female mate choice in red Jungle Fowl (*Gallus gallus*). The authors commented that the low

cost of choice in this mating system was likely to allow even low condition females to express their mate preference.

Examples of changes in female preference with condition are far more obvious in systems with direct benefits to mate choice. In a number of species of cricket and grasshopper, females are the choosy sex when environmental conditions are high, but males become the choosy sex when food becomes scarce (Gwynne 1985; Gwynne and Simmons 1990; Simmons 1994; Ritchie *et al.* 1998). This is because males produce a spermatophore which has a spermatophylax attached to it. A male attaches the combined spermatophore to the females genitalia. The spermatophylax is a proteinous mass that the female eats. When finished the female often then removes the spermatophore (Gwynne 1982). Brown (1997) found that females fed on low quality food mated more quickly and were less choosy than females on high quality food. Simmons (1994) found a similar effect when female condition was reduced by a gut Protozoan parasite in bush crickets. Females no longer chose between males but mated at very high frequency to compensate for the nutritional loss to infection.

The most convincing evidence for condition-dependent mate preference was found by Bakker *et al.* (1999) in sticklebacks. Female laboratory bred sticklebacks (*Gasterosteus aculeatus*) were allowed to choose between computer animated courting males. Full-sib families of approximately equal size were raised under standardised conditions. The average family condition was calculated

as the mean body mass divided per unit body length of six randomly sampled males from each full-sib family. Even though rearing conditions were standardised, the average family condition differed significantly between full-sib groups. The preference of two females from each full-sib family was assessed when given the choice between a male with red-throated breeding colouration and a male that was orange throated. Overall, females did not show a significant preference for either male, but female preference for the red throated male correlated positively with average family condition. Females from high average condition families preferred red throated females whilst females from low average condition families preferred orange throated males. This experiment differs from those above in that females in high and low condition still preferred a male phenotype, but differed in their preferred phenotype.

Where else might we expect to find condition-dependent mate choice? The cost of producing some of the sensory organs required to assess males may be high (Jennions and Petrie 1997). We can expect the development of these organs to be condition-dependent and may also be subject to temporary or permanent injury through infection.

For example, Jennions *et al.* (1994) found condition-dependent sensitivity to male calls in the African painted reed frogs when carrying out a repeatability of mate choice experiment. Females were subjected to artificial calls from two equidistant speakers. Simulated male calls differed in frequency by 400Hz.

Almost all the females tested preferred the deeper male call. When the difference in male calls was reduced to 200Hz, females overall showed no preference. Individuals who preferred the deeper call were on average of greater body size (measured as snout to vent length). This suggests that the sensitivity of females to the male call be in some way related to body size. In this species, large males have deeper calls (Dyson and Passmore 1988) and so this may be seen as evidence of assortative mating for male size. Dyson *et al.* (1992) however have shown that in the wild females do not mate assortatively for male size and so the importance of Jennions *et al.*'s experiment as an example of condition-dependent mate choice is unproven.

Females from other species have been shown to have considerable variation in their sensitivity to male sexual signals. In female katydids the size of a female thoracic spiracles determines auditory sensitivity to male calls (Bailey 1998). Gwynne and Bailey (1999) showed that female katydids have larger spiracles than males and that females with larger spiracles have a pairing advantage. This trait is probably under direct sexual selection as females compete for nuptial gifts from males and such females will be able to locate males more quickly. No correlation of spiracle size with body size or mass was found so this is not an example of condition-dependent mate choice, but it does emphasise that variation in female sensitivity to male signals may produce assortative mating. If

the development of the sensory organs required for mate choice is costly, we might expect to find condition-dependent mate choice.

From the current literature it appears that female mate preferences can be dependent upon individual condition. We may expect condition-dependent mate preferences to evolve wherever there are significant costs to mate choice.

Other condition-dependent effects

Because condition represents such a large proportion of individual fitness we expect condition-dependent mate choice to be directly associated with other fitness components such as fecundity and viability. Linkage between such traits will have consequences for direct and indirect benefit models of sexual selection. If choosy females are also the most fecund we might expect sexual selection through mate choice to occur more rapidly than current models suppose as the offspring of these individuals will be over represented in the next generation.

For many years, condition indices have been used to predict the health, productivity and fecundity of cattle and have been used for similar purposes in fisheries (Le Cren 1951; Bolger and Connelly 1989). In just the last ten years there have been numerous examples linking condition and female fertility and fecundity in fish (Demartini 1991; Munthali and Ribbink 1998; Ali and Wootton

1999; Lambert and Dutil 2000) and cattle (Sawyer *et al.* 1991; Dominguez 1995; Heuer *et al.* 1999; Loeffler *et al.* 1999). In addition condition is positively related to milk yield in dairy cattle which may be indicative of offspring survival in mammals (Waltner *et al.* 1993; Markusfeld *et al.* 1997; Heuer *et al.* 1999). Female condition has also positively related to seasonal mortality in cod (Lambert and Dutil 2000) and the willow ptarmigan (Robb *et al.* 1992).

This strong relationship between condition, fertility and fecundity is still apparent if we limit evidence to populations outside of fisheries and farms. In female Moose, body condition (measured as the percentage body fat of their individual) was found to explain much of the variation found in pregnancy, pregnancy duration, twinning and calving rate (Testa and Adams 1998). Females in greater condition were more likely to become pregnant, have twins, have shorter pregnancies and be less likely to lose their calf. Similar effects are found in Baltic Herrings. Here females in good condition had greater egg hatchling success (Laine and Rajasilta 1999). Female toads (*Bufo bufo*) in high condition are more fecund than low condition females (Reading and Clarke 1995). Infection of sticklebacks with trematode larvae also revealed a reduction in female fertility (Fitzgerald *et al.*1993).

Poulin (1994) however, found no such effect of infection in upland bullies even though the infection reduced other measures of female condition. Knell (1999) suggested that sexually transmitted parasite infections would be selected to

have little or no effect on individual vigour, as this will in turn reduce the parasites infectious capability. Many parasites are horizontally transmitted that do not require host mating activity and these parasites are selected to have increased virulence (Møller 1996). Such parasites will be those most detrimental to health of individuals in the population and the basis for parasite mediated sexual selection (Knell 1999).

Differential costs of reproduction in collared flycatchers has been found to be dependent upon female condition (Cichon *et al.* 1998). Brood size was manipulated by adding or removing chicks from nests. The survival of poor condition females from the season of manipulation to the next successive breeding season was negatively related to the increase in brood size. For high condition females there was no effect of brood manipulation. Simmons (1994) also found parasite infection to reduce female fecundity in bush crickets. Similar effects in females of the sex-role reversed pipe-fish when infected with trematodes have been found (Rosenqvist and Johansson 1995).

Males can directly affect female nutritional status and therefore condition and fecundity in species where females gain nuptial food gifts or nutrition from male ejaculates and spermatophores. In the seed beetle females that mate large males, which produce more nutritious spematophores, produce more eggs. These eggs are also larger (Fox *et al.* 1995), which may give increase the survivorship of the larvae.

Although evidence has not yet been presented where low condition females have different mate preferences to high condition females and are also less fecund, condition and fecundity do appear to be positively correlated in many diverse species. The consequences of the linkage of condition-dependent mate choice and fecundity and fertility has not been thoroughly investigated but may compensate for the reduction in the strength of sexual selection caused by poor condition females.

Consequences for female mate choice

Bakker (1999) suggested that condition-dependent female mate choice required further investigation as this may be a means by which genetic variation in female preference is maintained. In addition, condition-dependent mate choice may link female mate preference and other female fitness components such as fertility and fecundity. Linkage disequillibrium between genes for fitness and preference is likely to have a marked effect on the strength of sexual selection. If the choosiest females in a population also have the most offspring, we would expect sexual selection to act much faster than has been presently hypothesised by theoretical studies. These models presently assume equal fecundity for all females (Andersson 1994). As has been shown above, female condition tends to be

directly linked to fecundity wherever such a link is looked for. Direct links between the strength of mate choice and female fecundity have now started to appear (Simmons 1994).

Two theoretical studies have recently investigated two of the issues raised in this review. Poulin and Vickery (1996) investigated the effect of parasites on female mate choice. A number of models were investigated. Parasite infection of females was assumed to reduce female mate preferences (females tending to mate randomly if infected) and hence selection on the male ornament became weaker with higher rates of parasitism. In these models no account was taken of the reduced fecundity expected amongst parasitised females (Rolff 1998). Poulin and Vickery (1998) suggested that such effects were due to natural selection and so uninteresting in this context. Poulin and Vickery are of course correct in their analysis of what is sexual and what is natural selection, but one has always been analysed with the other in handicap models. Application of reduced female fecundity into their model revealed that sexual selection was no longer reduced by parasite infection in females under realistic parameters (Vickery and Poulin 1998).

The effect of viability differences between females on the evolution on mate choice has also been investigated theoretically (Tomlinson and O'Donald 1996). In this model it was assumed that females of higher viability were choosier than unfit females. The investigation found that variation in female viability did

not adversely effect the evolution of female mating preference and male trait. However, the model only considered the spread of a single, high viability allele, so after a period of time, variation in viability was exhausted. It is unclear whether the results of the model depend on this assumption. If variation in viability could be maintained, for example by fluctuating selection, the model may then produce different results.

Summary

Substantial variation in female preference is seen in most species where indirect female mate choice is operating. This variation in the most part appears to be due to social and environmental variation. Links between genetic variation in mate preferences and other sources of variation may occur if female mate choice is costly. This will produce selection for condition-dependent expression of female mate preferences. Evidence for condition-dependent female mate preference is slowly accumulating. Females in high condition also appear to be more fertile and have greater fecundity. If fecundity and the strength of mate choice are linked, we might expect stronger directional sexual selection than has been currently predicted under indirect and direct benefit models of sexual selection.

Thesis structure

I have written up my experimental work in the form of a series of manuscripts (chapters two to five). Already, chapter two has been accepted for publication in Animal Behaviour; chapters three, four and five are being prepared for submission to PNAS, short communications in Animal Behaviour and Conservation Biology respectively.

The work in this thesis was carried out under the supervision of Kevin Fowler and Andrew Pomiankowski, but the design and execution of all experiments was by the author.

Chapter two investigates the effect of female eyespan on mate preferences. Eyespan is strongly correlated with body size and so is an index of individual condition. We compared large and small eyespan female mate preference when males differed in eyespan by large, intermediate or small amounts.

Chapter three investigates the effect of a transient stress that effects a female fitness component. Females were fed sucrose media that was found to reduce the number of mature and developing eggs in the ovaries. Female ovarian activity was found to recover when females were placed back on the normal media (pureed sweetcorn). The same females were fed corn and sucrose and female preference was tested on each media. The design of the experiment

allowed female age, experience, and the order of exposure to the different media to be excluded as possible confounding factors for any changes in preference we might observe.

Chapter four follows up the results of chapter two. Chapter two used large and small eyespan females isolated in different cages and found that large eyespan females had stronger preferences for large eyespan males when males differed by intermediate amounts. Here large and small eyespan females were mixed in cages at a strongly female biased sex ratio. The aim was to see if females competed for copulations. Such an effect may have implications for eyespan-dependent female mate choice observed in chapter two.

Chapter five was part of a larger investigation of fluctuating asymmetry (FA) by Kevin Fowler and Andrew Pomiankowski (David *et al* 1998, Bjorksten *et al.* 2000a, 2000b). I am a co-author on the David *et al.* (1998) manuscript. I expand the approach of David *et al.* (1998) by adding a second measurement of male sexually selected trait FA in chapter five, which was analysed and written by myself. Chapter five investigates the use of sexual trait FA as a means of monitoring endangered populations and breeding programs. Sexual trait FA has been proposed as a sensitive measure of environmental and genetic stress during development. Sexual trait FA has also been associated with a number of fitness components. This has led to many investigators in this field suggesting that sexual trait FA could be used by conservation biologists to identify populations and

individuals under stress. We tested a number of morphological and sexual trait FA hypotheses by exposing stalk-eyed fly larvae to differing amounts of nutritional stress by varying larval food density.

Chapter six summarises the findings of the experimental chapters and relates them to my overall objective of investigating the role of phenotype and condition on female mate preference.

REFERENCES

Ahnesjö, I., Vincent, A., Alatalo, R., Halliday, T. and Sutherland, W.J. 1993. The role of females in influencing mating patterns. Behavioral Ecology, 4: 187-189.

Alatalo, R.V., Hoglund, J., Lundberg, A. and Sutherland, W.J. 1992. Evolution of black grouse leks – Female preferences benefit males in larger leks. Behavioral Ecology, 3: 53-59.

Ali, M. and Wootton, R.J. 1999. Effect of variable food levels on reproductive performance of breeding female three-spined sticklebacks. Journal of Fish Biology, 55: 1040-1053.

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

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

Arnqvist, G. 1992. Pre-copulatory fighting in a water strider: inter-sexual conflict or mate assessment? Animal Behaviour, 43: 559-567.

Bailey, W.J. 1998. Do large bushcrickets have more sensitive ears? Natural variation in hearing thresholds within populations of the bushcricket *Requena verticalis* (Listrocelidinae: Tettigoniidae). Physiological Entomology, 23: 105-112.

Bakker, T.C.M. 1999. The study of intersexual selection using quantitative genetics. Behaviour, 136: 1237-1265.

Bakker, T.C.M. and Pomiankowski, A. 1995. The genetic basis of female mate preferences. Journal of Evolutionary Biology, 8: 129-171.

Bakker, T.C.M., Künzler, R. and Mazzi, D. 1999. Condition related mate choice in sticklebacks. Nature, 401: 234.

Beach, F.A. and LeBoeuf, B.J. 1967. Coital behaviour in dogs. I. Preferential mating in the bitch. Animal Behaviour, 15: 546-558.

Berglund, A. 1993. Risky sex: Male pipefishes mate at random in the presence of a predator. Animal Behaviour, 46: 169-175.

Bjorksten, T., David, P., Pomiankowski, A. and Fowler, K. 2000a. Fluctuating asymmetry of sexual and non-sexual traits in stalk-eyed flies. Journal of Evolutionary Biology, 13: 89-97.

Bjorksten, T.A., Fowler, K. and Pomiankowski, A. 2000b. What does sexual trait FA tell us about stress? Trends in Ecology and Evolution, 15: 163-166.

Bolger, T. and Conolly, P.L. 1989. The selection of suitable indices for the measurement and analysis of fish condition. Journal of Fish Biology, 34: 171-182.

Borgia, G. and Collis, K. 1989. Female choice for parasite-free male satin bowerbirds and the evolution of bright male plumage. Behavioral Ecology and Sociobiology, 25: 445-454.

Brookes, R. 1996. Copying and the repeatability of mate choice. Behavioral Ecology and Sociobiology, 39: 323-329.

Brown, W.D. 1997. Female remating and the intensity of female choice in black-horned tree crickets, *Oecanthus nigricornis*. Behavioral Ecology, 8: 66-74.

Bulmer, M.G. 1989. Structural instability of models of sexual selection. Theoretical Population Biology, 35: 195-206.

Burkhardt, D. and de la Motte, I. 1983. How stalk-eyed flies eye stalk-eyed flies: Observations and measurements of the eyes of *Cyrtodiopsis whitei*. Journal of Comparative Physiology A, 151: 407-421.

Burkhardt, D. and de la Motte, I. 1987. Physiological, behavioural, and morphometric data elucidate the evolutive significance of stalked eyes in Diopsidae (Diptera). Entomologia Generalis, 12: 221-233.

Burhardt, D. and de la Motte, I. 1988. Big 'antlers' are favoured: female mate choice in stalk-eyed flies (Diptera, Insecta), field collected harems and laboratory experiments. Journal of Comparative Physiology A, 162: 649-652.

Cameron, R.D., Smith, W.T., Fancy, S.G., Gerhart, K.L. and White, R.G. 1993. Calving success of female caribou is related to body weight. Canadian Journal of Zoology, 71: 480-486.

Cichon, M., Olejniczak, P. and Gustafsson, L. 1998. The effect of body condition on the cost of reproduction in female Collard Flycatchers *Ficedula albicollis*. Ibis, 140: 128-130.

Clutton-Brock, T.H., Guinness, F.E., and Albon, S.D. 1982. Red Deer: Behavior and Ecology of Two Sexes. Edinburgh University Press. Edinburgh.

Collins, S.A. 1995. The effect of recent experience on female choice in zebra finches. Animal Behaviour, 49: 479-486.

Cox, C.R. and LeBouef, B.J. 1977. Female incitation of male competition: A mechanism in sexual selection. American Naturalist, 111: 317-335.

Darwin, C.R. 1859. On the origin of species by means of natural selection. Murrey, London.

David, P., Bjorksten, T., Fowler, K. and Pomiankowski, A. 2000. Condition-dependent signalling of genetic variation in stalk-eyed flies. Nature, 406: 186-188.

David, P., Hingle, A., Greig, D., Rutherford, A., Pomiankowski, A. and Fowler, K. 1998. Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. Proceedings of the Royal Society of London, Series B, 265: 2211-2216.

Day, T.H., Foster, S. and Engelhard, G. 1988. Mating behaviour in seaweed flies (*Coelopa frigida*). Journal of Insect Behaviour, 3: 105-120.

Demartini, E.E. 1991. Annual variations in fecundity, egg size, and the gonadal and somatic conditions of queenfish *Seriphus politus* (Sciaenidae). Fishery Bulletin, 89: 9-18.

Dominguez, M.M. 1995. Effects of body condition, reproductive status and breed on follicular population and oocyte quality in cows. Theriogenology, 43: 1405-1418.

Droney, D.C. 1998. The influence of the nutritional content of the adult male diet on testis mass, body condition and courtship vigour in a Hawaiian *Drosophila.* Functional Ecology, 12: 920-928.

Dugatkin, L.A. 1992. Sexual selection and imitation: Females copy the mate choice of others. American Naturalist, 139: 1384-1389.

Dugatkin, L.A. 1996a. Interface between culturally based preferences and genetic preferences: Female mate choice in *Poecilia reticulata*. Proceedings of the National Academy of Sciences, USA, 93: 2770-2773.

Dugatkin, L.A. 1996b. Genes, copying, and mate choice: Shifting thresholds. Behavioral Ecology, 9: 323-327.

Dugatkin, L.A. and Godin, J.J. 1992. Reversal of female mate choice in the guppy (*Poecilia reticulata*). Proceedings of the Royal Society of London, Series B, 249: 179-184.

Dyson, M.L. and Passmore, N.I. 1988. Two-choice phontaxis in *Hyperolius marmoratus* (Anura, Hyperoliidae): the effect of temporal variation in presented stimuli. Animal Behaviour, 36: 648-652.

Dyson, M.L., Passmore, N.I., Bishop, P.J. and Henzi, S.P. 1992. Male behaviour and correlates of mating success in a natural population of African painted reed frog (*Hyperolius marmoratus*). Herpetologica, 48: 232-242.

Falconer, D.S. and Mackay, T.F.C. 1996. Introduction to quantitative genetics. Longman. Essex.

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

Fisher, R.A. 1930. The genetical theory of natural selection. Clarendon Press: Oxford.

Fitzgerald, G.J., Fournier, M. and Morrissette, A. 1994. Sexual selection in an anadromous population of three-spined sticklebacks – no role for parasites. Evolutionary Ecology, 8: 348-356.

Forsgren, E. 1992. Predation risk affects mate choice in a Gobiid fish. American Naturalist, 140: 1041-1049.

Fox, C.W., McLennan, L.A. and Mousseau, T.A. 1995. Male body size affects female lifetime reproductive success in a seed beetle. Animal Behaviour, 50: 281-284.

Freeburg, T.M., Duncan, S.D., Kast T.L. and Enstrom, D.A. 1999. Cultural influences on female mate choice: an experimental test in cowbirds, *Molothrus ater*. Animal Behaviour, 57: 1099-1106.

Gerhart, K.L., White, R.G., Cameron, R.D. and Russel, D.E. 1996. Estimating fat content of caribou from body condition scores. Journal of Wildlife Management, 60: 713-718.

Gong, A. and Gibson, R.M. 1996. Reversal of a female preference after visual exposure to a predator in the guppy, *Poecilia reticulata*. Animal Behaviour, 52: 1007-1015.

Godin, J.J. and Dugatkin, L.A. 1995. Variability and repeatability of female mating preference in the guppy. Animal Behaviour, 49: 1427-1433.

Gotthard, K., Nylin, S. and Wiklund, C. 1999. Mating system evolution in response to search costs in the speckled wood butterfly, *Pararge aegeria*. Behavioral Ecology and Sociobiology, 45: 424-429.

Grafen, A. 1990. Biological signals as handicaps. Journal of Theoretical Biology, 144: 517-546.

Grant, J.W.A. and Green, L.D. 1995. Mate choice copying verses preference for actively courting males by female Japanese medaka (*Oryzias latipes*). Behavioral Ecology, 9: 534- 539.

Gwynne, D.T. 1985. Role-reversal in katydids: habitat influences reproductive behaviour (Orthoptera: Tettigonidae, *Metaballus* sp.). Behavioral Ecology and Sociobiology, 16: 355-361.

Gwynne, D.T. and Bailey, W.J. 1999. Female-female competition in katydids: sexual selection for increased sensitivity to a male signal? Evolution, 53: 546-551.

Gwynne, D.T. and Simmons, L.W. 1990. Experimental reversal of courtship roles in an insect. Nature, 346: 172-174.

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

Harvey, P.H., Stenning, M.J., and Campbell, B. 1985. Individual variation in seasonal breeding success of pied flycatchers (*Ficedula hypoleuca*). Journal of Animal Ecology, 54: 391-398.

Hedrick, A.V. and Dill, L.M. 1993. Mate choice by female crickets is influenced by predation risk. Animal Behaviour, 46: 193-196.

Heuer, C., Schukken, Y.H. and Dobbelaar, P. 1999. Postpartum body condition score and results from the first test day milk as predictors of disease, fertility, yield, and culling in commercial dairy herds. Journal of Dairy Science, 82: 295-304.

Höglund, J. and Alatalo, R.V. 1995. Leks. Princeton University Press. Princeton, New Jersey.

Hölldobler, B. 1976. The behavioural ecology of mating in harvester ants (Hymenoptera: Formicidae: pogonomyrmex). Behavioral Ecology and Socibiology, 1: 405-423.

Holmes, J.C. and Zohar, S. 1990. Pathology and host behaviour. In: Parasitism and host behaviour (Ed. By C.J. Barnard and J.M. Behnke), pp. 34-63. Taylor-Francis. London.

Houde, A.E. 1997. Sex, colour, and mate choice in guppies. Princeton University Press. Princeton, New Jersey.

Houle, D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. Evolution, 45: 630-648.

Houle, D. 1992. Comparing the evolvability and variability of quantitative traits. Genetics, 130: 195-204.

Houle, D., Hughes, K.A., Hoffmaster, D.K., Ihara, J., Assimacopoulos, S., Canada, D. and Charlesworth, B. 1994. The effects of spontaneous mutation on quantitative traits. I. Variances and covariances of life history traits. Genetics, 138: 773-785.

Houle, D., Morikawa, B. and Lynch, M. 1996. Comparing mutational variabilities. Genetics: 143: 1467-1483.

Howard, R.D. and Young, J.R. 1998. Individual variation in male vocal traits and female mating preferences in *Bufo americanus*. Animal Behaviour, 55: 1165-1179.

Isoherranen, E., Aspi, J. and Hoikkala, A. 1999. Variation and consistency of female preferences for simulated courtship songs in *Drosophila virilis*. Animal Behaviour, 57: 619-625.

Jakob, E.M., Marshall, S.D. and Uetz, G.W. 1996. Estimating fitness: a comparison of body condition indices. Oikos, 77: 61-67.

Jennions, M.D. and Petrie, M. 1997. Variation in mate choice and mating preferences: A review of causes and consequences. Biological Reviews, 72: 283-327.

Jennions, M.D., Backwell, P.R.Y. and Passmore, N.I. 1995. Repeatability of mate choice: the effect of size in the African painted reed frog, *Hyperolius marmoratus*. Animal Behaviour, 49: 181-186.

Johnsen, T.S. and Zuk, M. 1996. Repeatability of mate choice in red jungle fowl. Behavioral Ecology, 7: 243-246.

Johnstone, R.A. 1995. Sexual selection, honest advertisement and the handicap principal: reviewing the evidence. Biological Review, 10: 1-65.

Kempenaers, B. 1994. Polygyny in the blue tit: unbalanced sex ratio and female aggression restrict mate choice. Animal Behaviour, 47: 943-957.

Kirkpatrick, M. 1982. Sexual selection and the evolution of female choice. Evolution, 36: 1-12.

Kirkpatrick, M. 1986. Sexual selection and cycling parasites: A simulation study of Hamilton's hypothesis. Journal of Theoretical Biology, 119: 263-271.

Kirkpatrick, M. and Ryan, M.J. 1991. The paradox of the lek and the evolution of mating preferences. Nature, 350: 33-38.

Knell, R.J. 1999. Sexually transmitted disease and parasite-mediated sexual selection. Evolution, 53: 957-961.

Koga, T., Blackwell, P.R.Y., Jennions, M.D. and Christy, J.H. 1998. Elevated predation risk changes mating behaviour and courtship in the fiddler crab. Proceedings of the Royal Society of London, Series B, 265: 1385-1390.

Kotrba, M. 1996. Sperm transfer by spermatophore in Diptera: new results from the Diopsidae. Zoological Journal of the Linnean Society, 117: 305-323.

Külling, D. and Milinski, M. 1992. Size-dependent predation risk and partner quality in predator inspection in sticklebacks. Animal Behaviour, 44: 949-955.

Lafleur, D.L., Lozano, G.A. and Sclafani, M. 1997. Female mate choice in guppies, *Poecilia reticulata*: a re-evaluation. Animal Behaviour, 56: 513-514.

Laine, P. and Rajasilta, M. 1999. The hatching success of Baltic herring eggs and its relation to female condition. Journal of Experimental Marine Biology and Ecology, 237: 61-73.

Lambert, Y. and Dill, J.D. 2000. Energetic consequences of reproduction in Atlantic cod (*Gadus morhua*) in relation to spawning level of somatic energy reserves. Canadian Journal of Fisheries and Aquatic Sciences, 57: 815-825.

Lande, R. 1981. Models of speciation by sexual selection on polygenic traits. Proceedings of the National Academy of Sciences, USA, 78: 3721-3725.

LeCren, E. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in perch (*Perca fluviatilis*). Journal of Animal Ecology, 66:1504-1512.

Lesna, I. and Sabelis, M.W. 1999. Diet-dependent female choice for males with 'good genes' in a soil predatory mite. Nature, 401: 581-584.

Loeffler, S.H., de Vries, M.J. and Schukken, Y.H. 1999. The effects of time of disease occurrence, milk yield, and body condition on fertility of dairy cows. Journal of Dairy Science, 82: 2589-2604.

López, S. 1999. Parasitized female guppies do not prefer showy males. Animal Behaviour, 57: 1129-1134.

Losey Jr., G.S., Stanton, F.G., Telecky, T.M. and Tyler III, W.A. 1986. Copying others, an evolutionary stable strategy for mate choice: A model. American Naturalist, 128: 653-664.

Magnhagen, C. 1991. Predation risk as a cost of reproduction. Trends in Ecology and Evolution, 6: 183-186.

Magurran, A.E. and Seghers, B.H. 1994. Sexual conflict as a consequence of ecology: evidence from guppy, *Poecilia reticulata*, populations in Trinidad. Proceedings of the Royal Society of London, Series B, 255: 31-36.

Markusfeld, O., Galon, N. and Ezra, E. 1997. Body condition score, health, yield and fertility in dairy cows. Veterinary Record, 141: 67-72.

Maynard Smith, J. 1976. Sexual selection and the handicap principal. Journal of Theoretical Biology, 57: 239-242.

Maynard Smith, J. 1985. Sexual selection, handicaps and true fitness. Journal of Theoretical Biology, 115: 1-8.

McComb, K. and Clutton-Brock, T. 1994. Is mate choice copying or aggregation responsible for skewed distributions of females on leks? Proceedings of the Royal Society of London, Series B, 255: 13-19.

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

Milinski, M. and Bakker, T.C.M. 1990. Female sticklebacks use male coloration in mate choice and hence avoid parasitised males. Nature, 344: 330-333.

Milinski , M. and Bakker, T.C.M. 1992. Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. Proceedings of the Royal Society of London, Series B, 250: 229-233.

Møller, A.P. 1996.ffects of host sexual selection on the population biology of parasites. Oikos, 75: 340-344.

Morse, D. 1988. Relationship between crab spider *Misumena vatia* nesting success and earlier patch-choice decisions. Ecology, 69: 1970-1973.

de la Motte, I. & Burkhardt, D. 1983. Portrait of an Asian stalk-eyed fly. Naturwissenshaften, 70: 451-461.

Munthali, S.M. and Ribbink, A.J. 1998. Condition and fecundity of translocated rock-dwelling cichlid fish in Lake Malawi. Journal of Zoology, 244: 347-355.

Oeting, S. and Bischof, H.J. 1996. Sexual imprinting in female zebra finches: changes in preferences as an effect of adult experience. Behaviour, 133: 387-397.

Palokangas, P., Alatalo, R.V. and Korpimaki, E. 1992. Female choice in the kestral under different availability of mating options. Animal Behaviour, 43: 659-665.

Panhuis, T.M. and Wilkinson, G.S. 1999. Exaggerated eye span influences male contest outcome in stalk-eyed flies. Behavioral Ecology and Sociobiology, 46: 221-227.

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

Patriquin-Meldrum, K.J. and Godin, J.J. 1998. Do female three-spined sticklebacks copy the mate choice of others? American Naturalist, 151: 570-577.

Pocklington, R. and Dill, L.M. 1995 Predation on females or males: who pays the price for bright male traits? Animal Behaviour, 49: 1122-1124.

Pomiankowski, A. 1987a. Sexual selection: The handicap principle does work-sometimes. Proceedings of the Royal Society of London, Series B, 231: 123-145.

Pomiankowski, A. 1987b. The costs of choice in sexual selection. Journal of Theoretical Biology, 128: 195-218.

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

Pomiankowski, A., Iwasa, Y. and Nee, S. 1991. The evolution of costly mate preferences. I. Fisher and biased mutation. Evolution, 45: 1422-1430.

Poulin, R. 1993. Age-dependent effects of parasites on anti-predator responses in two New Zealand freshwater fish. Oecologia, 96: 431-438.

Poulin, R. 1994. Mate choice decisions by parasitized female upland bullies, *Gobiomorphus breviceps*. Proceedings of the Royal Society of London, Series B, 256: 183-187.

Poulin, R. and Vickery, W.L. 1996. Parasite-mediated sexual selection: just how choosy are parasitized females? Behavioural Ecology and Sociobiology, 38: 43-49.

Presgraves, D.C., Severance, E. and Wilkinson, G.S. Sex chromosome meiotic drive in stalk-eyed flies. Genetics, 147: 1169-1180.

Price, T. and Schulter, D. 1991. On the low heritability of life history traits. Evolution, 45: 853-861.

Reading, C.J. and Clarke, R.T. 1995. The effects of density, rainfall and environmental-temperature on body condition and fecundity in the common toad, *Bufo-bufo*. Oecologia, 102: 453-459.

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

Ritchie, M.G., Sunter, D. and Hockham, L.R. Behavioural components of sex role reversal in the tettigoniid bushcricket *Ephippiger ephippiger*. Journal of Insect Behaviour, 4: 481-491.

Robb, L.A., Martin, K. and Hannon, S.J. 1992. Spring body condition, fecundity and survival in female willow ptarmigan. Journal of Animal Ecology, 61: 215-223.

Rolff, J. Parasite mediated sexual selection: parasitized non-choosy females do not slow down the process. Behavioral Ecology and Sociobiology, 44: 73-74.

Rosenqvist, G. and Berglund, A. 1992. Is female sexual behaviour a neglected topic? Trends in Ecology and Evolution, 7:174-176.

Rosenqvist, G. and Houde, A.E. 1997. Prior exposure to male phenotypes influences mate choice in the guppy, *Poecilia reticulata*. Behavioral Ecology, 8, 194-198.

Rosenqvist, G. and Johansson K. 1995. Male avoidance of parasitised females explained by direct benefits in a pipefish. Animal Behaviour, 49: 1039-1045.

Rowe, L. 1992. Convenience polyandry in a water strider: foraging conflicts and female control of copulation frequency and guarding duration. Animal Behaviour, 44: 189-202.

Rowe, L. and Houle, D. 1996. The lek paradox and the capture of genetic variance by condition-dependent traits. Proceedings of the Royal Society of London, Series B, 263: 1415-1421.

Ryan, M.J. 1985. The Tungara Frog, a study in sexual selection and communication. Chicago University Press. Chicago.

Sawyer, G.J., Barker, D.J. and Morris, R.J. 1991. Performance of young breeding cattle in commercial herds in the SouthWest of Western-Australia. 1. Liveweight, body condition, conception and fertility in heifers. Australian Journal of Experimental Agriculture, 31: 431-441.

Schillito, J.F. 1971. Dimorphism in flies with stalked eyes. Zoological Journal of the Linnean Society, 50: 297-305.

Sih, A., Krupa, J. and Travers, S. 1990. An experimental study on the effects of predation risk and feeding regime on the mating behaviour of the water strider. American Naturalist, 135: 284-290.

Sikkel, P.C. 1998. Competitor intrusion and mate-search tactics in a territorial marine fish. Behavioral Ecology, 5: 439-444.

Simmons, L.W. 1994. Reproductive energetics of the role reversing bushcricket, *Kawanaphila nartee* (Orthoptera: Tettigoniidae: Zaprochilinae). Journal of Evolutionary Biology, 7: 189-200.

Slagsvold, T., Lifjeld, J.T., Stenmark, G. and Breiehagen, T. 1988. On the cost of searching for a mate in female pied flycatchers *Ficedula hypoleuca.* Animal Behaviour, 36: 433-442.

Testa, J.W. and Adams, G.P. 1998. Body condition and adjustments to reproductive effort in female moose (*Alces alces*). Journal of Mammology, 79: 1345-1354.

Tomlinson, I.P.M. 1988. Diploid models of the handicap principal. Heredity, 60: 283-293.

Tomlinson, I.P.M. and O'Donald, P. 1996. The influence of female viability differences on the evolution of mate choice. Heredity, 77: 303-312.

Trivers, R. 1985. Social Evolution. Benjamin Cummings Publishing. California.

Vickery, W.L. and Poulin, R. 1998. Parasitized non-choosy females do slow down the process: a reply to J. Rolff. Behavioral Ecology and Sociobiology, 44: 75-76.

Waage, J.K. 1979. Adaptive significance of postcopulatory guarding of mates and nonmates by male *Calopteryx maculata* (Odonata). Behavioral Ecology and Sociobiology, 6: 147-154.

Wade, M.J. and Pruett-Jones, S.G. 1990. Female copying increases the variance in male mating success. Proceedings of the National Academy of Sciences, USA, 87: 5749-5753.

Wagner, W.E., Murray, A.M. and Cade, W.H. 1995. Phenotypic variation in the mating preferences of female field crickets, *Gryllus integer*. Animal Behaviour, 49: 1269-1281.

Waltner, S.S., McNamara, J.P. and Hillers, J.K. 1993. Relationships of body condition score to production variables in high producing holstein dairy-cattle. Journal of Dairy Science, 76: 3410-3419.

Wildman, E.E., Jones, G.M., Wagner, P.E., Boman, R.L., Troutt, H.F. & Lesch, T.N. 1982. A dairy cow body condition scoring system and its relationship to selected production characteristics. Journal of Dairy Science, 65: 495-501.

Wilkinson, G.S. 1993. Artificial sexual selection alters allometry in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). Genetical Research, 62: 213-222.

Wilkinson, G.S. & Reillo, P.R. 1994. Female response to artificial selection on an exaggerated male trait in a stalk-eyed fly. Proceedings of the Royal Society of London, Series B, 255: 1-6.

Wilkinson, G.S. & Taper, M. 1999. Evolution of genetic variation for condition-dependent traits in stalk-eyed flies. Proceedings of the Royal Society of London, Series B, 266: 1685-1690.

Wilkinson, G.S., Kahler, H. & Baker, R.H. 1998. Evolution of female mating preferences in stalk-eyed flies. Behavioral Ecology, 9: 525-533.

Wilkinson, G.S., Presgraves, D.C. & Crymes, L. 1998. Male eye span in stalk-eyed flies indicates genetic quality by meiotic drive suppression. Nature, 391: 276-279.

Witte, K. and Ryan R.J. 1998. Male body length influences mate-choice copying in the sailfin molly *Poecilia latipinna*. Behavioral Ecology, 9: 534-539.

Zahavi, A. 1975. Mate selection - a selection for a handicap. Journal of Theoretical Biology, 53: 205-214.

Zahavi, A. 1977. The cost of honesty (further remarks on the handicap principal). Journal of Theoretical Biology, 67: 603-605.

Zuk, M., Tania, K., Robinson, S.I. and Johnsen, T.S. 1998. Parasites influence social rank and morphology, but not mate choice, in female red junglefowl, *Gallus gallus*. Animal Behaviour, 56: 493-499.

Chapter Two

Eyespan-dependent mate preference in the stalk-eyed fly

Cyrtodiopsis dalmanni

(Accepted for publication in Animal Behaviour)

ABSTRACT

We investigated eyespan-dependent mate choice in the stalk-eyed fly *Cyrtodiopsis dalmanni*. In this species, females prefer to mate with larger eyespan males. Female eyespan was experimentally manipulated by raising larvae at different densities. The mating preference of small and large eyespan females was compared using choice chambers containing two males that differed in eyespan. Neither small nor large eyespan females distinguished between males with small differences in eyespan ($\overline{X}$ = 1.45 mm), but both preferred the male with the wider eyespan when the difference between males was large ($\overline{X}$ = 3.17 mm). When presented with an intermediate difference in male eyespan ($\overline{X}$ = 2.40 mm), large eyespan females showed a significantly stronger preference for the wider eyespan

male than did small eyespan females. Eyespan-dependent mate choice in stalk-eyed flies is probably caused by small eyespan females being less able to discriminate differences between males. It may also reflect female condition as eyespan is highly correlated with body size, which is a strong predictor of developmental conditions.

INTRODUCTION

Darwin's (1859) theory of sexual selection has been intensively studied over the last twenty years (Andersson 1994). In particular, much attention has been given to the evolution of female mating preferences for exaggerated male ornamental characters and displays. This interest has largely centred on the signalling function, fitness consequences and other properties of exaggerated male ornaments (Andersson 1994). In contrast, there has been a relative neglect of female preferences (Rosenqvist and Berglund 1992; Ahnesjö *et al.* 1993).

In their review of variation in female mate choice, Jennions and Petrie (1997) suggested that many phenotypic factors relating to condition affect mate preferences. Evidence for phenotype-dependent mate choice has been found previously in a number of experimental studies. In the African painted reed frog, females prefer deeper, low frequency, calls when presented with two male calls with large frequency differences (Jennions *et al.* 1995). When the difference in call frequency was reduced, larger females were more likely to still show preference for the lower frequency call (Jennions *et al.* 1995). More recently, evidence for condition-dependent mate choice has been reported in three-spined sticklebacks (Bakker *et al.* 1999). Condition was measured using body mass to length ratios. Females from high condition families preferred red-throated males, whereas females from low condition families preferred orange-throated males.

Finally, there is evidence that parasite infection alters mate choice. López (1999) and Poulin (1994) found that the preferences of parasite infected females were weaker than those of uninfected females in guppies and upland bullies respectively. In both experiments infected females made fewer inspections of males, probably due to the energetic constraints imposed by infection.

Here we investigate eyespan-dependent mate choice in the stalk-eyed fly *Cyrtodiopsis dalmanni*. In wild populations of *C. dalmanni,* females roost at dusk on root threads overhanging streams (Burkhardt and de la Motte 1988). Females prefer to roost with larger males, which have correspondingly larger eyespan (Burkhardt and de la Motte 1988). At dawn, males mate with the females in their harem. As a result, the number of roosting females in a male's harem is a good predictor of mating success (Burkhardt and de la Motte 1988). In laboratory experiments with stalk-eyed flies, females have been shown to use difference in eyespan to choose between males (Wilkinson *et al.* 1998). However, there is considerable variation in female mate choice (Wilkinson *et al.* 1998). Phenotypic correlates underlying this variation in mate choice have not been investigated. In this study we investigated whether female eyespan affects female mate preference.

We made two measures of preference based upon the number of copulations and the roosting behaviour of females. We chose female eyespan as a measure of phenotypic variation primarily because there is good evidence in stalk-

eyed flies that female preference is based on visual assessment of males (Burkhardt and de la Motte 1988), and eyespan is probably associated with several aspects of visual perception (Burkhardt and de la Motte 1983). Eyespan can be measured accurately and is easily manipulated by altering larval food supply (David *et al.* 1998). In addition, female eyespan correlates strongly with body size which has been used previously as a measure of condition (Wilkinson and Taper 1999).

METHODS

Flies and Media

The flies used were from an outbred population collected in Ulu Gombak, Malaysia, in 1993 by Andrew Pomiankowski. It has been maintained since at a population size of at least 200 individuals in cage culture at 25°C. Lighting was on a 12hr dark:12hr light cycle with half hour dawn and dusk periods of reduced lighting. All flies and larvae were fed pureed sweetcorn containing the anti-fungal agent Nipogin.

Production of experimental flies

Flies were obtained by collecting eggs from the cage cultures over a two week period. To ensure a wide range of male and female eyespan amongst experimental flies, eggs were reared at low and high larval density (David *et al.* 1998). Upon eclosion, flies were separated according to sex and kept as virgins. This ensured that females did not vary in their early exposure to males. Variation in female experience of males is a potential confounding factor in the analysis of female preference in other species (Collins 1995).

When the flies reached sexual maturity at five to six weeks post eclosion, females were collected over ice and their eyespan was measured. Digitised microscope images were measured to the nearest 0.01 mm using NIH image software (Fig. 2.1a). Females were divided into small (<5.75 mm) and large (>6.00 mm) eyespan groups. Mean female eyespan was 6.12 ± 0.12 mm ($\overline{X}$ ± SD, N = 264) for large females and 5.33 ± 0.36 mm, (N = 264) for small females. Within each size category females were randomly divided into groups of six individuals.

Males were measured using a similar procedure (Fig. 2.1b) and were divided into two categories. Large males were greater than 9 mm in eyespan. Small males always had smaller eyespan than large males. The eyespan of the small males was varied to alter the mean difference in eyespan between small and

large males in each of three successive experiments. Males were then paired as one large and one small individual. The size of the difference between male pairs was a) large difference experiment, 3.17 ± 0.59 mm ($\bar{X}$ ± SD, N = 19), b) intermediate difference experiment, 2.40 ± 0.43 mm (N = 36) and c) small difference experiment, 1.45 ± 0.23 mm (N = 22). Note that because the eyespan of "large" males was fixed, "small" males in the small difference experiment had reasonably wide eyespan (7.81 ± 0.31 mm). Experiments were carried out sequentially in the order: large difference, small difference, intermediate difference experiment.

Copulation preference

Female preference was tested with the three groups of small and large males. Test cages were constructed from Perspex (30 × 14 × 14 cm). To prevent male-male competition obscuring female mate choice (Wilkinson *et al.* 1998), males were separated by a sheet of acetate fitted in the middle of each Perspex cage, punctured by 56 evenly spaced 5.8 mm holes cut using a cork borer (Fig. 2.2). The size of hole was chosen so as to restrict male movement but allow females (which have smaller eyespan) to move between sides. On the rare occasions when a male moved across the acetate barrier, it was returned to its original side and observations resumed. Plentiful food was available on both sides

at all times and was replaced every four days. At the same time males were swapped between sides to compensate for any bias of females to be on one side of the cage.

Each experimental cage housed either six small or six large test females. At the start of the experiment, three females were put on either side of the acetate divider. Flies were given three days to acclimatise to the cage conditions before observations began. Cages were observed for eight consecutive days for the first 1.5hrs of the day (1/2hr dawn and 1hr full illumination), which is when 95% of copulations are observed in the wild (Wilkinson and Reillo 1994). During this period the number of copulations with each male was recorded. A copulation was defined as a male mounting a female and engaging genitalia. The distribution of females within the cage was recorded at the end of the observation period. The distribution of females was recorded as the number of females roosting in each male's compartment. After a four day interval, observations were resumed for a further eight days, giving 16 observational days in total. During the four day interval, flies were kept under the same conditions but no observations were made. All observations were made by AH.

In each experiment, cages were observed in batches of twelve or less in order to avoid missing copulations (in copula pairs are conspicuous). In the small difference experiment two replicates of 12 cages were used, in the intermediate difference experiment three replicates of 12 cages were used, and in the large

difference experiment two replicates of 10 cages were used. Each replicate contained equal numbers of small and large female cages.

At the start of each replicate one reserve cage was set up for every three experimental cages. Reserve cages were used to replace dead male or female flies in experimental cages. Reserve cages were the same in every respect as the experimental cages but were not observed. This ensured that replacement flies had similar experience to those they replaced. Once a male or female fly was used from a reserve cage, that cage was removed from the reserve.

Males in some cages achieved less than one mating per day for the first eight days of observations. These males were replaced in the second eight days of observations using males from reserve cages (15 cages out of 80). If a replacement male failed to achieve more than one copulation per day over the second eight days, the cage was eliminated from the analysis (3 cages out of 80, leaving 77 cages). Males with low copulation rates were replaced because they were unrepresentative of normal males, often being found to have deformed genitalia or wounds which were not apparent upon a brief inspection at the start of the experiment. As the low copulation rate of these males reflected male incapacity rather than female mate choice, we decided to remove them from the experiment.

Data collected on the number of copulations by each male in a cage was used to calculate copulation preference. Copulation preference was measured

as $CP = C_L - C_S$, where C_L is the average number of copulations per day by the large male and C_S the average number of copulations per day by the small male. Cages with complete data (16 days of measurement) and cages with replacement males (8 days of measurement) were treated in the same manner.

Our measure of preference, CP, could be subject to a bias in favour of cages which had more copulations. Assuming two cages of females had the same small male: large male copulation ratio, the cage which had more copulations in total would show a stronger preference. With this in mind the relationship of absolute CP to total copulations within a cage was analysed using least squares regression. No regressions were significant which indicates that CP was independent of variation in total copulation number. As a precaution a relative preference measure was also used to analyse the data, where

$RCP = (C_L - C_S)/(C_L + C_S)$.

Roosting preference

Another method of measuring preference in stalk-eyed flies is to observe which male females roosted with (Wilkinson and Reillo 1994, using nearly identical apparatus as our own). This may be an efficient method of assessing female mate preference as females in the wild mate the males with which they roost. Roosting preference was measured as $RP = R_L - R_S$, where R_L is the

average number of females roosting per day with the large male and *Rs* is the average number of females roosting per day with the small male.

Both measures of preference were made at the level of the cage. They are measures of the average behaviour of groups of six females. Measurements of individual females could not be used, as any one female in a cage may have been influenced by the behaviour of the other females in the same cage. Hence, data from an individual female could not be assumed to be independent of any other in the same cage. Furthermore, following individuals would have greatly reduced the number of cages that could have been observed accurately in each replicate.

Statistical analysis

The dependency of copulation preference on the total number of copulations within a cage was analysed using least squares linear regression. Regressions were calculated for all females as well as small and large females separately for all experiments.

All copulation and roosting preference distributions were tested for normality using the Shapiro-Wilk *W* test (Shapiro and Wilk 1965) and were found to be normally distributed. Female roosting and copulation preference distributions for all females were tested for deviations from random mating using two-tailed *t* tests. The sample mean was compared to a null hypothesis of random

mating, with zero mean. Comparisons of copulation and roosting preference distributions of small and large females within each experiment were made in mixed model two-way ANOVAs with female size as a fixed factor and replicate as a random factor (Zar 1984). Using a two-way ANOVA allowed us to account for variation in female preference between replicates which otherwise could have obscured the effects of female size on preference. Copulation preference data from all three experiments were analysed in a model I, two-way ANOVA with experiment and female size as fixed factors. This allowed us to test for overall effects of difference in male eyespan and female size on female preference. All copulation preference analyses were repeated using the relative copulation preference statistic. JMP version 3.1.6 for the Macintosh (SAS 1996) was used for all statistical analysis.

RESULTS

Copulation preference

In the large difference experiment, the eyespan of large males were considerably greater than those of small males (mean = 3.17 mm). Small eyespan females gained 3.42 ± 1.09 ($\overline{X}$ ± SD) copulations per day and large eyespan females gained 3.33 ± 1.60 copulations per day. Females overall preferred to

copulate with large males ($t_{18} = 3.781$, $P = 0.001$). Both small and large females preferred to mate with large males (small females: $t_8 = 2.879$, $P = 0.021$; large females: $t_9 = 2.438$, $P = 0.038$). The mean copulation preference of small females was not higher than that of large females ($F_{1,1} = 1.000$, $P = 0.500$). There were no significant differences between replicates ($F_{1,15} = 0.005$, $P = 0.947$) and the interaction between female size and replicate was non-significant ($F_{1,15} = 0.005$, $P = 0.944$). The same relationships held when using relative copulation preferences. The lack of difference between small and large females suggested that large differences in male eyespan could be detected by both types of female.

It is possible that when the difference in male eyespan is reduced, small and large females behave differently. To test for this we performed the small difference experiment in which the average difference between males was only 1.45 mm. In this experiment small eyespan females gained 3.50 ± 0.83 ($\overline{X}$ ± SD) copulations per day and large eyespan females gained 4.76 ± 1.08 copulations per day. With a minor difference between males, females overall mated randomly ($t_{21} = 0.836$, $P = 0.413$). Neither small nor large females showed a significant copulation preference for small or large males (small females: $t_9 = -0.491$, $P = 0.635$; large females: $t_{11} = 1.178$, $P = 0.264$). The mean copulation preference of small and large females did not differ ($F_{1,1} = 1.566$, $P = 0.429$). There were no significant replicate ($F_{1,18} = 0.371$, $P = 0.550$) or interaction effects ($F_{1,18} = 0.890$, $P = 0.358$). The same relationships held when using relative copulation

preferences. When differences in male eyespan were small, both small and large females mated randomly.

Finally, we undertook a further experiment with an intermediate difference in male eyespan (mean difference = 2.4 mm). Small eyespan females gained 3.16 ± 1.04 ($\overline{X}$ ± SD) copulations per day in this experiment and large eyespan females gained 3.44 ± 1.19 ($\overline{X}$ ± SD) copulations per day. Here, females overall preferred to mate with large males ($t_{35} = 5.249$, $P < 0.001$). Both small and large females preferred to copulate with large males (small females $t_{17} = 2.161$, $P = 0.045$, large females, $t_{17} = 6.148$, $P < 0.001$). The mean copulation preference of small females was significantly weaker than that of large females ($F_{1,2} = 23.039$, $P = 0.040$). Again there were no significant replicate ($F_{2,30} = 2.340$, $P = 0.114$) or interaction effects ($F_{2,30} = 0.286$, $P = 0.753$). The same relationships held using relative copulation preferences.

Figure 2.3 displays the copulation preference functions of small and large females, for each of the three experiments. For small and large females, the strength of female copulation preference increases as the difference in the size of the male eyespan increases. To confirm this pattern, we combined the preference data from all three experiments. This revealed a significant effect of experiment ($F_{2,71} = 4.008$, $P = 0.023$), indicating that female preference changed when the average difference between male eyespan was varied. We also checked for the effect of female eyespan ($F_{1,71} = 3.047$, $P = 0.085$) and the interaction between

female eyespan and experiment ($F_{2,71} = 0.921$, $P = 0.403$). Neither of these were significant. Similar relationships were also found with using the relative preference measure.

Roosting preference

In the large difference experiment, females did not prefer to roost with small or large males ($t_{18} = 0.827$, $P = 0.419$). Neither small nor large females showed any roosting preference (small females: $t_9 = 0.212$, $P = 0.837$; large females: $t_8 = 1.278$, $P = 0.233$). Small and large females did not differ in the strength of roosting preference ($F_{1,1} = 1.366$, $P = 0.261$). Even when the difference between male eyespan is large, females showed no roosting preference in our apparatus.

A similar lack of roosting preference was found in the small difference experiment. Females did not show a roosting preference overall ($t_{21} = 0.388$, $P = 0.702$), or when split into small and large categories (small females: $t_9 = 0.080$, $P = 0.938$; large females: $t_{11} = 0.650$, $P = 0.529$). There was no difference between the strength of roosting preference in small and large females ($F_{1,1} = 0.073$, $P = 0.833$). Replicate and interaction terms were non-significant.

In the intermediate difference experiment there was again no roosting preference overall ($t_{35} = 1.882$, $P = 0.068$) or amongst small females ($t_{17} = 0.041$,

$P = 0.968$). Large females did show a roosting preference ($t_{17} = 2.626$, $P = 0.018$), but this was for small males, the opposite of our expectation. There were no differences in roosting preference between large and small females ($F_{1,2} = 4.653$, $P = 0.164$). The only significant effect was of replicate ($F_{2,30} = 6.077$, $P = 0.006$) due to both small and large females having much weaker roosting preference for small males in replicate two.

DISCUSSION

Presenting females with binary choices of males has become a standard means of measuring the intensity of female mate preferences. Using such a technique in the stalk-eyed fly *C. dalmanni*, we found female mate preferences for larger eyespan males became stronger when the two males presented had greater differences in eyespan. Preference was measured as the difference in the number of copulations with each male per day over 16 days. A similar result was found by Wilkinson *et al.* (1998) using *C. dalmanni* flies derived from the same region of Malaysia.

To investigate eyespan-dependent mate choice, females were split into two categories that varied in eyespan. Small females had eyespans less than 5.75 mm ($\overline{X}$ = 5.33 mm) and large females had eyespans greater than 6.00 mm ($\overline{X}$ = 6.12 mm). Both types of female mated at random when the difference between males was about 1.45 mm, and both showed strong copulation preference for the larger

male when the difference in eyespan was about 3.17 mm. However, when the difference in male eyespan was intermediate, in the range 1.65-3.13 mm ($\overline{X}$ = 2.40 mm), small females had weaker preference for large eyespan males than did large females.

The fact that the female preference functions diverged when males differed by intermediate values argues that large females were better able to distinguish between males with smaller differences in eyespan. However, it is possible that large females simply had stronger preferences than small females irrespective of differences in male eyespan. This interpretation gains more plausibility as the experiments varied in sample size, with the intermediate difference experiment being the largest in scale. It is possible that the larger sample size enhanced the power of the intermediate experiment to detect differences between small and large females.

This alternative interpretation was studied further by an analysis of the combined data from all three experiments. This indicated that there were significant differences in female preference between experiments indicating that female preference changed when the average difference between male eyespan was varied. However, the effect of female size and the interaction term were non-significant. So this analysis fails to distinguish whether large females have stronger preferences overall or stronger preferences just in relation to males of intermediate size difference.

The combined analysis is not strictly an appropriate test for our experimental design, as it combines data from three independent experiments which were performed sequentially. For instance, environmental fluctuations over time may have influenced female preferences. The need to make accurate observations of multiple behavioural interactions imposed a significant constraint on what could be measured at any one time. It would be of considerable interest to re-examine these findings by making simultaneous tests at each male size difference, with balanced sample sizes across male size classes.

Although in our analysis we tested for differences in the strength of large and small eyespan female *CP* using t-tests in each experiment, we did not correct α-values for repeated tests (a total of three; one in each experiment). We did not apply a Bonferonni or similar correction because the experiments did not test the same hypothesis: each experiment used a different 'difference' in male eyespan. Other authors may have been more conservative in their interpretation of the data and applied a correction to reduce the possibility of a type 1 error.

The range of differences in male (4.90-9.85 mm) and female (4.27-6.60 mm) eyespan in our experiments is within that found in the wild. Field collections of *C. dalmanni* have reported some populations having males of up to 17 mm in eyespan (Shillito 1971, Wilkinson pers. comm.), whereas the smallest eyespan can be as low as 4 mm.

Eyespan-dependent mate choice in *C. dalmanni* may be due directly to variation in female eyespan. Theoretical studies have shown that visual perception improves when the distance between each eye is increased (Burkhardt and de la Motte 1983). Stalk-eyed fly mating assessment is also known to be based on visual cues (Burkhardt and de la Motte 1988). When differences between males are small, both types of female may find it difficult to discriminate between males. When males differ greatly in eyespan, both small and large females may readily discriminate between males and show a preference for mating with large males. When differences are intermediate, small females may be more likely to make mistakes in discrimination. In contrast, females with wider eyespan and greater visual definition will be able to discriminate more strongly against small males. More experimentation is required to show whether eyespan is the causal factor behind eyespan-dependent mate choice in stalk-eyed flies.

An alternative interpretation is suggested by the strong correlation of female eyespan with body size (Wilkinson 1993). Body size in stalk-eyed flies has previously been used as a good estimate of condition (Wilkinson and Taper 1999). In this context, condition refers to the pool of resources available for allocation to the production and maintenance of traits (Rowe and Houle 1996). Individual stalk-eyed flies differ in condition due to genetic and environmental variation in the amount of resources they accrue during the larval stages, which are then partitioned amongst developing organs during pupation (Nijhout and Emlen

1998). Once an adult ecloses, its cuticle hardens and no further growth is possible. Consequently, adult body size reflects larval condition. So the different mate preferences of small and large eyespan females may be a form of condition-dependent behaviour.

The logic behind condition-dependent trait expression has been well explored for male sexual ornaments that are handicaps (Grafen 1990; Iwasa *et al.* 1991; Iwasa and Pomiankowski 1994; Rowe and Houle 1996). This logic may also apply to female mate preference. Female mate preference is likely to be costly not only because the sensory and morphological traits that enable females to make choices are costly but also because mate choice takes time, is energetically expensive and exposes females to predation risks (Pomiankowski 1987). These fitness costs may vary with female condition, leading to the evolution of condition-dependent expression of female mate preference.

There is no evidence that search costs are condition-dependent in stalk-eyed flies. It is more likely that female eyespan, and hence the strength of female preference, is restricted by the costs of growth and maintenance. In support of this, eyespan shows a strong allometric relationship with body size in female flies (de la Motte and Burkhardt 1983, David *et al.* 1998). However, as yet we do not have direct evidence of the fitness consequences of eyespan and body size growth.

Note that in our experiments, "large" male eyespan was fixed to be greater than 9 mm, with "small" males varying in size across experiments. Another

possible explanation of the results is that small eyespan females have a higher threshold for mate preference than large eyespan females. However, all evidence to date suggests that mate choice is dependent on the difference between male eyespan, rather than on thresholds (Wilkinson *et al.* 1998). Whatever the actual mechanism of mate choice, our experiments demonstrate that small eyespan females have weaker preferences than large eyespan males when differences between males are intermediate.

It is possible that rearing density may have confounded size (eyespan) effects in our experiment. As flies were separated by eyespan in this experiment and not by the larval density at which they were raised, this should have reduced the magnitude of any density effects on female behaviour. This was because, although greater larval density tended to reduce adult eyespan, large eyespan females were still found at high densities and small eyespan females at low densities. However, overall, small eyespan females tended to be from high larval densities and large eyespan females from low larval densities. As rearing density was not recorded during the experiment, it is impossible to exclude rearing density as having an effect on female preference.

Both the number of females roosting with a male and the number of copulations a male achieves have been used to measure mating preferences in stalk-eyed flies (Wilkinson and Reillo 1994, Wilkinson *et al.* 1998). We found that roosting was a poor indicator of female preference in our experiments. Only

one significant preference was found; this was a roosting preference for small males and is probably an experimental artefact. In contrast, copulation preference was a sensitive indicator of female mate choice in our experimental apparatus. In the wild, roosting is probably a good indicator of male mating success as males mate with the females with which they roost (Wilkinson and Reillo 1994). However, in our experimental apparatus, roosting appeared to be a random variable. This suggests that our experimental technique for measuring preference only measures one aspect of mate choice, and further investigation is needed of the cues used by females to choose males.

Our results have implications for a previous report of the covariance of female preference and male eyespan genes in stalk-eyed flies (Wilkinson and Reillo 1994). This study is important because a correlation of male eyespan and female preference genes is predicted by good genes and runaway models of sexual selection (Andersson 1994). Evidence for a genetic covariance comes from an artificial selection experiment in which lines were selected for small and large male eyespan (body size held constant). After 10 generations, male eyespan had increased in the high line and decreased in the low line relative to the control. Females from the high line retained the same strength of preference for large eyespan males, whereas low line females showed a marginal preference for males with smaller eyespan (same test males in each case; Wilkinson and Reillo 1994).

This correlated change in female preference was taken as evidence for a genetic correlation between male eyespan and female preference traits.

An alternative explanation for these observations is suggested by our experimental results. Female eyespan as well as female preference showed a correlated response to artificial selection on male eyespan. Females from the low line had significantly smaller eyespan than control females and high line females had significantly larger eyespan than control females (Wilkinson 1993). Differences between the eyespan of low and high line females were about the same as small and large females in our experiment. So differences in mating preference seen in the selection lines could be due simply to differences in female eyespan. Our results do not refute the evidence for genetic correlations between males and females, but suggest a simple cause due to eyespan changes in both sexes.

In summary, we have shown that female *C. dalmanni* stalk-eyed flies have strong female preference for males with larger eyespan. The strength of preference increases from random mating when differences between males are small, to strong preference for large eyespan males when differences between males are large. This change in preference is eyespan-dependent, as only females with large eyespan showed preference for the larger eyespan male when there was an intermediate difference in eyespan between males.

REFERENCES

Ahnesjö, I., Vincent, A., Alatalo, R., Halliday, T. and Sutherland, W.J. 1993. The role of females in influencing mating patterns. Behavioral Ecology, 4: 187-189.

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

Bakker, T.C.M., Künzler, R. and Mazzi, D. 1999. Condition related mate choice in sticklebacks. Nature: 401: 234.

Burkhardt, D. and de la Motte, I. 1983. How stalk-eyed flies eye stalk-eyed flies: Observations and measurements of the eyes of *Cyrtodiopsis whitei*. Journal of Comparative Physiology A, 151: 407-421.

Burkhardt, D. and de la Motte, I. 1988. Big 'antlers' are favoured: female choice in stalk-eyed flies (Diptera, Insecta), field collected harems and laboratory experiments. Journal of Comparative Physiology A, 162: 649-652.

Collins, S.A. 1995. The effect of recent experience on female choice in zebra finches. Animal Behaviour, 49: 479-486.

Darwin, C.R. 1859. On the Origin of Species. John Murray. London.

David, P., Hingle, A., Greig, D., Rutherford, A., Pomiankowski, A. and Fowler, K. 1998. Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. Proceedings of the Royal Society of London, Series B, 265: 2211-2216.

Grafen, A. 1990. Biological signals as handicaps. Journal of Theoretical Biology, 144: 517-546.

Iwasa, Y. and Pomiankowski, A. 1994. The evolution of mate preferences for multiple sexual ornaments. Evolution, 48: 853-867.

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

Jennions, M. D. and Petrie, M. 1997. Variation in mate choice and mating preferences: a review of the causes and consequences. Biological Reviews of the Cambridge Philosophical Society, 72: 283-327.

Jennions, M. D., Backwell, P.R.Y. and Passmore, N.I. 1995. Repeatability of mate choice: the effect of size in the African painted reed frog, *Hyperolius marmoratus*. Animal Behaviour, 49: 181-186.

López, S. 1999. Parasitized female guppies do not prefer showy males. Animal Behaviour, 57: 1129-1134.

de la Motte, I. and Burkhardt, D. 1983. Portrait of an Asian Stalk-eyed fly. Naturwissenshaften, 70: 451-461.

Nijhout, H.F. and Emlen, D.J. 1998. Competition among body parts in the development and evolution of insect morphology. Proceedings of the National Academy of Science, USA, 95: 3685-3689.

Pomiankowski, A. 1987. The costs of choice in sexual selection. Journal of Theoretical Biology, 128: 195-218.

Poulin, R. 1994. Mate choice decisions by parasitized female upland bullies, *Gobiomorphus breviceps*. Proceedings of the Royal Society of London, Series B, 256: 183-187.

Rosenqvist, G. and Berglund, A. Is female sexual behaviour a neglected topic? Trends in Ecology and Evolution, 7: 174-176.

Rowe, L. and Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proceedings of the Royal Society of London, Series B, 263: 1415-1421.

Shapiro, S.S. and Wilk, M.B. 1965. An analysis of variance test for normality. Journal of the American Statistics Association, 63: 1343-1372.

SAS Institute. 1996. JMP statistics for the Macintosh version 3.1.6.

Shillito, J.F. 1971. Dimorphism in flies with stalked eyes. Zoological Journal of the Linnean Society. 50: 297-305.

Wilkinson, G. S. 1993. Artificial sexual selection alters allometry in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). Genetical Research, 62: 213-222.

Wilkinson, G.S. and Reillo, P.R. 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. Proceedings of the Royal Society of London, Series B, 255: 1-6.

Wilkinson, G.S. and Taper, M. 1999. Evolution of genetic variation for condition-dependent traits in stalk-eyed flies. Proceedings of the Royal Society of London, Series B, 266: 1685-1690.

Wilkinson, G.S., Kahler, H. and Baker, R.H. 1998. Evolution of female mating preferences in stalk-eyed flies. Behavioral Ecology, 9: 525-533.

Zar, J.H. 1984. Biostatistical Analysis. 2nd ed. Prentice-Hall International. New Jersey.

FIGURE LEGENDS

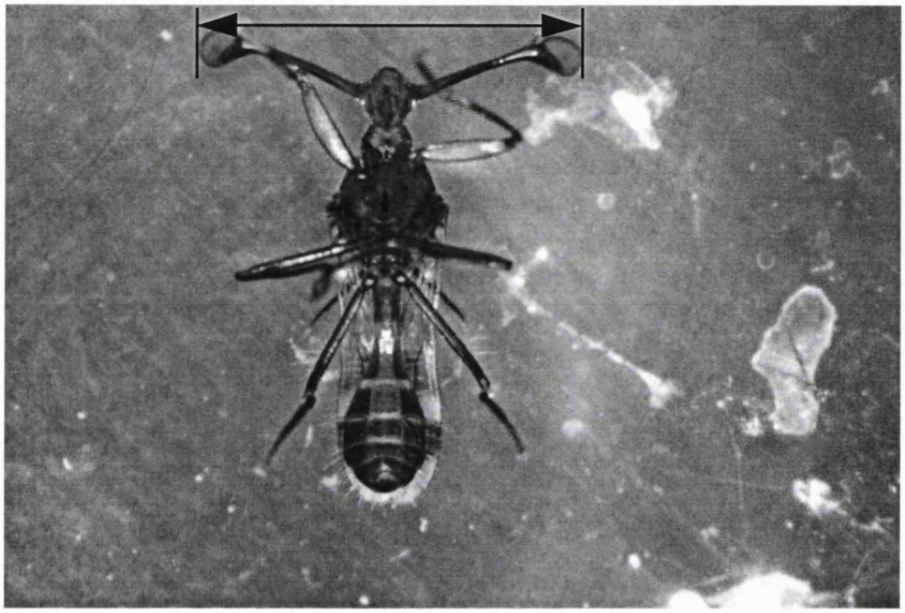
Figure 2.1. Measurement of eyespan in a) females and b) males. Eyespan was measured from the edge of the left eyebulb to the edge of the right eyebulb.

Figure 2.2. Cages used to measure female preference had two males, one with small and the other with large eyespan, separated by an acetate divider with small holes that prevented male movement between compartments. Six females were added to each cage to measure female copulation and roosting preference.

Figure 2.3. Copulation preferences for small (triangles) and large (open circles) females as a function of the difference in male eyespan. Each point represents the copulation preference of a cage of females. Filled circles represent preference distribution means.

Figure 2.1

a)



b)

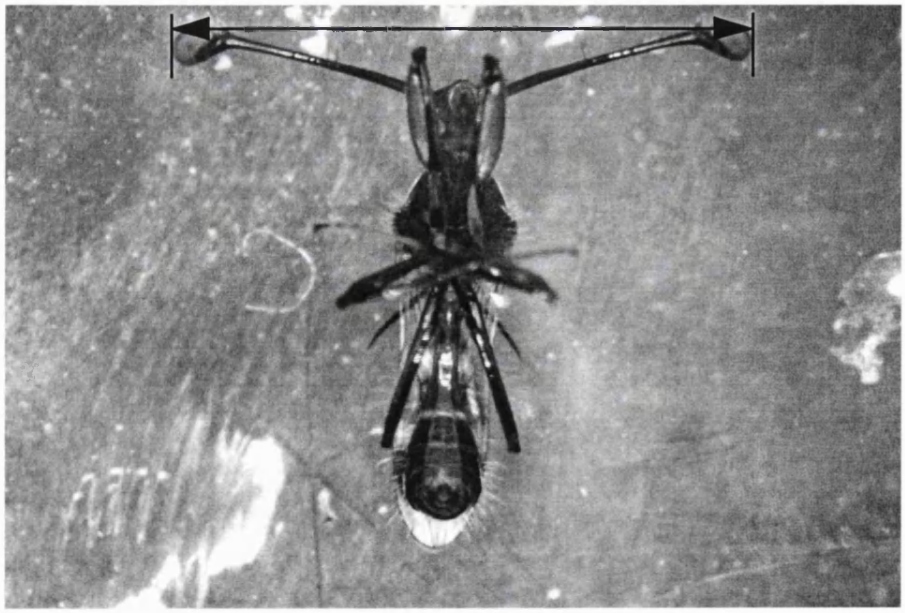


Figure 2.2

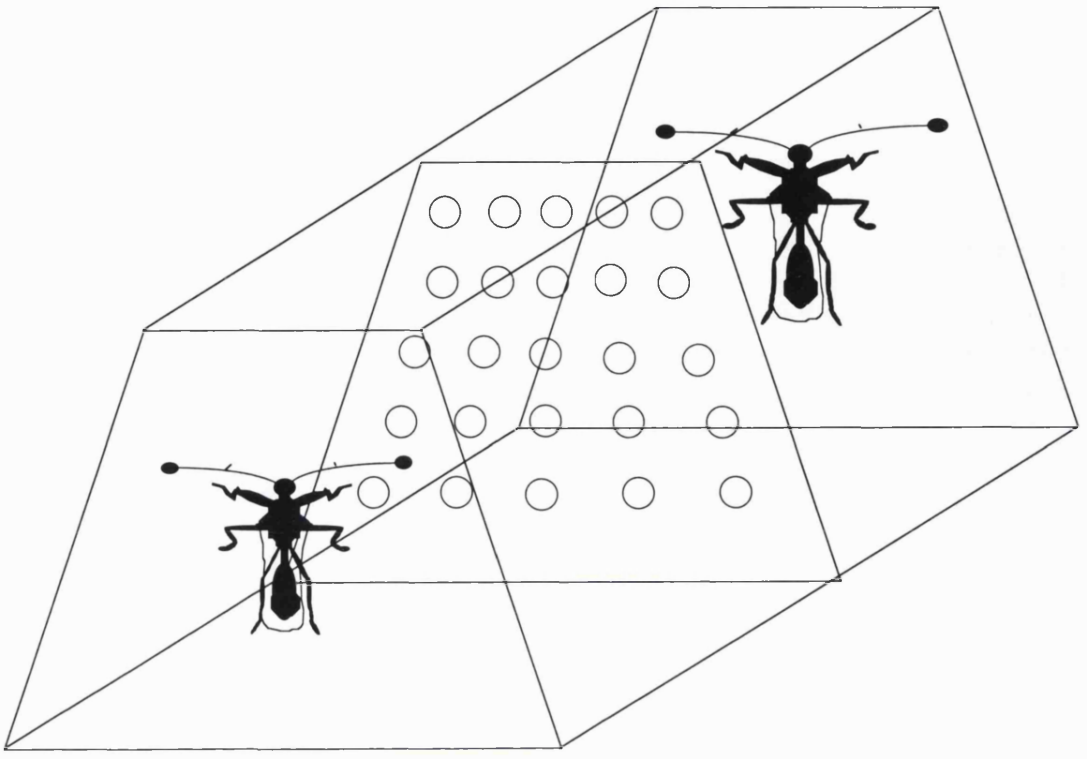
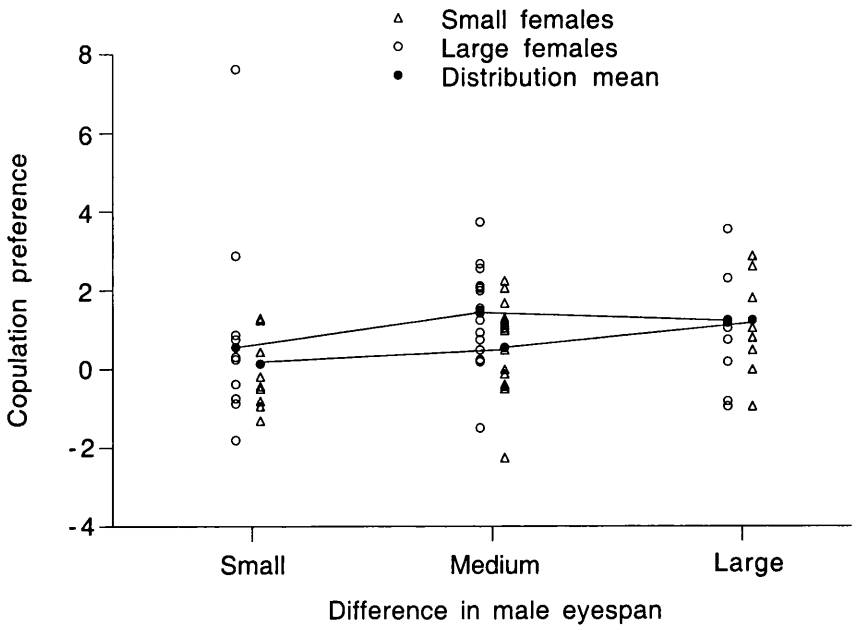


Figure 2.3


Small females
Large females
Distribution mean
Copulation preference
8
6
4
2
0
-2
-4
Small
Medium
Large
Difference in male eyespan

Chapter Three

The effect of transient food stress on female mate preference in the stalk-eyed fly *Cyrtodiopsis dalmanni*

ABSTRACT

In this study, we investigated the effect of a transient nutritional stress (sucrose culture medium) on female mate preference in the stalk-eyed fly *Cyrtodiopsis dalmanni*. We assessed the mate preference of groups of females that were exposed sequentially to different food media. Females were tested first on sucrose (novel medium) and subsequently on corn (standard medium), and in parallel, a second treatment assessed females first on corn and subsequently on sucrose. We found that females had much stronger preferences for large eyespan males when on corn than on sucrose. There were no effects on the strength of female mate preference due to age, experience or the order of exposure to culture media. Additional experiments demonstrated that maintenance on a sucrose diet reduced the number of mature and developing eggs in females without significantly increasing mortality. We discuss the adaptive significance of changes in female

preference due to transient alterations in diet and fertility, and the effect of changes in female preference on the strength of sexual selection.

INTRODUCTION

Until recently, little was known or understood about variation in female mating preferences despite the great attention paid to sexual selection over the last decade (Andersson 1994). There is some evidence for genetic variation in female preference (Bakker and Pomiankowski 1995) but preference genes have not yet been mapped. There is more evidence for environmental factors affecting preference, for example, two studies have measured variation in mate preference against variation in morhological phenotypes, whilst others have considered the effect of predation risk, parasitism, copying, age and experience.

There are only two experimental studies demonstrating that the morphological phenotype of a female affects mate preference. Jennions *et al.* (1995) found that large female African painted tree frogs were more likely to choose lower frequency sounds than smaller counterparts when given a choice between two artificial male calls which were made deliberately difficult to distinguish. Large females had greater powers of discrimination, probably because increased size improved sensitivity to variation in the frequency of sounds. More recently Hingle (chapter two) investigated eyespan-dependent mate choice in the stalk-eyed fly *Cyrtodiopsis dalmanni.* In this species females show mate preference for males with exaggerated eyespan (Wilkinson *et al.* 1998). Female eyespan was manipulated by rearing larvae at different larval densities thereby

creating a range of female eyespans (David *et al.* 1998). Given a binary choice between two males, large and small eyespan females did not differ in their preferences when the difference between males was large (both preferred the large eyespan male) or when the difference between males was small (both mated at random). However, at intermediate differences, large eyespan females had significantly stronger preferences for the large eyespan male.

Some morphological phenotypes may be closely related to condition (Andersson 1994; Jennions and Petrie 1997). Condition has been traditionally thought of as the nutritional state of an individual (Andersson 1982; Nur and Hasson 1984; chapter one). More recently condition has been redefined more loosely as the health and vigour of an individual which may be affected by factors other than nutritional state such as toxins, parasites or bodily injuries (Rowe and Houle 1996). As such, condition is closely linked to many fitness components such as fecundity and body size (chapter one). This has led some authors to suggest that the variation in female mate preference due to differences in body size may be indicative of condition-dependent mate choice (Jennions and Petrie 1997; Bakker 1999).

Other studies have used more direct methods to assess condition-dependent mate choice. Bakker *et al.* (1999) found that full-sib sisters of male sticklebacks with red bellies (which is strongly linked to condition), showed strong mate preference for red bellied males. In contrast, females from families

with lower condition preferred orange bellied males. López (1999) found that unparasitised female guppies showed stronger preferences for the attractive male in a binary choice apparatus. The lack of discrimination by infected females arose because they swam less frequently between males than control females and signalled acceptance of a mate more frequently. Although not measured directly it is likely that parasite infection reduced female condition.

Here we investigated how a component of condition affects mate preference in the stalk-eyed fly *C. dalmanni*. A nutritional stress was used to manipulate female ovarian activity. Sucrose was used in place of pureed sweetcorn, the normal food medium, to cause a reduction in egg production. We investigated a sucrose stress because it has previously been used to experimentally reduce egg laying in other insect species, such as *Drosophila melanogaster* (Ashburner 1989). However, the affect of sucrose is reversible, as females quickly recover their egg laying ability when they are moved back to normal food (Ashburner 1989). Using a transient stress allowed us to test the same females when in high and low condition.

We used a stress that affects egg laying because this is likely to be relevant to conditions experienced in the wild. In *Drosophila melanogaster* females show a wide range of variation in egg production under natural conditions, as many as 70% of females in the wild have no or few developing or mature eggs (Boulétreau 1978). This is probably due to nutritional stress and is usually abolished in

females reared in the laboratory with *ad libitum* food (Boulétreau 1978). Similar variation in ovarian activity has been found amongst wild caught *C. dalmanni* (Wilkinson and Reillo 1994).

We undertook three experiments. In the primary experiment, we investigated the effect of transient nutritional stress on female preference when females were allowed to choose between small and large eyespan males. We made the difference between small and large male eyespan great enough to expect female preference for the large eyespan male, given the results of previous mate choice experiments (chapter two). The second experiment assessed the effect of the transient nutritional stress on mature and developing eggs in *C. dalmanni.* This experiment allowed us to confirm that a sucrose diet reduced female fertility and that this effect was reversible. In the third experiment we examined how long it takes for prolonged exposure to a sucrose diet to affect female survivorship.

METHODS

Flies and rearing

Flies used in this study were derived from a laboratory population descended from a field collection made in 1993 by Andrew Pomiankowski in Ulu Gombak, Malaysia. The population has been maintained at a large population size

in cage culture at 25°C. Flies were kept on a 12hr light : 12hr dark light cycle. Half hour dawn and dusk periods were simulated by reflecting a 60W light beam off the ceiling of the rearing room so as to produce a reduced lighting level. All flies and larvae were fed pureed sweetcorn.

Experimental flies were obtained by collecting eggs from cage culture over a two week period. Damp paper towels were placed on the floor of stock cages, and left for 24hrs before being removed. Eggs laid on the paper were removed and transferred to plastic containers which were lined with cotton wool and filter paper. The filter paper in each container was moistened with water and covered with a thin layer of pureed sweetcorn. After 15 days pupae were removed from the filter paper and placed in 20 × 20 × 40 cm fly cages to eclose. Adult flies were fed on pureed sweetcorn which was changed twice a week, until sexual maturity at six weeks. At two weeks post eclosion, flies were separated into virgin male and female groups to prevent mating.

Experiment 1 - Female preference

Sexually mature females (6-8 weeks old) were collected over ice and their eyespan were measured using a digital camera attached to a monocular microscope and computer. Images were recorded and analysed using NIH image software. Any females with eyespans less than 6 mm were discarded. Small

females were discarded because we have evidence that they have weaker preferences than large females (chapter two). The remainder were assigned to one of 24 cages (14 × 14 × 30 cm), until each cage contained six females. Cages were lined with damp cotton wool covered with filter paper.

Cages of females were divided equally between two treatments. One treatment was fed a sucrose solution twice weekly. Sucrose was administered as a solution made of 65 g of food grade sucrose dissolved in 260 ml of boiled water. The solution was allowed to cool and then placed in small weighing boats with a layer of filter paper to prevent flies sticking to the solution. The second treatment was fed sweetcorn twice weekly. Sweetcorn was prepared from pureed kernals and then placed in similar small weighing boats.

Flies were kept for 30 days on their initial food regime. Feeding regimes were then reversed: sucrose females were given corn, and corn females were given sucrose. Flies were kept for a further 30 days on the new food regime. Each 30 day period was split into two periods. For the first 14 days, flies were allowed to acclimatise to the food regime. For the following 16 days, female preference was measured.

A total of twenty cages were observed, ten sucrose-corn cages and ten corn-sucrose cages. When females from experimental cages died they were replaced using females from the replacement cages that followed exactly the same food regime but were not observed. 21 replacement females were required for

sucrose-corn cages (13 when fed corn media and 8 when fed sucrose media). 14 replacement females were required for corn-sucrose cages (9 when fed corn media and 5 when fed sucrose media).

Sexually mature males (6-8 weeks old) were collected at the same time as females for use in mate preference trials. Males were measured using the same procedure as above. They were divided into two groups of fifty. One group had large eyespan (all > 9.0 mm) and the other small eyespan (all < 7.5 mm). The mean and standard deviation of large male eyespan was 9.13 ± 0.03 mm ($\overline{X}$ ± SD), and of small eyespan males was 6.67 ± 0.08 mm. From previous mate choice experiments (chapter two), we predicted that the females would show mate preference in favour of large eyespan males given this mean difference between males of 2.46 mm.

Large and small males were further subdivided into holding (N = 30) and replacement cages (N = 20). The replacement cages were used as a source to replace dead males from the experimental group, so as to maintain experimental males at a constant density.

Female mate preference was measured on a daily basis. At dusk (last half hour before lights off) on the first day, large eyespan males were taken from their holding cage and put into 5 cages from the sucrose-corn treatment and 5 cages from the corn-sucrose treatment. Similarly, small eyespan males were taken from

their holding cage and put into the remaining 5 sucrose-corn cages and 5 corn-sucrose cages.

Mating behaviour was observed from dawn on the following day, for one and a half hours. During this period the number of copulations by the male in each cage was recorded. We defined a copulation as a male mounting a female and engaging genitalia. All observations were made by AH. After this period, males were removed and put back into the large and small holding cages. At dusk on the second day, female cages that had been tested with large eyespan males were tested with small eyespan males and those that had been tested with small eyespan males were tested with large eyespan males. This procedure was repeated for a total of 16 observational days in each stage of the experiment.

Our technique exposed females to males at dusk and through the night, and tested for mate preference at dawn. This simulates conditions in the wild (de la Motte and Burkhardt 1983; Wilkinson and Reillo 1994). Field observations show that females aggregate in harems controlled by males at dusk and mate with the harem male at dawn (de la Motte and Burkhardt 1983). Very little mating activity occurs at other times of the day (Wilkinson and Reillo 1994).

Males could not be left in cages all day as this may have led to variation in male condition when exposed to the different food media. We could not add both large and small eyespan males to the same cage at dusk as this would have lead to male-male competition and so obscured the effects of female mate choice. Given

this constraint, we decided that the best solution was to place large and small eyespan males in experimental cages on alternate days.

Female mate preference was calculated at the level of the cage using the total number of copulations with large and small males, separately for corn and sucrose food media. Copulation preference was measured as $CP = C_L - C_S$, where C_L is the number of copulations by the large male and C_S the number of copulations by the small male, over the full 16 days of observation. Using this measure of preference, values of zero indicate random mating, positive values a preference for large eyespan males and negative values a preference for small eyespan males.

A number of further checks on the data were carried out, so as to search for and exclude possible confounding factors. Our measure of preference, CP, could be subject to a bias in favour of cages that had more copulations. For example, if two female cages had the same ratio of small eyespan male to large eyespan male copulations, the cage with the greater total copulations would be assigned a stronger preference. So we tested for any dependence of absolute copulation preference on the total number of copulations. In addition, we looked for changes in the number of copulations through time, and differences in the total number of copulations of flies on sucrose and corn diets. Finally, as a precaution, we reanalysed all CP tests using a relative preference measure $RCP = (C_L - C_S)/(C_L + C_S)$.

To interpret the results of the main experiment, two further experiments were undertaken to measure the effect of changes in food regime on components of female fitness.

Experiment 2 - Egg production

We measured female reproductive potential by counting the number of eggs in female ovaries when fed corn or sucrose. Six cages were set up, each containing 30 sexually mature females and 5 sexually mature males. Flies were fed corn during three days of acclimatisation to cage conditions. Two of the cages were assigned to each of three treatments: sucrose-corn, corn-sucrose and a control. Sucrose-corn flies were fed sucrose from day 1 to day 8 and then switched to corn until day 22. Corn-sucrose flies were fed corn from day 1 to day 8 and then switched to sucrose until the end of the experiment (day 22). Control treatment flies were fed corn throughout the experiment.

Starting on day 8 and then on every second day (i.e., day 10, 12 etc. until day 22), three females were randomly sampled from each cage and placed on ice. The ovaries were dissected from these flies and placed on a microscope slide. Digitised images of the ovaries were recorded using a digital camera attached to a microscope and computer with NIH image software. Images were printed off and the number of mature and developing eggs were counted. Mature eggs were

defined as stages 12-14, and developing eggs were defined as stages 8-11 using King's standard stages of oogenesis (King 1970; Ashburner 1989). The number of developing eggs was included in our measure because in other species of Diptera, females can absorb early egg stages in order to recover the protein contained within them (Wilson 1985). It was thought that counts of developing eggs may be more sensitive to a sucrose diet compared to estimates based upon numbers of mature eggs (Wilson 1985). A secondary advantage of direct counts of eggs was that it allowed a more accurate estimate of fertility than estimates based on actual eggs laid. Pilot experiments demonstrated that females laid eggs at many different sites within cages. Most sites were not readily accessible so that counts were inaccurate and time-consuming. All ovaries were dissected and scored by AH.

Experiment 3 - Survivorship

We quantified the effect on survival of prolonged feeding on sucrose. Two cages were set up, each containing 35 sexually mature females and 10 sexually mature males. One cage of flies was fed sucrose and the other corn until all the flies in one of the cages were dead. Every two days the number of dead females in each cage was recorded. Twice weekly the food in each cage was replaced with fresh media.

Statistical Procedures

CP distributions of sucrose-corn and corn-sucrose females on each food media were tested for normality using the Shapiro-Wilk *W* test (Zar 1984) and were found to be normally distributed. Distributions of *CP* for all females together (regardless of food media or treatment), both treatments (regardless of food media) and for each food treatment within each treatment were tested for deviations from random mating using two-tailed *t*-tests. The sample mean was compared to a null hypothesis of random mating, with a mean of zero. The mean *CP* of each the sucrose-corn treatment was compared with that of the corn-sucrose treatment using a two tailed *t*-test to see if the order of exposure to food media effected female *CP*. In addition the mean *CP* of corn fed females in the sucrose corn treatment was compared with the mean *CP* of the corn fed females from the corn-sucrose treatment using a two tailed *t*-test. This test allowed us to determine if there was any effect of age on *CP* on corn and was repeated for sucrose fed females. The effect of food on female *CP* was analysed using a paired two-tailed *t* test with female *CP* on corn and sucrose paired at the level of the cage for all females (both treatments combined) and for each treatment separately. We used a Bonferonni correction (Sokal and Rohlf 1995) where it was necessary to adjust for multiple comparisons. The Bonferroni method produces a corrected α-value for repeated testing of a hypothesis by dividing α by the number of repeated tests.

To further clarify the preference dataset, a model I two-way ANOVA of *CP* with experimental treatment and diet as fixed factors was used. This allowed for a direct test for an effect of the order of the nutritional stress on *CP* on each food type.

The dependency of *CP* on copulation number was analysed using least squares regression for sucrose fed flies separately for each treatment and for corn fed flies separately for each treatment. All *CP* analysis was repeated using the relative preference measure, *RCP*.

All total copulations distributions of sucrose-corn and corn-sucrose females on each food were tested for normality using the Shapiro-Wilk *W* test. Differences in copulation frequency between food media and experimental stages were analysed in a model I two-way ANOVA with experimental stage and food as fixed factors.

Ovarian activity was compared between sucrose-corn and corn-sucrose treatments and the control treatment in the egg production experiment. The number of mature and developing eggs in the ovaries of females were compared between treatments using two tailed *t*-tests for all days that dissections were taken (day 8, 10 etc. to day 22).

Differences between sucrose and corn fed female survivorship were tested using the Log-Rank test. This test also allows the partitioning of mortality data to test for differences during different time periods.

JMP version 3.1.6 for the Macintosh (SAS Institute 1996) was used for all statistical analysis.

RESULTS

Experiment 1 - Female preference

Pooling over both treatments (sucrose-corn and corn-sucrose), females preferred to copulate with large eyespan males (mean ± *SD* *CP* = 26.65 ± 13.24, *t* test: $t_{19} = 9.002$, $P < 0.001$). Previous studies have shown that females prefer to mate with larger eyespan males when given a binary choice between a small and large eyespan male (Wilkinson *et al.* 1998; chapter two). Our method differs from these previous studies as small and large eyespan males were presented on alternate days. But nonetheless our way of presenting males revealed similar preference for large eyespan males.

Similar mate preference was seen when analysing each treatment separately. Sucrose-corn females (*CP* pooled across food media) preferred large eyespan males (*CP* = 26.50 ± 14.96, $t_9 = 5.601$, $P < 0.001$), as did corn-sucrose females (*CP* = 26.80 ± 12.09, $t_9 = 7.010$, $P < 0.001$), and there was no difference in preference due to the order in which females were fed sucrose and corn diets (t_{18} =0.346, P =0.564).

Female preference on the different food sources was analysed by splitting the two treatments into their two food classes, and considering preference on corn and sucrose separately. Corn fed females, pooled from both treatments, showed a strong preference for large eyespan males ($CP = 22.05 \pm 9.34$, $t_{19} = 10.553$, $P < 0.001$). This preference on corn was present in both the sucrose-corn females ($CP = 20.80 \pm 7.05$, $t_9 = 9.327$, $P < 0.001$) and corn-sucrose females ($CP = 23.30 \pm 11.45$, $t_9 = 6.435$, $P < 0.001$; Fig. 3.1) when analysed separately. The sucrose-corn females were 30 days older than corn-sucrose flies when their mate preference was tested. However, this increase in age and experience did not make a detectable difference in the strength of female preference ($t_{19} = 0.346$, $P = 0.564$).

Sucrose fed females, pooled from both treatments, showed a weak preference for large eyespan males ($CP = 4.60 \pm 7.37$, $t_{19} = 2.790$, $P = 0.012$), that was significant in sucrose-corn females ($CP = 6.00 \pm 7.97$, t test: $t_9 = 2.38$, $P = 0.041$), but not different from random mating in corn-sucrose females ($CP = 3.20 \pm 6.84$, $t_9 = 1.479$, $P = 0.173$; Fig. 3.1). None of these relationships were significant after Bonferroni adjustment, indicating that the female preference on a sucrose diet for large eyespan males was of marginal significance. Preference on sucrose did not differ significantly between the two treatments ($t_{19} = 0.710$, $P = 0.411$), which again indicates that the strength of preference does not change with female age or experience.

The same cages of females were fed sequentially corn then sucrose (or vice versa), allowing us to directly compare preference of the same females on different food. Females had stronger preferences for large eyespan males when fed corn rather than sucrose (paired *t* test: $t_{19} = 7.508$, $P < 0.001$). Sucrose-corn and corn-sucrose treatment females showed similar effects when analysed separately (sucrose-corn: $t_9 = 5.530$, $P < 0.001$; corn-sucrose: $t_9 = 5.220$, $P < 0.001$; Fig. 3.1) which again indicates that the reduction in strength of preference from corn to sucrose was not affected by changes in female age and experience.

A further analysis was performed which allowed for the direct testing of an interaction between the experimental treatment and the food type on female preference. Using this ANOVA technique females had stronger preference for large males on corn than on sucrose ($F_{1,36} = 41.819$, $P < 0.001$) and there was no effect of the order of stress (experimental treatment) on female preference ($F_{1,36} = 0.003$, $P = 0.956$). The interaction term was also non-significant ($F_{1,36} = 0.964$, $P = 0.333$), indicating that the preference on each food type was not affected by the order they were given to females. These results concur with all our previous analyses, emphasising the lack of any age, experience or order of nutritional stress effects on female preference during the experiment.

A number of further tests were carried out to test for confounding factors relating to changes in the total number of copulations in different groups. First, we tested for dependence of the strength of the copulation preference ($CP = C_L - C_S$)

on the total number of copulations ($C_L + C_S$). However, the copulations preference *CP* did not increase with the total number of copulations for corn fed or sucrose fed flies in either treatment (least squares regression, all $P > 0.05$).

Second, we checked for changes in the number of copulations through time using a model I two-way ANOVA with experimental stage (the first or second 16 days of preference observations) and food (corn or sucrose) as fixed factors (Table 3.1). There was no effect of experimental stage, indicating that females did not change their copulation rate as they got older. There was also no effect of food, indicating that the copulation rate was the same for corn and sucrose fed flies. The interaction term was also non-significant, showing that the age of females did not effect their copulation rate differently on corn and sucrose.

Finally, all *CP* analyses presented above were repeated using *RCP*, a relative copulation preference measure (dividing preference by the total number of copulations for each cage). The results were exactly the same as with *CP*. This second measure of preference controls for variation in the total number of copulations between cages, confirming our primary finding that females had stronger preferences for large eyespan males when fed corn rather than sucrose.

Experiment 2 - Egg production

Pooled dissection counts from six females through time are shown in Figure 3.2. For the first 8 days sucrose-corn flies were fed sucrose, corn-sucrose flies were fed corn and control flies were fed corn. The mean number of mature eggs in the ovaries sucrose-corn females on day 8 was less than the control group ($t_{10} = 3.796$, $P = 0.004$), but in corn-sucrose females did not differ from the control group ($t_{10} = 1.210$, $P = 0.254$, Fig. 3.2a). A similar pattern was seen for the number of developing eggs (Fig. 3.2b); sucrose-corn females had significantly fewer developing eggs than control females on day 8 ($t_{10} = 5.053$, $P < 0.001$), but the corn-sucrose females was not different from control females on day 8 ($t_{10} = 0.065$, $P = 0.950$).

Food was changed on day 8 in the sucrose-corn and corn-sucrose treatments. An increase in ovarian activity in response to the new corn diet was seen in the sucrose-corn females relative to control females on day 18 for mature eggs ($t_{10} = 0.072$, $P = 0.944$, Fig. 3.2a) and on day 16 for developing eggs ($t_{10} = 1.736$, $P = 0.113$, Fig. 3.2b). The new sucrose diet of corn-sucrose females caused a reduction in the number of mature and developing eggs relative to control females, which became significant on day 22 for mature eggs ($t_{10} = 2.373$, $P = 0.039$) and on day 18 for developing eggs ($t_{10} = 3.282$, $P = 0.008$).

Experiment 3 - Survivorship

Measurement of survivorship was terminated after all sucrose fed flies died, 74 days after the start of the experiment (Fig. 3.3). Females fed on corn survived significantly longer than those fed on sucrose (mean time of death, $\overline{X} \pm SD$: sucrose = 47.58 ± 2.91 days, corn = 56.91 ± 5.17 days; Log-rank test: $\chi^2 = 14.988$, $df = 1$, $P = 0.0001$). Even though sucrose females were likely to die earlier than corn females, there was no significant difference in survivorship up to and including day 54 (Log-rank test: $\chi^2 = 2.671$, $df = 1$, $P = 0.119$). On day 56 and every day thereafter sucrose fed females were likely to die earlier than corn fed females (day 56, Log-rank test: $\chi^2 = 3.851$, $df = 1$, $P = 0.049$). This suggests that there is no survival cost of sucrose in the short term. However, prolonged feeding on a sucrose diet causes premature death.

DISCUSSION

Our experimental design allowed us to investigate the effect of a transient food stress on female mate preference in the stalk-eyed fly *C. dalmanni*. Preference was measured as the difference in the number of copulations with large and small eyespan males. Sucrose fed females showed a weak preference for

large eyespan males. The same females had a significantly stronger preference for large eyespan males when fed on corn.

This result provides strong evidence that diet can alter the strength of female preference. The experiment was designed to test for a number of other factors, but none of these could account for the data observed. Females were either switched from sucrose to corn, or from corn to sucrose. The order of the food stress had no effect on female preference. The change in preference was the same, though in the opposite direction, for flies moved from sucrose to corn as for those moved from corn to sucrose. This further showed that food stress did not cause any permanent change in female preference. There was also no detectable change in female preference that could be explained by age or experience. Nor was there any evidence of changes in copulation rate due to the different diets.

To further elucidate the observed change in female preference, female egg production on sucrose and corn media was examined. Variation in the number of developing and mature eggs was large. However, it was clear that females on sucrose tended to have reduced numbers of mature and developing eggs. This reduction was apparent after 8-14 days on the sucrose diet (Fig. 3.3). The effect of sucrose on reducing egg production was reversible. Once put back on a corn diet, egg counts returned to the level seen in control flies.

Two types of interpretation are consistent with the results obtained in our experiments. The first kind of explanation is that females reduce the effort

invested in mate preference when they have no eggs to fertilise. This makes adaptive sense as females are unlikely to benefit from choosing between males when they are not laying fertile eggs. Such an explanation makes a number of assumptions, in particular that females gain fitness benefits from mating with large eyespan males and avoid fitness costs when they fail to exert mate preference. There is evidence that male eyespan is a sensitive indicator of male genetic quality in stalk-eyed flies (David *et al.* 1998, 2000) but there are no direct or indirect measures of costs associated with female mate choice.

A second kind of explanation is that sucrose caused an overall change in female behaviour, of which female preference is just one aspect. This again could make adaptive sense if optimal female behaviour varies with the amount of protein in their diet. Against this hypothesis, females did not change their copulation rate as a result of sucrose exposure. Likewise, females fed on sucrose did not appear to be any less active or show any obvious abnormal behaviour. We also showed that there was no effect of the sucrose diet on mortality in the short term (for the first 54 days, Fig. 3.3). So we have no evidence for an overall change in female behaviour. Further systematic measures of female behaviour when fed sucrose, such as changes in foraging rate or the frequency of aggressive interactions, might be useful. It would also be illuminating to study other interventions that alter egg production to see whether there is a direct association of this with changes in female preference. As yet we know too little about the

fitness consequences of female preference in stalk-eyed flies to distinguish these explanations.

One other study has shown that the female mate preference is diet-dependent (Lesna and Sabelis 1999). In this study females changed their preference for males depending upon the male genotype which would give their offspring the most favourable genotype for the current substrate. Females specifically choose males with good genes - conferring a fitness advantage (population growth rate) on their offspring. However, the mating system of the predatory mite used in this study differs significantly from ours and changes in preference did not reflect changes in female condition.

In the wild there is substantial variation in egg number among female stalk-eyed flies (Wilkinson and Reillo 1994). Mature egg numbers ranged from zero to 35 per female. The reduction of ovarian activity in females fed sucrose therefore mimics the range of ovarian activity observed in the wild. This suggests that the changes in female preference observed in this study are relevant to female behaviour in the wild.

The effect of transient nutritional stress in the wild will be to weaken individual female mating preference. However, the net effect may be neutral or even increase the overall selective pressure on male eyespan. Stressed females mated randomly but produced few offspring, whereas unstressed females had

strong preferences and produced many eggs. So productive females tend to be those that mate with preferred males.

Our previous study of phenotype-dependent female preference in stalk-eyed flies showed that females with wider eyespan have stronger preferences when the difference between potential mates was relatively slight (chapter two). The present study shows that transient stresses that affect fitness components (and hence condition) over short periods of time can also drastically alter female preference. If condition-dependent mate preference is a general phenomenon, and high condition females also tend to be the most fecund, the strength of sexual selection may be greater than has been previously hypothesised. The link between mate choice and female fecundity is in need of further investigation.

REFERENCES

Andersson, M. 1982. Sexual selection, natural selection and quality advertisement. Biological Journal of the Linnean Society, 17: 375-393.

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

Ashburner, M. 1989. Drosophila. A laboratory handbook. Cold Spring Harbor University Press, New York.

Bakker, T.C.M. 1999. The study of intersexual selection using quantitative genetics. Behaviour, 136: 1237-1265.

Bakker, T.C.M., Künzler, R., and Mazzi, D. 1999. Condition related mate choice in sticklebacks. Nature, 401: 234.

Bakker, T.C.M. and Pomiankowski, A. 1995. The genetics of female mate preferences. Journal of Evolutionary Biology, 8: 129-171.

Boulétreau, J. 1978. Ovarian activity and reproductive potential in a natural population of *Drosophila melanogaster*. Oecologia, 35: 319-342.

Burkhardt, D. and de la Motte, I. 1988. Big 'antlers' are favoured: female choice in stalk-eyed flies (Diptera, Insecta), field collected harems and laboratory experiments. Journal of Comparative Physiology A, 162: 649-652.

Darwin, C.R. 1859. On the origin of species. John Murray. London.

David, P., Hingle, A., Greig, D., Rutherford, A., Pomiankowski, A. and Fowler, K. 1998. Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. Proceedings of the Royal Society of London, Series B, 265: 2211-2216.

David, P., Bjorksten, T., Fowler, K. and Pomiankowski, A. 2000. Condition-dependent signalling of genetic variation in stalk-eyed flies. Nature, 406: 186-188.

Iwasa, Y. and Pomiankowski, A. 1994. The evolution of mate preferences for multiple sexual ornaments. Evolution, 48: 853-867.

Jennions, M.D. and Petrie, M. 1997. Variation in mate choice and mating preferences: a review of the causes and consequences. Biological Reviews, 72: 283-327.

Jennions, M.D., Blackwell, P.R.Y. and Passmore, N.I. 1995. Repeatability of mate choice: the effect of size in the African painted reed frog, *Hyperolius marmoratus*. Animal Behaviour, 49: 181-186.

King, R.C. 1970. Ovarian development in *Drosophila melanogaster*. Academic Press, New York.

Kotbra, M. 1996. Sperm transfer by spermatophore in Diptera: new results from the Diopsidae. Zoological Journal of the Linnean Society, 117: 305-323.

Lesna, I. and Sabelis, M.W. 1999. Diet-dependent female choice for males with 'good genes' in a soil predatory mite. Nature, 401: 581-584.

López, S. 1999. Parasitized female guppies do not prefer showy males. Animal Behaviour, 57: 1129-1134.

Lorch, P.D., Wilkinson G.S. and Reillo, P.R. 1993. Copulation duration and sperm precedence in the stalk-eyed fly *Cyrtodiopsis whitei* (Diptera: Diopsidae). Behavioral Ecology and Sociobiology, 32: 303-311.

Milinski, M. and Bakker, T.C.M. 1992. Costs influence sequential mate choice in sticklebacks, *Gasterosteus aculeatus*. Proceedings of the Royal Society of London, Series B, 250: 229-233.

de la Motte, I. and Burkhardt, D. 1983. Portrait of an Asian Stalk-eyed fly. Naturwissenshaften, 70: 451-461.

Nijhout, H.F. and Emlen, D.J. 1998. Competition among body parts in the development and evolution of insect morphology. Proceedings of the National Academy of Sciences, USA, 95: 3685-3689.

Nur, N. and Hasson, O. 1984. Phenotypic plasticity and the handicap principle. Journal of Theoretical Biology, 110: 275-297.

Partridge, L., Green, A. and Fowler, K. 1987. Effects of egg-production and exposure to males on female survival in *Drosophila melanogaster*. Journal of Insect Physiology, 33: 745-749.

Rowe, L. and Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proceedings of the Royal Society of London, Series B, 263: 1415-1421.

SAS Institute. 1996. JMP statistics for the Macintosh version 3.1.6.

Sokal, R.R. and Rohlf, F.J. 1995. Biometry. W.H. Freeman and Company. New York.

Wilkinson, G.S. and Reillo, P.R. 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. Proceedings of the Royal Society of London, Series B, 255: 1-6.

Wilkinson, G.S. and Taper, M. 1999. Evolution of genetic variation for condition-dependent traits in stalk-eyed flies. Proceedings of the Royal Society of London, Series B, 266: 1685-1690.

Wilkinson, G.S., Kahler, H. and Baker, R.H. 1998. Evolution of female mating preferences in stalk-eyed flies. Behavioral Ecology, 9: 525-533.

Wilson. T.G. 1985 Determinants of oocyte degeneration in Drosophila melanogaster. Journal of Insect Physiology, 31: 109-117.

Zar, J.H. 1984. Biostatistical Analysis, 2nd edn. Prentice-Hall International, New Jersey.

FIGURE LEGENDS

Figure 3.1. Mean preference of each treatment in each stage of the experiment (i.e. when fed sucrose or corn), error bars indicate standard errors. Significant difference from random mating, after Bonferonni adjustment, are indicated by NS, $P>0.05$, *** $P<0.001$. Sucrose-corn and corn-sucrose flies did not differ in preference when fed the same diet. Both treatments had significantly weaker preferences on sucrose than on corn.

Figure 3.2. Mean number of (a) mature and (b) developing eggs in the ovaries of six females from one of three treatments: sucrose-corn, corn-sucrose and control (always corn). Error bars indicate the standard error of the mean. Food type was changed on day eight.

Figure 3.3. Survival curves of two cages of 35 females. One cage was fed sucrose the other corn. Mortality was measured every two days. Sucrose and corn fed flies did not differ in survivorship up to and including day 54. Thereafter sucrose fed females suffered greater mortality.

Figure 3.1

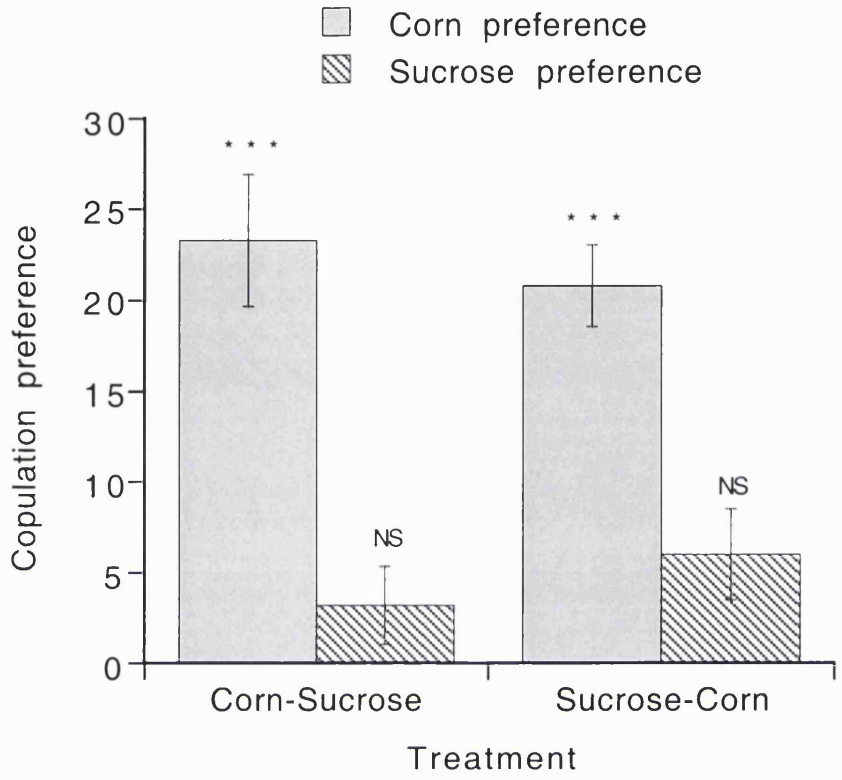
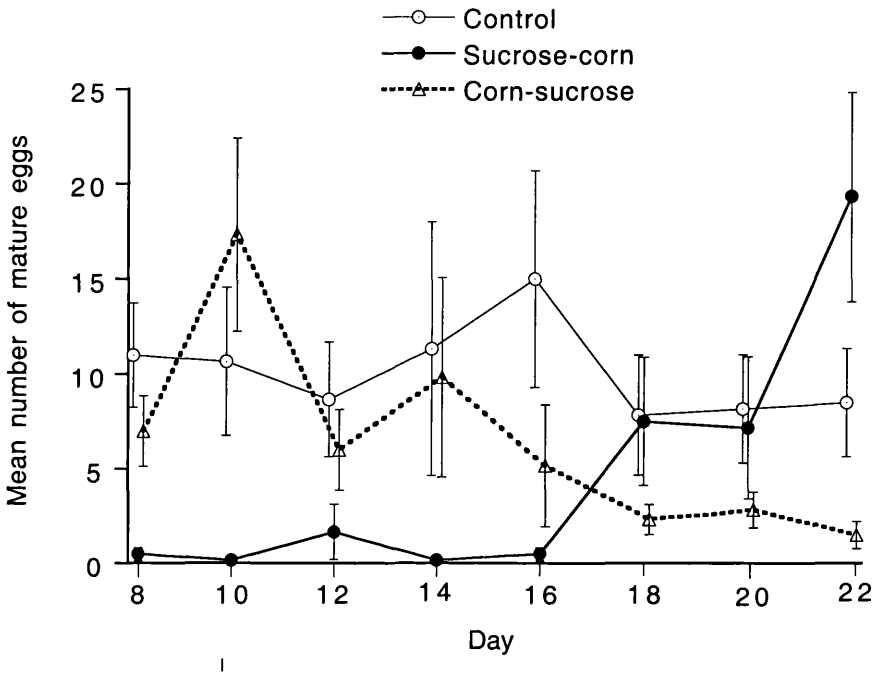

Corn preference
Sucrose preference
Copulation preference
30
25
20
15
10
5
0
* * *
* * *
NS
NS
Corn-Sucrose
Sucrose-Corn
Treatment

Figure 3.2

(a)


Control
Sucrose-corn
Corn-sucrose
Mean number of mature eggs
25
20
15
10
5
0
8
10
12
14
16
18
20
22
Day

(b)

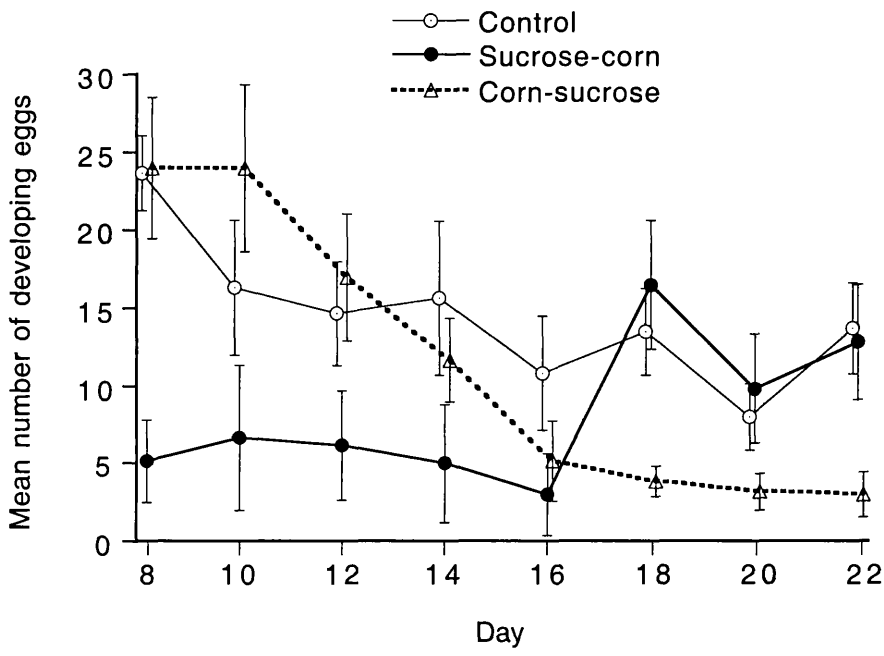

Control
Sucrose-corn
Corn-sucrose
Mean number of developing eggs
30
25
20
15
10
5
0
8
10
12
14
16
18
20
22
Day

Figure 3.3

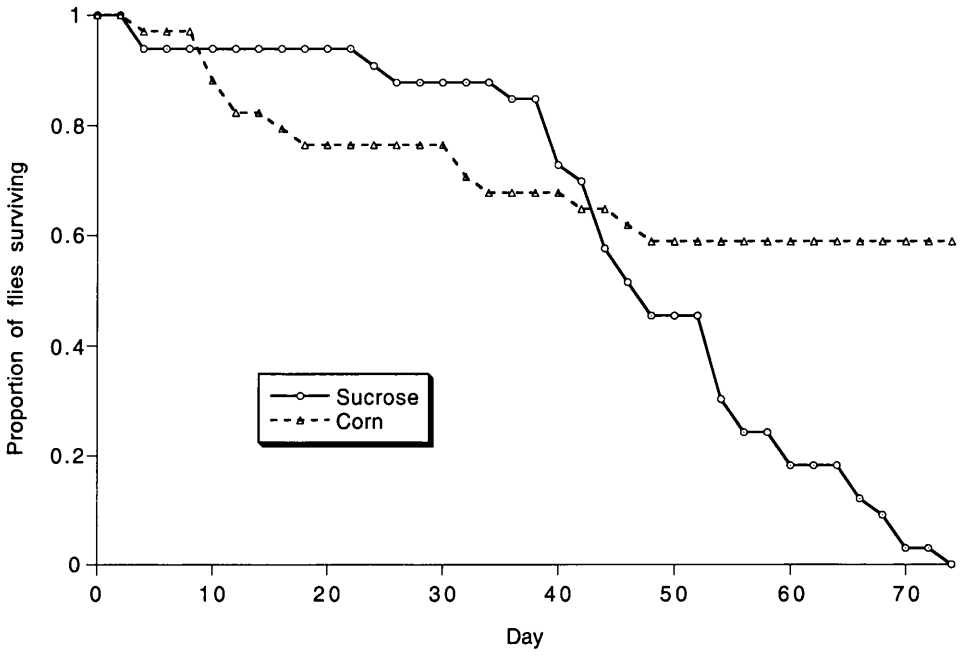

Proportion of flies surviving
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
70
Day
Sucrose
Corn

Table 3.1. Variation in female mating frequency with experimental stage and food as fixed factors. Stage refers to the feeding stage of the experiment (e.g. corn-sucrose flies were fed corn in stage one and sucrose in stage two).

Source of variation	*df*	*SS*	*P*-Value
Stage	1	164.0	0.134
Food	1	235.2	0.074
Stage × Food	1	156.0	0.143
Error	36	2506.1	

Chapter Four

Do stalk-eyed fly females compete for mates?

ABSTRACT

The strength of female mate choice in the stalk-eyed fly *Cyrtodiopsis dalmanni* depends on female eyespan, with large eyespan females having stronger preferences than small eyespan females (chapter two). We further investigated mate choice by comparing the mating behaviour of large and small eyespan females when they were kept in the same choice environment and were able to compete for copulations. Females were held in mixed groups and sequentially presented with males that differed in eyespan. Under these conditions, we expected to see eyespan-dependent mate choice caused by competition for access to males. Both large and small eyespan females preferred to mate with large eyespan males. But there was no evidence that female eyespan affected the strength of preference. Nor did female eyespan affect mating activity with large or small eyespan males. These results do not provide evidence that female stalk-eyed flies compete for access to mates.

INTRODUCTION

The study of the evolution of female mate preferences for males with exaggerated ornaments and displays has shifted in emphasis in recent years. Traditional studies of female preference investigated the fitness benefits and costs of the male signal (Andersson 1994). Recent attention has moved on to consideration of variation in female preference (Rosenqvist and Bergland, 1992; and Ahnesjö *et al.* 1993). Both environmental and phenotypic variation could affect female mate choice, and in a few cases this has been demonstrated using experimental investigations (Poulin 1994; Jennions *et al.* 1995; Bakker *et al.* 1999; López 1999). In their review of variation in female preference, Jennions and Petrie (1997) suggested that many phenotypic traits affecting female choice may also be representative of condition. However, the evidence to date for condition-dependent mate choice is limited (Bakker 1999; chapter one).

We have carried out several studies looking for evidence of phenotype and condition-dependent mate choice in the stalk-eyed fly *Cyrtodiopsis dalmanni*. In chapter two the preferences of large and small eyespan females were compared in a binary choice apparatus containing two males that differed in eyespan. When male eyespan differed to a large degree, both large and small eyespan females preferred large eyespan males and did not differ in their strength of preference. When males differed only slightly, both types of females mated randomly and

preferences did not differ in strength. When presented with intermediate differences between males, large and small eyespan females both preferred large eyespan males, but large eyespan females had stronger preferences. Female mate choice is visually based in *C. dalmanni* (Burkhardt and de la Motte 1988). As a number of visual characters improve with increasing eyespan (Burkhardt and de la Motte 1983), it is possible that the difference in mate choice observed was due to poor vision in small eyespan females.

Further to this, chapter three reports an investigation of the effect of a transient nutritional stress (sucrose media) on female mate choice. When fed pureed sweetcorn (normal medium) females had strong preferences for large eyespan males. But the same females had mated randomly when fed sucrose. The sucrose stress reduced female condition. Sucrose fed females produced significantly fewer mature and developing egg than corn fed flies. This suggests that females do not exert mate preference when there is no or little reproductive benefit in doing so.

Here we extend these investigations by considering the effect of competition on female mate choice. In the wild, large and small eyespan females mix and can often be seen engaging in competitive interactions (de la Motte and Burkhardt 1983). Females are known to compete for copulations in other species (Bush and Bell 1997; Dale *et al.* 1992), so we expected to see evidence of competition in *C. dalmanni*. Competition for mates is also predicted because the

most matings occur in a short period of time each morning (Wilkinson and Reillo 1994). We also expected competition for mates to escalate with the value of the male involved, as several lines of evidence suggest that females gain genetic benefits from their mate choice (Wilkinson 1993; David *et al.* 1998, 2000; Wilkinson *et al.* 1998).

We examined mate choice using a strongly female biased sex ratio (10 females: 1 male) to encourage competition amongst females for mates. Such strongly biased sex ratios are common in the wild where sex ratios at copulation sites can be in excess of this (Burkhardt and de la Motte 1988). A sequential choice procedure was used for observations of female mating behaviour, where females were presented with large and small eyespan males on alternate days over a ten day period. This resembles conditions in the wild where females choose a male to roost with at dusk prior to mating on the following morning.

Observations were made for one and a half hours each morning and a number of measurements of female mating behaviour were recorded. These included the number of copulations by each female in a cage, the size of the male and female copulating, the duration of the copulation and the time at which the copulation started. From this data we calculate the total number of copulations, copulation preference, mean copulation duration and copulation duration preference of large and small eyespan females from each cage. Changes in the

mating behaviour as the mating period progressed were also analysed to look for evidence of competition for early copulations.

METHODS

Flies and media

Flies used in this study were from a laboratory population reared from wild-caught individuals collected from Ulu Gombak, Malaysia, in 1993 by Andrew Pomiankowski. Since founding of the laboratory cultures, flies have been fed pureed sweetcorn containing the anti-fungal agent, Nipagin. Flies were kept at 25°C on a 12 hr light : 12 hr dark cycle with half hour dawn and dusk periods of reduced lighting. Flies were cultured in Perspex stock cages lined with moist cotton wool and filter paper to prevent desiccation.

Production of experimental flies

Experimental flies were obtained by collecting eggs from stock cages over a two-week period. Moist paper towels were placed on the floor of the stock cages and left for 48 hours. Eggs laid on the paper towels were harvested and placed in plastic containers. The number of eggs per pot was held constant but the amount

of food per pot was varied to create a range of larval food densities. Variation in larval food density and quality has been shown to produce adult flies with a wide range of eyespan measures (David *et al.* 1998; Knell *et al.* 1999).

After 14 days all pupae from the plastic egg pots were transferred to storage cages. After eclosion, adult flies were fed twice weekly on pureed sweetcorn. Two weeks after eclosion, before sexual maturity, flies were separated by sex to prevent variation between females in their degree of exposure to males. This precaution was taken because studies in other species have shown variation in female exposure to males can alter female mate preferences (Collins 1995). All flies were at least six weeks old at the start of observations in order to ensure sexual maturity.

Sexually mature females were collected over ice and measured for eyespan using a monocular microscope attached to a digitising camera and computer with NIH image software (chapter two). Females were divided into large and small eyespan groups. Large eyespan females were more than 5.8 mm in eyespan ($\overline{X} \pm SD = 6.01 \pm 0.14$ mm, N = 80), and small eyespan females were all less than 5.8 mm in eyespan ($\overline{X} = 5.42 \pm 0.25$ mm, N = 80). The mean difference in eyespan between large and small eyespan female categories was 0.59 ± 0.28 mm between size categories. From these stocks, sets of 5 large and 5 small eyespan females were randomly selected and placed in 16 experimental cages ($30 \times 14 \times 14$ cm).

Each female in a cage was given an individual two-colour identifier. This was painted on the thoraces of females with Humbrol Airfix™ paints. Two extra cages of females were designated as reserves. Females from these reserves had a similar history to experimental females but were not observed and remained unpainted. In the event of an experimental female dying, a reserve female was used as a replacement. One large and nine small eyespan females were replaced during the course of the experiment.

Males were measured using the same procedure as for females. Males were divided into large and small eyespan groups. All large eyespan males were more than 8.5 mm in eyespan ($\overline{X}$ = 9.00 ± 0.21 mm, N = 40) and all small eyespan males were less than 7.5 mm in eyespan ($\overline{X}$ = 6.81 ± 0.64 mm, N = 40). The mean difference in eyespan between large and small eyespan males was 2.19 ± 0.68 mm.

Large and small eyespan males were divided into experimental and reserve flies. For each size category thirty males were randomly selected and placed in a large holding cage (40 × 25 × 25 cm) to provide a source of experimental males. The remaining ten males were retained to act as reserves. In the event of an experimental male dying, it was replaced by a reserve male.

Copulation preference

After three days acclimatisation, half of the experimental cages had a single large eyespan male placed in them at dusk. A small eyespan male was placed in the remaining cages. At dawn the next morning, all experimental cages were observed for ninety minutes (thirty minutes reduced lighting, sixty minutes full illumination). We recorded the size of the male in the cage, the number of copulations, the copulation order, the identity of the female with which the male mated, and the time of the copulation.

A copulation was defined as a male mounting a female and engaging genitalia. The copulation order was the sequence of copulations in the morning. The first copulation of a morning would be designated a copulation rank of 1, the second a copulation rank of 2 etc. The distribution of copulations through time was measured by splitting the observation time into three equal periods, each thirty minutes long, and recording all copulations in each period. Thirty minute periods were chosen because they coincided with differences in lighting level, which changed from reduced to full lighting after thirty minutes. At the end of the ninety minutes of observation, all males were returned to their holding cages. That evening, experimental cages that had contained a large eyespan male now received a small eyespan male and cages which had contained a small eyespan

male now had a large eyespan male placed in them. This procedure was repeated for 10 days.

We calculated copulation preference (*CP*) using the total number of copulations by large and small eyespan males in each cage over the ten observational days, $CP = C_L - C_S$, where C_L is the total number of copulations by the large eyespan males in the cage and C_S was the total copulations by the small eyespan males in the cage. Copulation preference was calculated for a cage of females and for large and small eyespan females separately. In addition CP was calculated for females as a whole and for large and small females for each time period and rank.

CP is an absolute measure of preference and so might be biased if there is significant variation among cages in the total number of copulations. We tested for such bias by performing least squares regressions of absolute (unsigned) *CP* on the total number of copulations for large and small eyespan females and for all females in a cage over the whole observation period. The regression was non-significant ($P > 0.05$) which indicates that *CP* was not dependent upon the total number of copulations. However, when *CP* was analysed when split into time periods, it was dependent upon the total number of copulations ($b = 0.265$, $F_{1,94} = 7.535$, $P = 0.007$). *CP* was not dependent on total copulations for copulation ranks ($b = 0.116$, $F_{1,203} = 2.357$, $P = 0.126$). As a precaution all analyses of preference across ranks and time periods used a relative copulation preference measure,

where $RCP = (C_L - C_S/C_L + C_S)$. To maintain consistency, analysis of preference using all copulations were also repeated using *RCP*.

Copulation duration preference

During the observation period the duration of each copulation was recorded in addition to the factors described above. Copulation duration was defined as the time between a male engaging genitalia and dismounting. This allowed us to calculate a second measure of preference, which tests for a different mode of mate choice. Females may have exerted their preference by mating for longer with desirable males. Copulation duration preference (*CDP*) was calculated using the copulation duration of matings with large and small eyespan males separately within a cage averaged over all copulations for the ten observational days, $CDP = CD_L - CD_S$, where CD_L was the mean copulation duration of the large eyespan males within a cage and CD_S was the mean copulation duration of the small eyespan males within a cage. Copulation duration preference was calculated for a cage of females and for large and small eyespan females separately.

Statistical Procedures

Four types of data were analysed: total copulations, copulation preference, copulation duration, and copulation duration preference. For each type of data, distributions of cage values, as well as those of large and small eyespan females separately, were tested for normality using the Shapiro-Wilk *W* test (Shapiro and Wilk 1965). Only distributions of total copulations were non-normal. Most cages gained about the same number of copulations while a minority achieved greater numbers of total copulations. This resulted in positively skewed distributions of total copulations for large eyespan females, small eyespan females and large and small eyespan female data combined. In addition, when divided into copulation ranks or time periods, many categories contained zero entries. Such data does not lend itself to transformation to normality. All analysis of total copulation data therefore used non-parametric methods. Consequently we used a maximum likelihood logistic regression technique to analyse this data.

Total copulation data was assumed to be an ordinal variable. Ordinal data is considered discrete (i.e. not continuous), with a natural order (i.e. freezing being lower in temperature than warm which is lower in temperature than hot). A logistic regression model was constructed for the analysis, similar in design to ANCOVA, with female size and male size as nominal factors (Sokal and Rohlf 1995). Likelihood ratio tests were computed for each factor in the analysis and for

the interaction term. These tests compute twice the difference of the log likelihoods (G) between the full model and the model without the factor being tested.

Total copulations were also analysed across time periods. The ninety minute observation period was divided into three thirty minute time periods and the total copulations per cage were calculated for each time period. To test for changes in female total copulations through time, logistic regressions of total copulations with nominal factor female size and ordinal factor time period were performed. In addition, this test was repeated when data was separated into copulations with the large eyespan male and copulations with the small eyespan male. Wald tests (Sokal and Rohlf 1995) were used to make comparisons between categories within a factor if a significant G- test was found for that factor.

Total copulations were also analysed using copulation rank as a factor. The copulation order was divided into ranks. The fifth rank contained the fifth and any subsequent copulations in the morning. These late ranked copulations were pooled as they were infrequent compared to earlier ranks. Logistic regression of total copulations with nominal factor female size and ordinal factor copulation ranks was performed. This test was repeated when the data was divided into copulations with large eyespan males and with small eyespan males. Again, Wald tests were used to find any significant differences between categories within factors with significant G-tests.

The rest of the data was normally distributed and this allowed the use of a standard parametric analysis. *CP* (large eyespan, small eyespan and combined female data) distributions were tested for deviations from random mating using two-tailed *t*-tests. The mean of the preference distributions was compared to a null hypothesis of random mating, with mean zero. Two tailed *t*-tests were used to compare large and small eyespan female's *CP*.

Female preference (*RCP*) was analysed across time periods using a model 1 two-way ANOVA with female eyespan and time period as fixed factors. In addition, *RCP* was analysed across copulation ranks using a model 1 two-way ANOVA with female eyespan and rank as fixed factors. If significant variation was found for a factor with more than two categories, a Tukey-Kramer test was used to find which means differed from each other. This test compares all pairs of means and corrects for the number of tests applied.

Large and small eyespan females mean copulation duration was compared in a model 1 two-way ANOVA with female eyespan and male eyespan as fixed factors.

To test for changes in mean copulation duration across time periods, copulation duration was analysed in a model 1 two-way ANOVA with female eyespan and time period as fixed factors. This analysis was repeated for copulations with the large eyespan male and for copulations with the small eyespan male.

To test for changes in mean copulation duration across copulation ranks, copulation duration was analysed in a model 1 two-way ANOVA with female eyespan and rank as fixed factors. This analysis was repeated for copulations with the large eyespan male and for copulations with the small eyespan male. Tukey-Kramer tests were used to identify differences between means for any significant rank or time period factors.

CDP (large eyespan, small eyespan and combined female data) distributions were tested for deviations from random mating using two-tailed *t*-tests. The mean of the preference distributions was compared to a null hypothesis of random mating, with mean zero. A two tailed *t*-test was used to compare large and small eyespan female's *CDP*.

RESULTS

Total copulations

Large and small eyespan females did not differ in the number of copulations they gained over the full 10 days of the experiment ($\overline{X} \pm SD$, large eyespan females 10.50 ± 4.46 copulations per cage; small eyespan females 11.69 ± 3.79 copulations per cage; $G = 0.634$, $df = 1$, $P = 0.426$). When the observations were divided into three thirty minute time periods, large and small eyespan

females gained the same number of copulations ($G = 0.664$, $df = 1$, $P = 0.415$; Fig. 4.1). The number of copulations by all females combined decreased through the observation period ($G = 26.264$, $df = 2$, $P < 0.001$; Fig. 4.1). This was because females gained more copulations in the first half hour period than in the second and third half hour periods (1st vs. 2nd time period, $\chi^2 = 25.69$, $P < 0.001$; 2nd vs. 3rd time period, $\chi^2 = 0.97$, $P = 0.323$; Fig. 4.1).

When copulations with large eyespan males were analysed separately, large and small eyespan females did not differ in the number of copulations they gained ($G = 1.123$, $df = 1$, $P = 0.289$) but the number of copulations achieved by females was still dependent upon the time period ($G = 22.565$, $df = 2$, $P < 0.001$). Females gained fewer matings with large eyespan males in the second and third half hour time periods (1st vs. 2nd time period, $\chi^2 = 16.77$, $P < 0.001$; 2nd vs. 3rd time period, $\chi^2 = 0.09$, $P = 0.764$). For copulations with small eyespan males, large and small eyespan females did not differ in the number of copulations obtained ($G = 1.095$, $df = 1$, $P = 0.295$). Females as a whole varied in mating activity between the time periods ($G = 31.022$, $df = 4$, $P < 0.001$). Females gained more matings with small eyespan males in the first half hour time period than in the second and third (1st vs. 2nd time period, $\chi^2 = 8.41$, $P < 0.003$; 2nd vs. 3rd time period, $\chi^2 = 3.42$, $P = 0.064$).

Analysing the total copulations across copulation ranks is an alternative method to analysing changes in female mating behaviour during the observation period. The number of copulations females achieve will decrease with increasing copulation rank. This is because a third copulation in a morning could not occur without there first being a first and second copulation. Using ranked data, large and small eyespan females did not differ in the number of copulations they achieved ($G = 2.089$, $df = 1$, $P = 0.148$; Fig. 4.2).

When copulations with large eyespan males were considered separately, large and small eyespan females gained similar numbers of copulations ($G = 0.771$, $df = 1$, $P = 0.380$). Large and small eyespan females also gained equal numbers of copulations with the small eyespan male ($G = 1.306$, $df = 1$, $P = 0.253$).

Using ordinal logistic regression allows us to test for interactions between the factors in our models. These interactions identify if there is any effect of female eyespan on the decrease in copulation number observed across ranks and time periods. None of these interactions were significant, giving further evidence that large and small eyespan females do not differ in their mating behaviour.

Copulation preference

Combining large and small eyespan female data, there was significant preference for large eyespan males ($CP = 5.56 \pm 7.35$, $\overline{X} \pm$ SD; $t_{15} = 3.038$, $P = 0.009$). Analysed separately, large and small eyespan females both preferred large eyespan males (large eyespan females $CP = 3.00 \pm 4.80$, $\overline{X} \pm$ SD; $t_{15} = 2.499$, $P = 0.025$; small eyespan females $CP = 2.56 \pm 4.59$, $\overline{X} \pm$ SD; $t_{15} = 2.233$, $P = 0.041$). However, large and small eyespan females did not differ in the strength of preference for large eyespan males ($t_{15} = 0.299$, $P = 0.769$).

When the morning observation period was divided into thirty minute periods, *CP* became dependent upon the total number of copulations ($b = 0.265$, $F_{1,94} = 7.535$, $P = 0.007$). Further analysis of preference data was therefore carried out using the relative preference measure *RCP*, which takes into account differences in total copulations between categories. *RCP* was also used to re-analyse the complete mating data. The results of this analysis did not differ from those given above.

When *RCP* was analysed across the time periods, large and small eyespan females did not differ from each other in their preference ($F_{1,87} = 0.315$, $P = 0.576$; Fig. 4.3) and *RCP* of females overall did not vary between time periods ($F_{2,87} = 2.367$, $P = 0.100$; Fig 4.3). The interaction term between female eyespan and time period was not significant ($F_{2,87} = 0.275$, $P = 0.761$) indicating that the decrease

mating rate through the time periods was the same for large and small eyespan females.

When analysed across copulation ranks, large and small eyespan females had similar preferences ($F_{1,125} = 0.004$, $P = 0.951$; Fig. 4.4) and *RCP* of females as a whole did not vary across copulation ranks ($F_{4,125} = 0.996$, $P = 0.412$; Fig. 4.4). The interaction term was significant ($F_{4,125} = 2.789$, $P = 0.029$), revealing that changes in *RCP* through the copulation order were dependent upon female eyespan. Examination of this trend revealed that small and large eyespan female *RCP* fluctuated between ranks, sometimes with large female eyespan having greater *RCP*, sometimes with small eyespan females having greater *RCP*.

Copulation duration

Large and small eyespan females did not differ in mean copulation duration (large eyespan females 41.85 ± 6.95 secs; small eyespan females 43.07 ± 8.25 secs; $F_{1,30} = 0.087$, $P = 0.770$). Large and small eyespan males also copulated for similar lengths of time ($F_{1,30} = 3.240$, $P = 0.082$). The interaction term was not significant, indicating that the mean copulation duration of large and small eyespan females was not dependent upon the type of male with which they were mating ($F_{1,30} = 0.000$, $P = 0.990$). When copulation duration was divided into time periods, large and small eyespan females did not differ in their mean copulation

duration ($F_{1,87} = 0.588$, $P = 0.446$; Fig. 4.5) and mean copulation duration of females as a whole did not vary between mating periods ($F_{2,87} = 0.083$, $P = 0.921$; Fig. 4.5). The interaction term was non-significant ($F_{2,87} = 1.962$, $P = 0.147$).

When copulation duration with large eyespan males was analysed separately, large and small eyespan females had similar mean copulation duration ($F_{1,80} = 0.097$, $P = 0.757$; Fig. 4.5). Copulation duration did not vary between time periods ($F_{2,80} = 0.887$, $P = 0.416$; Fig. 4.5) and the interaction term was not significant ($F_{2,80} = 0.412$, $P = 0.664$). The same results were found with small eyespan males. Large and small eyespan females had the same mean copulation duration ($F_{1,59} = 0.503$, $P = 0.481$) and copulation duration did not change between time periods ($F_{2,59} = 1.014$, $P = 0.369$). The interaction term between female size and time period was not significant ($F_{2,59} = 0.451$, $P = 0.639$).

Mean copulation duration was also calculated for copulation ranks (Fig. 4.6). Here, large and small eyespan females did not differ in mean copulation duration ($F_{1,126} = 0.367$, $P = 0.530$), but copulation duration did vary between ranks ($F_{4,126} = 2.545$, $P = 0.043$). Analysis of the variation in copulation duration between ranks using the Tukey-Kramer test revealed that no ranks differed significantly from each other. The significance of the rank factor was only marginal and it is therefore likely that the Tukey-Kramer tests did not have sufficient power to detect the small variation of rank means. The interaction term

was not significant ($F_{4,126} = 0.487$, $P = 0.745$) indicating that variation in copulation duration between ranks was not dependent on female eyespan.

When large eyespan male copulation duration was analysed separately, large and small eyespan females did not differ in copulation duration ($F_{1,109} = 0.038$, $P = 0.845$) and copulation duration did not vary between copulation ranks ($F_{4,109} = 0.808$, $P = 0.523$). The interaction term was not significant ($F_{4,109} = 0.255$, $P = 0.906$). The same pattern was seen with small eyespan males. Large and small eyespan females had similar mean copulation duration ($F_{1,71} = 0.881$, $P = 0.351$) and copulation duration did not vary between ranks ($F_{4,71} = 1.538$, $P = 0.200$). Again, the interaction term was not significant ($F_{4,71} = 0.592$, $P = 0.670$).

Copulation duration preference

An alternative method of measuring preference compared to using the number of copulations is to measure the duration of copulations. When using this method females as a whole mated randomly ($CDP = 0.13 \pm 10.76$: $t_{12} = 0.044$, $P = 0.965$). Large and small eyespan females both mated randomly when analysed separately (large eyespan females $CDP = -1.73 \pm 11.72$; $t_{13} = 0.552$, $P = 0.591$; small eyespan females $CDP = 0.997 \pm 14.15$; $t_{14} = 0.273$, $P = 0.789$) and did not differ in their copulation duration preference ($t_{12} = 0.617$, $P = 0.549$).

DISCUSSION

We investigated the occurrence of eyespan-dependent mate choice in *C. dalmanni* stalk-eyed flies, when females could compete for copulations with males. We might expect female competition to be the most intense for access to large eyespan males, as there are indirect benefits to mating these individuals (Wilkinson 1993; David *et al.* 1998, 2000; Wilkinson *et al.* 1998). If large and small eyespan females do compete for copulations we would expect large eyespan females to gain the most access to males, especially preferred males. In *C. dalmanni*, aggressive interactions between females are frequently observed and are usually resolved in favour of the large females (personal observation, AH).

Mating early may also be adaptive as females might gain fertility benefits from early matings with males. One possibility is that males suffer from temporary loss of fertility in later copulations due to the exhaustion of sperm supplies. Another possibility is that later copulations are less fertile due to reductions in accessory gland material (Leferve and Jonsson 1962). Both effects are known in *Drosophila melanogaster* (Kvelland 1965; Markow *et al.* 1978). Measurements of copulation duration may allow us to investigate this phenomena as unsuccessful sperm transfer in stalk-eyed flies is marked by short copulations (Lorch *et al.* 1993).

Contrary to our expectations, large and small eyespan females did not differ in the number of copulations they gained and this was also true when copulations were divided into those with large eyespan males and those with small eyespan males. We failed to find any evidence for eyespan-dependent competition between stalk-eyed fly females for mates. However, we cannot exclude this possibility altogether without direct observations of female interactions.

When the data was split into three time periods, there was a general decline in female mating activity through the mating period (Fig. 4.1). This trend was also found when analysing large and small eyespan male copulations separately. This trend tallies well with field observation data suggesting that nearly all matings occur in the hour after dawn (Wilkinson and Reillo 1994).

As expected, females preferred to mate with large eyespan males. However, we failed to find evidence that large and small eyespan females differed in the strength of preference. Nor did large and small eyespan females differ in the strength of preference through time, or in the order in which they copulated.

We found no evidence in our experiment of decreasing copulation duration through the observation period, but variation in copulation duration was found across the copulation ranks. No differences between means were highlighted by further analysis, but this result is likely to be due to a slight decrease in copulation duration in the third copulation rank compared to the

others (Fig. 4.6). These results would tend to suggest that males did not become infertile with successive copulations in the morning, but this is far from conclusive evidence. CDP was not found to be a sensitive measure of female preference as copulation duration showed very little variation.

In summary we found no evidence for female eyespan-dependent competition for access to mates. Additionally we failed to find any evidence for competition for early copulations that may have contained more sperm. Copulation duration was extremely consistent across all parameters measured and was therefore an unusable method of measuring female mate preference.

REFERENCES

Ahnesjö, I., Vincent, A., Alatalo, R., Halliday, T. and Sutherland, W.J. 1993. The role of females in influencing mating patterns. Behavioral Ecology, 4: 187-189.

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

Bakker, T.C.M. 1999. The study of intersexual selection using quantitative genetics. Behaviour, 136: 1237-1266.

Bakker, T.C.M., Künzler, R. and Mazzi, D. 1999. Condition related mate choice in sticklebacks. Nature, 401: 234.

Burkhardt, D. and de la Motte, I. 1983. How stalk-eyed flies eye stalk-eyed flies: Observations and measurements of the eyes of *Cyrtodiopsis whitei*. Journal of Comparative Physiology A, 151: 407-421.

Burkhardt, D. and de la Motte, I. 1988. Big 'antlers' are favoured: female choice in stalk-eyed flies (Diptera, Insecta), field collected harems and laboratory experiments. Journal of Comparative Physiology A, 162: 649-652.

Bush, S.L. and Bell, D.J. 1997. Courtship and female competition in the Majorcan midwife toad, *Alytes muletensis*. Ethology, 103: 292-303.

Collins, S.A. 1995. The effect of recent experience on female choice in zebra finches. Animal Behaviour, 49: 479-486.

Dale, S., Rinden, H. and Slagsvold, T. 1992. Competition for a mate restricts mate search of female pied flycatchers. Behavioral Ecology and Sociobiology, 30: 165-176.

David, P., Hingle, A., Greig, D., Rutherford, A., Pomiankowski, A. and Fowler, K. 1998. Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. Proceedings of the Royal Society of London, Series B, 265: 2211-2216.

David, P., Bjorksten, T., Fowler, K. and Pomiankowski, A. 2000. Condition-dependent signalling of genetic variation in stalk-eyed flies. Nature, 406: 186-188.

Jennions, M.D. and Petrie, M. 1997. Variation in mate choice and mating preferences: a review of the causes and consequences. Biological Reviews, 72: 283-327.

Jennions, M.D., Backwell, P.R.Y. and Passmore, N.I. 1995. Repeatability of mate choice: the effect of size in the African painted reed frog, *Hyperolius marmoratus*. Animal Behaviour, 49: 181-186.

Knell, R.J., Fruhauf, N. and Norris, K.A. 1999. Conditional expression of a sexually selected trait in the stalk-eyed fly *Diasemopsis aethiopica*. Ecological Entomology, 24: 323-328.

Kotrba, M. 1996. Sperm transfer by spermatophore in Diptera: new results from the Diopsidae. Zoological Journal of the Linnean Society, 117: 305-323.

Kvelland, I. 1965. Some observations of the mating activity and fertility of *Drosophila melanogaster* males. Hereditas, 53: 281-306.

Lefevre, G. and Johnsson, U.B. 1962. Sperm transfer, storage, displacement, and utilisation in *Drosophila melanogaster*. Genetics, 47: 1719-1736.

López, S. 1999. Parasitized female guppies do not prefer showy males. Animal Behaviour, 57: 1129-1134.

Lorch, P., Wilkinson, G.S. and Reillo, P.R. 1993. Copulation duration and sperm precedence in the Malaysian stalk-eyed fly, *Cyrtodiopsis whitei* (Diptera: Diopsidae). Behavioral Ecology and Sociobiology, 32: 303-311.

Markow, T.A., Quad, M. and Kerr, S. 1978. Male mating experience and competitive courtship success in *Drosophila melanogaster*. Nature, 276: 821-822.

de la Motte, I. and Burkhardt, D. 1983. Portrait of an Asian Stalk-eyed fly. Naturwissenshaften, 70: 451-461.

Nijhout, H.F. and Emlen, D.J. 1998. Competition among body parts in the development and evolution of insect morphology. Proceedings of the National Academy of Sciences, USA, 95: 3685-3689.

Panhuis, T.M. Wilkinson, G.S. 1999. Exaggerated eye span influences male contest outcome in stalk-eyed flies. Behavioral Ecology Sociobiology, 46: 221-227.

Pomiankowski, A. 1987. The costs of choice in sexual selection. Journal of Theoretical Biology, 128: 195-218.

Poulin, R. 1994. Mate choice decisions by parasitised upland bullies, *Gobiomorphus breviceps*. Proceedings of the Royal Society of London, Series B, 256: 183-187.

Rosenqvist, G. and Berglund, A. 1992. Is female sexual behaviour a neglected topic? Trends in Ecology and Evolution, 7: 174-176.

SAS Institute. 1996. JMP statistics for the Macintosh version 3.1.6.

SAS Institute. 1998. JMP statistics and graphics guide version 3.

Shapiro, S.S. Wilk, M.B. 1965. An analysis of variance tests for normality. Journal of the American Statistical Association, 63: 1343-1372.

Shillito, J.F. 1971. Dimorphism in flies with stalked eyes. Zoological Journal of the Linnean Society, 50: 297-305.

Sokal, R.R. and Rohlf, F.J. 1995. Biometry. W.H. Freeman and Company. New York.

Wilkinson, G.S. 1993. Artificial selection alters allometry in the stalk-eyed fly *Cyrtodiopsis dalmanni* (Diptera: Diopsidae). Genetical Research, 62: 213-222.

Wilkinson, G.S. and Reillo, P.R. 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. Proceedings of the Royal Society London, Series B, 255: 1-6.

Wilkinson, G.S. and Taper, M. 1999. Evolution of genetic variation for condition-dependent traits in stalk-eyed flies. Proceedings of the Royal Society of London, Series B, 266: 1685-1690.

Wilkinson, G.S. Kahler, H. and Baker, R.H. 1998. Evolution of female mating preferences in stalk-eyed flies. Behavioral Ecology, 9: 525-533.

FIGURE LEGENDS

Figure 4.1. Histogram of total copulations on time period for large and small eyespan females. Each time period consisted of 30 minutes. The number of copulations gained by large eyespan female decreased from the first to the last 30 minutes of the observation period. Small eyespan female mating rate did not change through the mating period. Large and small eyespan female total copulations did not differ when data from each time period was analysed separately. Error bars indicate standard error of the mean.

Figure 4.2. Histogram of total copulations on copulation rank for large and small eyespan females. Large and small eyespan female total copulations did not differ within copulation ranks. Error bars indicate the standard error of the mean.

Figure 4.3. Mean *RCP* per cage in each time period for large and small eyespan females. Mean *RCP* did not change through the mating period for large or small eyespan females. Large and small eyespan females did not differ in *RCP* within time periods. Error bars indicate the standard error of the mean.

Figure 4.4. Mean *RCP* per cage with copulation rank for large and small eyespan females separately. Mean *RCP* did not change across the copulation order. Large

and small eyespan females did not differ in *RCP* within any copulation ranks. Error bars indicate the standard error of the mean.

Figure 4.5. Mean copulation duration per cage with time period for large and small eyespan females. Copulation duration did not change through the mating period. Large and small eyespan females did not differ in copulation duration within any of the time periods. Error bars indicate the standard error of the mean.

Figure 4.6. Mean copulation duration with copulation rank for large and small eyespan females separately. Copulation duration did not change through the copulation order. Large and small eyespan females did not differ in copulation duration within any of the copulation ranks. Error bars indicate standard errors of the means.

Figure 4.1

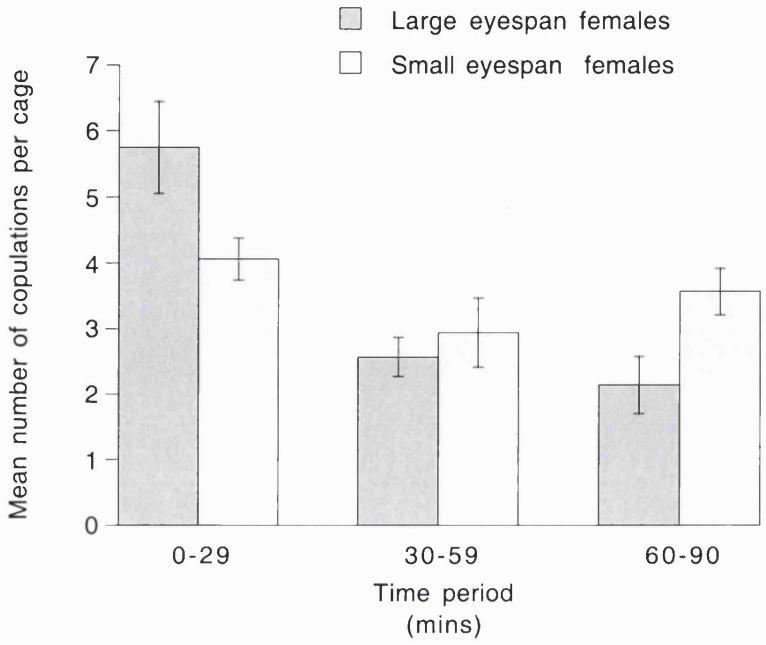

Large eyespan females
Small eyespan females
Mean number of copulations per cage
0
1
2
3
4
5
6
7
0-29
30-59
60-90
Time period
(mins)

Figure 4.2

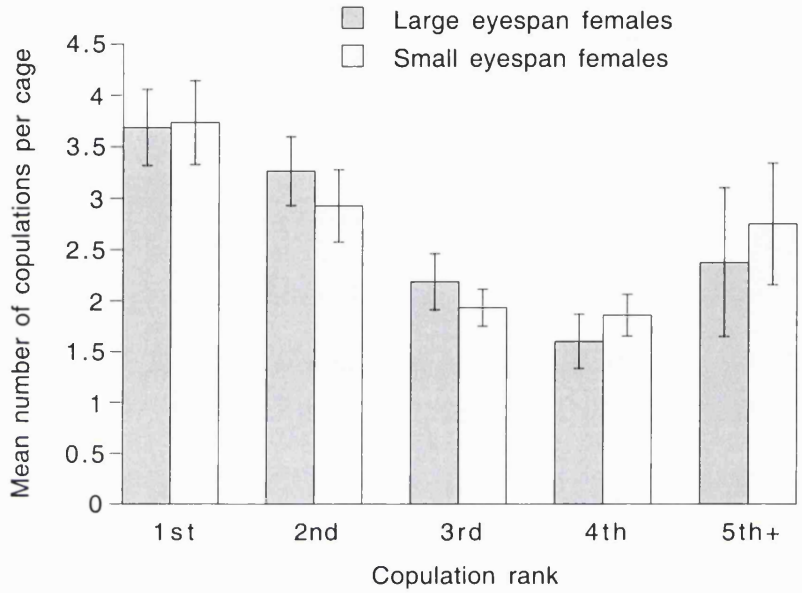

Large eyespan females
Small eyespan females
Mean number of copulations per cage
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
1st
2nd
3rd
4th
5th+
Copulation rank

Figure 4.3

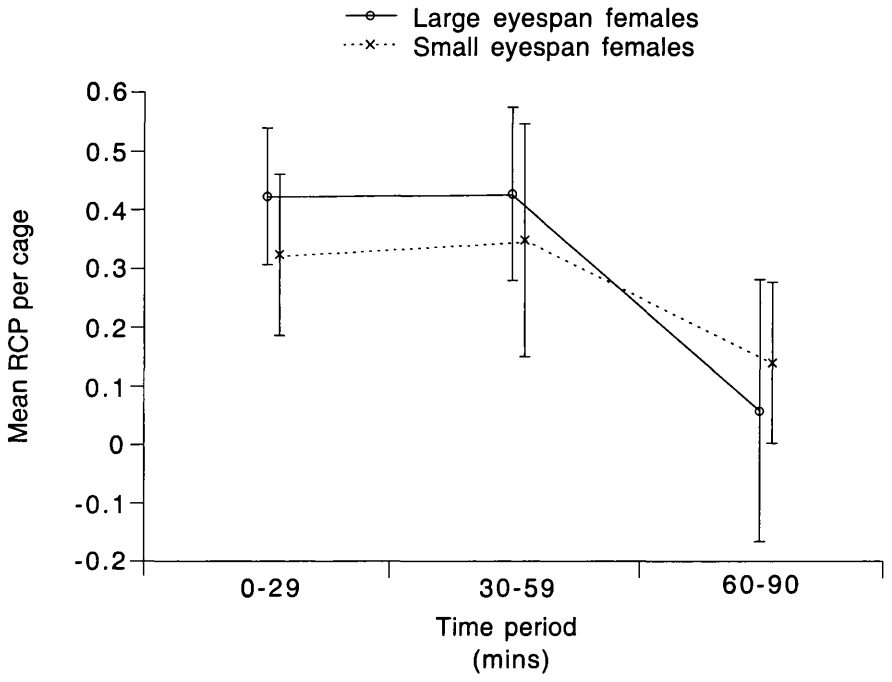

Large eyespan females
Small eyespan females
Mean RCP per cage
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
0-29
30-59
60-90
Time period
(mins)

Figure 4.4.

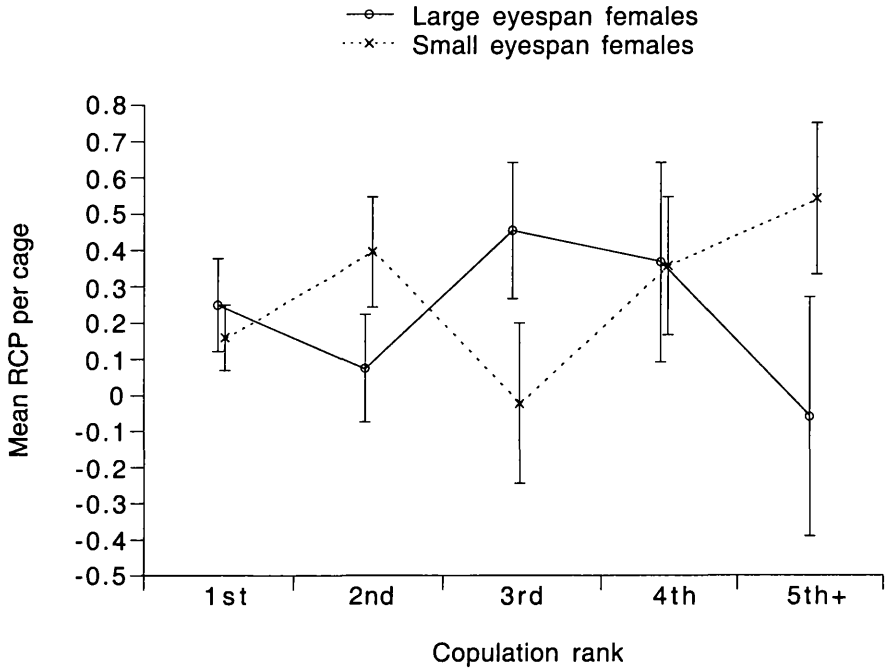

Large eyespan females
Small eyespan females
Mean RCP per cage
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
1st
2nd
3rd
4th
5th+
Copulation rank

Figure 4.5.

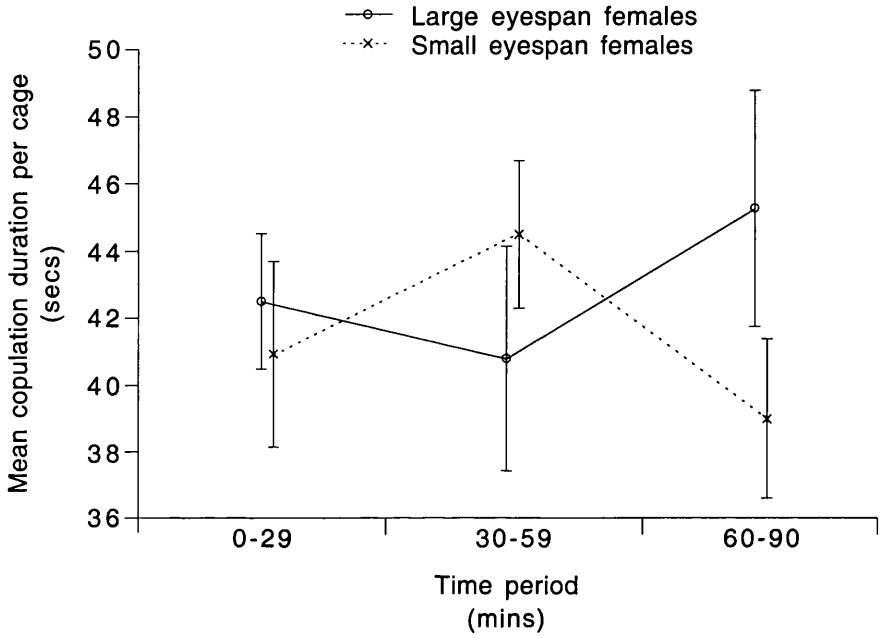
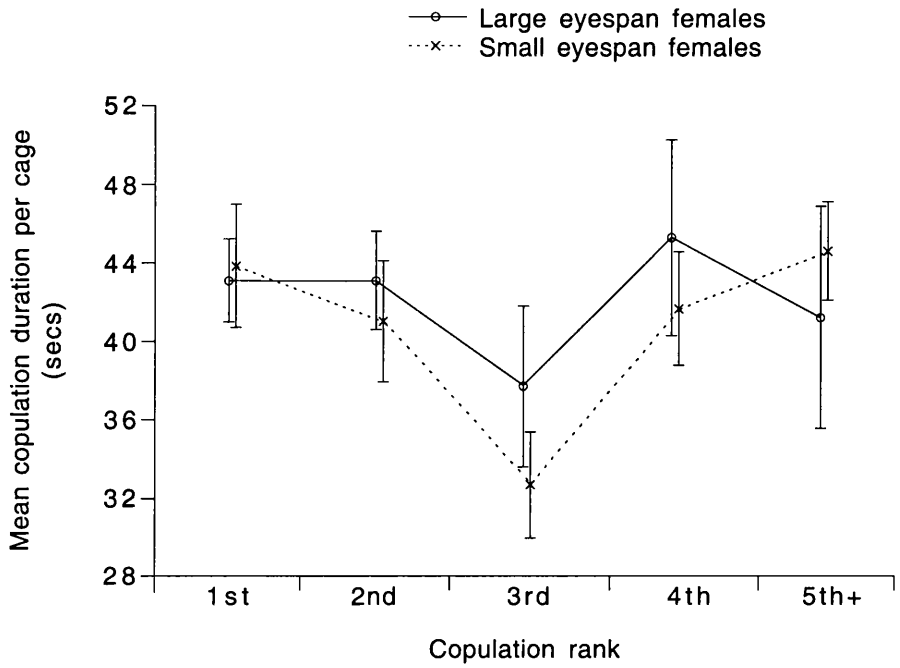

Large eyespan females
Small eyespan females
Mean copulation duration per cage (secs)
50
48
46
44
42
40
38
36
0-29
30-59
60-90
Time period (mins)

Figure 4.6.


Large eyespan females
Small eyespan females
Mean copulation duration per cage (secs)
52
48
44
40
36
32
28
1st
2nd
3rd
4th
5th+
Copulation rank

Chapter Five

Fluctuating asymmetry is an unreliable measure of environmental stress for conservation biologists

ABSTRACT

Fluctuating asymmetry (FA), the random deviation of bilateral traits from perfect symmetry, has been promoted as a sensitive measure of population level environmental and genomic stress. This has led to the proposal of its use as a viable monitoring technique for endangered populations by conservation biologists. In particular, sexual traits are thought to be sensitive to stress. We tested these hypotheses in the stalk-eyed fly *Cyrtodiopsis dalmanni,* under controlled laboratory conditions using stress caused by varying the level of larval density. Sexual (male inner and outer eyestalk) and non-sexual (female inner and outer eyestalk, wing width and wing length) traits were compared. Our data showed that sexual trait FA did not increase under larval density stress and was not more sensitive to stress than similar non-sexual traits. Mean sexual trait FA was lower then mean non-sexual trait FA when corrections for trait size were

made. Sexual trait FA did not show any evidence of a negative relationship with trait size. In contrast, all traits, especially sexual traits, decreased in size with increasing larval density, confirming that the stresses had been effective. Our results demonstrate that FA is not a reliable measure of environmental stress and is of limited value to conservation biologists.

INTRODUCTION

Fluctuating asymmetry (FA), the random deviation of a bilateral trait from perfect symmetry, has been suggested to be a reliable and sensitive indicator of environmental and genetic stress (Soule 1979, Leary and Allendorf 1989; Parsons 1992). The ease of measurement of FA and its cost-effectiveness compared to other approaches of population monitoring have led to increasing interest in its use in addition to other biomonitoring methods (Clarke 1995; Møller and Swaddle 1997).

Negative relationships between FA and a number of fitness components have been reported (Clarke 1995; Møller 1997; Møller 1999). In Møller's (1997) review, symmetrical individuals had better survival, higher fecundity and faster growth. These associations could arise directly, if individuals in poor condition also manifest higher FA. Or they could arise indirectly, if individuals with high FA attract more predation, have reduced foraging efficiency or do less well in competition with more symmetric individuals. Due to this strong relationship with fitness components, FA has been proposed to be useful to conservation biologists to identify populations subjected to stress, provide an early indicator of impending falls in population fitness and to monitor captive breeding populations for reintroduction to the wild (Clarke 1995).

However, many recent studies have raised doubts about the strength of evidence supporting a strong relationship between FA and fitness. Many studies included in review articles are based on small sample sizes, and were not carried out under controlled conditions (Clarke 1998). A number of well controlled, experimental studies with large sample sizes have reported no relationship between FA and stress or fitness components (e.g. Clarke 1998, Vøllestad *et al.* 1999, Bjorksten *et al.* 2000a, Bourguet 2000). Other studies have measured a number of traits and find that the FA of some traits responded to stress while others did not (Campbell *et al.* 1998, Roy and Stanton 1999, Woods *et al.* 1999, Clarke *et al.* 2000, Putman *et al.* 2000). In addition, few studies have compared the sensitivity of FA to that of other measures such as immune activity or body size, so as to see whether FA is a better measure of stress or fitness (David *et al.* 1998; Lagesen and Folstad 1998; Bjorksten *et al.* 2000). In addition, recent theoretical work (Houle 2000) suggests that FA is in fact a poor measure of the underlying developmental mechanisms of trait stability as it estimates a variance (developmental stability) with just two data points, the left and right sides of the trait.

Owing to the ambiguity surrounding the relationship between FA and fitness, attention has focused on traits thought to be more reliable indicators. In particular, exaggerated sexually selected traits have been suggested to be more sensitive to stress than morphological characters because of their recent history of

directional selection (Møller and Pomiankowski 1993; Watson and Thornhill 1994). Three basic hypotheses have been formulated concerning sexual trait FA (Bjorksten *et al.* 2000b). First, sexual trait FA is predicted to be more sensitive to stress than other traits because sexual traits are expected to be weakly canalised and have strong condition-dependent characters. Second, for the same reasons, sexual trait mean FA should be greater than the mean FA of similar non-sexual traits. Third, it has been proposed that sexual trait FA will show a negative relationship with trait size because only the highest quality individuals are able to grow large ornaments while maintaining bilateral symmetry. Few studies have investigated these hypotheses and at the same time compared sexual traits to morphological traits within the same individual.

Here we use the stalk-eyed fly *Cyrtodiopsis dalmanni* to investigate each of these hypotheses. *C. dalmanni* is sexually dimorphic for eyespan; male flies have eyestalks that are greater than body length (de la Motte and Burkhardt 1983). Greater eyespan has been shown to increase female attraction (Burkhardt and de la Motte 1988, Wilkinson *et al.* 1998) and be used by males in intra-sexual competition (Panhuis and Wilkinson 1999).

We subjected stalk-eyed fly larvae to a range of nutritional stress by varying larval density (David *et al.* 1998). By using controlled conditions we avoided confounding factors between treatment groups such as age,

environmental and genetic variation. Our study used large sample size with multiple replication of all stress levels to reduce the effect of sampling error.

Two measures were taken of the male sexual trait, the length of the inner and outer eyestalk. These were compared to the same measurements of female eyespan which is not thought to be subject to sexual selection. Using two measures of sexual trait FA, from different regions of the eyestalk, enhances our likelihood of detecting stress induced variation in sexual trait FA and evaluate the consistency of sexual trait FA hypotheses. A further comparison was then made to two measures of a non-sexual trait in both sexes. We chose two wing measures, wing length and wing width, as these are on the same scale as the eyestalk measurements. In addition, the wing measures could be made accurately from easily located landmarks. Once the FA in each trait was calculated, we created a number of composite FA measures as these have been advocated as being more sensitive measures of stress than individual FA values (Leung *et al.* 2000).

METHODS

Manipulation of environmental stress

Experimental manipulations were carried out on a laboratory population of *C. dalmanni* founded from collections made from Gombak, Malaysia in 1993.

The population has been maintained in population cages of at least 200 individuals since founding, at 25°C, on a 12h:12h light:dark cycle and fed pureed sweetcorn.

Flies were subjected to a range of nutritional stress by varying the amount of food available during larval development. Groups of 15 females were isolated from stock cages and allowed to lay eggs on damp filter paper for a 5 hr period. Eggs were then divided into groups of 13 and added to one of five quantities of pureed sweetcorn: 6.5g, 3.25g, 1.3g, 0.78g, 0.39g (equal to 0.03, 0.06, 0.1,0.25 and 0.5 g per egg respectively). Groups of eggs were then cultured until adult flies eclosed. Eclosed adults were left until their cuticles hardened and were then collected and frozen. Later, flies were sexed before their heads and wings were dissected.

Few egg pots showed the maximum survival of all 13 eggs to adult emergence, so food stress was calculated for each pot as the total number of adult flies that emerged per gram of corn. This measure takes account of mortality during development. As mortality is likely to be most common in the early stages of larval growth, this measure is likely to be a better measure of stress caused by variation in larval density than the original amount of corn. Mortality was found to show no significant variation over the five food levels.

Measurements

All measurements of morphological traits were made using a monocular microscope attached to a camera lucida and digitising tablet. The inner eyestalk, outer eyestalk, wing length, wing width and thorax length were measured using distances between easily defined landmarks (Fig. 5.1). Two replicate measurements were made on different days to allow partitioning of measurement error from true FA (David *et al.* 1999). Each replicate consisted of left and right measurements (except for thorax length). Damage to fly wings, heads and thoraxes during dissection and manipulation meant that sample sizes were unequal.

Great care was taken during the course of our study to measure trait FA accurately. We used a monocular dissecting microscope to measure specimens as binocular microscopes can suffer from systematic error due to the non-perpendicular relationship of the microscope eyepiece and stage. Our experience showed that such errors are not easily correctable as they vary with the orientation and size of the specimen in the field of view. During measurements and later when analysing the data, individuals that were damaged, miss-measured traits or outliers were identified. Such individuals were unrepresentative of normal growth or the true level of FA of an individual, and so were removed from the dataset.

Statistical procedures

Trait FA

Fluctuating asymmetry was calculated as the signed difference between left minus right $(L - R)$. The FA of each trait was calculated as the mean of the FA values in each of the two replicate measurements. To check that the asymmetry was representative of fluctuating asymmetry (FA) and not antisymmetry or directional asymmetry, we tested for normality and whether the mean asymmetry was equal to zero (Palmer and Strobeck 1986). Normality was tested using the Shapiro-Wilk *W* test for each sex and food level (Zar 1999). Deviations of distribution means from zero were tested using two-tailed *t*-tests, for each sex and sweetcorn food level.

Due to its non-normal distribution, absolute FA could not be used in parametric analysis so the transformation $|L - R|^{0.4}$ was applied (Swaddle *et al.* 1994). This efficiently normalised all absolute FA distributions. Error variance imitates FA. To check that error variance did not obscure the measurement of FA, we followed the procedure of David *et al.* (1998). This procedure uses a one-way ANOVA on absolute trait FA ($|L - R|$) measurements to partition the total variance into variance between individuals and variance between replicates within individuals. The *F*-ratio between these two variances measures true FA relative to

measurement error (David *et al.* 1999). We also assessed the repeatability of FA measurements by calculating the correlation coefficient between replicates of signed FA and comparing it to a null hypothesis of no association between replicates ($r = 0$) using two tailed t-tests.

FA and larval density

The sensitivity of trait FA to environmental stress was analysed using least squares linear regression of absolute trait FA on larval density for each trait in each sex. Absolute trait FA for each sex was also regressed on trait size to see if any size-dependent effects existed, as these may have masked any larval density effects on FA. Sex differences in the effect of larval density were estimated as sex × density interactions from ANCOVAs with sex as the nominal factor and density as the continuous factor, each trait being analysed separately. The response to larval density of different traits was compared using trait × density interactions in ANCOVAs with trait as the nominal factor and density as the continuous factor.

Mean FA

The hypothesis that sexual traits have higher FA values than non-sexual traits was tested by comparing absolute FA from male inner and outer eyestalk

length with the equivalent measures in females. In addition, male inner and outer eyestalk FA were compared to male wing width and length FA. Two tests were performed. Firstly the absolute FA from male and female eyestalk traits were compared in a one-way ANOVA with sex as the nominal factor. Secondly the same test was performed using relative absolute FA (absolute FA divided by trait size), as suggested by Bjorksten *et al.* (2000b), to eliminate the effect of the sexual traits being larger in males than female.

Trait size

Trait size was calculated as $(L + R)/2$. All trait size distributions were tested for normality for each sex and food level using the Shapiro-Wilk W test. Repeatability of trait measurements was assessed using the correlation coefficient between replicates. The sensitivity of a trait to environmental stress was analysed using least squares linear regression of trait size on larval density. Sex differences in the effect of larval density were estimated as sex $\times$ density interactions from ANCOVAs with sex as the nominal factor and density as the continuous factor.

Composite FA

Composite FA values have been suggested to be more sensitive measures of stress than individual trait FA by itself (Leung *et al.* 2000). A composite measure of FA was made by dividing absolute FA by the mean population FA for each trait and then summing the resultant values across traits for each individual. This gave each trait FA distribution a mean of zero and a standard deviation of one; so each trait FA made an equal contribution to the composite FA measure.

A male sexual trait composite FA was constructed for the sexual traits (male inner and outer eyestalk). The equivalent composite FA for females was made using the homologous eyestalk traits. Composites for wing FA (wing width and wing length) and an overall composite (inner eyestalk, outer eyestalk, wing width and wing length) were made as well. Composite FA were regressed on larval density to test for the response to stress. Sex-dependent and trait-dependent effects in FA response to larval density were tested as the interaction term in an ANCOVA with sex or trait type as the nominal factor and larval density as the continuous factor.

Correlation of FA across traits

Absolute FA between traits was compared using pairwise correlation within individuals to test for developmental relationships within growing organs (either eyestalks or wings). Comparisons were made for males and females separately.

Removal of outliers

Particular care was taken to remove individuals from the dataset that may not have been typical of normal development (i.e. with asymmetries not representative of FA). Possible causes of unrepresentative data in our dataset were damage during freezing or handling, abnormalities during development or adult emergence, and measurement errors. These causes are likely to artificially inflate FA estimates.

A number of procedures were used to remove atypical flies. At the measuring stage, individuals that had obviously damaged traits were excluded. After measuring, outliers were identified from the distributions of trait size or trait FA. Outliers were defined as datum more than the upper quartile plus 1.5 times the interquartile range or less than the lower quartile minus 1.5 times the interquartile range (SAS 1996). Lastly, outliers from correlation plots of trait size

and signed FA measures between replicate measurements were removed as these individuals were representative of either measurement error in at least one replicate or of traits damaged between replicates. These outliers were visually identified as any extreme datum conspicuously beyond the 95% confidence limits of correlation scatter plots.

RESULTS

Trait FA

Signed FA was calculated as the mean over two replicates of left minus right trait size values. Repeatability of FA measurements between replicates ranged from $r = 0.898$ (male wing length) to $r = 0.721$ (female outer eyespan), all $P < 0.001$. All signed FA distributions were found to be normal with means equal to zero, indicating the presence of fluctuating asymmetry rather than antisymmetry or directional asymmetry.

F-ratio tests for FA versus error variance were high for each trait and sex indicating that measurement error did not obscure true FA (numbers in square brackets indicate the proportion of total variance due to measurement error: male inner eyestalk, $F_{309,310} = 10.297$ [0.09]; male outer eyestalk, $F_{128,129} = 10.695$

[0.09]; male wing width, $F_{425,426} = 12.044$ [0.08]; male wing length, $F_{411,412} =$ 19.644 [0.04]; female inner eyestalk, $F_{298,299} = 5.562$ [0.15]; female outer eyestalk, $F_{104,105} = 5.209$ [0.16]; female wing width, $F_{361,362} = 12.35$ [0.08]; female wing width, $F_{337,338} = 17.391$ [0.05], all $P < 0.001$).

FA and larval density

We expected that sexual trait FA would be sensitive to stress. However, neither male inner or outer eyestalk FA showed any response to increasing larval density (Fig. 5.2; male inner eyestalk, $F_{1,289} = 0.736$, $P = 0.392$; male outer eyestalk, $F_{1,117} = 1.317$, $P = 0.253$). The homologous non-sexually selected trait FA in females also failed to show any sensitivity to larval density (Fig. 5.2; female inner eyestalk $F_{1,268} = 0.186$, $P = 0.667$; female outer eyestalk, $F_{1,86} =$ 1.234, $P = 0.270$), and there was no difference between the sexes in how eyestalk FA responded to larval density (inner eyestalk, $F_{1,605} = 0.049$, $P = 0.825$; outer eyestalk, $F_{1,230} = 0.006$, $P = 0.940$).

This lack of response was also evident in the non-sexual wing trait FA, as they did not respond to increasing larval density (Fig. 5.2; male wing width, $F_{1,405}$ = 1.948, $P = 0.164$; male wing length, $F_{1,409} = 1.915$, $P = 0.167$; female wing width, $F_{1,336} = 2.089$, $P = 0.149$; female wing length, $F_{1,336} = 0.621$, $P =$0 431). The response of wing trait FA to larval density did not differ between the sexes (wing

width, $F_{1,783} = 0.130$, $P = 0.719$; wing length, $F_{1,745} = 2.286$, $P = 0.131$). Within males, sexual trait FA did not differ from wing trait FA in their response to larval density (inner eyestalk vs. wing width, $F_{1,730} = 0.004$, $P = 0.950$; inner eyestalk vs. wing length, $F_{1,716} = 2.031$, $P = 0.155$; outer eyestalk vs. wing width, $F_{1,550} = 2.645$, $P = 0.104$; outer eyestalk vs. wing length, $F_{1,536} = 0.121$, $P = 0.7281$).

Size dependence of FA on trait size may have obscured any effect of larval density on FA values. Only male wing length FA showed a dependence on trait size ($b = 0.050$, $F_{1,410} = 5.779$, $P = 0.017$). To eliminate the positive relationship between male wing length and trait FA we took residuals from the regression line and re-tested for density dependence of residual FA. Eliminating size-dependence in this way reveals that male wing length FA, like all other traits measured, did not respond to larval density (residual male wing length, $F_{1,409} = 0.182$, $P = 0.670$).

Mean FA

The mean FA of sexual ornaments has been proposed to be greater than the mean FA of non-sexual traits (Møller 1990). We tested for this by comparing the mean FA of the two male sexual traits with the homologous traits in females. Both male sexual trait FA were greater (inner eyestalk, $F_{1,608} = 26.438$, $P < 0.001$; outer eyestalk, $F_{1,232} = 20.859$, $P < 0.001$). Similarly, within males, both sexual

trait FA were greater than the two measures of wing FA (inner eyestalk vs. wing width, $F_{1, 734} = 103.292$, $P < 0.001$; inner eyestalk vs. wing length, $F_{1,720} = 68.741$, $P < 0.001$; outer eyestalk vs. wing width, $F_{1,553} = 101.044$, $P < 0.001$; outer eyestalk vs. wing length, $F_{1,539} = 70.544$, $P < 0.001$).

These comparisons ignore size differences between traits. Therefore the analysis was repeated using relative FA to compensate for the greater size of the male sexual traits giving a value of the FA per unit trait size. Using relative trait FA, females had greater relative FA than males for the eyestalk measures (Fig. 5.3; inner eyestalk, $F_{1,608} = 68.432$, $P < 0.001$; outer eyestalk, $F_{1,232} = 6.248$, $P = 0.013$). Similarly, wing width relative FA was greater than eyespan relative FA in males (inner eyestalk vs. wing width, $F_{1,734} = 310.376$, $P < 0.001$; outer eyestalk vs. wing width, $F_{1,553} = 235.394$, $P < 0.001$), but wing length relative FA was smaller than eyespan relative FA in males (inner eyestalk vs. wing length, $F_{1,720} = 324.555$, $P < 0.001$; outer eyestalk vs. wing length, $F_{1,539} = 77.092$, $P < 0.001$).

FA and trait size

Sexual trait FA is predicted to decrease with increasing trait size if superior individuals are able to grow large traits whilst maintaining symmetry. However, regressions of sexual trait absolute FA on trait size showed no effect of trait size (male inner eyestalk, $b = 0.020$, $F_{1,286} = 1.145$, $P = 0.285$; male outer

eyestalk, b = - 0.020, $F_{1,115}$ = 0.000, P = 1.000). In addition the relationship between male eyestalk FA and trait size did not differ from the relationship found in the homologous traits in females (inner eyestalk, $F_{1,606}$ = 0.025, P = 0.875; outer eyestalk = $F_{1,230}$ = 0.071, P = 0.790).

Composite FA

The male sexual trait composite FA (inner and outer eyestalk) showed no response to larval density ($F_{1,122}$ = 1.084, P = 0.300), nor did the homologous female eyestalk composite FA ($F_{1,101}$ = 1.466, P = 0.229). There was no evidence that the male sexual trait composite FA was more sensitive than the homologous female non-sexual eyestalk composite FA ($F_{1,223}$ = 0.209, P = 0.648).

Male and female wing composite FA also failed to show any response to larval density (males, $F_{1,405}$ = 0.002, P = 0.968; females, $F_{1,336}$ = 2.125, P = 0.146). Comparing within males, there was no evidence that the sexual composite FA was more sensitive to larval density than the wing composite FA ($F_{1,527}$ = 0.931, P = 0.335)

The overall composite FA (wing and eyestalk traits) did not respond to changes in larval density (males, $F_{1,288}$ = 0.693, P = 0.407; females, $F_{1,85}$ = 0.473, P = 0.494). Sample sizes were slightly reduced due to the inclusion of outer eyestalk FA, which had a much smaller sample size than the other traits. But

excluding outer eyestalk FA from the composite measure had no effect on the results (males, $F_{1,111} = 0.693$, $P = 0.407$; females, $F_{1,268} = 3.857$, $P = 0.051$). There was also no difference between the sexes in the overall composite FA response to larval density (overall composite FA, $F_{1,196} = 0.001$, $P = 0.973$; overall composite FA excluding outer eyestalk FA, $F_{1,556} = 0.445$, $P = 0.505$).

Correlation of FA across traits

Sexual selected traits showed no correlation in FA (male inner eyestalk and outer eyestalk, $r = -0.139$, $N = 124$, $P = 0.124$) nor did the homologous female traits (female inner eyestalk and outer eyestalk, $r = -0.164$, $N = 103$, $P = 0.098$). Male wing traits also failed to show a correlation between width and length (male wing length and wing width, $r = -0.026$, $N = 304$, $P = 0.536$), but female wing width and wing length FA were negatively correlated ($r = -0.137$, $N = 338$, $P = 0.012$). No relationships existed in any of the eight possible between wing and eyestalk correlations apart from female wing width and outer eyestalk FA ($r = +0.247$, $N = 96$, $P = 0.015$). However, under Bonferonni correction, this correlation would not be significant (after correction new $\alpha = 0.006$).

Trait size

All trait size distributions were normal (all $P > 0.05$). The repeatability of trait size measurements was very high in both sexes (all $r > 0.99$, $P < 0.001$). All traits, in both sexes, showed decreases in size with increasing larval density (male inner eyestalk, $F_{1,307} = 447.79$; male outer eyestalk, $F_{1,127} = 264.56$; male wing width, $F_{1,423} = 646.57$; male wing length, $F_{1,409} = 573.49$; male thorax length, $F_{1,402} = 529.14$; female inner eyestalk, $F_{1,298} = 184.44$; female outer eyestalk, $F_{1,103} = 83.97$; female wing width, $F_{1,360} = 335.62$; female wing length, $F_{1,337} = 346.69$; female thorax length, $F_{1,356} = 274.67$, all $P < 0.001$).

Sex dependent effects of larval density on trait size were found in all traits, and were particularly strong for the two eyestalk traits (inner eyestalk, $F_{1,605} = 127.12$, $P < 0.001$; outer eyestalk, $F_{1,203} = 65.66$, $P < 0.001$; wing width, $F_{1,783} = 28.84$, $P < 0.001$; wing length, $F_{1,746} = 11.05$, $P = 0.001$; thorax length, $F_{1,758} = 9.28$, $P = 0.002$).

To control for differences in allometry due to sexual dimorphism, relative values of each trait were calculated using thorax length as a measure of body size. When the two eyestalk traits were regressed on larval density, a conspicuous negative effect of density was still apparent in the male sexual traits (inner eyestalk, regression coefficient, $b = -0.0039$, $F_{1,286} = 168.46$, $P < 0.001$; male outer

eyestalk, b = -0.0033, $F_{1,115}$ = 41.53, $P < 0.001$) and to a lesser degree in the female homologues (inner eyestalk, b = -0.0005, $F_{1,285}$ = 8.02, P = 0.005; outer eyestalk, b = 0.0002, $F_{1,98}$ = 0.56, P = 0.454). The response of relative wing measurements were smaller in males (wing width, b = -0.0003, $F_{1,385}$ = 8.28, P = 0.004; wing length, b = 0.0010, $F_{1,374}$ = 15.03, $P < 0.001$) and females (wing width, b = -0.0001, $F_{1,337}$ = 0.31, P = 0.581; wing length, b = 0.0013, $F_{1,315}$ = 17.34, $P < 0.001$).

Using relative trait size measurements, both sexual traits in males showed a greater response to larval density than their homologues in females (inner eyestalk, $F_{1,571}$ = 87.53, P<0.001; outer eyestalk, $F_{1,213}$ = 30.90, $P < 0.001$). This greater response was also seen within males when the male sexual traits were compared to wing measurements (inner eyestalk vs. wing width, $F_{1,671}$ = 161.07; outer eyestalk vs. wing length, $F_{1,660}$ = 157.40; inner eyestalk vs. wing width, $F_{1,500}$ = 83.54; outer eyestalk vs. wing length, $F_{1,489}$ = 69.87; all $P < 0.001$).

DISCUSSION

Fluctuating asymmetry is widely accepted as a sensitive measure of environmental and genomic stress and has even been proposed to be a good proxy for fitness (Leary and Allendorf 1989; Møller 1997; Møller 1999). This has led to the promotion of FA as a tool for the monitoring of endangered populations and

the detection of individuals at risk (Leary and Allendorf 1989; Parsons 1992; Clarke 1995).

These ideas have been extended to a particular subset of traits, those subject to sexual selection. FA in sexual traits has been proposed to be more sensitive to environmental stress than FA in conventional morphological traits because of their recent history of directional selection (Møller and Pomiankowski 1993, Watson and Thornhill 1994). Møller and Pominakowski (1993) suggested that this recent history of directional selection leads to reduced canalisation of sexual traits, making their development more sensitive to environmental stress. As such these traits are potentially of great interest to conservation biologists when monitoring population stress using FA.

In their review of sexual trait FA, Bjorksten *et al.* (2000b) identified three testable hypotheses that have been made about sexual trait FA. Here we have examined each hypothesis under controlled laboratory conditions and with a defined range of stresses. The first hypothesis predicts that sexual trait FA should be more sensitive to stress than other morphological traits (Moller and Pomiankowski 1993). We found that FA in the two sexual traits (male inner and outer eyestalk) showed no sensitivity to larval density. Neither sexual trait FA were more responsive to larval density than the homologous non-sexual trait FA in females or than non-sexual morphological trait FA in males (wing length and width).

The second hypothesis predicts that mean sexual trait FA should be greater than the mean trait FA for the homologous trait in females. Again the history of directional selection on sexual traits has been evoked to explain this (Møller and Pomiankowski 1993; Watson and Thornhill 1994), the suggestion being that it has selected for weak canalisation. In line with the hypothesis, we found that sexual trait mean FA was higher for both the male inner and outer eyestalk. However, this effect disappeared once we took account of the larger size of these male traits using relative FA. Mean relative FA in the male sexual traits was smaller than either the female homologous traits (Fig. 5.3) or the male non-sexual traits.

The third hypothesis predicts that sexual trait FA should decrease with trait size because only the highest quality individuals are able to grow large ornaments while maintaining bilateral symmetry (Møller and Pomiankowski 1993). Contrary to this prediction, we found that both male sexual traits showed no relationship between size and FA. Nor were the relationships between FA and size more negative in the sexual traits than either the female homologous traits or the male non-sexual traits.

This lack of support for any of the hypotheses about sexual trait FA is consistent with a recent review of other relevant experimental studies (Bjorksten *et al.* 2000b). Møller's (1990) original study of barn swallows stands alone in supporting the notion of greater sensitivity in sexual traits. All other studies have found contradictory or inconsistent patterns (Bjorksten *et al.* 2000b). In addition

to the lack of response to larval density in sexual trait FA, we found a similar lack of response amongst the non-sexual trait FA that were measured. Our data shows a consistent failure of trait FA to respond to larval density and that this is not dependent on the type of trait measured, be it sexual or morphological.

In a study similar to ours, Hunt and Simmons (1997) manipulated the amount of dung available to developing beetle larvae. Adult weight increased with increasing dung mass as did the size of male horns, the sexually selected character. In contrast to our study, horn asymmetry was found to be greater in broods with more dung. Although not all sexual trait FA hypotheses were tested, beetle horn asymmetry was found to increase with increasing horn size, the reverse relatioinship of that expected.

Previous studies that have failed to find consistent relationships between trait FA and stress have used composite FA measures, summing FA scores across several traits (Whitlock 1993; Dufour and Wetherhead 1996). Composite FA has been suggested to be far more sensitive to stress than individual trait FA (Leung *et al.* 2000). We used a number of composite FA measures: a male sexual trait composite FA, a female eyestalk composite FA, a male wing composite FA, a female wing composite FA, and an overall composite FA combining information from all traits. None of these composite FA measures showed an increase with larval density. Nor was the sexual trait composite FA more sensitive to larval density than the other composite measures. Our results are consistent in failing to

provide any support for widely cited hypotheses about the FA of sexual traits. Furthermore the patterns that we describe here are in strong agreement with those documented in parallel studies on this species conducted by us (David *et al.* 1998; Bjorksten *et al.* 2000a).

Very little is known about the mechanisms that influence bilateral development and how these may lead to FA (Møller and Swaddle 1997) beyond a few studies (Swaddle and Witter 1997, Klingenberg and Nijhout 1998). These studies have suggested that multiple measures of the same traits should have related FA, as they rely on the same developmental pathways during their growth. However, we found that there was no correlation between FA measures within eyestalks or wings. This result provides further evidence that the FA we measured was unrelated to the stressful conditions under which flies developed.

In contrast to these negative results with FA, all morphological traits decreased in size as nutritional stress increased. This shows that our choice of variable amounts of food and the range over which it was applied was genuinely stressful. In addition, we were able to demonstrate that the sizes of both male sexual traits (inner and outer eyestalk) were more sensitive to larval density than the homologous non-sexual traits in females or the non-sexual traits in males (wing length and width). These results accord with previous studies that have demonstrated strong condition-dependence of male eyespan variation in this species (David *et al.* 1998, 2000, Wilkinson and Taper 1999).

These results raise doubts over the utility of FA to conservation biologists as a measure of populations or individuals under stress. A single negative study cannot overrule the large body of data that has been accumulated over the last few years about FA and stress. However, much of this data does not come from well controlled, experimental studies, and so is potentially confounded by other factors. In addition, amongst experimental studies, there are many that have reported inconsistent responses (Bjorksten *et al.* 2000b). Some traits in some species respond to some stresses, but it is hard to predict whether general patterns will emerge. Attempts have been made to resolve inconsistencies between and within studies of FA using meta-analysis (Leung and Forbes 1996; Møller and Thornhill 1997, 1998; Møller 1999; Vøllestad *et al.* 1999). However the use of meta-analysis in this context is controversial because of concerns that it is inappropriate (Leamy 1997; Palmer and Strobeck 1997; Whitlock and Fowler 1997; Clarke 1998).

The meta-analysis technique requires the pooling of comparable studies which represent the testing of a single global hypothesis. However, often meta-analyses of the heritability, or sources of variation in FA pool data from many different species, types of trait, stresses and experimental procedures, which would seem to contradict assumptions of this type of analysis (Palmer 1999). In his review of Moller and Thornhills' (1998), Palmer found significant bias in sample exclusion and selective reporting which inflated effect sizes in the meta-

analysis. Further to this, Simmons *et al.* (1999) found that the results of studies investigating male sexual trait FA and its relationship with female mate choice were dependent upon when they were published. Studies were more likely to find a significant result (females preferring males with symmetrical ornaments) if they were published in the early nineties rather than the late nineties. Simmons *et al.* suggested that the decline in significant results through the nineties was due to the introduction of stringent statistical methods for analysing FA and more thorough testing of theoretical ideas. Could the susceptibility of trait asymmetry to stress be another example of this phenomena?

Unless further studies present a different picture, conservation biologists would be ill advised to rely on sexual trait FA as a diagnostic tool is assessing populations or individuals under stress.

REFERENCES

Bjorksten, T., David, P., Pomiankowski, A. and Fowler, K 2000a. Fluctuating asymmetry of sexual and non-sexual traits in stalk-eyed flies. Journal of Evolutionary Biology, 13: 89-97.

Bjorksten, T.A., Fowler, K. and Pomiankowski, A. 2000b. What does sexual trait FA tell us about stress? Trends in Ecology and Evolution, 15:163-166.

Bourguet, D. 2000 Fluctuating asymmetry and fitness in *Drosophila melanogaster.* Journal of Evolutionary Biology, 13: 515-521.

Campbell, W.B., Emlen, J.M. and Hershberger, W.K. 1998. Thermally induced chronic developmental stress in coho salmon - integrating measures of mortality, early growth and developmental instability. Oikos, 81: 398-410

Clarke, G.M. 1995. Relationship between developmental stability and fitness: Application for conservation biology. Conservation Biology, 9: 18-24.

Clarke, G.M. 1998. Developmental stability and fitness: The evidence is not quite so clear. The American Naturalist, 152: 762-766.

Clarke, G.M., Yen J.L. and McKenzie, J.A. 2000. Wings and bristles: character specificity of the asymmetry phenotype in insecticide-resistant strains of *Lucilia cuprina*. Proceedings of the Royal Society of London, Series B, 267: 1815-1818.

David, P., Hingle, A., Greig, D., Rutherford, A., Pomiankowski A. and Fowler, K. 1998. Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. Proceedings of the Royal Society of London, Series B, 265: 1-6.

David, P., Hingle, A., Fowler, K., and Pomiankowski, A. 1999. Measurement bias and fluctuating asymmetry estimates. Animal Behaviour, 57: 251-253.

Dufour, K.J., and Wetherhead, P.J. 1996. Estimation of organism wide asymmetry in red-winged blackbirds and its relation to studies of mate selection. Proceedings of the Royal Society of London, Series B, 263: 769-775.

Houle, D. 2000. A simple model of the relationship between asymmetry and developmental stability. Journal of Evolutionary Biology, 13: 720-730.

Hunt, J., and Simmons, L.W. 1997. Patterns of fluctuating asymmetry in beetle horns: an experimental examination of the honest signalling hypothesis. Behavioral Ecology and Sociobiology, 41: 109-114.

Klingenberg, C.P., and Nijhout, H.F. 1998. Competition among growing organs and developmental control of morphological asymmetry. Proceedings of the Royal Society of London, Series B, 265: 1135-1139.

Lagesen, K., and Folstad, I. 1998. Antler asymmetry and immuntiy in reindeer. Behavioral Ecology and Sociobiology, 44: 135-142.

Leamy, L. 1997. Is developmental stability heritable? Journal of Evolutionary Biology, 10: 21-29.

Leary, R.F., and Allendorf, F.W. 1989. Fluctuating asymmetry as an indicator of stress: Implications for conservation biology. Trends in Ecology and Evolution, 4: 214-217.

Leung, B., and Forbes, M.R. 1996. Fluctuating asymmetry in relation to stress and fitness: Effects of trait type as revealed by meta-analysis. Ecoscience, 3: 400-413.

Leung, B., Forbes, M.R., and Houle, D. 2000. Fluctuating asymmetry as a bioindicator of stress: Comparing efficacy of analyses involving multiple traits. American Naturalist, 155: 101-115.

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

Møller, A.P. 1997. Developmental stability and fitness: a review. The American Naturalist, 149: 916-932.

Møller, A.P. 1999. Asymmetry as a predictor of growth, fecundity and survival. Ecology Letters, 2: 149-156.

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

Møller, A.P. and Swaddle, J.P. 1997. Asymmetry, developmental stability and evolution. Oxford University Press, Oxford.

Møller, A.P. and Thornhill, R. 1997. A meta-analysis of the heritability of developmental stability. Journal of Evolutionary Biology, 10: 1-16.

Møller, A.P. and Thornhill, R. 1998. Bilateral symmetry and sexual selection: A meta-analysis. American Naturalist, 151: 174-192.

de la Motte, I. and Burkhardt, D. 1983. Portrait of an Asian Stalk-eyed fly. Naturwissenshaften, 70: 451-461.

Palmer, A.R. 1999. Detecting publication bias in meta-analysis: A case study of fluctuating asymmetry and sexual selection. American Naturalist, 154: 220-233.

Palmer, A.R., and Strobeck, C. 1986. Fluctuating asymmetry: measurement, analyses, patterns. Annual Review of Ecological and Systematics, 17: 391-421.

Palmer, A.R. and Strobeck C. 1997. Fluctuating asymmetry and developmental stability: heritability of observable variation vs. heritability of inferred cause. Journal of Evolutionary Biology, 10: 39-49.

Panhuis, T.M. and Wilkinson, G.S. 1999. Exaggerated eye span influences male contest outcome in stalk-eyed flies. Behavioral Ecology and Sociobiology, 46: 221-227.

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

Putman, R.J., Sullivan M.S. and Langbein, J. 2000. Fluctuating asymmetry in antlers of fallow deer (*Dama dama*): the relative roles of environmental stress and sexual selection. Zoological Journal of the Linnean Society, 70: 27-36.

Roy, B.A. and Stanton, M.L. 1999. Asymmetry of wild mustard, *Sinapis arvensis* (Brassicaceae) in response to severe physiological stresses. Journal of Evolulationary Biology, 12: 440-449.

SAS Institute. 1996. JMP statistics for the Macintosh version 3.1.6.

Simmons, L.W., Tomkins, J.L. Kotiaho, J.S., and Hunt, J. 1999. Fluctuating paradigm. Proceedings of the Royal Society of London, Series B: 266, 593-595.

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

Swaddle, J.P., and Witter, M.S. 1997. On the ontogeny of developmental stability in a stabilised trait. Proceedings of the Royal Society of London, Series B, 264: 329-334.

Swaddle, J.P., Witter, M.S. and Cuthill, I.C. 1994. The analysis of fluctuating asymmetry. Animal Behaviour, 48: 986-989.

Vøllestad, L.A., Hinhar, K. and Møller, A.P. 1999. A meta-analysis of fluctuating asymmetry in relation to heterozygosity. Heredity, 83: 206-218.

Watson, P.J., and Thornhill, R. 1994. Fluctuating asymmetry and sexual selection. Trends in Ecology and Evolution, 9: 21-25.

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

Whitlock, M.C. and Fowler, K. 1997. The instability of studies of instability. Journal of Evolutionary Biology, 10: 63-67.

Wilkinson, G.S. and Taper, M. 1999. Evolution of genetic variation for condition-dependent traits in stalk-eyed flies. Proceedings of the Royal Society of London, Series B, 266: 1685-1690.

Wilkinson, G.S. Kahler, H. and Baker, R.H. 1998. Evolution of female mating preferences in stalk-eyed flies. Behavioral Ecology, 9: 525-533.

Woods, R.E., Sgro, C.M., Hercus, M.J. and Hoffmann, A.A.1999. The association between fluctuating asymmetry, trait variability, trait heritability and stress: a multiply replicated experiment on combined stresses in *Drosophila melanogaster.* Evolution, 53: 493-505.

Zar, J.H. 1999. Biostatistical Analysis (fourth edition). Prentice-Hall Inc. New Jersey.

FIGURE LEGENDS

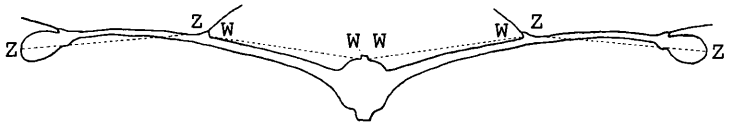
Figure 5.1. Measurements of (a) inner (Z) and outer (W) eyestalk length, (b) wing dimensions corresponding to wing width (X) and wing length (Y) and (c) thorax length (A). All measurements were highly repeatable.

Figure 5.2. Regressions of trait FA on larval density for males (dotted line) and females (solid line), (a) inner eyestalk, (b) outer eyestalk, (c) wing width and (d) wing length.

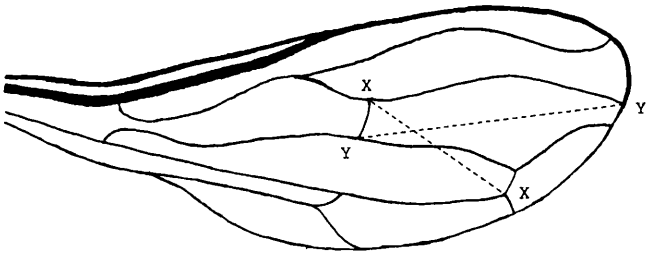
Figure 5.3. Histogram showing the mean relative FA of male and female inner and outer eyestalk FA. Error bars indicate standard errors. For both traits, mean female relative eyestalk FAs are larger.

Figure 5.1.

a)



b)



c)

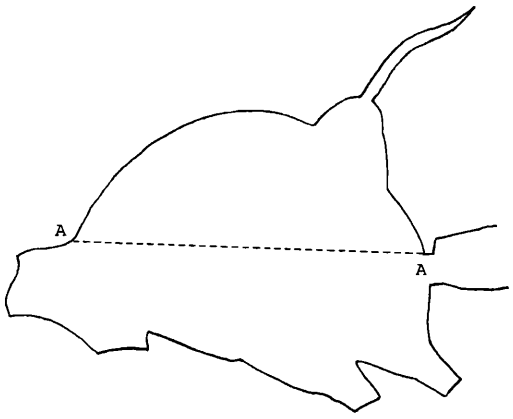
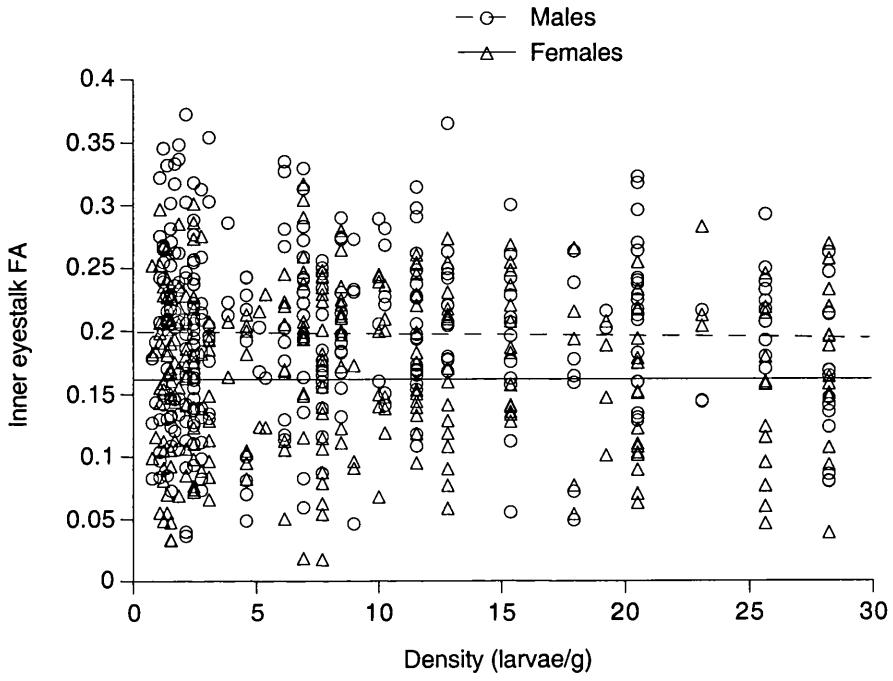
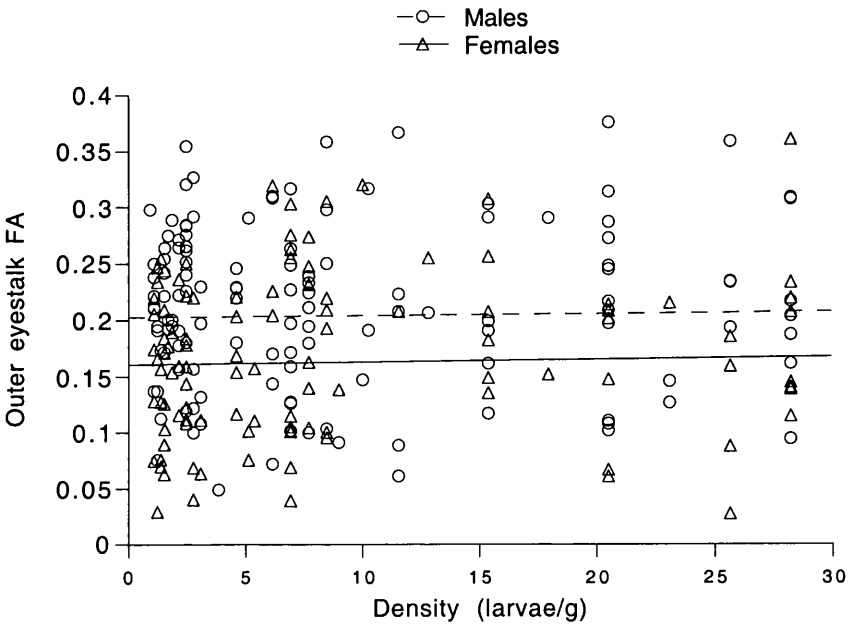


Figure 5.2.

(a)


Males
Females
Inner eyestalk FA
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
Density (larvae/g)

(b)


Males
Females
Outer eyestalk FA
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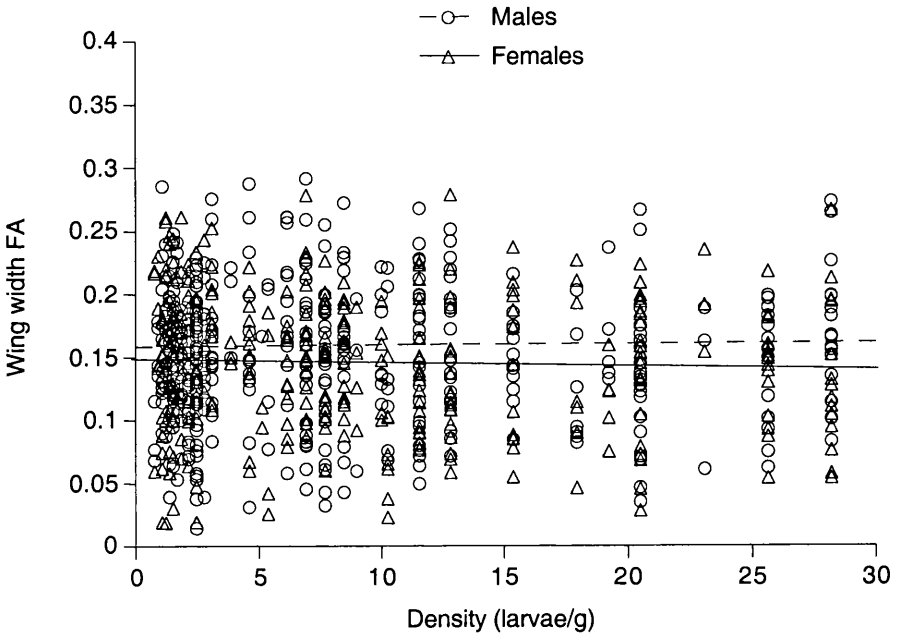
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
Density (larvae/g)

(c)


Males
Females
Wing width FA
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
Density (larvae/g)

(d)

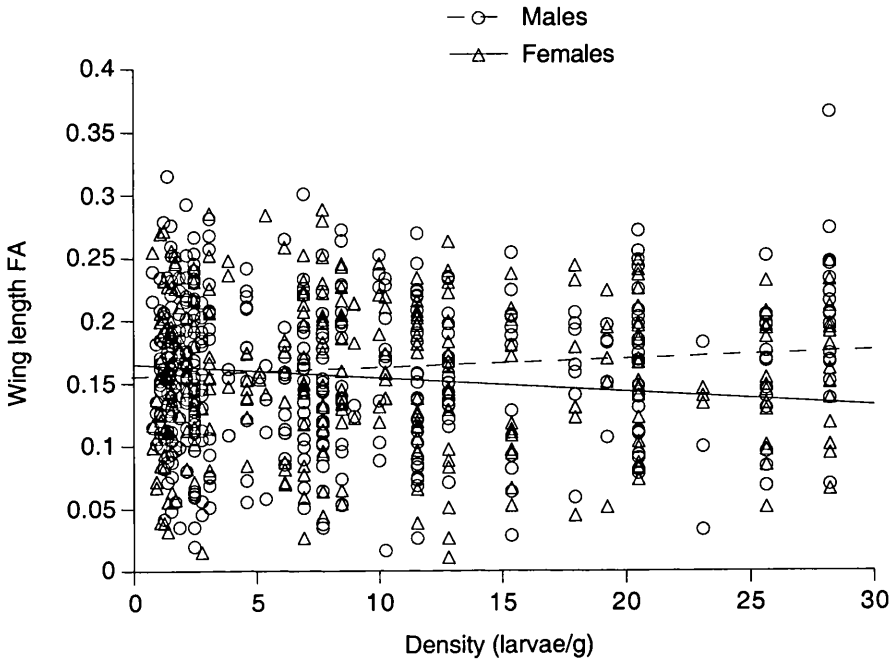
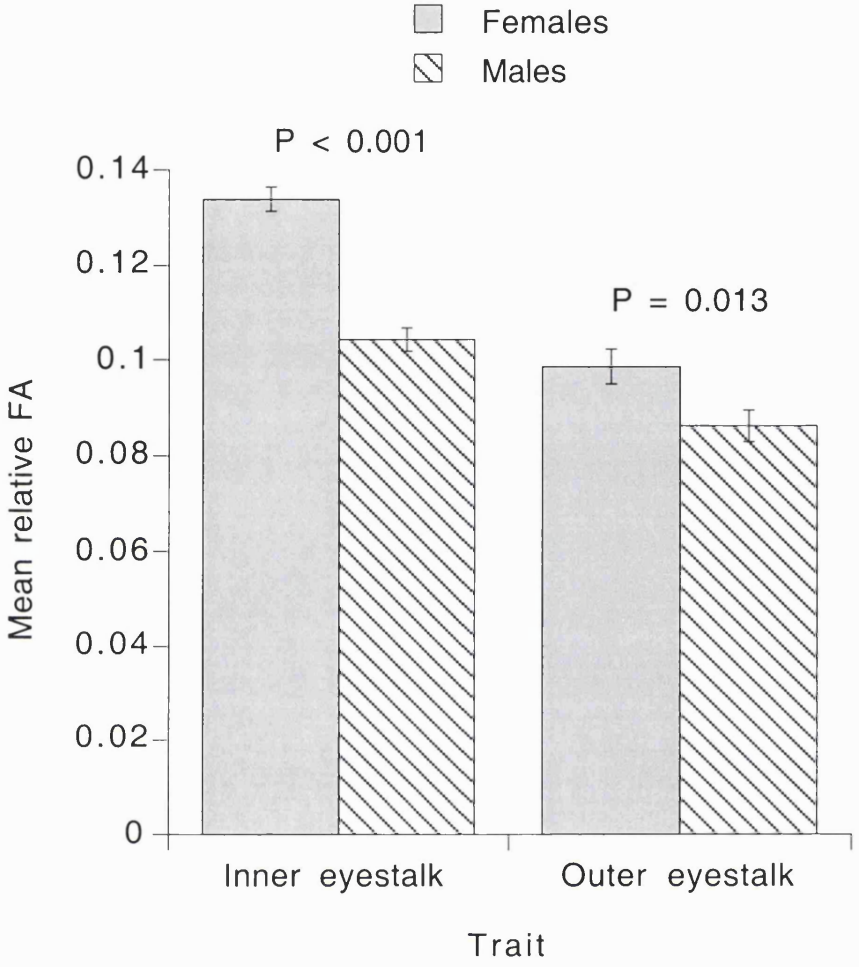

Males
Females
Wing length FA
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
30
Density (larvae/g)

Figure 5.3.


Females
Males
P < 0.001
P = 0.013
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Mean relative FA
Inner eyestalk
Outer eyestalk
Trait

Chapter Six

General discussion

The main objective of this thesis was to investigate condition-dependent mate preference. Condition-dependence of mate choice may play an important role in the rate and direction of evolution of male traits and female preferences (chapter one). Evidence for this phenomenon has been mostly limited to studies of the effect of parasite interactions on female mating behaviour (Poulin 1994; López 1999; but see also Zuk *et al.* 1998). Other examples of condition-dependent mate choice concentrate on variation in particular phenotypes that may be linked to condition (Jennions *et al.* 1994; chapter two).

I manipulated condition in female stalk-eyed flies either permanently or transiently. Permanent changes were achieved by culturing larvae at different larval food densities. This produced flies of varying eyespan. Transient changes in condition were achieved by feeding adult flies sucrose media instead of their normal corn diet.

Both means of manipulating condition resulted in a reduction in female preference. In the permanent stress experiment (chapter two), the reduction in

female preference appeared to be dependent upon the difference between the males females had to choose between. This may be because small females had poorer vision, making it more difficult for them to distinguish between large and small eyespan males. In the transient stress experiment (chapter three), the reduction in female preference in sucrose fed flies was accompanied by a reduction in female egg production. This loss of discrimination may be an adaptive change if female choice is costly as sucrose fed flies have very low fertility and are unlikely to benefit from mating with large eyespan males.

Recent research has also shown that large *C. dalmanni* females produce more eggs than their small counterparts and that their eggs are also larger (Claire Grant per. comm.). This again links condition-dependent mate choice to fecundity. Is this a general phenomenon? I looked at two manipulations of female condition and in both cases the decrease in mate choice was associated with a decrease in fertility. Investigation of these links requires more work. In particular, the consequences for theoretical models of sexual selection has not been analysed. One theoretical paper has shown that even if parasitism reduces female condition and hence mate choice, this has little effect on sexual selection if infected females also have reduced reproductive success (Vickery and Poulin 1998). But it is unclear how general this result is, and whether it applies to other causes of condition variation.

My investigations into the effects of condition on female mate preference conform to the presently held belief that low condition females will have weaker preferences compared with high condition females (Bakker 1999). In only one study, using sticklebacks, has a reduction in female condition led to females preferring a different type of male to high condition females (Bakker *et al.* 1999). The costs of choice in this experiment were removed to the best of the ability of the investigators so even low condition females could choose between males. Could it be that high and low condition females have different optimum phenotype mates? This may be true if the benefits of mating a desirable male are dependent upon female condition. This type of variable benefit for a male phenotype is found in the collard flycatcher. In this species females prefer to mate males with large white head patches late in the breeding season (Qvarnstrom *et al.* 2000). Late in the breeding season male head patch size correlates with reproductive success. However females do not prefer males with large head patch size early in the season when this trait does not correlate with reproductive success. Bakker *et al.'s* sticklebacks may be another example of this type of adaptive plasticity in mate choice.

In my investigations, the costs of mate choice were also reduced as far as possible. Why then, if there was no cost to being choosy did females not exert their preferences? In part this may be because it is energetically costly for females to reject males. This appears to be the means by which females exert their choice

in *C. dalmanni* (personal observation AH). In the restricted space of the choice chamber, females often appear to exert their choice by physically repulsing a male or by running away. In the wild, avoiding males may be easier as females can simply fly away. To see if this form of rejection behaviour is energetically costly, metabolic rate could be assessed using CO_2 monitoring equipment attached to fly cages. Potentially it is possible to find out the relative cost of mating versus refusal behaviour.

Competition amongst females for copulations with males, and in particular with large eyespan males, may have increased the effects of eyespan-dependent mate choice if large eyespan females had a competitive advantage (chapter four). I found no evidence for competition for access to mates, suggesting that the results found in chapter two would be unaffected by interactions between large and small eyespan females. The female competition experiment failed to find an effect of female eyespan on mate choice. This was surprising as differences in mate choice were found in chapter two when males differed by about the same amount as the males used in chapter three. This may be due to many procedural differences between the experimental designs. As such it is difficult to conclude that female competition will not affect eyespan-dependent mate choice.

Finally, I also investigated the effects of nutritional stress on sexual trait fluctuating asymmetry (FA). It has been suggested that FA may be used by conservation biologists to monitor endangered populations as FA has been

correlated with a number of fitness components (Clarke 1995; Møller 1999). Sexual trait FA has been proposed to be particularly sensitive to environmental and genomic stress (Bjorksten *et al.* 2000). I found that a permanent reduction in condition induced by larval food density had no effect on sexual trait FA or any other trait FA measured (chapter five). It would appear that conservation biologists would be ill advised to rely on measurements of FA to monitor stress.

What aspects of my investigations do I believe warrant further study? I would be particularly interested in investigating the role of post-copulatory mate choice in determining paternity in this species. This may be achieved in a number of ways, particularly radio-labelling males of certain phenotypes. More accurately, microsatellites may be used to type offspring and sperm from the spermathecae. In such a high mating rate species, where even choosy females are likely to mate with several males in a day under laboratory conditions, it is imperative to identify the reproductive success conferred by these matings. Field observation have shown that females often eject spermatophores soon after copulation and this may be a means of post-copulatory female mate choice (Wilkinson and Reillo 1994). Although I have never observed such behaviour, I did not look directly for it. Sperm competition is likely to be intense in this species and this also warrants further investigation.

Finally, the relevance of my studies to mate choice needs to be assessed in the wild. Every effort was made during the investigations to use parameters which

fall within the range found in the wild. Nevertheless it would be useful to have observations of copulations in the wild, rather than just roosting parameters. Measurement of variation in female eyespan and mate preference for entire leks of flies would be particularly pertinent to my research, as would egg counts of preferring females compared to randomly mating females.

REFERENCES

Bakker, T.C.M. 1999. The study of intersexual selection using quantitative genetics. Behaviour, 136: 1237-1265.

Bakker, T.C.M., Künzler, R. and Mazzi, D. 1999. Condition related mate choice in sticklebacks. Nature, 401: 234.

Bjorksten, T.A., Fowler, K. and Pomiankowski, A. 2000. What does sexual trait FA tell us about stress? Trends in Ecology and Evolution, 15:163-166.

Clarke, G.M. 1995. Relationship between developmental stability and fitness: Application for conservation biology. Conservation Biology, 9: 18-24.

Jennions, M.D., Backwell, P.R.Y. and Passmore, N.I. 1995. Repeatability of mate choice: the effect of size in the African painted reed frog, *Hyperolius marmoratus*. Animal Behaviour, 49: 181-186.

López, S. 1999. Parasitized female guppies do not prefer showy males. Animal Behaviour, 57: 1129-1134.

Møller, A.P. 1999. Asymmetry as a predictor of growth, fecundity and survival. Ecology Letters, 2: 149-156.

Poulin, R. 1994. Mate choice decisions by parasitized female upland bullies, *Gobiomorphus breviceps*. Proceedings of the Royal Society of London, Series B, 256: 183-187.

Qvarnstom, A., Part, T. and Sheldon, B.C. 2000. Adaptive plasticity in mate preference linked to differences in reproductive effort. Nature, 405: 344-347.

Vickery, W.L. and Poulin, R. 1998. Parasitized non-choosy females do slow down the process: a reply to J. Rolff. Behavioral Ecology and Sociobiology, 44: 75-76.

Wilkinson, G.S. and Reillo, P.R. 1994. Female choice response to artificial selection on an exaggerated male trait in a stalk-eyed fly. Proceedings of the Royal Society of London, Series B, 255: 1-6.

Zuk, M., Tania, K., Robinson, S.I. and Johnsen, T.S. 1998. Parasites influence social rank and morphology, but not mate choice, in female red junglefowl, *Gallus gallus*. Animal Behaviour, 56: 493-499.