Sexual selection and the benefits of mating with attractive males in *Drosophila simulans*

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signed: M L Taylor

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ABSTRACT

Over the last century, sexual selection has grown from a controversial theory into a vast field of theoretical and empirical research. Although Darwin outlined two major mechanisms within his theory, male-male competition and female mate choice, the latter has promoted a wealth of research by virtue of its complexity. Despite decades of research into how female preferences and sexually selected traits have evolved, there is still little consensus as to why females prefer the males they do. Preferences are thought to evolve from either direct selection on the preference, as females themselves benefit directly from mating with a preferred male, or through indirect selection on the preference via offspring fitness. In all cases however, female preferences should compensate for the costs of discriminating between potential mates, if they are to remain overall beneficial. The fitness benefits of mating with preferred males were investigated here using the fruitfly *Drosophila simulans,* employing a range of behavioural, phenotypic and quantitative genetic approaches. The findings presented here indicate that female *Drosophila simulans* do not gain directly from mating with a preferred male. Multiple mating can increase fecundity, although costs from male harassment can reduce the net benefit. They also indicate that females may benefit indirectly from mating with attractive males as attractiveness is heritable and sons of preferred males are themselves preferred. There is also evidence that attractive males are successful in both the pre- and post-copulatory sense, as preferred males are better sperm competitors than less-preferred males. However, although there appear to be benefits from preferred males via their sons, there appear to be no benefits from males via their daughters' fitness. These findings collectively indicate that female preferences in *Drosophila simulans* are driven by indirectly selected benefits (via Fisherian sons), and that females benefit directly from mating multiply.

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AUTHOR'S DECLARATIONS

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The views presented here represent my own survey and interpretation of the current literature, under the guidance of Dr David Hosken and Dr Nina Wedell.

CHAPTER 2: Sexual selection and female fitness in *Drosophila simulans*

Dr David Hosken and Dr Nina Wedell provided guidance for planning and structure of all experimental procedures and preparation of the manuscript. I collected the data, conducted the analysis and am first author on the manuscript.

CHAPTER 3: Multiple mating increases female fitness in *Drosophila simulans*

Dr David Hosken and Dr Nina Wedell provided guidance for planning and structure of all experimental procedures and preparation of the manuscript. I collaborated with Clare Wigmore to collect the data. I conducted the analysis and am first author on the manuscript.

CHAPTER 4: The heritability of attractiveness

Dr David Hosken and Dr Nina Wedell provided guidance for planning and structure of all experimental procedures and preparation of the manuscript. I collected the data, conducted the analysis and am first author on the manuscript.

CHAPTER 5: Attractive males have greater success in sperm competition

Dr David Hosken and Dr Nina Wedell provided guidance for planning and structure of all experimental procedures and preparation of the manuscript. I collaborated with Katherine Hoyle to collect the data, and am second author on the manuscript.

CHAPTER 6: Attractive males do not sire superior daughters

Dr David Hosken and Dr Nina Wedell provided guidance for planning and structure of all experimental procedures and preparation of the manuscript. I collected the data, conducted the analysis and am first author on the manuscript.

CHAPTER 7: Conclusions and prospects: sexual selection and the benefits of mating with attractive males in *Drosophila simulans*

The conclusions presented in this Chapter represent my own interpretation of the data presented in the previous chapters, under the guidance of Dr David Hosken and Dr Nina Wedell.

CHAPTER 1

Introduction: sexual selection and female mate choice

Sexual selection and female mate choice

It has now been over a century since Darwin first proposed his general theory of sexual selection: “the advantage which certain individuals have over others of the same sex and species solely in respect of reproduction” (Darwin 1871). His was prompted by his observations of male armaments used in male-male competition and absurdly elaborate ornaments used by males to display to an audience of females, which seemed counterintuitive to his theory of natural selection. He reasoned that such traits could still be favoured by selection if they gave their owners greater success at securing a mate and felt that this form of selection deserved a distinction from natural selection: ‘Sexual selection… depends, not on a struggle for existence, but on a struggle between the males for possession of the females; the result is not death to the unsuccessful competitor, but few or no offspring’ (Darwin 1859, pp 88, reviewed in Andersson 1994). His concept of male traits evolving under female choice in particular was not immediately popular, partly because it was widely assumed that monogamy was the prevailing mating system. If all individuals paired monogamously, there would be no benefit to mating with a competitive or attractive mate, and most mating behaviour could be interpreted from a naturally selected impetus to find a mate of the right species (Cronin 1991). The recognition of widespread polygamy and variation in reproductive success generated a vast field of theoretical and empirical investigations of Darwin’s ideas, drawing on fields as diverse as evolutionary biology, ethology, population genetics, phylogenetics, speciation, molecular biology and behavioural ecology (reviewed in Cronin 1991; Andersson 1994). Darwin himself characterized female choice as the ability of the male ‘to excite or charm….. the females, which no longer remain passive, but select the most agreeable partners’ (Darwin 1871, pp 398). Female mate choice is now widely accepted as the behavioural, sensory and structural properties in females which bias mating/fertilization success towards males of certain phenotypes (Jennions & Petrie 1997).

However, while the benefits to a victorious male who successfully defeats his rival and secures himself mating access to a female are immediately apparent, the benefits to choosy females are not at all as intuitive. Exercising any discriminatory behaviour in itself incurs costs in terms of energy expenditure in searching for and sampling mates, time lost from other activities (e.g. foraging), and risks such as encountering predators or disease or even the risk of not mating at all (Andersson 1994). Any chosen male must then surely at least compensate the female for these costs, for the preference to persist.

How might females benefit?

The simplest explanation for the evolution of female preferences is direct selection on the preference via a direct benefit to female reproductive success. For example, females may have greater fecundity from mating with preferred males, or gain nutritional benefits from nuptial gifts that males provide (Halliday 1978; Thornhill & Alcock 1983). Females may benefit because preferred males provide better material resources, such as breeding territories or parental care to offspring (Reynolds & Gross 1990; Andersson 1994). In all cases, the reproductive success, i.e. fecundity or fertility, of females is directly elevated by mating with a preferred male.

In some species there appear to be no apparent opportunities for direct benefits, for example where all females receive sufficient sperm from a mating episode, or where males provide no material resources. This has inspired a suite of explanations that suggest female preferences evolve through indirect selection via benefits accrued through females' offspring (reviewed in Andersson 1994; Jennions & Petrie 2000;

Arnqvist & Rowe 2005). All of these models necessarily involve a genetic correlation between the preference and the sexually selected trait. Fisher's 'runaway process' (Fisher 1930; Lande 1981; Kirkpatrick 1982) was the first genetic-based model to describe mating advantages to males with preferred traits purely on the basis of the genetic correlation between the male trait and female preference. The preference itself was assumed to be initiated by a naturally selected advantage to female fitness, but proliferates solely in conjunction with the male trait as females produce sons with their father's preferred trait(s), and daughters who prefer the trait. The Fisher process may also be characterized by rapid, directional selection on the trait, to the point where the trait size becomes balanced by the naturally selected costs of producing the trait and its sexually selected mating advantage (Lande 1981; Kirkpatrick 1982; Arnold 1983). 'Indicator processes' is the collective term for models that predict male traits to be indicators of the condition and/or genetic quality of the male (reviews in Andersson 1994; Jennions & Petrie 2000; Neff & Pitcher 2005; Andersson 2006). In mating with a highly ornamented (higher quality) male, females will produce offspring that inherit these apparent 'good genes' from their father, and thereby gain indirect fitness benefits for themselves (Williams 1966). Zahavi's 'handicap' principle (Zahavi 1975) encapsulated this general idea on the specific premise that male traits are honest signals, i.e. that only males of higher quality and condition are able to fully produce and display costly sexually selected traits. Related versions of this idea also posit male traits as indicators of specific fitness traits such as immunocompetence (Hamilton & Zuk 1982), or non-additive genetic benefits such as genetic compatibility with the female (Zeh & Zeh 1996; Tregenza & Wedell 2000; Neff & Pitcher 2005). The potential for any indirect benefit to sustain selection on female preferences is reliant on the persistence of heritable variation in sire quality or attractiveness. Theory predicts that strong, directional selection via female choice erodes the variation in male fitness, leaving females with little to gain from choosing between males (Kirkpatrick & Ryan 1991; Andersson 1994). This so-called ‘lek paradox’, inspired by the highly skewed mating success of males in lekking species, could be resolved by any number of solutions to the problem of maintaining additive genetic variation, (e.g. Hamilton & Zuk 1982; Rowe & Houle 1996; Tomkins et al. 2004; Radwan 2008) and many studies do report significant heritability in sexually selected traits (Pomiankowski & Møller 1995).

Some authors have recently pointed out that ‘good genes’ may be selected incidentally by females preferring traits evolved under a ‘Fisherian runaway’ process, as investment in sexual traits may be so costly that they function in the same manner as ‘indicator’ traits, or have incidental effects on other expressions of viability, e.g. parasite load (Balmford & Read 2001). Equally, benefits akin to ‘Fisher’s sons’ may appear from the genetic linkage that develops between preference and ‘good genes’ fitness under assortative mating (Kirkpatrick & Ryan 1991). This has led some to suggest that the conventional dichotomy in experimental attempts to prove either process is currently in effect is flawed, and that both processes should be regarded as part of a continuum of indirect benefits that evolve in response to the strength of direct selection on the preference (Kokko 2001; Kokko et al. 2002). However, this specifically invokes the assumptions that traits are currently costly and condition-dependent, and that there is a mechanism to maintain heritable variation in male fitness. A fundamental distinction between these two processes, however, is that male traits evolving under the Fisher process do so solely via their linkage with the preference, and at equilibrium average males have the highest fitness. In contrast, ‘indicator processes’, by virtue of acting over a much larger portion of the genome and indicating overall male quality, represent constant selection for increased male fitness, independent of the preference itself (Andersson 1994; Cameron et al. 2003; Arnqvist & Rowe 2005). It is doubtful then whether these two processes inevitably effect qualitatively similar evolutionary dynamics, although one merit of this debate is that researchers now have a better insight into how benefits via offspring fitness may appear to the experimental observer (Kokko 2001; Cameron et al. 2003).

A recent addition to the repertoire of explanations for female mate choice is sexually antagonistic coevolution driven by sexual conflict (Rice & Holland 1997; Holland & Rice 1998; Gavrilets et al. 2001; and reviewed in Arnqvist & Rowe 2005). This stems from the fundamental difference in reproductive investment by each sex, in a sexually reproducing species (Parker 1979; Lessells 1999; Parker 2006). Males and females investing differentially in traits to optimise their respective fitness inevitably leads to the potential for sexual conflict over such issues as mating rate, re-mating rate or parental investment, as traits that increase fitness in one sex have a directly negative effect on the fitness of the other, by virtue of pulling fitness towards one optimum and away from the other (Parker 1979). As with other sexual selection processes, the intensity of conflict is influenced by the potential to find alternative mates arising from inequalities in parental investment (Trivers 1972), the operational sex ratio (Emlen & Oring 1977) or rates of reproduction (Clutton-Brock & Vincent 1991). In this context female choice serves to *avoid* the costs of males that depress their fitness by causing them to behave suboptimally (e.g. mating too frequently). Attractive males are then those better able to force or coerce females into decisions that benefit themselves, irrespective of the costs to females. These costs are broadly categorised as phenotype-dependent and phenotype-independent respectively (Parker 1979; Arnqvist & Rowe 2005). The prominent feature of female preferences evolving under sexually antagonistic coevolution is the depressed direct fitness of the female from mating with a ‘preferred’, or perhaps more suitably termed ‘manipulative’ male.

Other considerations

More recently, the focus of the benefits of mate choice have widened to consider the interactive effects with other forms of sexual selection, e.g. the impact that male-male competition may have on the availability of males from which females can choose. There is some evidence that processes directly affecting male-male competition and female choice may not always be complimentary (reviewed in Wong & Candolin 2005). Polyandry also introduces another conundrum to sexual selection, that of processes occurring post-copulation. Females mating multiply in one reproductive cycle inevitably leads to sperm from more than one male competing for fertilizations of available ova. Sperm competition (Parker 1970) and cryptic female choice (Eberhard 1996) are the post-copulatory equivalents of male-male competition and female choice respectively, and are potentially explained through the same array of benefits as pre-copulatory female choice (Eberhard 1996; Birkhead & Møller 1998; Jennions & Petrie, 2000). Empirically disentangling pre- and post- copulatory effects requires a more sophisticated approach than measuring the outcome of standard mate choice tests, and the use of morphological markers, molecular techniques and artificial insemination techniques have begun to demonstrate the prevalence of post-copulatory selection on male and female traits (reviewed in Jennions & Petrie, 2000; Pizzari & Birkhead 2002; Evans et al. 2003; Hosken et al. 2003).

The potential impact of so many interacting processes and mechanisms of selection on the benefits of female mate choice might be cause for simply distilling studies of mate choice into establishing whether the net outcome of female preferences are beneficial or costly. Certainly much debate has centred on the relative influences of direct and indirect benefits in offsetting direct costs (Kirkpatrick 1996; Kirkpatrick & Barton 1997; Cameron et al. 2003; Chapman et al. 2003; Cordero & Eberhard 2003; Kokko et al.

2003; Hosken & Tregenza 2005). For example, the ‘sexy sons’ effect, often aligned with the Fisher process, suggests that attractive males that directly depress female fitness can still be beneficial for females if their sons inherit the attractiveness, and thereby mating success, of their fathers (Weatherhead & Robertson 1979). This particular case, however, has been found to be unsustainable under direct selection (i.e. costs) on female preferences (Kirkpatrick 1985) and indirect benefits in general are likely to be relatively ineffectual unless direct selection on female preferences is weak or neutral (Kirkpatrick 1996; Kirkpatrick & Barton 1997). One approach has been to use experimental evolution to examine the net consequences of sexual selection over time (Holland & Rice 1999; Holland 2002; Martin & Hosken 2003; Stewart et al. 2005). While this is a valuable approach for explaining the net consequences of female mating preferences at a population level, it still remains that examining the relative component parts of benefits from preferred males in individuals provides the insight into which process(es) may be currently in effect (Arnold 1983; Wade 1987).

Evidence for benefits of female choice

Evidence for direct benefits to females has now been found across a diverse range of taxa including birds, insects, fish, amphibians and mammals (reviewed in Andersson 1994). A meta-analysis of the effect sizes of direct benefits by Møller & Jennions (2001) concluded relatively small effect sizes of direct benefits. For example, although 39 studies across 51 species (insects and birds) reported a positive association of female fecundity and preferred male traits, only 2.3% of the variance in female fecundity could be attributed to preferred male traits. They concluded that direct benefits had a relatively small effect on female mating preferences in the studies reviewed. There is less evidence for indirect selection on female preferences than for direct selection. A meta-analysis of studies investigating indirect benefits by Møller & Alatalo (1999) concluded an even smaller effect size than direct benefits – just 1.5% of the variance in offspring viability (in this case survivorship) was attributable to preferred male traits. In this study 20 of 22 studies showed a positive effect of preferred male traits on offspring viability. However, since most of the studies reviewed in both meta-analyses considered benefits accrued from mating with only one male, similar reviews of the direct and indirect benefits of multiple mating may reflect a truer picture. A review of polyandry in insects (Arnqvist & Nilsson 2000) reported an effect size of 5% for female fecundity from mating multiply, suggesting that direct benefits may accrue over several males. No effect sizes for indirect benefits from polyandry were available in the review by Jennions & Petrie (2000). One possible explanation for the relatively weak evidence for 'good genes' effects is that viability benefits may be traded-off against the costs of producing sexually selected traits, making them appear more 'Fisherian' in nature (Kokko 2001), and yet a meta-analysis by Jennions et al. (2001) found that most sexually selected traits correlated positively with male adult longevity. Much of the current evidence then appears to advocate the predominance of male traits as honest signals of condition, although this does not distinguish between largely environmentally and genetically determined variation in condition among males. However, the small effect size of 1.7% variance in survivorship attributable to sexually selected traits, again, advocates a relatively small effect of indirect viability benefits in general. Studies investigating the Hamilton-Zuk (1982) parasite-load hypothesis (that higher quality males carry fewer parasites) indicate that there are many instances where evidence conflicts with the predictions of the original 'handicap' principle (Getty 2002). A recent re-iteration of the predictions from Zahavi's (1975) theory of honest signalling emphasizes that the original handicap principle specifically predicted a positive correlation between the quality of the male and the investment in the sexually selected trait, and yet the marginal returns from an increased investment in the trait are specifically dependent on the cost and preference functions, which remain largely unknown and apparently taxon specific (Getty 2002). The outcome of this crucial difference is that *a priori* predictions about the relationship between the apparent quality of the male and the quality of his offspring are not currently possible. Consequently investigations into indirect benefits might be best served by simultaneously accounting for several manifestations of indirect benefits using daughters' fitness and sons' mating success (e.g. Jones et al. 1998).

The model system

Sexual selection and the benefits of mating with attractive males were investigated here using *Drosophila simulans*. This fly is thought to have originated in sub-Saharan Africa and separated from its sibling species *D. melanogaster* around 2 million years ago (Powell 1997). Both now occupy a ubiquitous geographical range across temperate and tropical climes. They overlap in geographical range, ecology and behaviour and have been used extensively in comparative studies into speciation events and adaptation (Powell 1997; Capy & Gibert 2004). *D. simulans* presents a suite of characteristics that not only make it amenable to studying aspects of sexual selection and the benefits related to female preference, but also provides comparative evidence to an extremely well-known genus of insects. It has a polygamous mating system, both males and females routinely copulate with multiple mates, and there is no parental care beyond selecting a suitable oviposition site, usually in decaying and fermenting fruit and vegetation. Males and females are sexually dimorphic; males are typically smaller, are more elongated in body shape and have sex-combs on their forelegs, which they use to grip the female during copulation. Females assess males via their courtship ritual of wing raises, 'songs' from wing vibrations, and mounting attempts, but copulation only occurs with female cooperation – there is no forced copulation with sexually mature females (Markow 1996, but see Markow 2000 for evidence of forced copulations in teneral females). Female mating preferences across *Drosophila* have been well-studied (e.g. Pitnick 1991; Barth et al. 1997; Hoikkala et al. 1998; Ritchie et al. 1999; Hine et al. 2002; Pitnick & Garcia-González 2002; Rybak et al. 2002; Droney 2003; Friberg & Arnvqist 2003), but there is no clear consensus as to the net fitness consequences. In some instances, preferred males carry a cost of reduced fecundity or longevity (Pitnick 1991; Pitnick & Garcia-González 2002; Droney 2003; Friberg & Arnqvist 2003), and in others preferred males provide indirect benefits to female fitness (e.g. Partridge 1980; Taylor et al. 1987; Hoikkala et al. 1998; Hine et al. 2002). Some studies have also documented a cost to female fitness of persistent courtship from non-preferred males (Partridge & Fowler 1990; Chapman & Partridge 1996; Friberg & Arnqvist 2003). There is evidence for a female preference based on male body size, but again, previous work has shown mixed results (Partridge & Farquhar 1983; Partridge et al. 1987; Pitnick 1991; Markow et al. 1992; Markow 1996; LeFranc & Bundgaard 2000). There are also only a few explicit tests of the compensatory effect of indirect benefits on costly males in *D. melanogaster* (Orteiza et al. 2005; Stewart et al. 2005). Female *D. simulans* do show considerable aversion to courtship from males after their first mating (Pitnick 1991; Gromko & Markow 1993; personal observations), suggesting multiple mating may be costly.

The fitness consequences of sexual selection are investigated here using a mixture of behavioural observations, ecological manipulations, phenotypic correlations and quantitative genetics. Chapter Two tackles the issue of direct (fecundity) benefits to females from mating with attractive males and examines the impact of male-male competition on female choice. Chapter Three continues on from this to examine the benefits and costs of multiple matings. Chapter Four examines a crucial assumption of indirect benefits models, that sons inherit their father's attractiveness and thereby mating success. Chapter Five examines the relationship between pre- and post-copulatory processes by investigating the sperm competitiveness of attractive males. Chapter Six addresses indirect genetic benefits of attractive males specifically via daughters. Chapter Seven draws together the implications of the preceding chapters and outlines further questions prompted by the findings.

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CHAPTER 2

Sexual selection and female fitness in *Drosophila simulans.*

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ABSTRACT

There is a current debate over the net fitness consequences of sexual selection. Do preferred males increase female fitness or are these males manipulating females for their own benefit? The evidence is mixed. Some studies find that mating with attractive males increases female fitness components, while others show that preferred males decrease measures of female fitness. In this study, we examined some of the fitness consequences of pre-copulatory sexual selection in *Drosophila simulans*. Virgin females were either paired with one male and given an opportunity for one copulation or were exposed simultaneously to two males. This allowed us to compare female preference (copulation latency) and fitness (longevity, lifetime productivity and rate of offspring production) both with and without the influence of male-male competition. When females had access to a single male, neither female longevity, productivity, nor short-term rate of productivity were associated with female preference, and although females mated more quickly with larger males, male size was also not associated with any female fitness measure. Inclusion of male-male competition showed that female longevity was negatively affected by preference, while productivity and rate of productivity was unaffected. This latter experiment also indicated that females preferred larger males, but again, male size was not associated with female fitness. These results indicate that females may not benefit from mating with preferred males, but they may incur survival costs.

Keywords: female preference; male-male competition; fitness; costs and benefits.

INTRODUCTION

There is much current debate about the net fitness consequences of sexual selection (Holland and Rice 1998; Pizzari and Snook 2003; Cordero and Eberhard 2003). Traditional sexual selection models assume that females either benefit from their mate choices or at least suffer no net fitness cost by mating with preferred males (Andersson 1994), and a large body of evidence indicates that females can gain direct and indirect benefits from mating with preferred males. For example, mating with attractive males increases offspring survival in frogs and peacocks (Petrie 1994; Welch et al. 1998), and it has been suggested that sexual selection invariably becomes linked to good genes, so sexual selection not only increases sexual fitness but also naturally selected fitness components (Jennions and Petrie 2000). Frequently, however, studies only investigate one component of fitness (e.g. survival) and/or one component of sexual selection (e.g. female choice). The potential problems of this approach are illustrated by the cockroach *Nauphoeta cinerea* where male-male competition and female choice oppose one another (Moore and Moore 1999*)*. Furthermore, there is no *a priori* reason to expect male-male competition and female choice to act in a reinforcing manner (Moore and Moore 1999; also see Bonduriansky and Rowe 2003).

More recently, it has been suggested that sexual conflict drives sexual selection, with male traits serving to manipulate females in ways that benefit male fitness, irrespective of the cost to females. If so, there may be net female fitness costs to mating with attractive males (Holland & Rice 1998; and see Parker 1979), with sexual selection resulting from females trying to minimise naturally selected costs rather than attempting to maximise sexually selected benefits (Holland and Rice 1998). While the net fitness consequences of sexual selection may be taxon specific, evidence for costly sexual selection is accumulating (Arnqvist and Rowe 2005; and see Arnqvist and Rowe 1995;

2002; Martin & Hosken 2003; Martin et al. 2004; Crudgington et al. 2005; Parker 2006).

Arguably the best-studied species in this context is *Drosophila melanogaster*. This fly has been the subject of intense investigation using a range of approaches. Experimental evolution studies using cytogenetic techniques show that when females are prevented from co-evolving with males, males rapidly become more damaging to them, reducing female lifespan (Rice 1996; also see Wigby and Chapman 2004). Fitness assays using various mutants have shown that male ejaculate components are toxic to females (Chapman et al. 1995; Wigby and Chapman 2005), and these toxins have been implicated in female-lifespan reductions during laboratory evolution (Rice 1996). In the wild, mating males are typically larger than unpaired males (Partridge et al. 1987a,b; Markow 1988), and there is also laboratory evidence that females prefer larger males (Pitnick 1991). However, mating with preferred males decreases female lifetime-reproductive success (Pitnick 1991; Pitnick and Garcia-González 2002; Friberg and Arnqvist 2003). One of the major criticisms of this type of work is that fitness assays do not take into account potential indirect benefits to females (Cordero and Eberhard 2003; Pizzari and Snook 2003). However, large direct fitness costs to females are unlikely to be compensated for by indirect benefits for many reasons (Kirkpatrick 1985; Cameron et al. 2003; Hosken and Tregenza 2005), and recent work indicates that at least in the laboratory, indirect fitness benefits do not compensate for the direct costs of sexual selection in this species (Stewart et al. 2005; Orteiza et al. 2005).

While sexual selection in *D. melanogaster* has been intensely investigated, less is known about its close relative *Drosophila simulans*. This fly has a similar mating system to *D. melanogaster*, with female cooperation required for copulation and male-male competition for mates (Markow 1996). Females are courted by males before copulation and display rejection behaviour by flying or moving away from the male (Gromko and Markow 1993). As with *D. melanogaster*, large male mating advantage has been reported, with bigger males more likely to be in copula (Markow and Ricker 1992; also see Markow et al. 1996). In this study, we investigate some of the fitness consequences of pre-copulatory sexual selection in *D. simulans*. As females determine whether copulation occurs or not, they are predicted to respond to preferred males by copulating after shorter courtship duration. We therefore used the time it took for copulation to begin (copulation latency) as our measure of male attractiveness and female preference (hereafter preference). This fits with definitions of preference - preference reflects females' propensity to mate with certain males (Jennions & Petrie 1997). The use of copulation latency as a measure of preference and attractiveness is also consistent with previous studies, which show that females take longer to mate with hybrid or heterospecific males (i.e. they are less preferred/attractive: Ritchie et al. 1999; Acebes et al. 2003). Additionally, copulation latency is associated with male size and characteristics of courtship song (Ritchie et al. 1999) and has been used extensively in studies of female mate preference in *Drosophila* (e.g. Barth et al. 1997; Ritchie et al. 1999). We assessed some of the fitness consequences of female preference using two components of female fitness (longevity and lifetime progeny production), and conducted this assessment both with and without the influence of male-male competition. While we did not determine the precise details of the male trait(s) preferred by females, we examined whether female *D. simulans* prefer larger males. Specifically we ask: (1) Does mating with a preferred male (in the absence of male-male competition) elevate female fitness? (2) Does pre-copulatory sexual selection in total elevate measures of female fitness? (3) Do *D. simulans* females prefer larger males, and does the inclusion of male-male competition alter female preference?

MATERIALS AND METHODS

Flies

Stock populations of *D. simulans* were derived from 20 isofemale lines supplied by the Centre for Environmental Stress and Adaptation Research, La Trobe University, Australia. Isolines had been cultured from individuals caught from a wild population at Tuncurry, Eastern Australia in March, 2004. Isolines were mixed in a population cage and reared on '*Drosophila* quick mix medium' (supplied by Blades Biological, UK) with yeast and water at 25°C and 12:12 light/dark cycle, to initiate an outbred stock population, which has subsequently been maintained at a population size of >200 individuals for about ten generations. Subsequent housing conditions follow this regime unless reported otherwise. We used wing length as a measure of body size (Gilchrist and Partridge 1999). Wing length was measured with a Leica dissecting microscope connected to a PC digital image analysis system (SPOT basic), and measures were repeatable: 50 flies were measured twice (blind), remounting the wings between measures, and regression of measure one on measure two showed they were very strongly associated ($ß = 1.008$; $r^2 = 0.99$; $p < 0.0001$) and are highly correlated with another structural size measure, hind tibia length ($n = 87$; $r = 0.77$; $p = < 0.001$). We also tested for the effects of storage on wing length (frozen or in ethanol) and found no storage effect: regression analysis shows that measures before and after storage were strongly associated (fresh-frozen: $n = 50$; $ß = 1.015$; $r^2 = 0.99$; $p < 0.0001$; fresh-ethanol: $n = 50$; $ß = 0.99$; $r^2 = 0.99$; $p < 0.0001$).

Fitness benefits of female preference with no male-male competition

Emerging virgin adults from the stock population were collected every 12 h, separated and housed by sex with an excess of the culture medium for 3 days before experimental pairings. A total of 120 females were used for the experiment, split over two blocks.

Matings were conducted between 09:00-12:00 (equivalent to the first 3 h of 'daylight' the flies would normally experience and corresponding with the period of peak mating activity in natural populations (Gromko and Markow 1993)). All flies were 3 days old at the time of pairing to ensure full sexual maturity, and therefore negate any effects of female receptivity (Spieth 1974). The evening before mating, one male was haphazardly selected and aspirated into each mating vial (40ml) containing culture medium. On the day of mating, one female was added to each vial and continuously observed for 3 h or until a single mating had concluded, at which time males were removed and stored for future wing measurements. Ambient temperature and the time of female introduction and the start and end of copulation were recorded. Copulation latency (the time from female introduction to commencement of copulation) was used as an indicator of a female's willingness to mate with the given male (i.e. female preference). We used this overall interval as a pragmatic measure of female response to male courtship effort, which can stop and start and is otherwise difficult to measure (personal observation). This measure of latency is significantly correlated with the time from first courtship to copulation ($n = 67$; $r = 0.63$; $p < 0.001$), justifying its use as our measure of female preference.

Females were transferred to fresh laying vials, with excess culture medium, after 24 h, after 6 days and then every 7 days until vials ceased to contain developing larvae. Females were checked daily for death and once dead, stored for wing measurements. Female longevity was recorded in days since eclosion. Lifetime reproductive success (LRS) equalled the number of eclosed progeny on the eighth day after the first eclosed progeny in each vial. This is because *D. simulans* larvae take between 8-9 days to develop and eclose, so that 7 days worth of eclosing time allows for almost all of the progeny to be accounted for without including grandchildren. LRS was therefore the summed progeny count from all of the vials relevant to each female. We also assessed the efficacy of this measure of female fecundity by mating a separate batch of virgin females and counting eggs laid over 7 days, and then counting the final number of progeny that emerged from the same vials. Progeny emerging from each vial was significantly positively related to the original number of eggs counted ($n = 19$; $r = 0.711$; $p = 0.001$). We additionally investigated the possibility that females mating with preferred males could reproduce faster, as this could also represent a fitness benefit. To do this, we looked at the number of eggs laid in the first 24 h after mating. This time period was chosen because effects of male ejaculate components on female oviposition can be rapid and transitory (Chapman 2001).

Female preference and fitness with male-male competition

All housing and virgin adult collection procedures were as outlined above. A total of 60 females were used again split over two blocks. The evening before mating, two males were haphazardly chosen and aspirated into each mating vial containing culture medium. On the day of mating, one female was added and observed as above. Copulation latency, duration and temperature were recorded. When copulation commenced, the unmated male was removed and frozen for future measurement. Mating males were similarly removed and stored within 30 min of copulation ending. Females were transferred to new laying vials every 5 days, until vials ceased to contain developing larvae. Female longevity, LRS and male and female body sizes were all recorded as described above. We again investigated the possibility that females mating with preferred males reproduced faster. However, this time, we looked at the number of eggs laid in the first 5 days after mating. This time period was chosen because we found no effects after 24 hours in experiment 1, but preliminary data indicated that most females were still fully fertile at 5 days. Furthermore, most females remate at around this time

(Markow 1996), and in *D. melanogaster*, female oviposition rates remain elevated for about 7 days post-mating (Manning 1967). For each experimental block, we also carried out ten pairings of single females with single males as per experiment one. These allowed us to see if any differences in mating behaviour (preference or copulation duration) were due to different treatments (access to one male or two males) or merely due to a blocking effect (across experiments). As a result, only data on preference and copulation duration were collected for these pairings.

Statistical Analyses

Data analysis was conducted using SPSS v 11.5. Raw data were tested for normality using Shapiro-Wilks tests. Raw data were log-transformed to improve normality as necessary. Sample sizes vary somewhat across analyses due to missing data; for example, some females did not copulate or produce any progeny, and we did not have a complete set of male and female wing sizes for all pairs. Four females in the one-male experiment that produced less than five offspring in total were not included in the analyses.

RESULTS

Fitness benefits of female preference with no male-male competition

Summary statistics for copulation duration, female preference (time to copulate) and fitness measures are given in Table 1. We used a multivariate analysis of covariance (MANCOVA) to initially investigate the main effects only (there were no significant interactive effects) of all explanatory variables (preference, copulation duration, female body size, male body size and block) on the two fitness measures, LRS and female longevity. There was no effect of female preference, copulation duration, or female body size on either fitness measures (MANCOVA: $n = 89$; all Wilks lambda < 0.99; F

$_{2,83} < 0.57$; $p > 0.5$; post-hoc ANCOVA: all $F_{1,84} < 2.4$; all $p > 0.13$), but there was a significant block effect, which univariate analysis showed was driven by effects on LRS (MANCOVA: $n = 89$; Wilks Lambda 0.87; $F_{2,83} = 6.1$; $p = 0.003$; post-hoc ANCOVA: $F_{1,84} = 11.13$; $p = 0.001$). As female longevity and LRS potentially influence each other, we also conducted two separate analyses of covariance, but as the results are essentially identical, only the first analysis is presented in this paper. A separate MANCOVA also indicated that females mating with attractive males did not produce offspring at a faster rate (i.e. in the first 24 h of laying), but there were significant, positive effects of female size and block in both the MANCOVA and post-hoc ANCOVAs (all $F > 2.83$; all $p < 0.003$) (all other associations NS: $F < 1.9$; $p > 0.09$). In sum, these analyses found no significant association between preference and female fitness (Fig. 1).

Previous work on *D. simulans* indicates females may prefer larger males (Markow and Ricker 1992). We examined this possibility and found that time to copulate was significantly negatively related to male body size, although this relationship was weak ($n = 112$; $r = -0.191$; $p = 0.04$; Fig. 2). When we substituted male size for female preference and re-ran the fitness analyses above, we again found no statistically significant relationships between the explanatory variables and either fitness measure (MANCOVA: $n = 89$; all Wilks Lambda < 0.99; $F_{2,83} < 0.68$; $p > 0.5$; post-hoc ANCOVA: all $F_{1,84} < 3.01$; $p > 0.08$), but there was still an effect of block on LRS (MANCOVA: $n = 89$; Wilks Lambda $= 0.84$, $F_{2,83} = 7.78$; $p = 0.001$; post-hoc ANCOVA: $F_{1,84} = 14.23$; $p < 0.001$). Analysis of offspring production in the first 24 h also revealed no effect of male size (data not shown).

Influence of male-male competition

Summary statistics for copulation duration, female preference (time to copulate) and fitness measures are given in Table 2 and were analysed as above. None of the predictor variables was significant in the multivariate analysis (MANCOVA: $n = 50$; all Wilks Lambda < 0.97; all $F_{2,44} < 2.6$; all $p > 0.08$), but univariate post-hoc tests indicated an effect of female preference on female longevity (post-hoc ANCOVA: $F_{1,45} = 5.118$; $p = 0.03$; see Fig. 3; all other predictors were not significant; ANCOVA: all $F_{1,45} < 3.1$; $p > 0.08$). This effect seemed to be driven by a single data point, but removal of this datum strengthened the association between preference and longevity (MANCOVA: $n = 49$; Wilks Lambda $= 0.86$; $F_{2,43} = 3.4$; $p = 0.04$; post-hoc ANCOVA: $F_{1,44} = 6.86$; $p = 0.01$). Therefore, it appears that females live longer after mating with less-preferred males, but their LRS was the same. Univariate analyses with each fitness measure as a covariate for the other (see above) produced the same results. We repeated these analyses with progeny produced in the first 5 days laying as the dependent variable to see if reproduction rate varied with preference, but other than a block effect and a negative association between preference and longevity in both the MANCOVA and post-hoc ANCOVA (all $F > 3.83$; all $p < 0.03$), there were no other significant associations (all $F < 1.77$; all $p > 0.18$).

While the size of the successful male was not associated with female preference across vials ($n = 58$; $r = -0.153$; $p = 0.25$), 69% of females copulated with the larger of the two males available ($\chi^2 = 8.34$; $p = 0.004$). We again examined whether male size influenced female fitness, and once more, there were no significant effects (MANCOVA: $n = 49$; all Wilks Lambda < 0.97; all $F_{2,43} < 1.89$; all $p > 0.16$; post-hoc ANCOVA: all $F_{1,44} < 3.8$; $p > 0.05$). We also compared the fitness of females that mated with the largest of the two males available with that of females mated with the smallest male. Again, there were no significant effects (LRS of females mated with the largest and smallest male: t (56) = -0.98; $p = 0.33$; longevity of females mated with the largest and smallest male: t (50) = 1.74; $p = 0.09$). Once more, we repeated these analyses with females rate of reproduction over the first 5 days of laying as the dependent variable, and other than the block effect in both MANCOVA and post-hoc ANCOVA (all $F > 10.39$; all $p < 0.001$), no significant associations were found (all $F < 3.65$; all $p > 0.06$).

Comparison of experiments

When we compared the two experiments, we found copulation duration was significantly longer in the second experiment, compared to the first (Tables 1 and 2: t (169) = -4.65; $p < 0.001$; ANCOVA: n = 151; $F_{1,147} = 31.47$; $p < 0.001$). However, when we compared the copulation duration from females paired with one and two males in experiment 2, we found no significant difference (Table 2, females paired with one and two males: t (72) = -1.33; $p = 0.26$), suggesting that the difference between experiments arose from a between-day effect rather than any experimental effect of number of males present. Similarly, when we compared copulation latency, we found that mating occurred more rapidly in experiment 2 (Tables 1 and 2: t (169) = 2.86; $p = 0.005$; ANCOVA: n = 151; $F_{1,145} = 4.5$; $p = 0.036$). This difference was not significant when we compared single and double male treatments from experiment 2 (Table 2, females paired with one- and two-males: t (73) = -1.86; $p = 0.07$).

DISCUSSION

While we found evidence of female preference for larger males, we found no fitness benefits to females that mated with preferred males. Females mating with less-preferred males were as likely to have as many progeny over their lifetime and produce as many offspring in the first 24 h of laying as those that mated with more preferred males. This contrasts somewhat with similar work in the closely related *D. melanogaster* (Pitnick 1991; Pitnick and Garcia-González 2002) and other *Drosophila* (e.g. Droney 2003) that report decreased female fitness from mating with attractive males. While there was also no effect on female LRS in our second experiment, females mating with attractive males tended to have shorter lifespans than those mating with less-preferred males. This may be because females in poorer condition are less able to resist male advance and die sooner. However, this was not the case in our first experiment where, with larger sample size, there were no effects of preference on longevity. Another potential proximate mechanism for the longevity cost we documented in the second experiment could be the increased copulation duration we recorded. However, smaller males always copulated longer, and male size was not associated with female longevity, so this does not appear to be the answer. Additionally, males that did not copulate in experiment 2 were removed once copulation began, and the average time females were exposed to two males was only 15 min; so it does not seem that additional harassment is the cause of the apparent longevity cost, but it seems that the addition of male-male competition amplifies longevity costs of reproduction to females. Longevity costs of mating have also been documented using *D. simulans-D. sechellia* introgression lines (Civetta et al. 2005), but like here, longevity and LRS were not associated. Interestingly, our longevity finding may explain some of the variation in longevity cost/benefits reported by Civetta et al. (2005): in their study, some females were housed with one male and some with two. Our longevity findings also reflect those found for *D. melanogaster* where mating with preferred males has longevity costs for females (Pitnick and Garcia-González 2002; Friberg and Arnqvist 2003), but we reiterate that male attractiveness had no LRS consequences for females. In any case, the longevity difference between our two experiments requires further investigation, but similar results have been found in studies of other insects (Martin and Hosken 2003), and in *D. melanogaster*, differences in longevity between females evolving with or without sexual selection were not manifest in the absence of males (Wigby and Chapman 2004). This also suggests that inclusion of male effects can be important. We also detected block effects. Potential explanations include difference in development times/eclosion dates across blocks, although all flies were the same age at copulation, and small differences in food quality across blocks. Nevertheless, the block effects were statistically controlled for when investigating other factors, and overall, we found no indication that pre-copulatory sexual selection increased female fitness.

One possible reason for the lack of association between our sexual selection components and LRS may be that we only allowed one copulation, and female *D. simulans* usually remate after 3-5 days (Markow 1996). How access to males and post-copulatory sexual selection influences female preference and LRS will be the subject of future investigation. In any case, one obvious conclusion to draw from our finding that there were no fitness benefits to females is that female preference may be based solely on indirect benefits that we have not assessed (e.g. Fisher's sons effect; reviewed in Andersson 1994). While indirect benefits are likely to be very small (e.g. Møller and Alatalo 1999), they are nevertheless a major factor invoked in sexual selection studies (Andersson 1994), and there is evidence for benefits of female mate choice through offspring (e.g. Wedell and Tregenza 1999; Head et al. 2005). Furthermore, because we found no LRS cost from mating with attractive males, even small indirect effects may be enough to maintain preference (all else being equal). This also contrasts with findings of large direct fitness costs in *D. melanogaster* (e.g. Pitnick and Garcia-González 2002) and no detectable indirect compensation (Orteiza et al. 2005; Stewart et al. 2005). More generally, large direct costs are unlikely to be compensated for through indirect avenues (Kirkpatrick 1985; Cameron et al. 2003; Hosken and Tregenza 2005). Benefits of female preference have also been reported in studies relating female preference to offspring survival (quality; e.g. Petrie 1994). By allowing progeny to develop, pupate and eclose in these two experiments, we included some aspects of offspring quality in our fitness assay (larval survival and development), but again we found no effects.

Partitioning larvae amongst many vials and supplying generous amounts of food inevitably draw the criticism that such benign environmental conditions are likely to mask true variation in larval quality, and therefore, female fitness, and the addition of stress may be needed to detect associations (Hoffmann and Parsons 1991). Such concerns may necessitate an imposed form of competition or environmental stress in future studies of sexual selection benefits. However, the addition of stress can also mask fitness variation if stresses are too strong, and we did see substantial variation in LRS across females. Nevertheless, there was no association between LRS and preference, although attractive males may have reduced female longevity in one experiment. Again, we need to specifically investigate the effects of stress on fitness components and mate preference in these flies.

The lack of association between our measure of female preference and life-time reproductive success could also be due to the fact that copulation latency does not adequately measure female preference. However, we do not think this is so for a range of reasons. Firstly, video analysis of courtship behaviour in *D. melanogaster* found that copulation occurs as a result of male courtship effort and the female signalling response, and when female response is statistically controlled for, all males had comparable copulation latency (i.e. delay reflects female preference for males, not male effort per se:

Barth et al. 1997). Secondly, as pointed out in the "Introduction", females mate faster with conspecifics compared to hybrid or heterospecific males, and mating speed is correlated with aspects of male song (Ritchie et al. 1999; Acebes et al. 2003). Finally, our measure of female preference was associated with male size: females mated more quickly with larger males. Female preference for large males is well documented in *Drosophila*, although the reasons for this are unresolved (reviewed in Markow 1996), and larger male mating advantage has been found previously in *D. simulans* (Markow and Ricker 1992) and in *D. melanogaster* (Partridge et al. 1987a,b).

While there is no *a priori* reason to expect female preference and male-male competition to be reinforcing (see "Introduction"), our results suggest they may well be in *D. simulans*. Females seemed to prefer larger males whether male-male competition was included or excluded, although the preference was weak. The broadly complementary nature of these two components of sexual selection contrasts with results from cockroaches, which demonstrate male-male competition working in opposition to female preference (Moore and Moore 1999; Moore et al. 2001). Matings also occurred, although statistically not significant, more rapidly when more than one male was present. In addition, copulation duration was longer when two males were present during courtship and copulation initiation. This suggested a behavioural response to sperm competition risk (Parker 1970), an interpretation made in other studies demonstrating male adjustment of copulation duration (e.g. Gage 1991; Pitnick 1991; Martin and Hosken 2002). However, copulation duration was also longer in males paired singly with females in experiment 2, indicating that the increase in copulation duration in this experiment was not due to the perceived risk of sperm competition.

In conclusion, it appears that male-male competition and female choice broadly coincide in *D. simulans* because larger males are usually preferred and enjoy a mating advantage in all the contexts we investigated. However, there may be some female longevity costs to mating with attractive males when pre-copulatory male-male competition occurs, although the precise reasons for this are unclear. Additionally, we found no female LRS benefits associated with mating with preferred, attractive males. Our findings therefore suggest females obtain no fitness benefit to mating with attractive males, but female preference could be maintained by indirect fitness benefits not included in this study. This possibility remains to be assessed.

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Table 1. Summary statistics for copulation duration, female preference (copulation latency) and fitness measures when females were exposed to a single male.

Variable	Mean (± SE)	Range	N
Preference (latency: mins)	25.8 (2.7)	2-121	114
Copulation duration (mins)	17.9 (0.49)	9-35	114
Female longevity (days)	45.2 (1)	10-60	107
Productivity (offspring no.)	230.96 (10.29)	17-493	105
Female size (mm)	1.74 (0.004)	1.60-1.85	102
Male size (mm)	1.53 (0.004)	1.40-1.66	112

Productivity here does not include those females that produced less than five offspring.

Table 2. Summary statistics for copulation duration, female preference (copulation latency) and fitness measures when females were exposed to two-males, and below, when females were exposed to a single male (which allowed us to compare these data with those from experiment 1 (Table 1)).

Variable	Mean (± SE)	Range	N
Females paired with two males:			
Preference (latency: mins)	14.5 (2.39)	1-104	58
Copulation duration (mins)	22.4 (0.89)	11-40	57
Female longevity (days)	38 (1.34)	13-52	53
Productivity (offspring no.)	265.7 (14.59)	42-540	58
Female size (mm)	1.77 (0.016)	1.39-1.98	52
Male size (winner; mm)	1.55 (0.013)	1.35-1.70	58
Male size (loser; mm)	1.52 (0.014)	1.21-1.73	58
Females paired with one male:			
Preference (latency: mins)	24.5 (6.6)	4-91	17
Copulation duration (mins)	25.9 (2.6)	10-53	17

Copulation here does not include one outlier of 77 minutes.

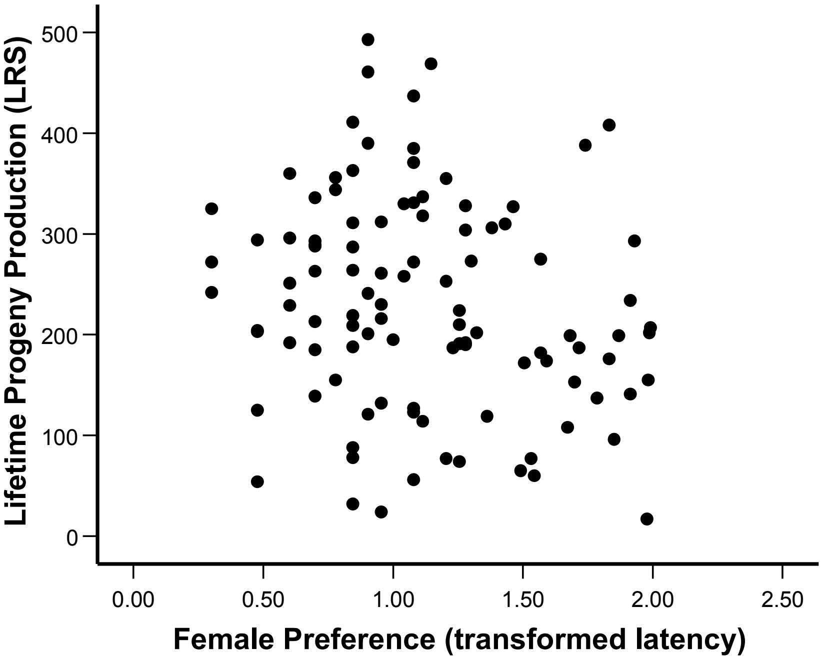

Fig. 1. Female preference (log-transformed copulation latency) was not associated with any fitness benefits to females. Shown here are the results from experiment 1 (when female had access to a single male) and note that increases on the x-axis represent decreased preference.

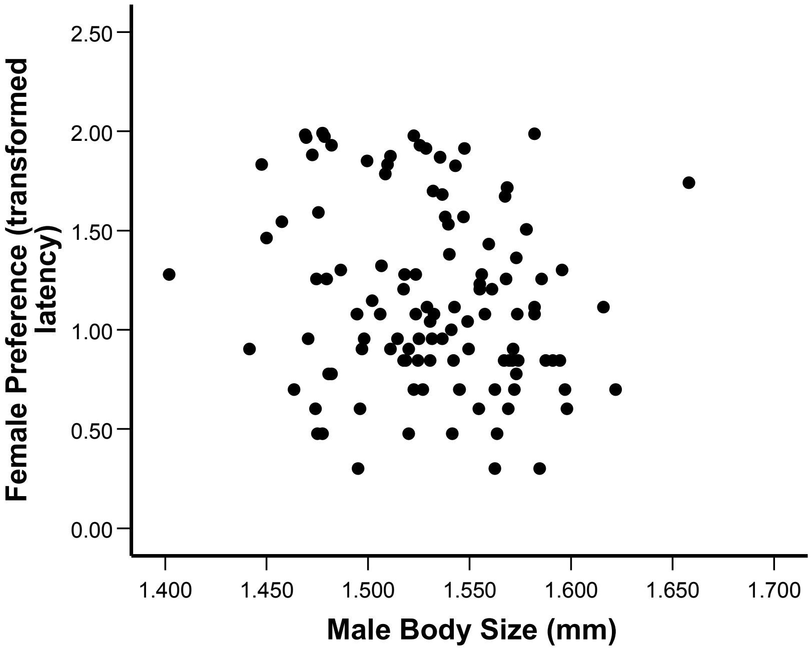

Fig. 2. Females tended to prefer (have shorter copulation latency with) larger males. Shown here are the results from experiment 1 (when female had access to a single male). Females mated faster with bigger males (note that increases on the y-axis represent decreased preference).

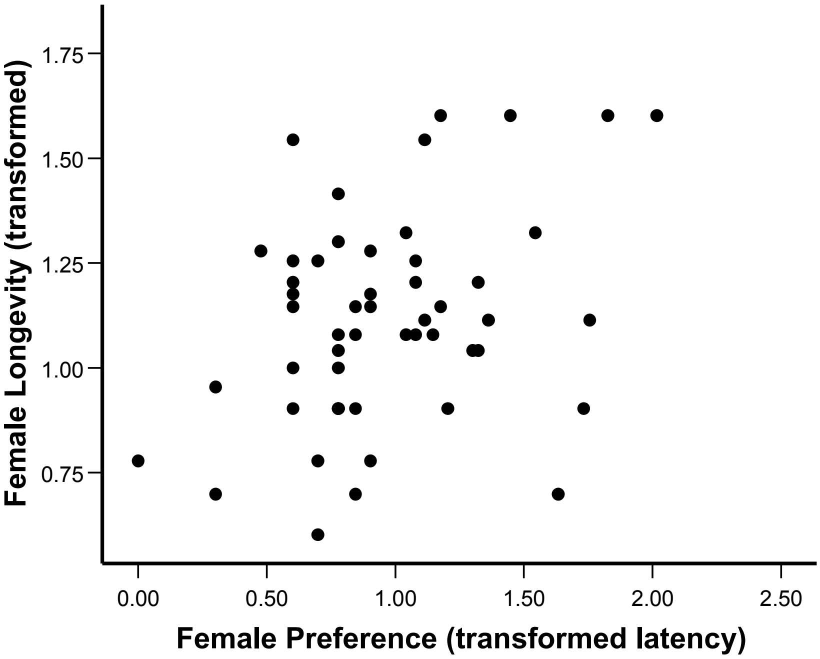

Fig. 3. In the second experiment, which included male-male competition, females that copulated with preferred males had reduced lifespan. Again, note that increases on the x-axis represent decreased preference.

CHAPTER 3

Multiple mating increases female fitness in *Drosophila simulans.*

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ABSTRACT

While polyandry is essentially ubiquitous across the animal kingdom, the net fitness consequences of multiple mating remain the subject of much debate. In some taxa the costs of multiple mating outweigh potential benefits, and large direct costs are unlikely to be compensated for by indirect benefits. Nevertheless, direct and indirect benefits potentially provide females with substantial fitness returns, and these are manifest in some species. We investigated some fitness costs and benefits of multiple mating in the fly *Drosophila simulans*. We compared the longevity and lifetime reproductive success of females with intermittent or continual exposure to males with those of singly mated females housed alone or housed with virgin females. We also compared the same fitness components in females mated once, twice and three times. We found no difference in the lifetime reproductive success of females housed intermittently with males and those housed continually with males, but females in these treatments produced more offspring than singly mated females (housed alone or with virgin females). However, females that were continually exposed to males died younger than females from any other treatment. We also found that females who mated more than once had higher lifetime reproductive success, and that number of matings had no influence on residual longevity. These results contrast somewhat with findings from *Drosophila melanogaster*, and suggest that while polyandry is beneficial for female *D. simulans*, male harassment can be costly.

KEYWORDS

Drosophila simulans; female preference; fitness costs; harassment; multiple mating; sexual conflict.

INTRODUCTION

Polyandry, where females mate with multiple males, is extremely widespread, but the precise reasons for female multiple mating remain the subject of much debate (Arnqvist & Nilsson 2000; Jennions & Petrie 2000; Hosken & Stockley 2003). Do females benefit from polyandry or is mating with multiple males a consequence of selection acting on males? For example, polyandry could be insurance against genomic incompatibility (Tregenza & Wedell 1998), or a means of avoiding inbreeding costs (Hosken & Blanckenhorn 1999) or enhancing offspring viability (Anderson et al. 2007; Gowaty et al. 2007). Where females are able to store sperm from more than one male, there is sperm competition and the potential for sperm selection (Parker 1979, 1984), with potential associated benefits to females (Zeh 1997; Hosken et al. 2003; Fisher et al. 2006). However, the most obvious potential fitness benefit to polyandry is through female fertility, and in a meta-analysis Arnqvist & Nilsson (2000) found that in insects with no nuptial feeding, remating increases fertility, fecundity and overall lifetime reproductive success. They concluded that direct fitness benefits drive the evolution of polyandry (Arnqvist & Nilsson 2000), although it is unclear whether the association they found is cause or effect.

While polyandry frequently appears to be beneficial for females, it could also be driven by male coercion resulting from sexual conflict over mating (Trivers 1972; Parker 1984; Holland & Rice 1998). If so, mating with multiple males may not elevate female fitness (Holland & Rice 1998), and there is evidence consistent with this. For example, in socially monogamous birds, female fitness components are often depressed by extra-pair copulations (Arnqvist & Kirkpatrick 2005), and evidence from experimental evolution studies of insects has shown that imposing monogamy often elevates female fitness (Holland & Rice 1999; Pitnick et al. 2001; Martin & Hosken 2003; Martin et al.

2004), consistent with the notion that polyandry need not always be beneficial. Additionally, costs to females of mating too frequently have been well documented, and include several examples of males physically damaging females during copulation (Crudgington & Siva-Jothy 2000; Blanckenhorn et al. 2002; Edvardsson & Tregenza 2005; Eady et al. 2007). In these situations, female resistance to copulation has been interpreted as a response to minimize mating costs (Holland & Rice 1998), an interpretation supported by instances where mating is not costly and females show no resistance behaviour (e.g. Martin & Hosken 2004). Comparative evidence also suggests that mating rate is typically negatively associated with female longevity, and is especially pronounced where females are continually housed with males (Arnqvist & Nilsson 2000).

We investigated potential fitness benefits and costs of polyandry in the fly *Drosophila simulans*. This ubiquitously occurring fly has a polygamous mating system, both males and females routinely copulate with multiple mates, and there is no parental care beyond selecting a suitable oviposition site, usually in decaying and fermenting fruit and vegetation (Powell 1997). This fly is also a close relative of the well-studied *Drosophila melanogaster*, but differs in a number of ways. For example, *D. melanogaster* females have reduced fitness when mating with preferred males (Pitnick 1991; Pitnick & Garcia-Gonzales 2002; Friberg & Arnqvist 2003), and there appears to be no indirect fitness advantage to females through their sons (Orteiza et al. 2005; Pischedda & Chippindale 2006). In contrast, male attractiveness is heritable and there are no reproductive costs to mating with preferred males in *D. simulans* (Taylor et al. 2007, 2008). Furthermore, *D. melanogaster* forced to evolve under monogamy have higher fitness than flies forced to evolve under polyandry (Holland & Rice 1999; but see Promislow et al. 1998), consistent with polyandry being driven by costs rather than benefits. Mating also reduces female life span in *D. melanogaster* (Fowler & Partridge 1989), and this seems to be caused by the seminal fluid (Chapman et al. 1995), and when females are prevented from coevolving with males, they incur even larger mating costs (Rice 1996). Finally, although female *D. melanogaster* mate with multiple males (Gromko et al. 1984; Ochando et al. 1996), there are no apparent benefits to polyandry in this species (Brown et al. 2004), and female fecundity is negatively associated with number of copulations (Kuijper et al. 2006). Currently, however, it is unclear what the fitness consequences of polyandry are for *D. simulans*. Females do mate more than once (Markow 1996), and yet they show considerable aversion to courtship from males after their first mating (Pitnick 1991; Gromko & Markow 1993; M.L. Taylor unpublished data), suggesting significant costs to multiple mating. Males are also persistent in their courtship of both virgin and nonvirgin females (personal observations), and several studies document costs to persistent male courtship distinct from the costs associated with multiple mating itself (Partridge & Fowler 1990; Clutton-Brock & Langley 1997; Shuker et al. 2006).

With this in mind, we compared the longevity and lifetime reproductive success of female *D. simulans* intermittently or continually exposed to males with those of singly mated females housed alone or with other (virgin) females. We also compared the same fitness components in females mated once, twice or three times. We note here that we are not concerned with distinguishing between number of matings and number of males mated as we think this is an artificial distinction; in nature female flies are unlikely to copulate multiply with one male. Instead we are concerned with the potential benefits of mating multiply, which in nature would be polyandrously. We ask, is multiple mating beneficial or costly for female *D. simulans*?

METHODS

Flies

Stock populations of *D. simulans* were derived from 20 isofemale lines supplied by the Centre for Environmental Stress and Adaptation Research, La Trobe University, Australia. Isolines had been cultured from individuals caught from a wild population at Tuncurry, Eastern Australia in March 2004. Isolines were mixed in a population cage and reared on '*Drosophila* quick mix medium' (supplied by Blades Biological, Edenbridge, Kent, U.K.) with yeast and water at 25 ^{o}C and a 12:12h light:dark cycle, to initiate a large outbred population, maintained at a population size of over 500 individuals for over 15 generations at the time of the experiment. Subsequent housing conditions followed this regime unless reported otherwise. For each experiment, emerging virgin adults from the stock population were collected every 12 h, separated and housed by sex with an excess of the culture medium for 3 days before experimental pairings.

Experiment 1: Housing With and Without Males

In this experiment, we tested whether females would increase their lifetime reproductive success (LRS) by remating, and how continuous exposure to males influenced LRS and longevity. A total of 200 females were used, split into three blocks, with 15-20 females per treatment per block. Females were paired with a single male of the same age and allowed a single copulation. Matings were conducted during the first 3 h of 'daylight' the flies would normally experience, which corresponds to the period of peak mating activity reported in natural populations (Gromko & Markow 1993). The evening before mating, one male was haphazardly selected and aspirated into each vial (40 ml) containing culture medium. On the day of mating, one female was aspirated into each vial and observed for 3 h or until a single copulation had occurred. We recorded the time taken for copulation to commence (copulation latency) and the length of copulation itself. Males were then removed and discarded and mated females allocated to one of four treatments: housed alone (T1); housed alone and given a 3 h exposure (as above) to two males every 5 days (intermittent male exposure: T2); housed with two males (T3); housed with two other virgin females of the same age (T4). All males used in these treatments were of corresponding age to the female, and any lost male flies were replaced with those from either the original collection date or as near to it as possible (i.e. all replacements were either the same age or within a few days of the female). Females were transferred to fresh vials with the culture medium after 24 h and then every 5 days, and treatment conditions were maintained until the female died. Females in the T2 treatment were exposed to two virgin males (of corresponding age) for 3 h on the day of transfer to a new vial and observed continuously for any further copulations. Lifetime reproductive success (LRS) was scored as the cumulative total of offspring from all vials for each female. Offspring from each vial were counted on the 8th day after the first day of eclosion (this is because *D. simulans* larvae take 8-9 days to develop and eclose, so that 7 days worth of eclosing time allows for almost all of the progeny to be accounted for without the overlap of grandchildren). We have previously assessed the efficacy of this measure of female fecundity, and found that the number of offspring that eclose from vials is significantly positively related to the number of eggs laid (Taylor et al. 2008). Females were checked daily for mortality, and longevity was scored in days since the first mating. Body size of females was measured using wing length, specifically from the humerocostal break to the distal end of the second vein (from Gilchrist & Partridge 1999). Both wings were mounted on a slide and measured using a Leica MZ dissecting microscope with a PC digital image analysis system (SPOT Basic, version 4.1, D. Taubman, University of New South Wales, Australia), and then averaged to give an overall estimate. We have previously found this method to be highly repeatable and to be a good surrogate of general body size (Taylor et al. 2008).

Experiment 2: Effects of Number of Matings

In this experiment we aimed to determine the effects of multiple matings on female fitness. A total of 116 females were used for this experiment. Virgin females were initially paired with a single virgin male and allowed a single copulation, under the same conditions as described above. Males were then removed and discarded and mated females were allocated to one of three mating treatments. From our observations of females in T2 above, we predicted that female *D. simulans* are likely to remate a maximum of two times, despite repeated opportunities for more. Therefore, after the first mating, females in this experiment were allocated to treatments of either one, two or three copulations. Those females allowed two and three copulations were then paired daily, as above, with fresh males and continuously observed until all had copulated a second time. Females allowed three copulations were then paired daily with fresh virgin males, and again observed to ensure all had copulated a third time. These second and third matings were conducted over both the 3 h 'dawn' and 'dusk' periods the flies would normally experience to maximize opportunity for copulation. All mated females were transferred to fresh vials with the culture medium every 3 days. The LRS was scored as above, using the cumulative total of offspring from each vial for each female. Females were checked daily for mortality, after which they were stored for wing measurements. Female longevity was measured in days since eclosion but was entered into the analysis as longevity after mating (residual longevity), as mating effects would be manifest only after females had copulated (see below). We used wing length as a general measure of body size of females, in this case taken from the anterior cross vein to the distal end of the second vein (from Partridge et al. 1987). We have previously found this method also to be a good surrogate of general body size (Taylor et al. 2008).

Statistical Analysis

Data analysis was conducted using SPSS version 14 (SPSS Inc., Chicago, IL, U.S.A.). Raw data were tested for normality using Shapiro-Wilks' tests and transformed to improve normality where appropriate. Sample sizes vary across analyses because of missing data (e.g. some females escaped) and those females that produced fewer than five offspring in total (and therefore represent a small cluster of unusually low offspring production) were removed from the analysis. In the first experiment all fitness measures are taken from first mating to death. In the second experiment, we were interested purely in the effects of remating on female fitness. Remating in *D. simulans* is highly variable, with some females remating after just a few days and others taking up to another 7 days. To compare the effects of these events on female longevity we calculated the median day on which all copulations in all treatments had been completed, and recalibrated all female longevity from this point. Therefore all female longevity measures in this experiment are from 15 days after the day they eclosed, that is, after the point at which fitness effects of remating can be seen (= residual longevity; cf Blanckenhorn et al. 2002). To reiterate, we used residual longevity here because effects of an event (mating) cannot be seen until the event has occurred, which in this experiment was after the third mating. For LRS, we counted all offspring produced as described above. For both experiments, we conducted a multivariate analysis of covariance (MANCOVA), using LRS and female longevity as dependent variables, and female body size as a covariate. However, owing to the potential influence of LRS on longevity and vice versa, we also conducted two separate ANCOVAs, using female body size again as a covariate. Treatment and block were also used as fixed factors where appropriate. The MANCOVA and ANCOVA approaches give qualitatively equivalent results, so we present only the results of the ANCOVAs.

RESULTS

Experiment 1: Housing With and Without Males

Overall LRS and rates of reproductive outputs are shown in Fig. 1a, b and survivorship of females is shown in Fig. 2. In general, females who had the opportunity to remate, both intermittently and continually, had higher LRS than females who mated only once (ANCOVA: mating treatment: $F_{3,153} = 36.81$, $P < 0.001$; see figure legend for comparison of treatment groups). We also found a block effect ($F_{2,153} = 12.17$, $P < 0.001$). Our treatments also influenced female longevity (ANCOVA: $F_{3,155} = 32.37$, $P < 0.001$), with the longevity of females who were continuously housed with males significantly reduced from that of females in all the other treatment groups. Female body size, used as a covariate in both analyses, had no significant effect on either LRS or longevity (ANCOVA: $F_{1,133} = 0.366$, $P = 0.54$ for LRS and $F_{1,133} = 1.18$, $P = 0.279$ for longevity), but LRS and longevity were significantly associated (ANCOVA: $F_{1,155} = 84.15$, $P < 0.001$; Pearson correlation: $r = 0.411$). Females in the 'male exposed' treatments (T2 and T3) had a later and higher peak of offspring production that continued to remain higher than for females who mated only once, although females in the continually exposed treatment always had a lower rate of production than females exposed only intermittently (Fig. 1b). This was also coincident with the decline in survivorship (Fig. 2), where survivorship of those females continually housed with males was always lower and began to drop off much earlier than that for females in the intermittent-exposure treatment, which followed the same rate of decline as females not exposed to males at all after their initial mating.

Females in the intermittent-exposure group (T2: exposed to males for 3 h every 5 days) were also scored for extra copulations observed during the exposure periods. Of the 47 females, 36 were observed to remate during these exposure times: 15 remated once, 11 remated twice and 10 were observed to copulate for a fourth time. Statistical tests could not be conducted on these data as females rather than experimenters determined which group was which, but they do show that when exposed to males regularly, females maximally mate only two to four times, with a mode of two.

In sum, females in this experiment were able to increase their LRS by remating, but we documented a longevity cost when females were continually housed with males. This was not seen in continual housing with other females or intermittent exposure to males.

Experiment 2: Effects of Number of Matings

Female mating treatment had a significant effect on LRS (ANCOVA: $F_{2, 42} = 8.93$; $P = 0.001$), with more than one mating elevating reproductive output, and a weak trend for females in the three-matings treatment to have greater LRS than those in the two-matings treatment (Fig. 3). Additionally, larger females also produced more offspring (ANCOVA: $F_{1,42} = 8.55$; $P = 0.006$; Pearson correlation: $r = 0.39$). However, residual female longevity (see Methods) was unaffected by the number of matings (Fig. 4), LRS or body size (ANCOVA: $F_{2,42} = 0.31$; $P = 0.73$ for number of matings; $F_{1, 42} = 1.10$; $P = 0.30$ for LRS; $F_{1, 42} = 3.6$; $P = 0.06$ for female body size).

In sum, these results also suggest that females significantly increased their lifetime reproductive success by mating more than once, and at no apparent cost to their longevity.

DISCUSSION

Our major findings were that mating with multiple males increased female lifetime reproductive success in *D. simulans*, and that longevity costs to reproduction were apparent only when females were housed continually with males. When females were only intermittently exposed to males, as would occur in nature, there were no longevity costs to multiple mating but females were able to reap its fitness benefits, and, finally, there were no associations between number of matings and residual longevity. These results contrast with findings from *D. melanogaster* and we discuss them further below.

Our results clearly demonstrated a net fitness advantage to those females who mated more than once as lifetime reproductive success was significantly increased in both experiments by remating, which is consistent with polyandry benefits in *D. simulans*. Again we note that in nature females are unlikely to mate multiply with the same male, and our investigations replicate this situation and find multiple mating is advantageous for females. Our findings are therefore consistent with general findings across insects (Arnqvist & Nilsson 2000). Our second experiment demonstrated that just one extra copulation was sufficient to increase LRS significantly, and although a third copulation elevated LRS even further, the increase was not as pronounced as from the first remating, and hence was not statistically significant. Had we enforced additional copulations, we may well have seen an asymptotic effect, as noted in many other insects (Thornhill & Alcock 1983; Arnqvist & Nilsson 2000), but we note that most of the females continuously observed for additional copulations in our first experiment did not mate more than three times (26 of 47 mated three times or less) even though they potentially could have mated much more often. Our findings contrast with one of the often-cited interpretations of Bateman's (1948) classic *D. melanogaster* study (that female reproductive success is maximized by mating with only one male; Snyder &

Gowaty 2007), and with recent work on this species that found a negative association between number of matings and female fitness (Kuijper et al. 2006). Brown et al. (2004) also found no benefits to polyandry over monandry in *D. melanogaster*, but they did not look at the fitness of singly mated females. Nevertheless, they concluded that sperm replenishment was the most likely benefit to multiple mating in *D. melanogaster* (Brown et al. 2004). It is possible that larval competition may have influenced offspring production in our study. However, larval density tends to influence individual traits such as body size and development time in *Drosophila*, rather than absolute viability (Ashburner et al. 2005). Since all females were transferred to fresh vials every 3 or 5 days and food medium was supplied ad libitum, we think impacts of larval competition are likely to be minimal in this context. Therefore, while our measure of female fitness includes some indirect fitness components (i.e. larval survival to adult), sperm replenishment remains the most likely explanation for the beneficial effect we found.

Females housed continually with males suffered a significant reduction in their life span compared to females housed alone or with other females or exposed intermittently to males. This is a widespread finding across insects (Arnqvist & Nilsson 2000; and see e.g. Clutton-Brock & Langley 1997), and has even been shown in reverse, where continuously receptive females have an adverse effect on male longevity (see Partridge 1988; Martin & Hosken 2004). Surprisingly, in the second experiment we found that female longevity was not at all affected by the number of copulations they experienced (females were exposed to males for two 3 h mating periods daily until they remated, but otherwise housed alone), supporting our suggestion that higher rates of courtship/harassment may be costly to females rather than mating per se. This contrasts with findings of some other fly studies that document an accelerating cost to the number of matings (Chapman et al. 1995; Blanckenhorn et al. 2002; but see Martin & Hosken 2004 for an exception). Partridge & Fowler (1990) documented a similar reduction in female life span in the presence of males in *D. melanogaster*; they used mutant males that were able to court females but not copulate. They speculated that perhaps males may have a similar effect on females as the presence of other females, for example, through feeding competition, contamination of food medium, disease etc. (Partridge & Fowler 1990). However, our results indicate a cost of male harassment rather than these alternatives because there was no reduction in life span when females were housed with other females, and LRS was the same whether females were with males continually or only intermittently so (i.e. it does not seem that males consumed resources needed by females). This is consistent with recent work on *D. melanogaster* where harassment had a much greater negative effect on female fitness than mating number (Kuijper et al. 2006). It is of course possible that we have not experimentally captured the relevant spectrum of mating rates required to see the negative effects of multiple mating reported in other studies, and the toxic effects of accessory gland proteins do act in a dose-dependent manner (Chapman et al. 1995). However, in other similar studies reporting costs of multiple mating on female fitness, the number of copulations that females had was comparable to here (Chapman et al. 1995; Chapman & Partridge 1996; Brown et al. 2004). We also used the number of copulations observed in the first experiment as an indication of how many copulations females would naturally engage in which was maximally four despite many more opportunities. All the above strongly suggests a nonmating cost to longevity, but we note the longevity costs to harassment (or at least being housed with males) may not be a net fitness cost when compensated for by the advantages of remating we recorded.

Surprisingly, we did not find a significant effect of female body size in most of our fitness measures. This may be because females were provided with food ad libitum, and some studies document fitness effects only under stressful conditions (e.g. Chapman & Partridge 1996). This is something we need to investigate further. We also found a difference between experimental blocks. Again, many studies have documented an influence of environmental effects on *Drosophila* reproduction (e.g. Barth et al. 1997; Basso da Silva & Valente 2000), and although we standardized our environmental rearing and mating conditions as much as possible, there will inevitably be some variation in females that have eclosed at different times, from different larval cohorts and mated on many different days, and we note that this has been statistically controlled for in the analysis.

Our primary motivation for this work was to investigate the potential benefits and costs of polyandry in *D. simulans.* We found that the benefits to females of multiple mating were sufficient to offset the costs of remating, but that male harassment can nevertheless be costly. Our results also suggest that a lack of female refugia, where females can escape male attention, may lead to an overestimation of reproductive costs to females.

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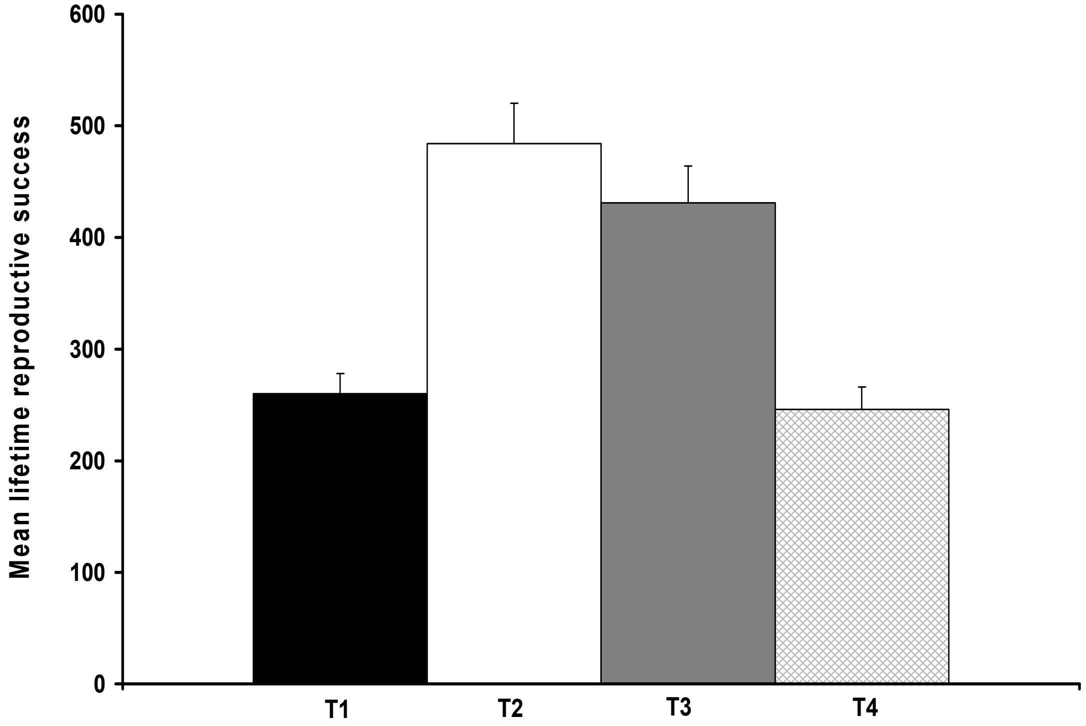

Figure 1.(a) Overall mean +SE lifetime reproductive success for females under different housing regimes: T1: females housed alone, N = 44; T2: females housed alone and exposed to two males for 3 h every 5 days, N = 47; T3: females housed with two males, N = 50; T4: females housed with two other virgin females, N = 34. This does not include 14 females that produced fewer than five offspring in total. Post hoc pairwise comparison of treatments shows that LRS in females housed alone or with other females (T1 and T4) was significantly lower than in females housed continually or intermittently with males (T2 and T3): Tukey test: mean difference: T1 versus T2 = -271.8, P < 0.001; T1 versus T3 = -290.6, P < 0.001; T1 versus T4 = -60.1, P = 0.09; T2 versus T3 = -18.8, P = 0.5; T2 versus T4 = 211.6, P < 0.001; T3 versus T4 = 230.5, P < 0.001.

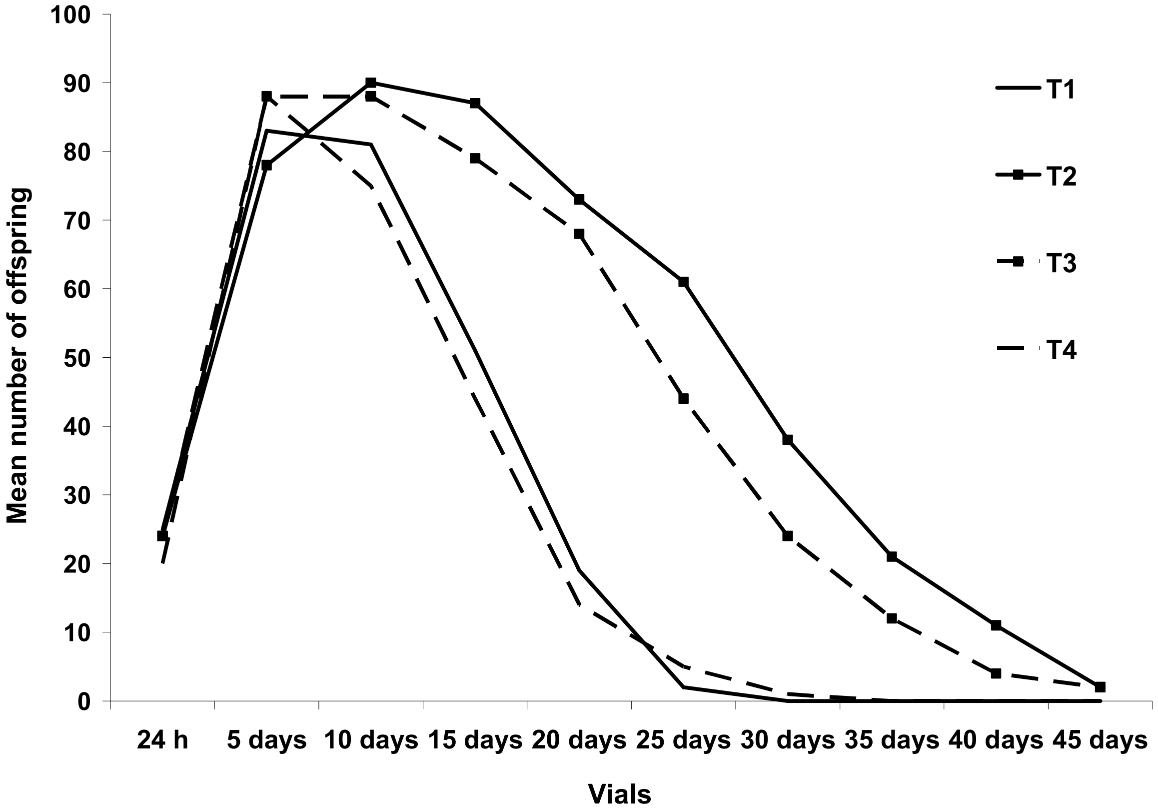

Figure 1.(b) Rate of reproductive output for females under different housing regimes: T1: females housed alone, N = 44; T2: females housed alone and exposed to two males for 3 h every 5 days, N = 47; T3: females housed with two males, N = 50; T4: females housed with two other virgin females, N = 34. This does not include females that produced fewer than five offspring in total.

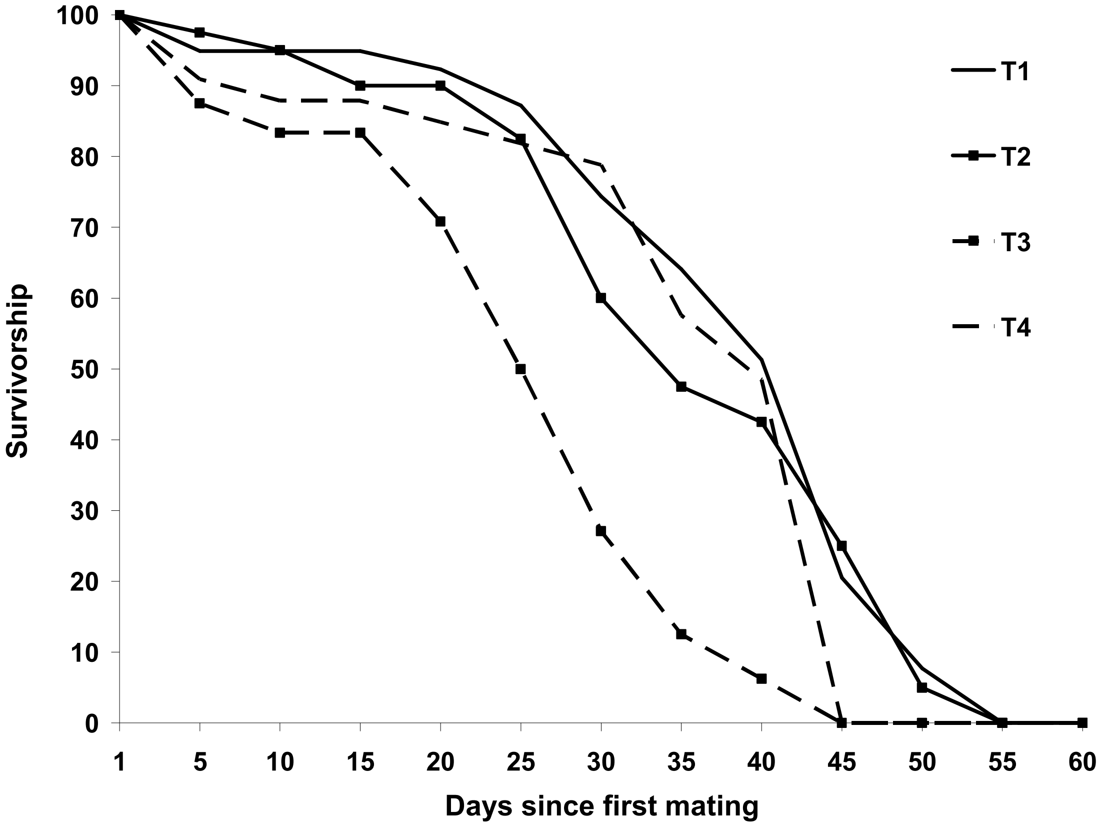

Figure 2. Percentage of females surviving as a function of housing regime (see legend to Fig. 1 for details) and time since first mating: N = 39 for T1 females; N = 44 for T2 females; N = 48 for T3 females; N = 33 for T4 females. This does not include females that produced fewer than five offspring in total. Post hoc pairwise comparison of treatments shows that female longevity in treatment 3 is significantly different from that in all other treatments: Tukey test: mean difference: T1 versus T2 = -1.3, $P < 0.001$; T1 versus T3 = -2.3, $P < 0.001$; T1 versus T4 = -.4, P = 0.1; T2 versus T3 = -.9, $P < 0.001$; T2 versus T4 = .9, P = 0.002; T3 versus T4 = 1.7, $P < 0.001$.

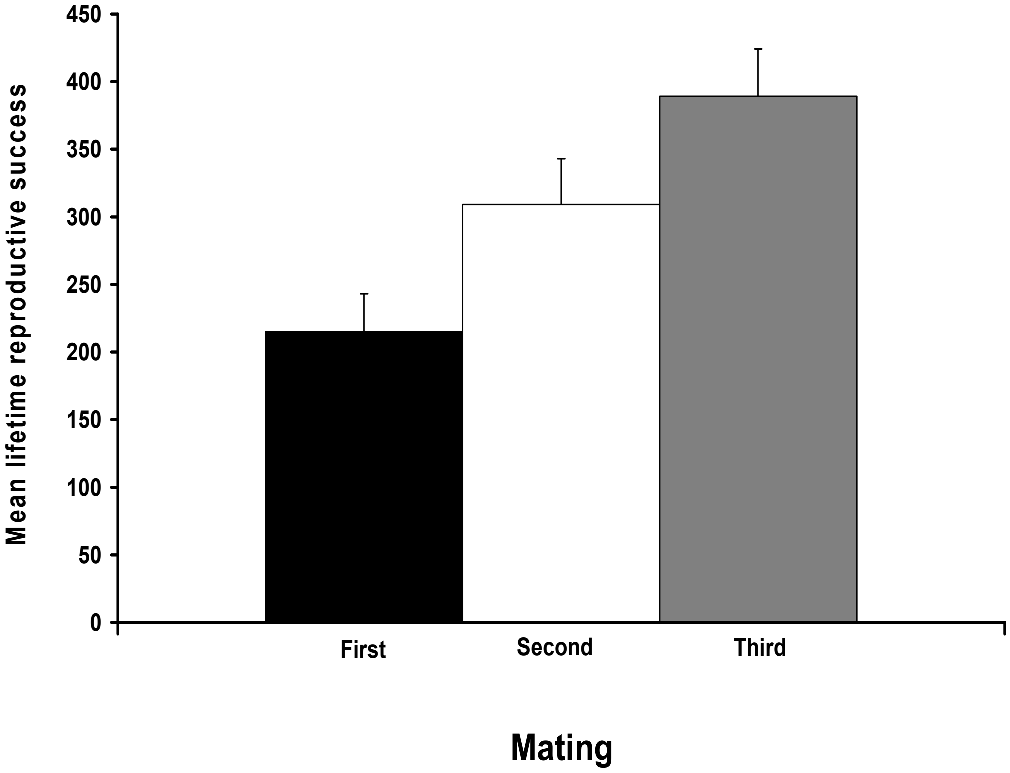

Figure 3. Mean lifetime reproductive success for females mating once, twice and three times. Bars show mean +SE for all females in each treatment group: N = 21 for one mating; N = 26 for two matings; N = 15 for three matings. This does not include three females that produced fewer than five offspring in total. Post hoc pairwise comparison of treatments shows that LRS in singly mated females is significantly lower than in multiply mated females, but that LRS is not significantly different between females mated twice and three times: Tukey test: mean difference: 1 versus 2 = -118.9, P = 0.01; 1 versus 3 = -207.7, P < 0.001; 2 versus 3 = -88.8, P = 0.09.

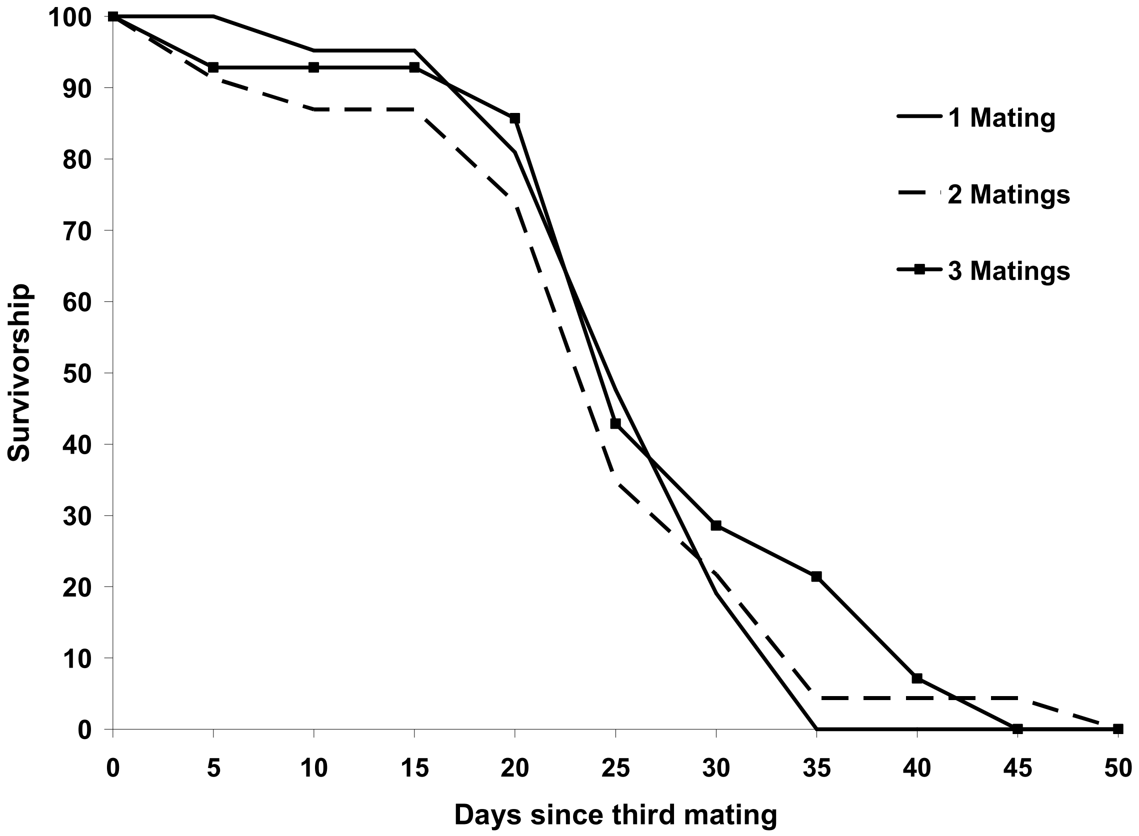

Figure 4. Survivorship of females mated once, twice and three times: N = 21 for one mating; N = 23 for two matings; N = 14 for three matings. Three females that produced fewer than five offspring in total have been removed from the analysis.

CHAPTER 4

The heritability of attractiveness

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Abstract

Sexual selection is responsible for the evolution of numerous conspicuous and extravagant displays observed in nature – from cricket choruses to peacock's tails. A key assumption of many models of sexual selection is that attractive males father attractive sons [1]. However, while particular traits under sexual selection have been shown to be heritable [1,2], the evidence for the heritability of attractiveness *per se* is far less compelling [1,3]. This dearth of information has led to disagreement about the existence and importance of this fundamental link between sire and sons' attractiveness [4]. Here we demonstrate in *Drosophila simulans* that attractive fathers sire attractive sons, as assumed by theory.

Introduction

That attractive males father attractive sons is assumed by many sexual selection models; both by models based on genetic benefits – whereby females gain fitness benefits through their offspring – and by models based on sexual conflict – whereby attractive males are manipulating females for male benefit irrespective of the fitness consequences for females. This crucial link has been convincingly established in guppies [5] and sticklebacks [6], but in general, there is a lack of evidence for this fundamental genetic association [1,3]. We, therefore, investigated the heritability of male attractiveness in *Drosophila simulans* using a full-sib/half-sib design. Briefly, 68 sires were each mated to 2-3 dams (n = 185) and we assessed the attractiveness of a total of 527 sons (mean per dam = 2.85). The sons from each dam were collected as virgins at emergence and housed together for three days before being placed into individual mating vials with a three day-old virgin female (one male and one female per vial) collected from our out-bred stock population founded from wild-caught females and maintained in large population cages since 2004. Vials were observed continuously until copulation began, and the delay between the introduction of females and copulation, the ‘copulation latency’ , was recorded. Copulation latency, a standard measure of female preference and male attractiveness in *Drosophila* [7-9], was used as our measure of attractiveness. Latency is associated with characteristics of courtship song, and females take longer to mate with hybrid or heterospecific males [7,8]. Importantly, males cannot force copulations while females can thwart male interests by ignoring them, walking away, or extruding their ovipositor; in addition, females must open their vaginal plates for copulation to occur [10]. Thus, copulation latency serves as a measure of preference, i.e. the propensity of females to mate with certain males [11]; females also take longer to mate with unattractive males [7,8]. The latency measure we employed is highly correlated with the delay from first courtship to copulation ($n = 67$; $r = 0.63$; $P < 0.001$), but is easier to accurately discern and measure. Analyses of genetic variation were conducted on sire and dam variance components estimated with Restricted Maximum Likelihood (SPSS) and G-tests were used to test the significance of the sire and dam estimates.

Results/Discussion

We found substantial additive genetic variation (V_A) for the attractiveness of sons through sires and also high evolvability (coefficient of additive genetic variation (CV_A)) ($V_A = 69.7$, $CV_A = 1.88$). Attractiveness was also significantly heritable through sires, with a narrow-sense heritability of 0.291 ± 0.15 ($G = 6.412$; $P = 0.011$). The dam estimate was lower and non-significant ($h^2 = 0.14 \pm 0.2$; $P = 0.48$). However, the sire estimate is more accurate [12] as it is untainted by common environment and dominance effects. Therefore, we can conclude that attractiveness is heritable. The precise characters conferring male attractiveness were not the focus of this study, but previous reports suggests that larger males are more attractive [9,13]. However, in the current study a large sample size indicated that there was no significant phenotypic association between male size and attractiveness (Spearman's rank correlation: n = 488; r = -0.03; P = 0.465). Thus, our finding does not seem to be driven by male body size, which is in agreement with another study reporting no association between male size and attractiveness [14]. Nevertheless, we found attractiveness was significantly heritable, and our results emphasise the fact that attractiveness is a composite trait that cannot be totally captured by simple measurements of single characters. That is to say, even if individual traits that are subject to sexual selection are heritable, this need not imply attractiveness in total is heritable and can evolve.

We have recently shown that females mating with attractive males produce the same number of offspring as those mating with unattractive males [9]. Our results presented here suggest that females mating with attractive males will produce attractive sons. In sum, this suggests that mating with attractive males could provide a net fitness benefit to female *D. simulans*. This assumes that indirect costs are minimal, and that attractive males do not produce poor quality offspring in other regards, but nevertheless, our results contrast starkly with recent findings in the closely related fly *D. melanogaster* [4,15]. For example, a recent hemiclonal investigation found that males with high reproductive success did not produce more attractive sons [15], which is very different to what we find here. Regardless of the net fitness outcome, however, our finding that sexy fathers sire sexy sons provides much needed evidence for a critical assumption of many models of sexual selection.

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CHAPTER 5

Attractive males have greater success in sperm competition

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Abstract

While sexual selection is responsible for the rapid evolution of many characters [1,2], the precise relationship between pre- and post-copulatory sexual selection is unclear [3]. In some species, the two are positively associated and reinforce each other, while in others, the two bouts of selection are antagonistic and cancel each other out. Here we assessed the relationship between female preference for males and male fertilization success during sperm competition in the fly *Drosophila simulans*. We find that attractive males sired more offspring and also find a positive genetic correlation between male attractiveness and siring success.

Introduction

The classical mechanisms of sexual selection are female mate choice and male-male competition for mates [1,2]. While Darwin [1] introduced the concept of sexual selection more than one hundred years ago, it became clear only very recently that sexual selection could continue after mating. Sperm competition, where the sperm of two or more males compete to fertilize a female's ova, represents post-copulatory male-male competition, whereas cryptic female choice, which is any post-intromission female biasing of resources or paternity toward certain males, is the post-copulatory equivalent of female choice [3]. There is now ample evidence for evolution via both pre and post-copulatory sexual selection [1-3]. What remains much less clear however, is how these two episodes of sexual selection relate to one another [3], and if males that are preferred in the pre-copulatory arena (attractive males), also perform best in the post-copulatory one.

It has been suggested – and there is some evidence – that post-copulatory sexual selection typically increases the variance in male reproductive success, and thus amplifies pre-copulatory sexual selection [4]. In red flour beetles, for example, male pre-copulatory attractiveness is positively associated with success during sperm competition [5], and in guppies, more ornamented males sire more offspring when the sperm of two males are artificially inseminated in equal numbers [6]. Male mating advantage more generally has also been shown to correlate with fertilization success [7]. However, in water-striders, larger males have higher mating success, but smaller males secure a greater share of paternity from each mating [8]. As a result, pre- and post-copulatory sexual selection cancel each other out in this system and reduce the variance in male reproductive success [8]. Similarly, in Soay sheep, males that copulate most sire fewer offspring toward the end of the rut when they become sperm depleted and lose out in sperm competition [9].

Here, we investigate in the fly *Drosophila simulans* the relationship between female mate preference – a major component of pre-copulatory sexual selection – and fertilization success of the second of two males to copulate with a female (= P2). Female preference, which reflects male attractiveness, was measured as copulation latency: the speed with which a female mates with a male. Our design allowed us to investigate both phenotypic and genetic associations between these characters (Supplemental data).

Results

To assess phenotypic correlations we used multiple regression with the duration of copulation 1 and 2, number of offspring produced before copulation 2, number of offspring produced after copulation 2, female age at second copulation and copulation latency of the second male as our predictor variables. P2 (male fertilization success) was the dependent variable and data were transformed as appropriate (Supplemental data). Copulation latency was significantly negatively associated with P2 ($F_{1,178} = 7.45$; $p = 0.007$) – second males which took longer to copulate (less preferred males), sired fewer offspring (Figure 1). If males that monopolised paternity as second males are excluded, the association is even stronger. All other associations were not statistically significant ($F < 0.94$; $p > 0.33$), which indicates that P2 variation was not due to variation in offspring survival – i.e. there were no associations between offspring production and P2. Therefore, the association between faster mating speed and P2 is unlikely to be due to low-quality, less preferred males siring offspring that showed decreased survival during the larval stage. The inclusion of the attractiveness of the first male (copulation latency of the first copulation) does not change these conclusions, neither does the use of relative copulation duration (duration of male 2 copulation-duration of male 1 copulation) or the inclusion of male age in the analysis. This lack of a male-age effect means associations are unlikely to be caused by older males with larger sperm reserves transferring more sperm and courting more. Regression of (sire) family means (P2 on copulation latency) also reveals a negative association between mating latency and paternity ($n = 38$; $ß = -0.401 \pm 0.15$; $r = 0.41$; $p = 0.01$). This is consistent with more attractive males (those that mate faster) also being more successful during sperm competition. This family-level association, which approximates genetic correlations [10], also means the phenotypic association reported above is unlikely to be caused by sperm depleted females remating faster, because each data point here is the mean of about five females. Finally, we used MANOVA to assess the genetic association between attractiveness and paternity. While the variance around such estimates is typically very large – they are variances of variances [10] – the sign of associations is informative [10]. MANOVA indicated there was a negative genetic correlation between paternity and copulation latency ($rG = -0.16 \pm 0.7$). Although the standard errors were large, as expected [10], it is the sign of the association between attractiveness and paternity we are primarily interested in and this is consistent with the phenotypic and family level associations reported above: more attractive males (those that mate faster) are more successful during sperm competition. We have previously shown that attractiveness is heritable [11] and here found that P2 was also significantly heritable ($h^2 = 0.22 \pm 0.2$; $F_{19,45} = 1.813 >$ critical $F_{(0.05)19,45} = 1.80$; $p < 0.05$).

We also examined whether females mating with more preferred males produced more offspring after their second copulation, again using multiple regression. Various models were investigated – predictors included copulation latency (of copulations 1 and 2), number of offspring produced before the second copulation, copulation durations, female age at the second copulation, and P2 – but there was no clear association between female preference of second males (copulation latency of second copulations) and the number of offspring produced after the second copulation. In the simplest, biologically plausible model with copulation latency of copulation 2, duration of the second copulation and offspring produced prior to the second copulation as predictors, there were no significant associations (all p-values > 0.11; all $F < 2.6$; $n = 189$).

Discussion

Our results indicate that attractive males (those that copulated more quickly) sire more offspring as second males during sperm competition, and that there appears to be a genetic basis to this association. Therefore, pre and post-copulatory sexual selection appear to reinforce one another in *D. simulans*. Additionally, the traits involved in both selection episodes can evolve, as both male attractiveness [11] and success in sperm competition are heritable, and the positive genetic correlation between them suggests they will evolve in concert. Interestingly, the attractiveness of first males had no impact on paternity (P2 and therefore P1), but our results clearly show that attractive males preferred by females are more successful in sperm competition when they are second to mate. This strongly parallels work with guppies and red flour beetles, where preferred males also have greater fertilization success [5,6].

The precise mechanism underlying this association is unknown, but two, non-mutually exclusive alternatives seem possible: either preferred males are intrinsically better sperm competitors, or females bias paternity toward preferred males. There is evidence for either mechanism in other taxa. For example, in guppies, more attractive males have greater siring success when equal numbers of sperm are artificially inseminated, suggesting intrinsic male effects [6]. Similarly, cryptic female choice against less preferred males has been documented in feral fowl, where females are more likely to eject the sperm of subordinate males [12]. Our previous work with *D. simulans* [13] and the current study suggest females do not directly benefit from mating with preferred males, but attractive males do sire attractive sons [11] that are also better sperm competitors. This contrasts with the closely related *D. melanogaster* where preferred, attractive males reduce female fitness [14] and do not sire more attractive sons [15].

In conclusion, attractive male *D. simulans* preferred by females had higher fertilization success during sperm competition, although the precise mechanism underlying this association is unknown. Additionally, females do not produce more offspring when mating with preferred males, but may reap indirect fitness benefits through the attractiveness and siring success of sons. These data are consistent with pre- and post-copulatory sexual selection acting in a reinforcing manner in this species.

Supplemental Data

Supplemental data are provided below

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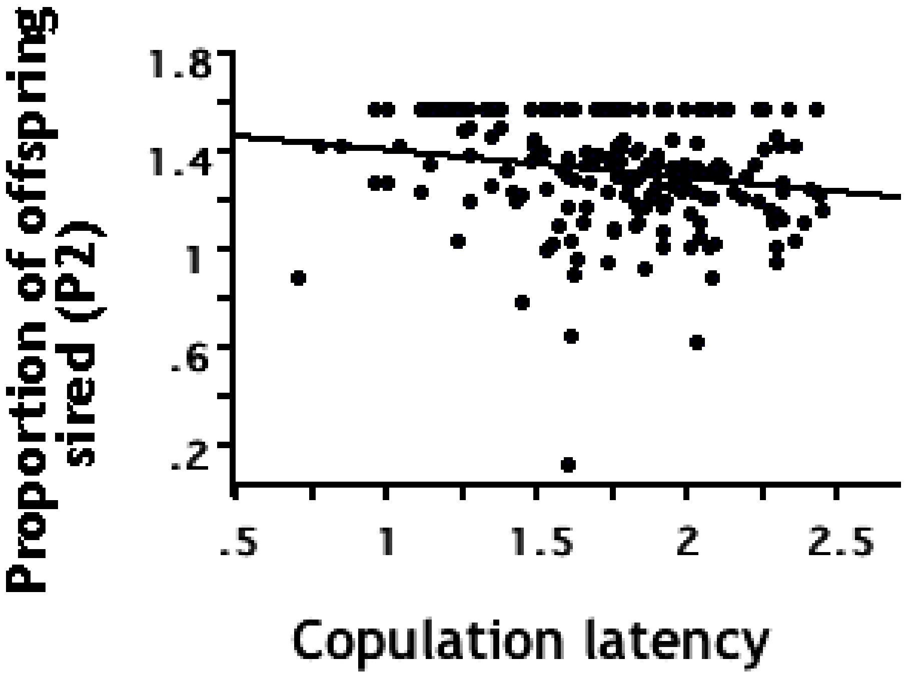

Figure 1. Female preference and paternity.

The association between copulation latency (log10 transformed), our measure of female preference for a male, and the proportion of offspring sired as the second of two males to mate with a female (arcsin square-root transformed). Males that mated faster sired more offspring. Note that the removal of the individual with the extremely low P2 value, and/or removal of the individual with the very fast copulation greatly increased the strength of the association, as does the use of logit transformation of the paternity data.

Supplemental Methods

Fly Stocks

Stock populations of *Drosophila simulans* were derived from twenty isofemale lines caught from a wild population at Tuncurry, Eastern Australia in March 2004. This cosmopolitan fly has a polygamous mating system, with males and females routinely copulating with multiple partners. There is no parental care beyond selecting a suitable oviposition site, usually in decaying and fermenting fruit and vegetation, and females are unreceptive for ca. 2 days after copulation [S1, S2]. Isolines were mixed in a population cage and reared on '*Drosophila* quick mix medium' (Blades Biological, UK) with yeast and water at 25°C and 12:12 light/dark cycle, to initiate a large (> 500 individuals) out-bred population. Flies were maintained in this way for more than 35 generations before this investigation. Our basic design is outlined in Figure S1. Briefly, virgin flies were collected from population cages and housed in single-sex groups for 3 days to ensure sexual maturity [S2]. Sires and dams were then paired haphazardly and were continuously observed until they copulated. When pairs had finished mating, the male was removed from the vial and placed with a new dam, while mated females were left undisturbed to lay for 3 days before being discarded. These vials were then monitored daily until offspring emerged. The sons (wild-type: WT) were then collected and housed separately, and were subsequently mated to a non-virgin female homozygous for the recessive ebony body marker to assess their sperm competitiveness and attractiveness (see below). This design (multiple dams/sire to produce sons for experimental matings) meant we had a standard full-sib/half-sib design [S3] to allow us to estimate genetic associations in addition to the phenotypic ones.

The ebony flies were obtained from the *Drosophila* Stock Centre (Tucson, Arizona), and had been collected from a wild population in Mexico in 2006. Since then they had been mass-bred in an outbred populations of > 200 flies. The ebony phenotype has been used as a phenotypic marker (black body colour) in previous *D. simulans* sperm competition studies [S4, S5].

Copulation latency, the time it took for mating to occur, was our measure of male attractiveness and female preference for a male. Preference measures females' propensity to mate with certain males [S6], and is therefore reflected by copulation latency because female *Drosophila* take longer to mate with hybrid or heterospecific males (i.e. less attractive males: [S7, S8]). *Drosophila* copulation latency is also associated with characteristics of courtship song [S7] and for all these reasons has been used extensively in studies of female mate preference in this taxon [S7, S9-S13]. Also note that male flies cannot force copulation so latency must reflect female cooperation [S14]. The number of offspring sired by the second of two males to mate (P2) [S4, S5, S15] was our indicator of post-copulatory reproductive success. Using these two measures – female preference and P2 – we were able to assess how key components of pre- and post-copulatory sexual selection were associated.

Experimental first matings (Ebony females with ebony males)

To generate non-virgin females to mate to our focal males, we mated virgin ebony-bodied females to virgin ebony-bodied males to provide a morphological marker for progeny sired by the first male (Figure S1). Virgin ebony-bodied flies were collected from population cages and the males and females housed separately as above. When these flies were 3-5 days old pairs of males and females were transferred into small vials. Pairs were continuously observed until they copulated and copulation had ceased, with copulation latency and duration recorded. Males were then removed and discarded, while females remained in these vials until they mated for a second time (with the focal male). These first vials were retained, until the offspring from the first copulations began to eclose. This ensured that all females had received fertile sperm from their first copulation. The date the offspring from this first copulation emerged was recorded, and then the number of progeny emerging in the next 7 days was recorded (this 7 day count was used because it takes about 8-9 days for larvae to develop and eclose, so 7 days after initial emergence allows for most offspring to emerge without potential for overlap with any grandchildren [S12]). Counting offspring from the first copulation enabled us to control for potential effects of sperm use from first mating prior to second copulations, assuming offspring number produced correlated with sperm use, as appears to be the case [S16]. Analyses at the level of sire family (where mean sire family values are used rather than treating each individual as a separate data point: see below) also ensure that any associations detected are unlikely to be due to some females remating rapidly when sperm depleted. We also recorded female age at the time of second copulations to control for the delay between the two copulations.

Experimental second matings (Ebony females with focal males)

After approximately 4 days (range 2-8 days), the singly mated ebony females were placed with two virgin 3-day old WT brothers in a new vial. We did this to increase the likelihood that females would remate. Note that attractiveness is heritable [S13], so brothers are similar in their attractiveness, and that the inclusion of male-male competition does not alter mating outcomes [S12]. Thus placing females with brothers will still reflect female preference/male attractiveness. Also note that by definition, more attractive males are more preferred males and because females have to co-operate for mating to occur in this taxon (see discussion in [S14]), this protocol measures attractiveness/preference. The flies were continuously monitored as above. For all copulations (i.e. a female's first and second copulation) the following data were recorded: copulation latency, copulation duration, the age of both the male and female flies, and the temperature of the room in which they mated. Copulation latency was measured from the time flies were placed into vials until copulations began. This measure is highly correlated with time from first courtship until copulation ($n = 67$; $r = 0.63$; $p < 0.001$), but is easier to measure accurately, justifying the use of time from introduction until copulation as our measure of female preference and male attractiveness [S10, S11]. When copulation ceased, males were removed. If after approximately 4 hours no copulation had occurred, the female was returned to her original vial, and copulation was attempted again with new virgin WT brothers 24 hours later.

After their second copulation, females were housed alone in a new vial for 24 hours and then were transferred to new vials every 3 days, until they were frozen 7 days after copulation. Productivity over this period is highly correlated with life-time offspring production [S12]. Offspring were collected and recorded as before (i.e. for 7 days after first emergence). All offspring were then counted and scored for body colour phenotype. These data were used to calculate P2. A previous larger study indicated that attractiveness was not associated with body size [S13], and in other flies P2 is unaffected by male size [S15]. To further assess this we measured body size for a sub-sample of our WT flies. Body size was measured as wing length, which is highly correlated with other structural size measures [S12]. We focused on P2 because strong second male sperm precedence [S4, S5] make detecting P1 (sperm defense) effects less likely. However, we could still investigate sperm defense because P1 = 1-P2.

Analyses

Multiple regression models were used to assess phenotypic relationships between our predictor variables and paternity scores, as well as between female preference and offspring number. The paternity of the second males (P2) varied from 0-1, with a mean of 0.88 (±0.13; $n = 189$). We conducted analyses including and excluding triads where the second male to mate sired no offspring as it was possible that in some of those instances (when second males sired no offspring) no sperm were transferred. The precise analysis performed had no effect on our conclusions and as a result, we only present the results here for those triads where we could be sure viable sperm were transferred in both copulations (i.e. females produced offspring after copulation 1, and male 2 sired some offspring after copulation 2). Additionally, in a subset of flies, we looked to see if body size influenced either paternity or copulation latency and no significant associations were detected ($N = 72$; all $F < 2.2$; all $p > 0.14$). The lack of association between body size and attractiveness (copulation latency) is also supported by a much larger study of close to 500 flies which also found no body size-copulation latency association [S13], and it therefore seems that body size has not driven the attractiveness-paternity association. Paternity data were transformed in two ways: 1) we calculated P2 and arcsine square-root transformed this value prior to analysis and, because P2 is a binomial proportion, 2) the number of offspring sired by the focal males were logit transformed [S4] and this value was used as the dependent variable. Results are the same regardless of the approach, and hence only the former are presented here. Other continuous variables were $\log_{10}$ transformed. Residuals were checked for normality with Kolmogorov-Smirnov tests and were found to be normal ($P > 0.17$). Genetic correlations were calculated from sums of cross-products obtained from a nested (dam within sire) MANOVA [S3] using an EXCEL spread-sheet provided by Allen Moore that implements the calculations of Becker [S17]. We have shown attractiveness to be heritable [S13], but have not previously assessed the heritability of P2. We used restricted maximum likelihood to calculate the sire and dam variance components and narrow sense heritabilities were calculated from these. For hypothesis testing we employed mixed model nested (dam within sire) ANOVA [S3] with Satterthwaite's approximation of the error term to account for the imbalanced design as recommended by Lynch and Walsh [S3] and implemented by Simmons and Kotiaho [S18]. Only the sire estimate of narrow sense heritability is reported because the dam term is confounded by dominance and environmental variance. Genetic estimates are based on 40 sires, 85 dams and approximately 2.5 sons per dam (189 sons). Means are reported ± 1 SE.

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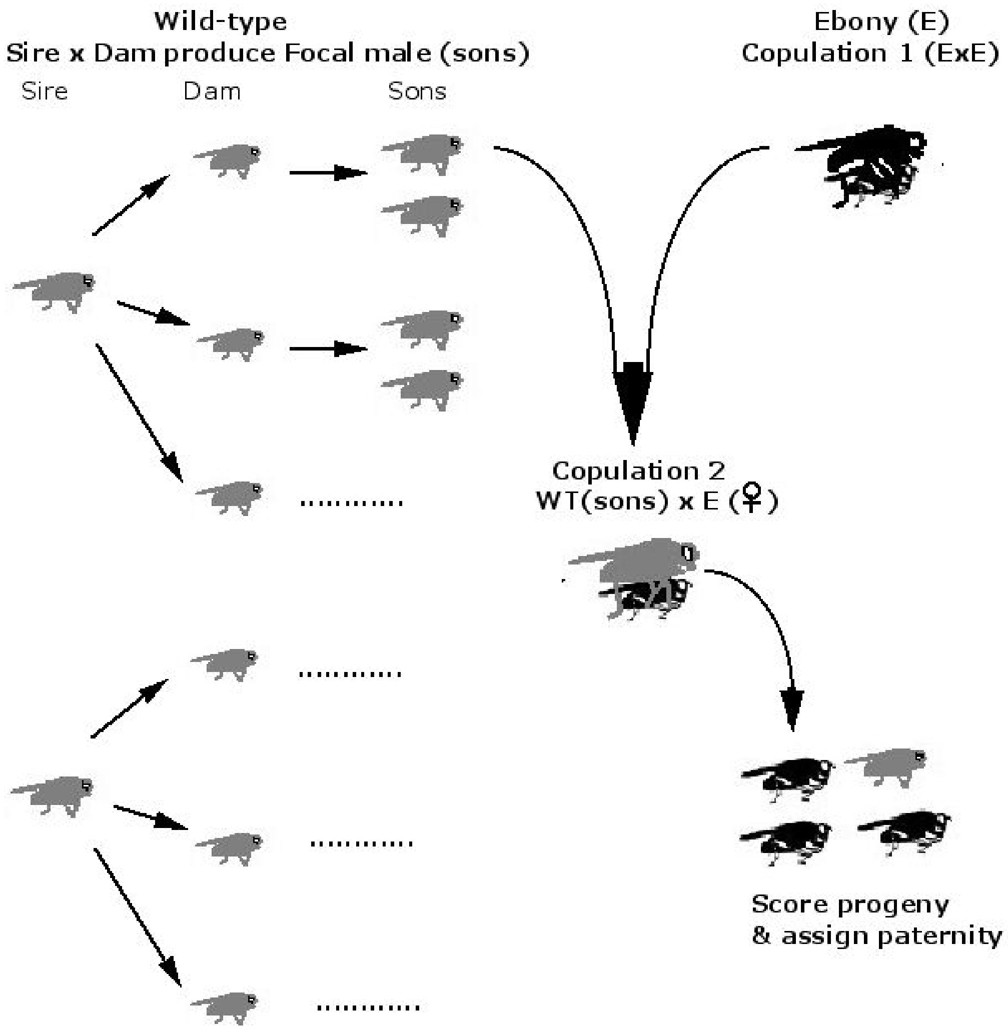

Figure S1. The basic experimental design. Sires are mated to dams to produce sons that are the focal males in the P2 and attractiveness assessment (40 sires, 85 dams (ca. 2.1/sire), 189 sons (ca. 2.5/dam)). This generates the full-sib/half sib pedigree structure used in genetic assessments. Sons are mated to ebony females (Copulation 2) that have previously copulated with ebony males (Copulation 1). Male attractiveness (copulation latency) and paternity (P2) are scored from the sons-ebony female pairings.

CHAPTER 6

Attractive males do not sire superior daughters

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ABSTRACT

Much of the recent work on the evolution of female choice has focused on the relative influence of direct and indirect benefits, and particularly whether direct costs can be offset by indirect benefits. Studies investigating whether attractive males benefit females by increasing the viability of their offspring often report mating advantages to sons consistent with the Fisher process, while detecting no or weak viability benefits. One potential reason for this is that sons may trade-off viability benefits with investment in costly traits that enhance mating success, leading to the suggestion that viability benefits may be better detected by examining daughters' fitness. Here we investigate the relationship between male attractiveness and daughters' fitness in *Drosophila simulans*. We measured daughter (and dam) lifetime reproductive success and longevity. We found no evidence that attractive males sire high fitness daughters. Additionally, neither daughters nor dams gained direct benefits from mating with attractive males. However, aspects of daughters' fitness were related to dam characters.

Key Words: female preference; indirect genetic benefits; daughters' fitness; maternal effects

INTRODUCTION

Much of the debate surrounding the benefits of female mate choice centres on the relative influences of direct and indirect fitness benefits, and the capacity of these benefits to sustain costly female preferences (Kirkpatrick 1996; Cameron et al 2003; Codero and Eberhard 2003; Hosken and Tregenza 2005). Theory predicts that direct benefits should have the greatest effect in offsetting costs, and that indirect effects may be weak and therefore relatively ineffectual in comparison (Kirkpatrick 1996; Kirkpatrick and Barton 1997). Meta-analyses of direct and indirect effects across a range of taxa also suggest that indirect benefits are smaller than direct benefits (Møller and Alatalo 1999; Jennions et al 2001; Møller and Jennions 2001). However, in species where direct benefits are absent, and females suffer no direct costs from their mate choices, small indirect benefits may be all that is required to maintain preference (Kirkpatrick 1996). Females may benefit indirectly by mating with attractive males in two general ways (reviews in Andersson 1994; Jennions and Petrie 2000). In the first, attractive males produce sons that inherit their fathers' attractive traits and therefore also have increased mating success, so female fitness is indirectly increased via sons' mating success (Fisherian mating advantages: Fisher 1930; Lande 1981; Kirkpatrick 1985). In the second, attractive males are themselves of superior genetic quality and hence viability, and offspring sired by these males also inherit these 'good genes'. In this case, female fitness is indirectly increased through the production of high viability offspring (reviews in Jennions and Petrie 2000; Andersson 2006). Several studies finding weak or no evidence for good genes effects conclude that females benefit largely via the Fisher sons process (Jones et al 1998; Tomkins and Simmons 1999; Head et al 2005; Hadfield et al 2006). However, it has been argued that all indirect benefits ultimately become linked to good genes (Rowe and Houle 1996; Jennions and Petrie 2000), or, put another way, that good genes effects are inevitable, although this does not preclude the possibility that either or both of these mechanisms may be distinctly influencing female mating preferences at any one time (Kokko et al 2003; Andersson 2006).

It has also been suggested that good genes effects – measured as increased offspring viability (Møller and Alatalo 1999; Jennions et al 2001) – may only be manifest in daughters since sons may trade-off viability with investment in traits that enhance mating success (Pitnick and Markow 1994; Droney 1998; Kokko 2001; Getty 2002). This has led some authors to suggest that empirical tests of good genes should utilise daughters' fitness as a more reliable measure of the inclusive fitness benefits from attractive males (Jennions and Petrie 2000; Hunt et al 2004). A little-explored theory by Seger and Trivers (1986), also predicts that, under sexually antagonistic fitness variation, preferences that benefit females specifically via their daughters' fitness, even at a cost to sons' fitness, should evolve. There is a growing body of evidence consistent with such sexually antagonistic effects (Rice 1984; Norris 1993; Fedorka and Mousseau 2004; Pischedda and Chippindale 2006; Foerster et al 2007; O'neal et al 2007). It would appear then, that indirect selection on female preferences may be most apparent by examining the effects of male attractiveness on daughters' fitness.

Here we investigate the indirect fitness benefits to females from mating with attractive males by examining the fitness of their daughters in *Drosophila simulans*. While it is currently unclear precisely which male traits females prefer, we have previously shown that mating with attractive males provides no direct benefits to females in this species (Taylor et al 2008). Additionally, we have found that male attractiveness is heritable and attractive males also have greater success in post-copulatory sexual selection, and that this success is also heritable (Taylor et al 2007; Hosken et al 2008). All this suggests that female mate preference is at least partly driven by Fisherian effects in this species. However, little is known of the indirect benefits to females via their daughters in *D. simulans*, but some evidence for offspring viability benefits associated with male attractiveness have been reported in *D. melanogaster* (Partridge 1980; Taylor et al 1987), *D. montana* (Hoikkala et al 1998) and *D. serrata* (Hine et al 2002). We measured female preferences for males and then examined the relationships between male attractiveness and daughters' lifetime reproductive success (LRS) and longevity. We also assessed associations between mothers' and daughters' fitness.

METHODS

Flies

Stock populations of *Drosophila simulans* were derived from twenty isofemale lines supplied by the Centre for Environmental Stress and Adaptation Research, La Trobe University, Australia. Isolines had been cultured from individuals caught from a wild population at Tuncurry, Eastern Australia in March, 2004. Isolines were mixed in a population cage and reared on 'Drosophila quick mix medium' (supplied by Blades Biological, UK) with yeast and water at 25^{o}C and 12:12 light/dark cycle, to initiate a large outbred population. Subsequent housing conditions follow this regime unless reported otherwise. The stock population had been maintained at 500-1000 flies for over 35 generations at the time of the experiment. For the experiment, emerging virgin adults from the outcrossed population were collected every 12hrs, anaesthetized with ice, separated by sex (using genital morphology visible at the tip of the abdomen) and kept in separate 180ml vials, with an excess of the culture medium for three days prior to experimental pairings.

Sire attractiveness

Female mate choice has been widely explored across *Drosophila* and female preferences for male traits such as courtship behaviour, male song frequency and large body size have been established using female responses to male courtship (e.g. Pitnick 1991; Droney 1996; Barth et al 1997; Ritchie et al 1999; Acebes et al 2003; Droney 2003). Since females largely determine whether copulation occurs or not in *Drosophila* (Markow 1996), they are predicted to respond to preferred males more rapidly and therefore, copulation should occur more rapidly with attractive males. The time taken for females to accept and copulate with a male (copulation latency) has been used as a standard measure of female preference across many *Drosophila* studies (see above). We therefore used the time it took for copulation to begin after introduction (copulation latency) as our measure of male attractiveness and female preference. Female preference in this instance follows the general definition established by Jennions and Petrie (1997) - preference reflects the propensity for females to mate with certain males. We measured female (hereafter dams) preferences for males (hereafter sires) and then assessed the relationship between the attractiveness of sires and their daughters' fitness.

Parent-offspring fitness

A total of 45 sires were used. Sires were taken from the virgin collections and paired with a single dam of the same age and allowed a 3hr exposure between 09:00-12:00 in which to copulate. This time period is equivalent to the first three hours of 'daylight' the flies would normally experience, and also corresponds with the time of peak mating activity reported in natural populations (Gromko and Markow 1993). We recorded the time taken for copulation to commence (copulation latency) and the length of copulation itself. Following copulation, sires were removed and frozen for future measurement. All dams that copulated were transferred to fresh vials with the culture medium every

24 hours for 7 days, and then once per week until they died. Dams' fitness was measured as for their daughters (see below). From each sire, a total of eight daughters (chosen at random from those emerging from the first 2 vials of each dam), were assayed for fitness; half were mated to a virgin male from the stock population, to measure LRS, and the other half kept as virgins to assess longevity. We measured longevity in virgin daughters as there are potentially costs to longevity from mating itself (e.g. Partridge and Fowler 1990; Chapman et al 1995; Taylor et al 2008) and daughters' mates may also distort any fitness benefits conferred from sires. Daughters to be mated were paired with a virgin male, of the same age collected from the stock population, and allowed a 3hr exposure in which to copulate, under the same conditions as their parents. We again recorded copulation latency and copulation duration. Mated daughters were transferred to fresh vials with the culture medium every 24 hours for 7 days, and then once per week until death. Virgin daughters were also transferred to fresh vials once per week until death.

Many studies of indirect benefits have utilised juvenile offspring survivorship (Møller and Alatalo 1999), however, some authors have suggested that indirect benefits should be measured over as many indices of fitness as possible (Hunt et al 2004). Therefore, our measures of fitness in daughters were a standardised measure of lifetime reproductive success (LRS) and adult longevity, which equates closely with total daughter fitness. Lifetime reproductive success (LRS) for daughters (and dams) was measured from the summed progeny count from the first 7 vials only, as previous work has shown this to be a good proxy for lifetime productivity from a single copulation (Taylor et al 2008). Longevity of virgin daughters (and dams) was measured in days from eclosion to death, and mortality checked on a daily basis. Body sizes of sires, dams, daughters and daughters' mates were measured using wing length (Gilchrist and

Partridge 1999), which we have previously found to be a good measure of general size (Taylor et al 2008).

Statistical Analysis

Data analysis was conducted using SPSSv15. We used individual daughters' fitness measures to assess the phenotypic relationship between parents and daughters' fitness. We conducted an analysis of covariance (ANCOVA), using parents' traits (copulation latency (sire attractiveness), copulation duration, dams' LRS, dams' longevity and both parents' body sizes) to examine the effects of parental traits on daughters' fitness. We also repeated the parent-daughter analyses at the family-level, using fitness measures averaged across daughters. Using average family fitness controls for the individual effects of the males that daughters mated with (and provides an estimate of genetic correlations - Lynch and Walsh 1998). Although our primary interest was the relationship between attractive sires and their daughters, we also considered the possibility of direct effects on dam and daughters' fitness. We again used ANCOVA with dams and daughters' own traits (copulation latency (sire and daughters' mates' attractiveness respectively), copulation duration, longevity and body sizes of males and females where appropriate) to examine the direct effects on dams' LRS and longevity, and daughters' LRS and virgin daughters' longevity. We also regressed mean daughters' LRS (mated sisters) against mean daughters' longevity (virgin sisters) as this also provides a relatively unbiased estimate of the genetic correlation (r*G*) between traits (Lynch and Walsh 1998). In all cases, all relevant variables were included in the model, and then backwards elimination used to simplify the final models. Variables were transformed to improve normality where appropriate and residual plots checked for normality. Sample sizes vary across analyses due to missing data (e.g. some females escaped, or wing sizes were not measurable, and 7 dams and 17 daughters that failed to produce any offspring were discounted from the analysis). We note here that although we could conduct dam-daughter regressions to estimate the heritability of female fitness, we do not include these analyses as dam estimates include maternal and dominance effects and can therefore be inaccurate. Additionally, the sample sizes we employ are rather small for accurate quantification of genetic parameters.

RESULTS

Phenotypic parent-daughter associations

Our primary aim was to see if females mating with attractive males produced higher quality daughters. The final models for parent-daughter analyses, derived after backwards elimination, are shown in Table 1. In these phenotypic correlations, we found no evidence that attractive sires produced daughters with higher fitness (LRS and virgin daughters' longevity) (ANCOVA: all $F < 0.66$; $p > 0.42$). Although dams' LRS was weakly negatively associated with daughters LRS, this was non-significant (ANCOVA: $ß = - 0.01$; $F_{1,116} = 3.48$; $p = 0.06$). However, we did find that dams' body size was significantly negatively correlated with the longevity of virgin daughters (ANCOVA: $ß = -33.48$; $F_{1,100} = 7.28$; $p = 0.008$) – larger mothers produced shorter-lived daughters.

Family-level associations

We examined the effects of parents' traits on averaged daughters' fitness, as this controls for the individual effects of daughters' mates. The final models derived after backwards elimination are also shown in Table 1. We, again, found no significant associations between fathers' attractiveness and average daughters' fitness, either LRS or longevity (ANCOVA all $F < 2.3$; $p > 0.14$; Figures 1a and 1b). However, we did find a significant negative association between dams' LRS and daughters' LRS (ANCOVA:

ß = -0.29; $F_{1,26}$ = 5.89; p = 0.02) and a significant positive association of dams' longevity and daughters' LRS (ANCOVA: ß = 1.79; $F_{1,26}$ = 6.89; p = 0.014). Dams' body size was, again, significantly negatively associated with virgin daughters' longevity (ANCOVA: ß = -33.68; $F_{1,27}$ = 9.31; p = 0.005). There was no significant genetic correlation (rG) between daughters' LRS and virgin daughters' longevity (ß = -0.49 (SE± 1.43); $F_{1,34}$ = 0.12; p = 0.73).

Direct effects

Whilst we did not find an association between sire attractiveness and daughters' fitness, we did find that sire copulation latency was significantly negatively correlated with both sire body size and copulation duration (ANCOVA: ß = -2.49; $F_{1,33}$ = 13.32; p = 0.001 for body size and ß = -.029; $F_{1,33}$ = 8.26; p = 0.007 for copulation duration). So larger sires were preferred and also copulated for longer, although, this did not translate into any direct effects on dams' LRS or longevity (ANCOVA: all F < 4.46; p > 0.05). The relationships between daughters' mates' attractiveness (copulation latency) and both body size and copulation duration were also negative, but non-significant (ANCOVA all $F_{1,119}$ < 1.99; p > 0.16). We found no evidence that daughters' LRS was directly affected by the attractiveness of the males they themselves mated with (ANCOVA: $F_{1,99}$ = 0.84; p = 0.36), or any other of their own traits (ANCOVA: all F <2.6; p > 0.12). Virgin daughters' longevity was also not significantly associated with their body size (ANCOVA: $F_{1,116}$ = 0.59; p = 0.44).

In sum, these results indicate no indirect benefits to dams from attractive sires via daughters' fitness, and no direct effects of attractive males on female LRS (either in dams or daughters).

DISCUSSION

Our primary interest was to establish whether attractive males produced daughters with higher fitness. While we have previously found evidence consistent with Fisherian mating advantages in this species (Taylor et al 2007; Hosken et al 2008), it has been suggested that good genes effects, likely manifest in daughters, are inevitable in sustaining female preferences (Jennions and Petrie 2000). However, at both a phenotypic and family level, neither sire attractiveness nor body size was significantly associated with daughters' LRS or survival. Therefore it seems that good genes effects are not manifest in this population. These results contrast with studies showing fathers' reproductive success negatively affecting their daughters' fitness (Fedorka and Mousseau 2004; Pischedda and Chippindale 2006; Foerster et al 2007; O'neal et al 2007), but are consistent with others reporting a neutral effect of father's reproductive success on their daughters' fitness alongside benefits via sons' fitness (mating success) (Norris 1993; Jones et al 1998; Tomkins and Simmons 1999; Rundle et al 2007). Although we did not directly measure sons' fitness here, our previous findings (see above) find attractive males sire attractive sons, and together with the results presented here suggest mate choice operates largely via the Fisherian model in this population. While it is also possible that a neutral father-daughter relationship signifies a benign laboratory environment where fitness variation in offspring was minimized (Qvarnström and Price 2001; Hunt et al 2004; Schmoll et al 2005), these are the same experimental conditions under which the benefits via sons were previously found. The lack of any direct benefits from attractive males is also consistent with previous findings, using the same experimental protocols and much larger sample sizes (Taylor et al 2008). A meta-analysis of good genes effects by Møller and Alatalo (1999) concluded that indirect benefits constitute a small effect size in general, so it is possible that our statistical power was not sufficient to detect a small good genes effect (Cohen 1988). However, our statistical power was sufficient to detect effects of dams' fitness on their daughters, which were consistent across the family-level analyses. So even if sire effects on daughters were present, they would have to be small and of relatively minor significance compared to other effects detected in our study population. An interesting comparison to our results is the finding in *D. serrata* that males with extreme blends of cuticular hydrocarbons were most preferred, but produced offspring of intermediate viability (Hine et al 2002). The authors suggest this may be evidence for a preference initiated under Fisherian mating advantages that is now stabilizing under trade-offs between physiologically expensive sexually selected traits and viability.

We also found that dams exhibited a significant preference for larger sires, but no significant preference for larger males was found in daughters. This is consistent with a generally inconclusive body of literature on male body size as a preferred trait in other *Drosophila* (Partridge and Farquhar 1983; Partridge et al 1987; Pitnick 1991; Markow et al 1992; Markow 1996; LeFranc and Bundgaard 2000; Avent et al 2008), and our previous work on this species (Taylor et al 2007; Taylor et al 2008). Additionally, there was some evidence for relationships between dam and daughter traits. Dams with higher LRS produced daughters with lower LRS in the family level analyses, and smaller dams produced longer-living virgin daughters. There was also some evidence that dams' longevity and daughters' LRS were positively correlated. Negative dam-daughter fitness associations are reminiscent of *D. pseudoobscura*, where females constrained to mate with non-preferred males produced more eggs than those paired with preferred males. This was explained as a compensatory effort towards a less-suitable mate (Gowaty et al 2007). In the same study, flies paired with a preferred mate produced offspring with higher overall viability. However, if this were the case here, we would also expect to find a relationship between sire attractiveness and daughters'

LRS, which we did not. It is also possible that fecundity and egg quality trade-off, so highly fecund dams produced daughters with lower fecundity. Nonetheless, other authors have rejected the possibility that quantity trades-off against quality in species where fecundity is generally high, as differences in reproductive effort amongst females are likely to be mediated via egg numbers rather than quality (Jones et al 1998).

The opposing dam-daughter fitness could also indicate sexually antagonistic effects (Holland and Rice 1998; Pischedda and Chippindale 2006; reviewed in Arnqvist and Rowe 2005). If females were allocating more fecundity to a manipulative/high fitness male then we would expect to see high fecundity in females, with low daughter fitness. Again however, we did not find a direct association with sire attractiveness in either dams or daughters, and previous work also indicates that there is no such direct effect on immediate fecundity in this species (Taylor et al 2008). Other potential explanations include, parental stress, differential stress across generations, and differences in larval rearing conditions, all of which could have influenced fitness (Sgro and Hoffman 1998; Reznick et al 2000; Ackerman et al 2001; Priest et al 2008a, b). A range of mate choice studies taking account of maternal effects have also demonstrated an effect of female condition on offspring fitness (Meikle et al 1995; Hewison and Gaillard 1999; Qvarnström and Price 2001) and an effect of amplifying sire effects on offspring fitness (Hunt and Simmons 2000; Kotiaho et al 2003). How maternal condition affects offspring fitness in *D. simulans* also requires further work.

This study was designed to specifically test for indirect fitness benefits to females via their daughters. Whilst the results provide no evidence that attractive males sired higher quality daughters, neither did they indicate that preferred males decreased female fitness. It is conceivable that environmental and maternal contributions to offspring fitness may have obscured the true nature of the relationship between male attractiveness and daughters' fitness, although this requires further work. Nevertheless, current evidence suggests Fisherian effects underlie mate preference in our populations.

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Table 1. Shown here are the final models derived after backwards elimination. Values for Copulation Latency are included to show the stage at which father's attractiveness was eliminated (α = 0.05). Results significant at p<0.05 are highlighted with *.

Traits	N	β (se)	F (df)	p
Phenotypic: Mated daughters' fitness (LRS), Minimal Adequate Model: R^2 = .03				
LRS (Dams)	**118**	**-0.01 (0.01)**	**3.48$_{(1,116)}$**	**0.06**
Intercept		**13.9 (1.11)**	**156.46$_{(1,116)}$**	**0.00**
(Copulation Latency (Dams)	111	0.01 (0.01)	0.19$_{(1, 105)}$	0.66)
Phenotypic: Virgin daughters' fitness (Longevity), Minimal Adequate Model: R^2 = .07				
Body Size (Dam)	**102**	**-33.48 (12.41)**	**7.28$_{(1, 100)}$**	**0.008***
Intercept		**99.52 (20.52)**	**23.51$_{(1, 100)}$**	**0.00**
(Copulation Latency (Dams)	83	-0.03 (0.04)	0.43$_{(1, 78)}$	0.52)
Family-level: Mated daughters' fitness (LRS), Minimal Adequate Model: R^2 = .27				
LRS (Dams)	**29**	**-0.29 (0.12)**	**5.89$_{(1, 26)}$**	**0.02***
Longevity (Dams)		**1.79 (0.68)**	**6.89$_{(1, 26)}$**	**0.01***
Intercept		**157.86 (31.64)**	**24.9$_{(1, 26)}$**	**0.00**
(Copulation Latency (Dams)	29	0.19 (0.24)	0.64$_{(1, 25)}$	0.43)
Family-Level: Virgin daughters' fitness (Longevity), Minimal Adequate Model: R^2 = .26				
Body Size (Dams)	**29**	**-33.68 (11.04)**	**9.31$_{(1, 27)}$**	**0.005***
Intercept		**99.87 (18.22)**	**30.03$_{(1, 27)}$**	**0.00**
(Copulation Latency (Dams)	27	-0.05 (0.03)	1.84$_{(1, 23)}$	0.18)

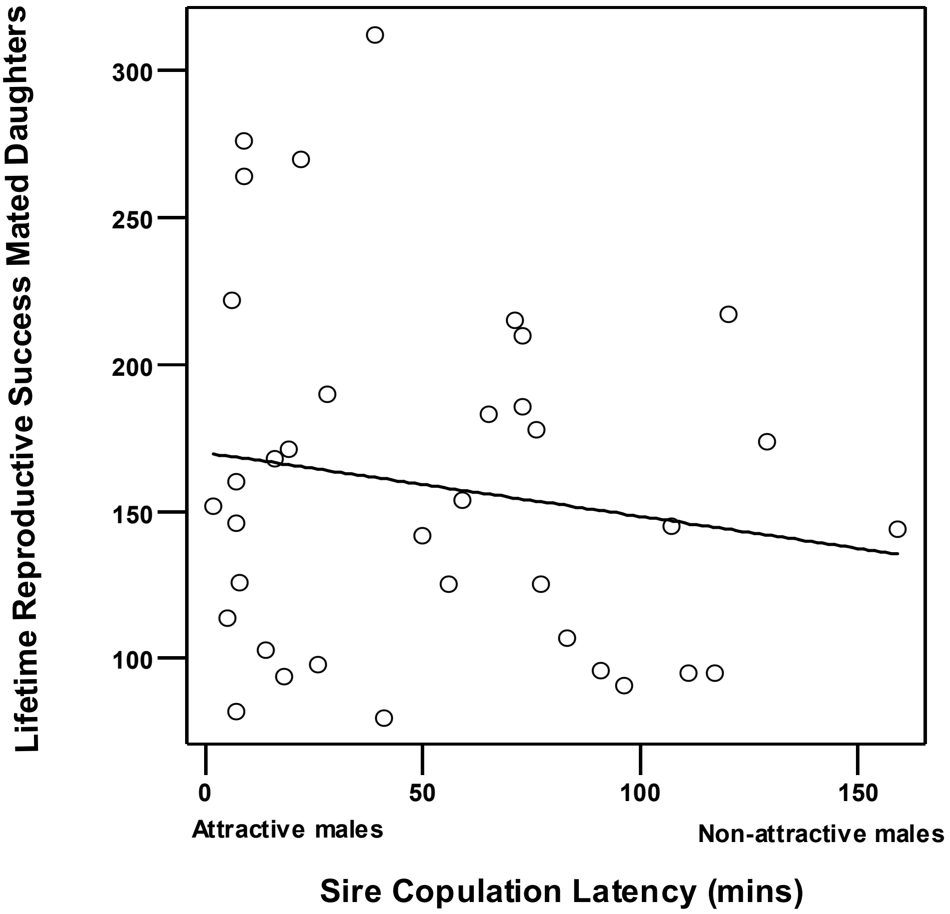

Fig. 1a. There was no significant association of father's attractiveness and daughters' fitness. Shown here is fathers' attractiveness (sire copulation latency – our measure of female preference for a male) and mated daughters' averaged LRS. Note that on this scale, longer copulation latency indicates less preferred males.

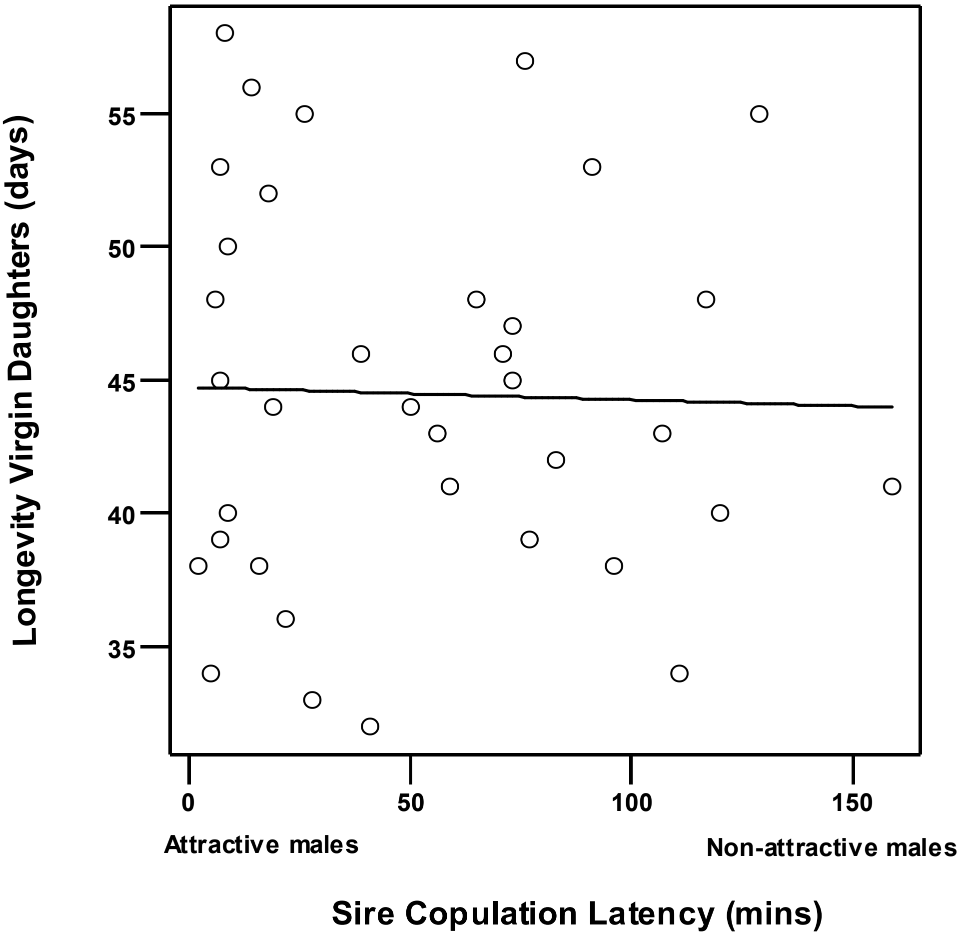

Fig. 1b. Shown here is fathers' attractiveness (sire copulation latency) and virgin daughters' averaged longevity. Note again that less preferred males are expected to take longer to copulate.

CHAPTER 7

Conclusions and prospects: sexual selection and the benefits of mating with attractive males in *Drosophila simulans*

The benefits of mating with attractive males in *Drosophila simulans*

Explanations for how and why females evolve to prefer any of a number of sexually selected traits in males have been the focus of evolutionary and behavioural ecology for decades (Andersson 1994). The preceding chapters presented a suite of investigations that highlight some of the possible ways that females benefit by mating with attractive males, although they are by no means exhaustive.

Chapter Two examined the simplest premise of benefits from preferred males – that a female mating with an attractive male can directly increase her fecundity or fertility. This requires nothing more than direct natural selection on the preference itself and formal modelling predicts that direct selection will produce female preferences for males who benefit their immediate reproductive success (Kirkpatrick & Barton 1997). Most of the current evidence also suggests that direct benefits are common in nature, but that the overall effect size is small (Møller & Jennions 2001). Contrary to this then is the finding that, in this population of *D. simulans*, there is no direct effect of preferred males on female fitness, even when measured over a variety of fecundity estimates. Females demonstrated a significant preference for larger males, and male-male competition reinforced this preference, but there was no apparent evidence that this behavioural response towards these males was associated with a direct increase in female fitness. Females in nature may benefit if males confer them greater material resources, however, this was not examined in this investigation as there is no parental care in these flies and males are unlikely to represent a large direct benefit in the form of superior territorial resources as mating takes place communally on the food/ oviposition source (Gromko & Markow 1993; Markow 1996). Since females do routinely mate multiply in nature, the most obvious caveat to this conclusion is that direct benefits may accrue over several matings, therefore differences between individual males from a single mating may need to be considerably larger to be detected (Møller & Jennions 2001). However, while there was no evidence of a direct benefit from one attractive male, there was some evidence that longevity was reduced in females mating with preferred males. Thus if the benefits of attractive males accrue over several matings, it follows that any costs of mating will also have a cumulative effect.

Following on from these findings, Chapter Three examined the fitness benefits of multiple mating. Multiple mating may benefit females in many of the same ways as a preferred male – for example, by directly increasing fecundity through availability of viable sperm, or by providing the opportunity to diversify indirect benefits (Jennions & Petrie 2000). Equally, if females are coerced into re-mating too frequently by attractive/manipulative males, the costs of mating may negate the benefits. This chapter presented robust evidence that multiple mating does benefit female *Drosophila simulans* directly, by increasing lifetime reproductive success, and that costs to longevity have little or no impact on this benefit. These results concur with a widespread finding across insect taxa (Arnqvist & Nilsson 2000), and contrasts with an often-cited interpretation of Bateman's work (Bateman 1948; and reviewed in Snyder & Gowaty 2007) that female fecundity is maximized after only one or a few matings. It is likely that direct selection on females to maintain adequate sperm supplies will overshadow any indirect effects of males of varying quality, and a study by Brown et al. (2004) has also suggested that there are no advantages to polyandry over monandry *per se* in *D. melanogaster*. However, since females in natural *Drosophila* populations are unlikely to mate repeatedly with the same male, polyandry may carry incidental benefits of high quality males, for example by promoting sperm competition and therefore the potential for sexual selection to continue post-copulation (see below).

In the absence of direct selection on preferences, indirect selection may be sufficient to maintain female mating preferences, although any benefits reliant on genetic correlations between female preferences and sexually selected traits involve a number of important assumptions (Andersson 1994). Chapter Four examined one of the assumptions of indirect benefits models, that of heritable variation in male attractiveness. Male attractiveness was significantly heritable and highly evolvable, suggesting that additive genetic variation in attractiveness exists in these flies, and sons of preferred males are themselves preferred. These results suggest that females may currently be benefiting indirectly via Fisherian mating advantages, which, in the absence of direct fitness benefits, also suggests minimal costs to female preferences (formal modelling of the Fisher process predicts that when direct selection (i.e. costs) on the preference is introduced, the lines of equilibrium collapse and females prefer males who benefit their immediate fitness (Lande 1981; Kirkpatrick 1982; Kirkpatrick 1985)). This finding concurs with other work on *D. melanogaster* (Rundle et al. 2007), crickets (Head et al. 2005) and sandflies (Jones et al. 1998), which also provide evidence for Fisherian mating advantages as the major benefit from mating with attractive males. Mating preferences based solely on benefits of a Fisherian nature are predicted to be rare in nature, since female preferences are unlikely to be entirely cost free, however, they may be more prevalent in laboratory populations where costs to females are minimal. Establishing the basis for the significant variation in male attractiveness in this study requires further work. For example, these investigations did not aim to determine specifically which male traits females preferred, or whether attractiveness was dependent on male condition as suggested by some as a solution to the lek paradox (Rowe & Houle 1996; Tomkins et al. 2004). There is also a growing body of evidence suggesting that males trade-off somatic maintenance against sexually selected traits, so that the traditional view of the relationships between Fisherian and indicator traits and male survival is flawed (Pitnick & Markow 1994; Droney 1998; Kokko 2001; Getty 2002). The costs to males of producing sexually selected traits in this population also require further exploration: costs associated with courtship vigour may be more influential than those associated with morphology, if for example, male mating success over a lifetime is largely governed by their persistence in approaching and displaying to females, as is the case in other *Drosophila* (Gromko & Markow 1993; Droney 1998).

Females mating multiply inevitably leads to sperm from more than one male competing for fertilization success in these sperm storing flies, so that sexual selection continues post-copulation (Parker 1970). Chapter Five examined the relationship between pre- and post-copulatory mating success, as it is not clear if these two processes necessarily coincide. Attractive males sired a greater proportion of offspring under sperm competition, strongly supporting the theory that preferred males also have greater success post-copulation, either because they are better sperm competitors or because cryptic female choice favours them (Parker 1970; Eberhard 1996). As with male attractiveness in the previous chapter, sperm competitiveness was also significantly heritable and positively genetically correlated with male attractiveness, so that these traits can potentially evolve in concert. Again, there was no relationship between sperm competitiveness and overall female fitness, so it appears that females in this *Drosophila* system are benefiting indirectly from mating with attractive males as their sons are successful in both the pre- and post- copulatory arenas. Whether this post-copulatory success was attributable to attractive males being intrinsically better sperm competitors or their sperm being selected in the Fisherian or ‘good genes’ sense remains unknown (Keller & Reeve 1995; Pizzari & Birkhead 2002; Evans & Simmons 2007), as the traits conferring greater fertilization success were not specifically determined. Much of the evidence for the roles of accessory gland proteins in *Drosophila* male seminal fluids suggests selection for traits that increase intrinsic male fertilization success under sperm competition. The high value of P2 also concurs with estimates of P2 in other *Drosophila* and suggests selection for sperm displacement ability, although there is also evidence for both male and female effects on P2 values (reviewed in Singh et al. 2002). Attractive males that induce females to re-mate sooner than is optimal under usual rates of sperm depletion might also be expected to be better intrinsic sperm competitors as they face greater risk of sperm competition by overlapping their sperm with that of previous males. It is likely then that attractive males are intrinsically better sperm competitors, rather than being preferred both pre- and post- copulatory, although one caveat of this assumption based on these results is that P2 was only tested against one phenotype of male, i.e. the ebony-bodied mutant.

Finally, if Fisherian mating advantages do represent the major benefit of mate choice in these flies, and multiple mating and sperm competition combine to effect a similar mating advantage in post-copulatory processes, then it is reasonable to assume that indirect benefits are sufficient to maintain preferences in these females. However, as aforementioned, 'good genes' effects are perhaps an inevitable consequence of preferences based on indirect effects, since Fisherian traits themselves are initiated under an element of 'good genes', and most male traits are expected to become costly and dependent on either environmentally or genetically determined condition (Balmford & Read 1991; Kirkpatrick & Ryan 1991; Jennions et al. 2001). Therefore, the presence of Fisherian mating advantages itself suggests that females may be receiving indirect benefits through the overall quality of their offspring, and that they may simply 'look' like Fisherian traits due to the trade-offs that males make between somatic maintenance and sexually selected traits (Kokko 2001). Preferences based on 'good genes' should also be expected under more realistic assumptions that female preferences in nature are invariably costly to some extent (Kirkpatrick 1996; Møller & Alatalo 1999). Since these findings cannot indicate at present just how male costs affect their preferred traits, it is reasonable to assume that daughters' fitness may indicate whether males are preferred for any 'good genes' effects. Perhaps disappointingly then, Chapter Six provides no evidence that attractive males increased daughters' fitness, although a definitive refute of 'good genes' effects requires further exploration into the effects of preferred males on male offspring viability, as well as the effects of maternal genotypes on offspring fitness. Suffice it to say that there is currently no evidence here that preferred males increase their daughters' fitness in these flies.

Costs

The potential for sexual conflict to effect evolutionary change has re-focused a lot of the attention in mate choice studies on the costs of mating, and particularly the costs from mating with preferred males themselves (Parker 1979; Parker 2006). Although examples of sexually antagonistic coevolution are common across insect taxa (reviewed in Arnqvist & Rowe 2005), and much of the evidence in *D. melanogaster* is concurrent with this, a consistent thread throughout these findings is that there is no indication of a strong phenotype-dependent cost of preferred males on female fitness. Chapter Two did present some evidence of a longevity cost to females when two males were present, suggesting costs may be associated with male-male competition. However, in this instance females stopped ovipositing long before they died, so the cost did not impact directly on fecundity. Similarly, in Chapter Three the costs attributed to male harassment were more than compensated for by the benefits of mating multiply, i.e. females that were harassed and died younger still had higher LRS than singly mated females. In nature, females are unlikely to spend large amounts of time confined in a space with a male as they were in these experimental conditions, and often a female's best response to a non-preferred male reported in natural populations is simply to fly away, suggesting this is a very effective means to avoid harassment (Gromko & Markow 1993). There was no evidence of the much-cited result by Chapman et al. (1995) that female longevity is reduced by multiple mating itself, so it seems that many of the well-known costs to *D. melanogaster* females from male seminal fluids are simply not manifest, at least not in this population of *D. simulans*. Although the lack of a direct LRS cost from preferred males in these studies does not refute the idea of phenotype-independent costs, such as manipulating females to mate too frequently, it certainly indicates that females in this population are receiving sufficient benefits to compensate. This is in direct contrast to other studies of mate choice in *Drosophila*, where preferred males directly depress the fitness of females (Pitnick 1991; Droney 2003; Friberg & Arnqvist 2003), and studies of experimental evolution that report that sexual selection represents a reproductive load on populations (Holland & Rice 1999; Martin & Hosken 2003).

Evolutionary effects of mating with attractive males in D. simulans

The findings presented in this thesis collectively suggest that females are currently selected to mate multiply by a naturally selected benefit of maintaining an adequate supply of sperm, and that female preferences are based largely on indirect Fisherian mating advantages, which may continue to be manifest in the post-copulatory arena. Although good genes effects are expected to be inevitable, there is so far no evidence of female preferences based on this form of indirect benefit. These conclusions are somewhat unusual in that most of the current evidence suggests that direct benefits are more prevalent than indirect benefits (Møller & Alatalo 1999; Møller & Jennions 2001), and that male traits function as honest signals of condition, as predicted by 'good genes' models (Jennions et al. 2001). Much of the current debate concerning the false dichotomy between indirect benefits processes, and the relative influences of direct and indirect benefits, is entirely contingent upon the costs to both males and females prevalent in the system (Kirkpatrick 1996; Kirkpatrick & Barton 1997; Kokko 2001; Kokko et al. 2002; Cameron et al. 2003; Chapman et al. 2003; Cordero & Eberhard 2003). In the *Drosophila* system explored here, preferences that are maintained by indirect benefits suggests that direct selection on females is either absent or very weak (Kirkpatrick & Barton 1997), and the lack of strong phenotype-dependent costs also supports this. If Fisherian traits are currently costly, then the population could already have reached an equilibrium where trait expression and female preferences are stable, i.e. not exaggerating. If traits are currently experiencing strong directional selection characteristic of a 'runaway' process, e.g. because ecological costs for males are also similarly absent as for females, then the system could be expected to move towards condition-dependence, which may reflect indirect benefits of a 'good genes' nature. Similarly, if all attractive males have similar success in post-copulatory sperm competition, then some measure of sperm selection might be expected to arise as pre-mating advantages resolve to equilibrium. Experimental evolution using these flies would indicate whether the net benefits reported throughout this study, particularly if based on exaggerating Fisherian traits, are sustained over time. Tests of whether indirect genetic benefits compensate for costly males has already suggested that in *D. melanogaster* this is not the case (Orteiza et al. 2005; Stewart et al. 2005).

Caveats

An often-cited criticism of results obtained under laboratory conditions is that they tell us little about what happens in nature (Harshman & Hoffmann 2000), and that different selection pressures exist under laboratory cultures than in nature (Ackermann et al. 2001). For example, these investigations are unable to account for ecological costs such as extensive search and sampling time, predation risks or disease. However, even if Fisherian mating advantages have arisen in this population over the generations since they were introduced into laboratory culture, because large ecological costs have been relaxed, this in itself is an important empirical finding that conforms to theoretical predictions about how evolution of female preferences proceeds. The challenge is not to catalogue all the current processes in nature by emulating the precise natural conditions for every species, but to determine what provokes evolutionary change. Laboratory-based investigations are extremely effective tools for extracting underlying evolutionary processes precisely because they can impose chosen selective pressures.

Another common criticism is that environmental variation may account for a large proportion of the variation in results observed, and that different assay protocols in different laboratories may account for disparate results (Ackermann et al. 2001). Environmental effects in *Drosophila* experiments are always likely to be a concern given the scale with which *Drosophila* may respond to environmental variation, and where the full range of selective pressures prevalent in nature is largely unknown. While environmental effects have been statistically controlled for where appropriate in these analyses, this indicates very little about the nature of environmental variation itself and this is an ongoing problem. Chapter Six in particular highlights a potential bias in comparing fitness estimates between parents collected from a stock population, where mating was uncontrolled and densities of non-related individuals were higher, and offspring that were reared in individual vials of full-siblings. Carry over effects have been found to affect fitness estimates across two successive generations in *D. melanogaster* (Sgro & Hoffman 1998). One way to solve this problem would be to rear focal females in individual vials (of siblings) and gather sample sizes from across many individual vials. This is a technique used in many experimental evolution studies, to control unintended effects of density on traits such as growth and body size (e.g. Holland 2002). However, this carries the risk of creating 'welfare state' conditions, so that any fitness effects are masked by the extreme benevolence of the environment. In fact, many laboratory studies conclude that including an effect of stress would perhaps bolster otherwise weak results. Many studies also report effects of condition on expression of sexually selected traits, which requires a degree of variation in the environment (reviewed in Qvarnström & Price 2001). Other suggestions for increasing accuracy of fitness estimates include a consideration of population parameters, mating behaviour exercised over a lifespan and inclusive fitness on estimates of fitness under laboratory conditions (Hughes 1995; Hunt et al. 2004; Priest et al. 2008a, 2008b). Chapters Three, Four, Five and Six by definition of investigating multiple mating and indirect benefits account for some of these caveats.

A further source of environmentally-based variation particularly relevant to sexually selected traits is indirect genetic effects, most commonly highlighted by maternal effects (Miller & Moore 2007). Again, Chapter Six hints at the potential for maternal effects to influence offspring fitness and maternal effects on sexually selected traits have been reported before (Hunt & Simmons 2000; Qvarnström & Price 2001). Full-sib/half-sib designs, as used in Chapter Four, are useful in partitioning genetic variance into additive and non-additive genetic effects. However, non-additive effects include all other genetic effects, plus gene-by-environment effects, which includes the social environment in which traits are selected and manifest (Moore et al. 1997; Wolf 2003). For example, a study of male body size in *Drosophila* concluded that, although they accounted for less than direct genetic effects, indirect genetic effects contributed significantly to phenotypic variance in body size (Wolf 2003). The negative covariance between direct and indirect genetic effects also clearly indicates that selection on one individual can have a significantly antagonistic effect on the phenotypes of conspecifics in the social environment, although the strength of this relationship is also dependent on the relatedness between individuals. As outlined above, in the *D. simulans* experiments presented here the social environments (density) of larvae were not standardized between generations or across females, which potentially introduced some unaccounted-for indirect genetic effects. Also, offspring were reared with full siblings, which potentially affected sexually selected traits that may have been based on competitive ability, for example (Moore et al. 1997; Wolf 2003). Supplying females with food *ad libitum* and moving them to fresh vials at standardized, frequent intervals may have alleviated some of this type of effect, although, again, this remains unquantified and warrants further attention in future work.

Despite the apparent complexity of potential environmental artefacts and the dilemma of creating suitably variable environments to detect fitness variation among individuals, it still remains that model systems such as *Drosophila*, and laboratory-based investigations in general, can tease apart some of the most complex selection processes involved in sexual selection, and suggest at the very least which processes may be operating under the conditions which females are currently evolving.

Further Work

Previous work has suggested that females prefer larger males in *Drosophila* (Markow 1996), however, in these studies male size proved to be an inconsistent proxy for male attractiveness. For example, Chapter 2 reported a significant but weak preference for larger males, whereas Chapter 4 reported no significant association of male size and their attractiveness. Appendix A presents a meta-analysis of the association between male attractiveness (copulation latency) and male body size across all of the investigations presented here. Body size was negatively associated with copulation latency in all 9 blocks of data, i.e. larger males copulated quicker and therefore were taken to be more attractive, but this relationship was significant in only 1 of these blocks. The effect size (weighted for varying sample sizes) explained only 1.44% of the variation in the overall relationship (Rosenthal 1991), so it appears that body size itself was not a strong predictor of male attractiveness but may be associated with other aspects of male attractiveness, such as courtship vigour. Further work examining the nature of the male traits that underpin female mating preferences in this species could indicate whether the Fisherian mating advantages detected are currently exaggerating or at equilibrium.

Most of the debate and research concerning female mate choice has been aimed at determining the benefits to females of mating with what are presumed to be the ‘one best male’ (Jennions & Petrie 1997). The assumption of heritable variation in male trait expression focuses attention on the influence of variation in males in directional selection on preferences. However, the reverse is equally applicable to explaining the evolution of sexually selected traits – if all females prefer the same males, there is no selective pressure for males to invest in a trait away from the preferred average, regardless of their ability to do so – variation in both female preferences and male traits is implied in directional selection on male traits (Arnold 1983). Females of many species do demonstrate relative rather than absolute choices, and in many species over half of females mate with the first male encountered (Jennions & Petrie 1997). Variation in female mating preferences is an important aspect of mate choice that is beginning to receive more empirical support, as researchers consider the circumstances that may alter or constrain female mating preferences in themselves, so that the choice of mate may not actually reflect a universal female preference across a population or species (Jennions & Petrie 1997). Appendix B presents the results of a preliminary investigation of female preferences across genetically distinct lines of females. The results indicate that females of different genetic backgrounds agree on which males are the ‘most’ and ‘least’ attractive males, but preference for intermediately attractive males depends on the genotype of the female. Idiosyncratic preferences across females satisfies a number of ‘problems’ related to the maintenance of variation among males, and may also explain why large body size itself may be an inconsistent proxy for male attractiveness in these flies, if male size is associated with other cues of male quality relative to the choosing female. The effects of non-additive genetic benefits, or genetic compatibility, on female mating preferences has also been receiving support recently (Tregenza & Wedell 2000; Neff & Pitcher 2005). Further work into the basis of variation in female mating preferences is likely to prove fruitful to a holistic view of how and why female preferences evolve and are sustained in this species.

Another possible route for the evolution of female preferences, which has not been specifically investigated here, is via sensory bias. This suggests that female mating preferences are initiated by biases already present in female sensory ecology, and then developed via the direct, indirect or sexual conflict models (reviewed in Endler & Basolo 1998). The most intuitive example of a sensory bias in *Drosophila* would be a male pheromone that mimics the cues that females use to detect fermenting fruit as a source of food and oviposition site. There are some explicit tests of female preferences for cuticular hydrocarbons (e.g. Droney & Hock 1998; Hine et al. 2002; Rybak et al. 2002; Grillet et al. 2006; Skroblin & Blows 2006) which have shown that females do show preferences for male pheromones, although it is not known to what extent they exploit pre-existing biases.

Finally, *Drosophila simulans* was chosen as a model system as, in addition to its own tractability for the questions at hand, it posed a direct comparison to an extremely well-researched sibling species, and in fact well-known genus of insects. One of the consistent features of the findings presented here is their contrast to findings in *D. melanogaster*. Capy & Gibert (2004) compared the evolutionary history of *D. melanogaster* and *D. simulans* and concluded that the genetic, ecological and behavioural differences between these two species are remarkable given their recent ancestry and relatively ubiquitous distribution. They suggest that, in general, genetic variation between populations is less in *D. simulans* than in *D. melanogaster*, possibly due to differences in overwintering and migratory behavior between the two. *D. melanogaster* is thought to have made its colonization debut into temperate climes shortly after the last ice age, whereas a ubiquitous distribution is a much more recent event in *D. simulans*. In short, it remains largely unknown why such closely related species are actually 'false friends' in many of the details of their genetics, ecology and behaviour, but this fact alone can explain at least how sexual selection processes may have diverged in each (Capy & Gibert 2004).

In summary, these findings indicate that female *D. simulans* benefit directly by mating multiply, although the costs of male harassment may reduce the overall net fitness effects. Females also benefit indirectly from mating with attractive males as they produce attractive sons. Preferred males are also better sperm competitors, so that pre- and post-copulatory processes work in concert. Daughters' fitness did not provide any evidence for 'good genes' effects, and there is no indication that sexual conflict has led to any currently negative net fitness effects in this population. These results are therefore most consistent with direct benefits from multiple mating and sexual selection via the Fisherian model of mate choice.

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APPENDICES

APPENDIX A: Meta-analysis of male attractiveness and male body size

Table 1. Summary of meta-analysis of the relationship between male attractiveness (as measured by copulation latency = CL) and male body size (as measured from wing lengths =BS) across 9 experimental blocks (experiments are those presented in Chapters 2-6), following procedures in Rosenthal (1991). CL represents the time (mins) taken for a 3-day old virgin male to initiate copulation with a 3-day old virgin female. BS (mm) was measured post-copulation using SPOT Basic digital image analysis software. Spearman rank correlation coefficient r was used as the effect size indicator to convert results to Z-scores and Fisher's Zr scores for comparison across blocks. The upper half of the table shows a summary of the data used in the analysis, and the lower half shows the results of the meta-analysis. While all blocks show a negative relationship between male size and attractiveness, (i.e. that larger males copulate quicker), only one of these blocks shows a significant relationship. The meta-analysis also indicates that these blocks are not significantly different in their significance values or effect sizes. These blocks also represent statistically robust results, and the effect size explains 3.61% (unweighted) and 1.44% (weighted) of the variance in attractiveness.

Block	**N**	**Mean(SD) CL (mins)**	**Mean(SD) BS (mm)**	**r**	**p**	**Z**	**Fisher's Zr**
1	57	31.8(29.8)	1.534(.04)	-.21	0.12	1.17	-.21
2	55	19.5(26.6)	1.528(.03)	-.19	0.16	0.99	-.19
3	28	13.1(13.7)	1.572(.1)	-.22	0.26	0.64	-.22
4	30	15.8(21.7)	1.540(.1)	-.18	0.34	0.43	-.18
5	44	14.4(14.3)	1.445(.08)	-.25	0.11	1.23	-.25
6	43	53.5(45.9)	1.453(.09)	-.40	**0.01**	2.46	-.42
7	30	20.3(24.1)	1.588(.1)	-.20	0.30	0.53	-.20
8	20	24.95(27.3)	1.537(.1)	-.02	0.92	1.39	-.02
9	342	10.39(10.2)	1.643(.05)	-.04	0.49	0.04	-.04
Total:	649	17.63(23.7)	1.585(.09)	-.28	0.00	4.00	-.28

Analysis:	**r**	**Z**	**$\chi2$**	**p**
Heterogeneity of significance values	-	-	3.96	0.86
Heterogeneity of effect sizes weighted by df (N-3)	-	-	8.13	0.42
Significance of combined blocks	-	2.96	-	0.002
Significance of combined blocks weighted by df (N-1)	-	1.00	-	0.15
Combined blocks effect size	-0.19	-	-	-
Combined blocks effect size weighted by df (N-3)	-0.12	-	-	-

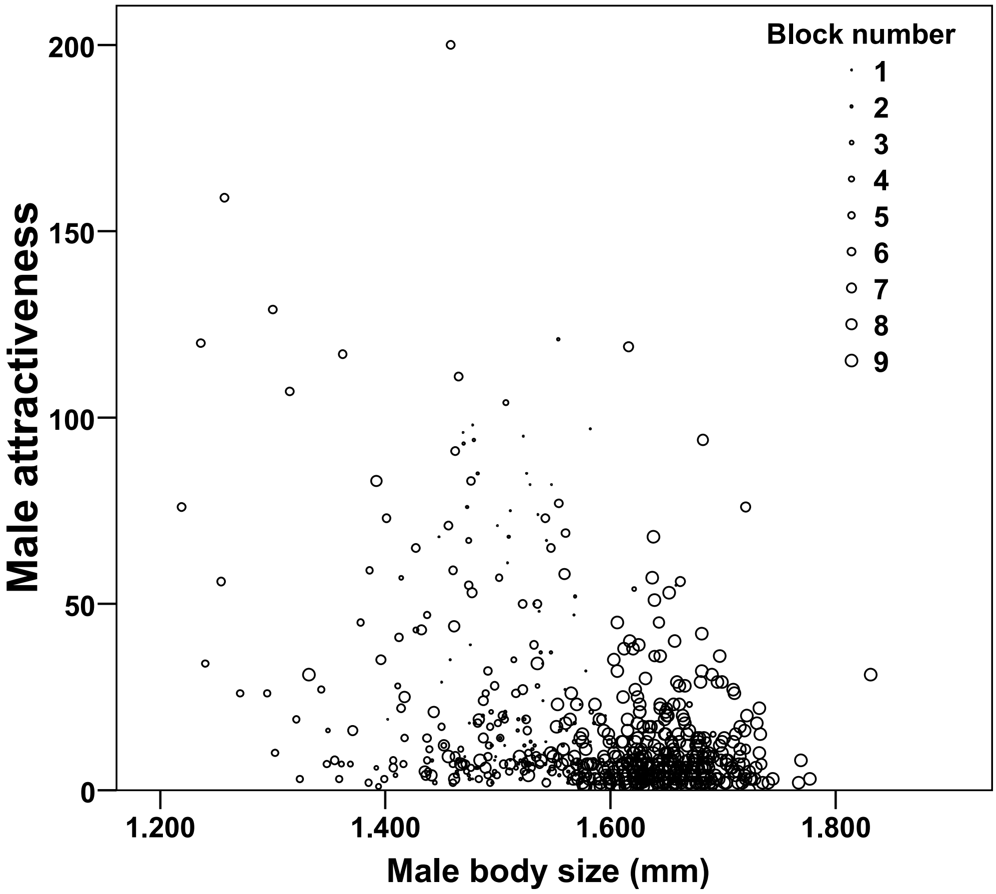

Fig. 1. There was a weak trend for larger males to copulate after a shorter duration of courtship, i.e. they were more preferred. However, only 1 block in 9 shows this relationship to be significant.

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APPENDIX B: Female preferences across isolines of females

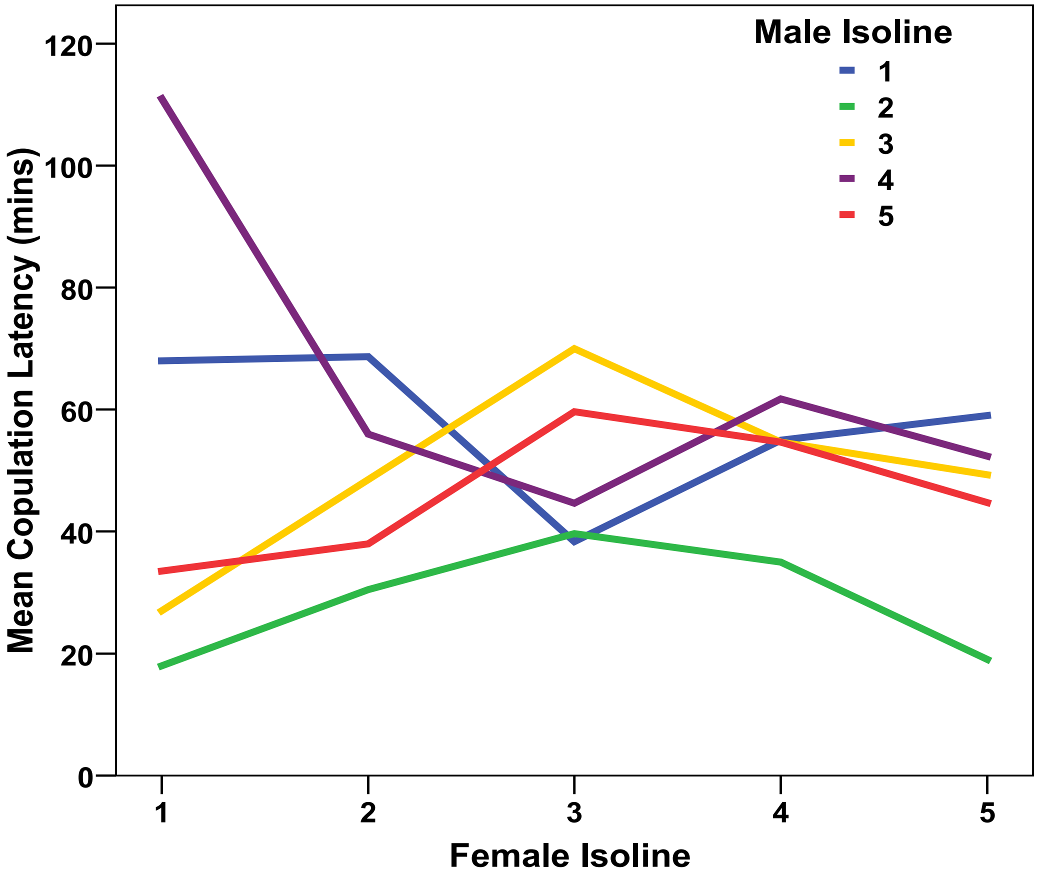

Fig. 1. Females from different isolines show varying preferences for males from different isolines. In brief, virgin females were paired one-on-one with a single virgin male and observed continuously until copulation. All possible combinations of isoline pairs were tested, with five replicates of each combination. The length of time taken for copulation to commence (copulation latency) was taken as a measure of female preference for the courting male, with shorter copulation latencies indicating more attractive males. Although this represents a relatively small sample size of just 25 matings, from this figure there are some patterns that indicate how female preferences vary for different males. For example, males from isoline 2 appear to be attractive to females from all isolines, whereas males from isoline 4 appear to be less preferred than other males. Interestingly, females in many instances prefer males from isolines different to their own.