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SEXUAL SELECTION AND NOVEL MUTATIONS: EMPIRICAL TESTS FOR GOOD GENES INDIRECT BENEFITS AND VARIABLE SEARCH EFFORT

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ABSTRACT

In this thesis, I use 10 populations of *Drosophila melanogaster*, each fixed for a different visible recessive mutation to investigate two outstanding issues in sexual selection research. First, to quantifty indirect benefits of female mate choice and explore the nature of genotype × environment interactions for different fitness components, I estimated the effects of these mutations on male sexual fitness and productivity in the population's ancestral laboratory environment, as well as a novel food environment. Indirect benefits in the ancestral environment were lacking, suggesting that a good genes process is not acting. Cross-environment correlations were not conducive to indirect benefits following colonization of a novel environment. Second, to investigate the contribution of variable search effort to variance among males in mating success, I conducted mate choice trials to compare the relative mating success of mutant males in small vs. large arenas. Sexual selection against mutant males was stronger when search effort was included than when it was excluded, indicating that varying ability to find mates may increase the strength of selection against deleterious alleles.

RÉSUMÉ

Dans cette thèse, j'utilise 10 populations de *Drosophila melanogaster*, chacune fixée pour une mutation récessive visible différente afin d'adresser deux sujets non-résolus dans la recherche sur la sélection sexuelle. Premièrement, afin de quantifier les avantages indirects du choix de partenaire des femelles et explorer la nature des interactions génotype × environnement pour différentes composantes de valeur sélective, j'ai estimé les effets de ces mutations sur la valeur sélective sexuelle des mâles et la productivité dans l'environnement de laboratoire ancestral de la population, ainsi que dans un nouvel environnement alimentaire. Des avantages indirects étaient absents dans l'environnement ancestral, suggérant que le processus des bons gènes est absent. Les corrélations entre les environnements n'étaient pas propicse aux bénéfices indirects au cours de la colonisation du nouvel environnement. Deuxièmement, afin d'explorer la contribution de l'effort variable investit par les mâles dans la recherche d'un partenaire au succès reproductif, j'ai effectué des évaluations pour comparer le succès reproductif relatif des mâles mutés dans des petites arènes et dans des grandes arènes. La sélection sexuelle contre les mâles mutés était plus prononcée lorsque les efforts de recherche furent inclus que lorsqu'ils étaient exclus, indiquant que la variabilité de la capacité à trouver un partenaire pourrait augmenter la force de sélection contre des allèles délétères.

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Chapter 1

An Overview of Sexual Selection

INTRODUCTION

Sexual selection is a process that occurs when there is a non-random relationship between variation in a trait and reproductive success. As Darwin (1859) described it, 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." As Darwin's quote suggests, it is most often the case that males compete for access to females and subsequent fertilization, while females choose amongst these males. There are, however, well documented species in which these sex roles are reversed (reviewed by Gwynne 1991; Bonduriansky 2001), although I focus here on the traditional scenario of male competition and female choice.

Darwin (1859, 1871) recognized two basic forms of sexual selection. First, intrasexual selection (often termed male-male competition) occurs when individuals of one sex (generally males) compete, either directly or indirectly, with one another for access to females. Intrasexual selection can result in the evolution of weapons of combat that often adorn the males of many species, as well as traits that enhance a male's ability to locate females during scramble competition. Second, intersexual selection (often termed 'mate choice' or 'female choice') occurs when females mate nonrandomly with respect to one or more traits in the males, preferring certain individuals (trait values) over others.

Intersexual selection can results in the evolution of showy display traits, including ornaments and courtship behaviours that are common in males of many species.

Although Darwin (1859) initially viewed sexual selection as being "less rigorous" than natural selection, much subsequent research has revealed it to be an important process in the origins of diversity both within and among species. The strength of selection arising from mating success, for example, is often greater than that of other fitness components including viability (Kingsolver et al 2001; Hoekstra et al 2001). Substantial evidence also supports a central role of sexual selection in the origin of new species (Price 1998; Panhuis et al. 2001).

Sexual selection arises from variance in reproductive success. In males, such variance can arise from many sources including a number that have come to be appreciated only recently (e.g., postcopulatory processes). To successfully reproduce, a male must first search for and locate (or attract) a female. Males potentially compete indirectly with other males in their ability to do this, a process known as scramble competition (Otte 1979). Scramble competition can favour a diverse array of traits in different taxa, including larger eyes and chemorecepting antennae in males of many arthropods (Thornhill and Alcock 1983), earlier male than female maturation and hatching in many animals (Moya-Larano et al 2007; Baughman 1991), and better male spatial memory in some animals (Lane et al 2009; Jacobs et al, 1990). Males may also compete directly with other males for access to females (or a breeding territory or site that is associated with subsequent access to one or more females), a process known as contest competition

(Andersson 1994). Contest competition has been well documented in a variety of animals and has been shown in specific cases to favour larger body size, weaponry, agility, and threat signals (Clutton-Brock & Albon 1979; Andersson 1994; Bonnet et al 2001).

After locating a female and gaining access to her, males may then have to convince, or otherwise coerce, her into mating. Females often assess males with respect to particular display traits and/or courtship behaviours, preferring certain phenotypes over others, and males of many species have evolved elaborate ornaments and behavioural signals in response to these preferences (reviewed in Jennions & Petrie 1997). Why female preferences evolve for such traits, thereby generating sexual selection on males, is an outstanding issue in sexual selection research to which I return below when discussing the good genes hypothesis.

Finally, after mating males may face postcopulatory sexual selection arising from sperm competition and cryptic female choice. Sperm competition refers to competition between the sperm of two or more males for fertilization of a given set of ova (Parker 1970) and is often referred to as the postcopulatory version of male-male competition. Sperm competition has led to the evolution of many traits including sperm size and motility, ejaculate size and male genital morphology (Parker 1998; Wedell et al 2002). Mate guarding is a male strategy that has evolved in many species to try to prevent females from remating, thereby reducing the risk of sperm competition (Beecher and Beecher 1979; Birkhead 1979). Males of different species have evolved various traits and/or behaviour to do this including physical mate guarding, sperm plugs, and the presence of seminal proteins within their ejaculate that reduce female mating rate (Alcock 1994; Kern et al 2004; Poiani 2006).

Cryptic female choice is broadly defined as any postcopulatory ability of females to favour one male of the same species over another (Thornhill 1983) and is the postmating version of female mate preferences. Females have multiple avenues for favouring certain males over others including biasing the amount of sperm transfer that occurs during mating, or by differentially storing or using the sperm of different males. Females may also alter egg laying rates and may even differentially invest in offspring sired by different males (Simmons 1987; Otronen 1997; Reviewed in Eberhard 1996). Several studies (reviewed by Eberhard 1996; 1997) have demonstrated that, in the absence of sperm competition, successful sperm transfer of a male does not guarantee paternity, suggesting an important role for cryptic female choice. Such choice appears to have influenced the evolution of sperm size and motility (Eberhard, 1996).

Although much of the research in sexual selection has focused on its phenotypic consequences in terms of trait evolution (i.e. extravagant sexual displays and weapons of combat), my thesis addresses two broader outstanding issues. The first concerns the role of indirect benefits in the evolution of female mate preferences that generate sexual selection, a process referred to as the good genes hypothesis. Although this topic has also received much attention, both from empirical investigation and theory, it remains a central unresolved issue in sexual selection research. The second concerns the role of variable male search effort in contributing to variance among males in their reproductive success, in particular with respect to the purging of deleterious mutations and the consequences for population mean fitness. This latter issue is one that has been largely overlooked in the literature to date. Below I provide some background relevant to each of these topics in turn.

GOOD GENES MATE CHOICE

Mate preferences exist when individuals of one sex (usually females) mate with certain males over others (Ritchie 1996; Wagner 1998). Such preferences are extremely widespread in natural populations (Houde 1994; Bakker 1993; Brooks & Couldridge 1999; Wilkinson and Reillo 1994; Jennions & Petrie 1997) and have been shown to be costly in a number of cases (e.g., Pomiankowski 1987; Reynolds & Gross 1990; Rundle et al. 2009). This implies that they must be maintained within populations by the direct and/or indirect benefits they provide to females.

In many species, males actively compete for mates by providing direct benefits to females. These benefits are varied and commonly include nuptial gifts and parental care of offspring (Clutton-Brock 1991; Vahed 1998; Boggs 1995; Sheldon 1993). Often overlooked, direct 'benefits' can include the minimization of male-induced costs such as those arising from sexual conflict (Chapman et al 2003). Sexual conflict occurs due to the divergent reproductive interests of males and females (e.g. the optimal mating rate for males is often higher than for females). The sexually antagonistic selection that results can favour traits in one sex that harm the other, such as adaptations in males to coerce females to mate at higher rates and counter-adaptations in females to resist this acquisition of benefits (Parker 1979; Holland & Rice 1998; Gavrilets et al. 2001). Whether via avoidance of male-induced costs or the acquisition of certain benefits, females can gain an immediate fitness increase as a result of their choice of mates. The evolution of mate preferences via such direct benefits is non-controversial: preferences will evolve whenever the benefits exceed the costs.

In contrast to direct benefits, indirect benefits occur if females prefer males with high breeding value for fitness. By preferentially mating with such males, females pass their partner's 'good genes' on to their offspring, benefiting indirectly by producing offspring of high genetic quality (Williams 1966; Kirkpatrick and Ryan 1991). The evolution of mate preferences via indirect benefits is known as the good genes hypothesis and a fundamental requirement is that the sexual display traits in males that are the target of female choice are honest indicators of male genetic quality (Iwasa and Pomiankowski 1999). This is suggested to be true whenever sexual display traits are costly to produce and their exaggeration increases male mating success because, once genetic variation in the male display trait is exhausted (as would be expected under persistent directional selection), variation in other loci that affect condition will be recruited in a process known as 'genic capture' (Rowe & Houle 1996). Because higher condition individuals can better pay the costs of trait exaggeration, display traits become honest indicators of overall male condition. The relative magnitude of such indirect benefits is the subject of much empirical interest in sexual selection research and is central to our understanding of the evolution and maintenance of mate preferences.

Evidence for Good Genes Mate Choice

The prevalence of good genes indirect benefits is unclear. Alternative theoretical analyses have suggested that indirect benefits may overcome direct costs (Houle & Kondrashov 2002), or that they are unlikely to do so (Cameron et al. 2003; Kirkpatrick 1996; Kirkpatrick & Barton 1997). Empirical studies are common and results are likewise mixed. Correlative evidence suggests that female mate choice often has positive effects on various components of offspring fitness (Reynolds and Gross 1992; Norris 1993; Petrie 1994; Wedell 1999; Welch et al 1998; Evans et al 2004), although in a number of cases other components also appear to suffer (Brooks 2000; Hine et al 2002; Wedell 1999). In many of these studies, however, one or a few select components of offspring fitness were measured, overlooking certain key components and making any conclusions about the net effect of mate choice on offspring fitness difficult or impossible to determine (Rundle et al 2007).

In a few cases, quantitative genetic studies have been undertaken to test for the presence of the genetic correlations necessary for good genes to occur (i.e. between female preference, male display and offspring fitness; Kirkpatrick 2009). Results of these studies are likewise mixed, with both positive and negative genetic correlations being reported (Brommer et al. 2007; Hine at al 2002; Qvanström 2006; Bakker 1993). Another approach has been to use experimental evolution to manipulate the opportunity for mate choice over one or more generations and then measure offspring fitness in the resulting treatments. Partridge (1980) used this approach in *Drosophila melanogaster* to compare the larval viability of progeny of females that were allowed to choose their mates (sexual selection treatment) with those of females under randomly assigned enforced monogamy. She found a 1-2% increase in larval viability in the progeny of females from the sexual selection treatment. However, in similar experiments using *D. melanogaster* and *D. pseudoobscura*, Schaeffer et al (1984) found no difference in the juvenile survival of progeny from monogamy and sexual selection treatments. Promislow et al. (1998) demonstrated an increase in adult survivorship in *D. melanogaster* when female mate choice was allowed; however they were not able to demonstrate any difference in larval competition between the selection and monogamy treatments (as in Partridge's original study). Holland and Rice (1999) measured net reproductive rate (a more inclusive measure of fitness) between selection and monogamy treatments in *D. Melanogaster* after 47 generations of experimental evolution. They found a 30% increase in fitness in the monogamy treatments, indicating that any indirect benefits obtained by females were not enough the counter strong sexual conflict known to exist in the system.

If females prefer mates with high breeding value for fitness, sexual selection should likewise promote adaptation to novel or changing environments (Lorch et al. 2003). Experimental evolution has been employed to test this prediction as well, with results of three recent studies also mixed. Using *D. melanogaster*, Holland (2002) manipulated the opportunity for mate choice in replicate populations adapting to a thermal stress environment. Though he found substantial adaptation, there was no difference between the monogamy and polyandry treatments, indicating that sexual selection did not promote adaptation. In a similar experiment, Rundle et al. (2006) varied both natural and sexual selection in *D. serrata* during adaptation to a novel food environment. Again sexual selection did not appear to speed adaptation. Using a similar experimental design, however, Fricke & Arnqvist (2007) found that the bruchid beetle, *Callosobruchus maculates,* had an accelerated rate of adaptation to a novel host plant in the polyandry treatment when compared with the monogamy treatment. Past results using this approach are therefore mixed and make conclusions about indirect benefits difficult. An important goal of future studies will be to understand the factors that affect the magnitude of indirect benefits.

SEXUAL SELECTION & MUTATION LOAD

It has been suggested by several authors that sexual selection may have beneficial effects on population mean fitness, including promoting the fixation of beneficial mutations (Proulx 1999, 2001, 2002; Lorch et al. 2003) and the purging of deleterious mutations (Whitlock 2000; Siller 2001; Agarwal 2001; Reviewed by Agarwal & Whitlock 2009). The basic idea is that if mutations have similar effects on sexual and nonsexual fitness, then sexual selection will act in concert with natural selection, increasing population mean fitness. In many species, males provide no parental care and a single male can fertilize many females. In such cases, population mean fitness is almost entirely determined by female fitness. In such a situation, if males carrying deleterious mutations have lower reproductive success, sexual selection can purge such mutations from the population without a corresponding reduction in population mean fitness (Whitlock & Agrawal 2009). Such effects may slow the process of mutation meltdown, decreasing the risk of extinction for small populations, and may even favour the evolution of sex itself (Agrawal 2001; Siller 2001). Although such benefits of sexual selection can arise from a good genes process (i.e. females prefer to mate with males carrying fewer deleterious mutations), it may also arise from other components of male sexual fitness if males of lower genetic quality perform more poorly (e.g., if males carrying deleterious mutations are less likely to win in a scramble competition). The majority of research into the benefits of sexual selection for population mean fitness have focused on the good genes scenario, with the involvement of other fitness components having received little attention.

THESIS OVERVIEW

The subsequent two data chapters of this thesis were written as stand-alone manuscripts in the style of academic journal articles. As such, there is some overlap in the background material presented, although I attempted to keep this to a minimum. Chapter 2 was a collaborative project involving Michael Whitlock (Department of Zoology, University of British Columbia), and my supervisor, Howard Rundle. In this chapter, I attempt to quantify indirect benefits of mate choice in the fruit fly *D. melanogaster.* Using ten separate mutations independently introgressed into a laboratory population, I estimate the effects on both male sexual fitness (mating success in choice trials) and an inclusive measure of nonsexual fitness (productivity). I also address genotype × environment interactions for the two components of fitness by performing the assays in both their 'ancestral' laboratory environment and a novel larval food environment. Doing so provides direct insight into how indirect benefits may vary following colonization of a novel environment. For reasons described in the chapter, submission of this work for publication is awaiting a repetition of the productivity assay using the alternative approach discussed therein.

Chapter 3 is a slightly modified version of the following article, also co-authored by Michael Whitlock and my supervisor, Howard Rundle:

MacLellan, K., M.C. Whitlock and H.D. Rundle. 2009. Sexual selection against deleterious mutations via variable male search success. Biology Letters: *In press*.

In this chapter, I quantify the contribution of search effort to sexual selection against the same ten mutants in *Drosophila melanogaster*. Specifically, I ask whether including a component of search effort lowers the reproductive success of mutant relative to wild-type males in replicate binomial mating trials. I do this by directly manipulating the opportunity for search effort by conducting the mating trials in two different sized arenas that vary approximately 600-fold in volume.

Although both chapters represent collaborative efforts, I was the primary contributor to all stages of this work. This included creating the introgressed mutant populations, collecting all of the data, analyzing and interpreting the results, and writing this thesis and the manuscript from Chapter 2.

Chapter 2

An empirical test of good genes indirect benefits in an ancestral and a novel environment

INTRODUCTION

Mate preferences exist when individuals of one sex (usually females) prefer certain trait values over others when choosing mates (Ritchie 1996; Wagner 1998). Such preferences are extremely widespread in natural populations and the phenotypic consequences of the sexual selection they generate have been well studied (Houde 1994; Bakker 1993; Brooks & Couldridge 1999; Wilkinson and Reillo 1994; Jennions & Petrie 1997), focusing on the evolution of the spectacular array of sexual displays and signals that characterize the males of many species. Mate preferences are generally assumed to be costly (Pomiankowski 1987; Reynolds & Gross 1990; Rundle et al. 2009; Byers et al. 2005; Cotton et al. 2006) and are therefore maintained within populations by the direct and/or indirect benefits they provide to females. The relative magnitude of such benefits is the subject of much empirical interest in sexual selection research and is central to our understanding of the evolution and maintenance of mate preferences (Kokko et al. 2003).

Direct benefits exist when a female's immediate fitness is increased by her choice of mates. Such benefits are widespread and varied, including such things as nuptial gifts, better parental care, and a reduced risk of transmission of parasites or sexually-transmitted diseases (Clutton-Brock 1991; Vahed 1998; Boggs 1995; Sheldon 1993). In such cases, the evolution and maintenance of costly preferences is easily understood as long as the benefit to females outweighs the cost of choosing. Often overlooked, direct 'benefits' can include the minimization of male-induced costs such as those arising from sexual conflict (Chapman et al 2003).

Indirect benefits exist if females prefer males of high breeding value for fitness. By preferentially mating with such males, females benefit indirectly by passing their partner's good genes on to their offspring (Williams 1966; Kirkpatrick and Ryan 1991). The evolution of mate preferences via indirect benefits is known as the good genes hypothesis and it requires that sexual display traits in males are honest indicators of overall genetic quality (Iwasa and Pomiankowski 1999). This is suggested to be true whenever sexual display traits are costly to produce and their exaggeration increases male mating success (Rowe & Houle 1996).

The extent of any good genes indirect benefits, and thus their role in preference evolution, is unclear. Alternative theoretical analyses have suggested that indirect benefits are capable of overcoming any direct costs (Houle & Kondrashov 2002), or that they are unlikely to do so (Cameron et al. 2003; Kirkpatrick 1996; Kirkpatrick & Barton 1997). Empirical evidence is likewise mixed. There is substantial evidence suggesting indirect benefits to mating with preferred males for some components of offspring fitness (Partridge 1980; Promislow et al. 1998; Hine et al 2002; Norris 1993; Petrie 1994; Sheldon et al 1997; Brooks 2000; Wedell & Tregenza 1999; Welch 1998; Evans et al 2004; Boake 1985; Reynolds & Gross 1992), however in some cases other components of offspring fitness appear to suffer (Pischedda & Chippindale 2006; Hine et al 2002; Chippindale et al 2001; Brooks 2000; Wedell & Tregenza 1999). Attempts to measure the effect of mate choice on net offspring fitness have similarly provided varied results, revealing weak indirect benefits that are overwhelmed by direct costs in some cases (Rice 1996; Holland and Rice 1999; Stewart et al 2008; Orteiza et al 2005), yet suggesting more substantial indirect benefits in others (Head et al. 2005; Rundle et al. 2007). Experimental evolution has also been used to investigate good genes by manipulating the opportunity for mate choice across one or more generations and then measuring offspring fitness. Results are again mixed (Promislow et al. 1998; Holland & Rice 1999; Radwan et al. 2004; Radwan 2004; Martin & Hosken 2003), although such manipulations alter both the opportunity for good genes and sexual conflict, both of which can affect population mean fitness (Whitlock & Agrawal 2009).

Female preference for males of high breeding value for fitness should help purge deleterious mutations and fix beneficial ones (Whitlock 2000; Lorch et al. 2003), potentially reducing the mutation load on a population (Agrawal and Whitlock 2009). In *Drosophila melanogaster*, the effect on male mating success has been investigated for a number of mutations shown to be deleterious to female fitness, with results providing some support for good genes (reviewed in Whitlock & Agrawal 2009). Good genes indirect benefits should likewise promote adaptation in altered environments (Proulx 1999, 2001, 2002; Whitlock 2000; Lorch et al. 2003). Support for this prediction, however, is weaker; evolution experiments that have manipulated the opportunity for sexual selection during adaptation to novel environments have found benefits to be small

(Fricke & Arnqvist 2007) or non-existent (Holland 2002; Rundle et al. 2006). Genotype × environment interactions provide one potential explanation for these results. Such interactions are common for performance traits (Hunt et al. 2004) yet little is known concerning the degree to which they may vary for different aspects of fitness, including sexual (e.g. male attractiveness) and non-sexual (e.g., productivity) components. Genotype × environment interactions may cause indirect benefits of mate choice in one environment from translating into another (Hunt et al. 2004; Rundle et al. 2006).

Here we are interested in whether females gain an indirect benefit from their choice of mates and how this changes in a novel environment. Using ten separate mutations independently introgressed into a laboratory population of *Drosophila melanogaster*, we estimate the effect of each on male sexual fitness (male mating success in choice trials) and an inclusive measure of nonsexual fitness (productivity). Indirect benefits are indicated if mutations have concordant effects on productivity and male attractiveness, increasing or decreasing both. To provide insight into genotype × environment interactions for different components of fitness, we perform these fitness measurements in two separate environments: the laboratory environment to which the population is well adapted (cornmeal food; termed their 'ancestral' environment for brevity) and a novel environment consistent of a different larval food (corn-flour food). Doing so provides direct insight into the indirect benefits of mate choice following colonization of a new environment.

MATERIAL & METHODS

Experimental populations

A stock population of *Drosophila melanogaster* was created from a sample of approximately 200 flies collected from multiple localities within a 5km radius of Dundas, ON, Canada in fall 2005, and supplemented by a second collection of similar size from the same area in fall 2006. This stock was maintained at a large population size in two cages under constant conditions (25°C, 70% relative humidity, 12L:12D photoperiod) with overlapping generations in the laboratory of R. Dukas at McMaster University, Canada. In February 2007, a large sample of this stock was transferred to the University of Ottawa, Canada, and was maintained in 16 half-pint bottles under constant conditions (25°C, 50% relative humidity, 12L:12D photoperiod) with discreet non-overlapping generations on a standard cornmeal-based food for 27 generations prior to the start of the experiment.

Ten mutant populations were obtained from the Bloomington stock centre in late 2007. Three of these, yellow (*y*), forked (*f*), and white (*w*), were fixed for different X-linked visible recessive mutations, and seven of them, brown (*bw*), claret (*ca*), cinnabar (*cn*), eyeless (*ey*), plexus (*px*), sepia (*se*), and speck (*sp*), were fixed for different autosomal visible recessive mutations. Six of these mutations affected eye phenotype (*bw*, *w*, *cn*, *ca*, *se*, *ey*), two affected wing phenotype (*sp* and *px*), and one each affected bristles (*f*), and body colour (*y*). Each mutation was crossed separately into the laboratory stock population via multiple rounds of introgression, thereby yielding ten populations that shared a similar wild-type stock genetic background but was each fixed for a different visible mutation. At least one round of introgression was initiated by a mating in each direction (i.e. stock female × mutant male and mutant female × stock male) to ensure that the final introgressed mutant populations carried mtDNA and Y chromosomes from the stock. After introgression, mutant populations were maintained under the same conditions as the stock.

For each autosomal mutation, a single round of introgression began with a mass cross involving approximately 50 virgin stock females and 50 mutant males in each of two bottles. The resulting F1 offspring were allowed to emerge as adults and mate among themselves to produce the F2 generation. From the F2 generation, 100 males exhibiting the mutant phenotype (i.e. mutant homozygotes) were then collected to proceed to the next round of introgression using 100 new virgin stock females. This procedure was repeated for four rounds, at the end of which 100 F2 virgin mutant females were collected for the fifth and final round of introgression. These females were crossed to 100 stock males and the resulting F1 offspring were allowed to emerge and mate among themselves to produce F2s. Virgin females and males exhibiting the mutant phenotype were then collected to form the final mutant population. After completion of this introgression, the average reduction in the mutant genetic background was approximately 97%.

For each X-linked mutation, the first round of introgression involved a mass crossing of approximately 100 virgin stock females with approximately 100 mutant males. From the resulting F1 offspring, 100 virgin females (heterozygous at the mutation locus) were then backcrossed with 100 stock males to produce the F2 generation. Males exhibiting the mutant phenotype were collected to start the next round of introgression. This procedure was repeated for three additional rounds, except the F2 individuals were created in these cases by allowing the F1 individuals to emerge and mate among themselves rather than backcrossing the F1 females to stock males. During the fourth and final round, F2 individuals were crossed with each other and then both male and female F3 offspring exhibiting the mutant phenotype were collected to found the final mutant population. After completion of this introgression, the average reduction in the mutant genetic background was approximately 97%.

Male mating-success assay

For each mutant population, the mating success of mutant relative to stock males was determined using a female choice mating assay. Each replicate involved 20 stock females placed together in a translucent plastic cage (14 x 14 x 14 cm) with 20 mutant and 20 stock males. Cages were shielded from sight to minimize disturbance and mating pairs were removed by aspiration at 10 min. intervals. To ensure that females had a choice between males of both types, only the first ten mating pairs were collected from any single cage, after which the cage was terminated and remaining individuals were discarded. Males from the mating pairs were identified as either stock or mutant by phenotype.

Forty replicate cages were performed for each mutant population, 20 in which both types of males were raised in the standard cornmeal (i.e. 'ancestral') environment and 20 in which they were raised for a single generation in the novel corn-flour-based food environment (see Rundle et al. 2005 for media recipe). Females were raised in the ancestral environment in all cases such that differences in the mating success of mutant relative to stock males in the two environments could be attributed to changes in the males and not the females. Individuals for use in the mating trials were collected as virgins upon emergence using light CO_2 anesthesia and held separately by sex (females in groups of ten, males in groups of five) in vials containing 5 ml of their respective food for five days prior to the assay. The assay was performed in blocks such that 1-3 mutant populations were tested within a single environment during any particular generation (Table 2.1).

Productivity assay

The productivity of each mutant population was determined in both the ancestral (cornmeal) and novel (corn-flour) food environments by counting the number of adult offspring produced from the eggs laid in a 24h period by a single female raised in that environment. Productivity therefore represents a combined measure of the fecundity of mutant females and the egg-to-adult survivorship of their male and female offspring. As with the mating-success assays, productivity was measured in blocks such that 1-3 mutant populations were tested in a given environment within a single generation (Table 2.1). The productivity of the stock population was measured alongside the mutant populations as part of each block to control for common environmental effects across generations. For a given mutation, the effects on productivity and male mating success in a given environment were tested contemporaneously.

All flies used in the assays were collected upon emergence as virgins using light CO_2 anesthesia and held separately by sex (males in groups of five, females in groups of ten) on 5 ml of their respective food (i.e. cornmeal or corn-flour) for five days prior to the assay. All male individuals for use in the novel environment had been raised for a single generation on the corn-flour food. For each population, 120-150 females were transferred singly to separate vials containing 10 ml of environment-appropriate food. A single stock male was then added to each vial. The use of standard stock males controls for any differential effects of males on female productivity. After 24 hours, the male and female were discarded. All offspring emerging from each vial were then counted.

Statistical analyses

The mating success of mutant relative to stock males was calculated as a proportion (p_{ms}) by dividing the number of mutant matings by the total number of matings within a cage (i.e. stock + mutant). The mating success of each mutant population was then calculated as the average proportion across all replicate cages of that mutation in a particular environment. The productivity (w_{prod}) of each mutant population relative to the stock was calculated in each environment by dividing the total number of offspring produced by all replicate females of that mutation by the total number of offspring produced by all replicate stock females from the same block. Calculating relative productivity in this way weights each female by her total fecundity, as would occur in nature.

Because we are primarily interested in whether females gain an indirect benefit from their choice of mates (i.e. good genes mate choice), we conducted a single overall test in each environment of whether natural and sexual selection acted in the same direction. To do this, in each environment the effects of each mutation were classified as either consistent with good genes (i.e. the mutation increased or decreased both male mating success and productivity relative to the stock population in that environment) or inconsistent (i.e. opposite effects on male mating success and productivity in that environment). An exact binomial test (i.e. sign test) was then used, treating the ten individual mutations as replicates, to determine whether the effects of natural and sexual selection on these mutations was concordant in both the ancestral and novel environments.

The effect of each individual mutation on male mating success was evaluated using a one-sample *t*-test, treating the population cages as replicates, to determine whether the success of mutant males differed significantly from 0.5 (i.e. equal mating success of mutant and stock males). Results did not change qualitatively when proportions were arcsine-square root transformed prior to this analysis, so the untransformed values are presented for simplicity. The effect of each individual mutation on productivity was evaluated using a non-parametric Kruskal-Wallis test, treating individuals as replicates, that compared the number of adult offspring produced by mutant females with that produced by the matched stock females within the same block. A non-parametric test was used because a number of females from every population produced few or no offspring, generating a bimodal distribution of productivity that violated the assumption of normality.

RESULTS

In the ancestral environment, the effects on male mating success and productivity of six of the 10 mutations were concordant, with each of these reducing both fitness components (Fig 2.1A). Six of 10 mutations, however, does not differ significantly from that expected by chance alone (binomial probability of six or more successes with $\hat{p} = 0.5$; $P = 0.377$). Within the individual mutations, eight had significant effects on productivity (six decreasing and two increasing relative to the stock), and seven mutations had significant effects on mating success (all of them causing a reduction relative to the stock; Table 2.2). Restricting the overall test to those eight mutations with significant effects on productivity, the effects of five of these are concordant. Five of eight mutations does not differ significantly from that expected by chance alone (binomial probability of five or more successes with $\hat{p} = 0.5$; $P = 0.363$)

In the novel (corn-flour food) environment, the effects on male mating success and productivity of five of the 10 mutations were concordant (Fig. 2.1B). Four of these mutations reduced both male mating success and productivity relative to the stock, while one increased both fitness components. Five of 10 mutations does not differ significantly from that expected by chance alone (binomial probability of five or more successes with $\hat{p} = 0.5$; $P = 0.623$). Within the individual mutations, four had significant effects on productivity (two of these causing a decrease and the other two causing an increase relative to the stock) and five had significant effects on male mating success (all of them causing a reduction relative to the stock; Table 2.3). Restricting the overall test to the four mutations with significant effects on productivity in this environment, the effects on mating success are concordant for three of these. Three of four mutations does not differ significantly from that expected by chance alone (binomial probability of three or more successes with $\hat{p} = 0.5$; $P = 0.312$).

DISCUSSION

There was little evidence in either environment that females would benefit indirectly from their choice of mates. In the environment to which the population was long adapted ('ancestral' environment), six of the ten mutations reduced both male mating success and productivity overall (five of eight when the analysis is restricted to those mutations with significant effects on productivity), while in the novel environment, five of 10 mutations had concordant effects (three of four when the analysis was restricted to those mutations with significant effects on productivity). In no case do these results differ from that expected by chance alone.

There are three main explanations for the apparent absence of indirect benefits in my experiment. First, there may be little role for good genes indirect benefits in the evolution of female mate preferences in this population of *D. melanogaster*. Although there is evidence from another population of *D. melanogaster* that mating with attractive males provided an indirect benefit to females (Rundle et al. 2007), direct costs of mate choice were not estimated in this case. Indirect benefits in a different laboratory population of *D. melanogaster*, however, were found to be weak and incapable of offsetting direct costs (Orteiza et al 2005; Stewart et al 2005, 2008), suggested that preferences have not evolved because of their indirect benefit.

Deleterious mutations have been investigated with respect to both sexual and non-sexual selection in a few other studies, all of which used *D. melanogaster*. Sharp & Agarwal (2008) measured selection arising from male reproductive success, viability, and female fecundity for eight dominant phenotypic mutations. For five of the mutations, selection arising from variance in male mating success was concordant with that arising from non-sexual components of fitness (viability, female fecundity). Of the remaining three mutations, two had ambiguous effects and one had increased both fecundity and reproductive success. Whitlock and Bourguet (2000) measured the effects of five recessive phenotypic mutations on male mating success and female productivity. Three of the mutations that had deleterious effects on productivity also had strong negative effects on male mating success, while two mutations had opposite effects on mating success and productivity.

The second potential explanation for the apparent absence of indirect benefits is a lack of statistical power. Based on the probability of the binomial distribution, 8 of 10 mutations would need to be consistent with good genes to achieve a $p < 0.5$. Thus, we only had sufficient power to detect a large effect size and it is therefore possible that a smaller signal was present but we but not detected here.

The third potential explanation for the apparent absence of indirect benefits in my experiment is that they may exist in this population but were obscured by population-specific environmental effects that were not controlled by my experimental design. For logistical reasons, productivity and mating success assays were performed in blocks of 1-3 mutants in each environment. For the productivity assay, a large sample of stock individuals (minimum of 136 females) were measured concomitantly within each block. The productivity of each mutant population was then calculated relative to the stock individuals within the same block, thereby controlling for any effects that were common to all populations within a given block (e.g., temporal variation in food or environmental quality). Population-specific effects (i.e. those affecting individual populations as opposed to the entire block, such as variation in larval density, naturally occurring epizootics, positional effects in the incubator, etc.) are not controlled by such a design, however, and appear to have been large for this assay; mean stock productivity ranged from 39-120 and 5-22 among blocks in the ancestral and novel environments respectively. This among-block variability in stock productivity is highly significant in both cases (one-way ANOVA; ancestral environment: $F = 198.6$, *d.f.* = 6, $P = <0.001$; novel environment: $F = 31.4$, *d.f.* = 4, $P = <0.001$). Consistent with population-specific effects, in both environments the mutations with the highest relative productivities derived from the blocks with the lowest stock productivity, suggesting that the poor absolute performance of the stock, as opposed to the effects of the mutation itself, may have been responsible. Although rarely controlled in *Drosophila* fitness assays, such population-specific effects on productivity may be substantial, adding considerable noise to the data. In my case, because mutant and stock individuals can be distinguished visually, an elegant (although labour-intensive) solution would be to raise mutant and stock individuals for use in a productivity assay in the same vial. While such a design would not reduce among-block variation, it would minimize within-block effects by unifying the microenvironment, including rearing density, of mutants and the matched controls used to calculate relative productivity.

In contrast to the productivity results, population-specific effects appear weak in the male mating success assay. The between-environments correlation for the effects of these mutations on relative male mating success was very high (Fig. 2.2a; discussed below). Such consistency is unlikely if population-specific environmental effects were large.

Genotype × environment interactions exist whenever environmental variation affects different genotypes in different ways (Fry et al 1996). Taking the productivity data at face value, results differed dramatically for the two fitness components, suggesting that any good genes indirect benefits from one environment are unlikely to translate to another. For male mating success, there was no significant effect of environment when measured across all mutations (t = -0.1858, $d.f.$ = 9, P = 0.857), indicating no reduction in average performance in the novel environment. All mutations that were deleterious or beneficial in the ancestral environment also remained so in the novel environment, and a strong correlation existed among their effects between the two environments (Fig. 2.2a: r^2 = 0.885, P = <0.001), indicating that their rank order remained similar. Although genotype × environment interactions for non-sexual fitness components are quite common (Lynch & Walsh 1998; Hunt et al 2004; Vieira et al 2000; Fry et al 2006), little is known about the degree to which male mating success is plastic with respect to environment. Our result suggests that male mating success was remarkably consistent across two larval foods, despite a dramatic differences in stressfulness (mean absolute productivity of the stock population was reduced by 78.8% in the novel corn-flour environment).

In contrast to the mating success data, the effect of the mutations on productivity varied dramatically between the ancestral and novel environments, with substantial changes in the rank order that produced a very low between-environment correlation (Fig. 2.2b: $r^2 = 0.069$, $P = 0.463$). Four of the mutations even changed from deleterious to beneficial or vice versa (Tables 2.2 & 2.3). Despite these changes, however, there was no evidence that the effects of the mutations become more deleterious overall in the more stressful novel environment ($t = -0.9628$, *d.f.* = 9, $P = 0.361$). Substantial genotype × environment interactions for productivity were also observed across five environments in 18 mutation accumulation lines in *D. melanogaster* (Fry et al 1996). Strong genotype × environment interactions for productivity but not male mating success suggest that good genes mate choice will not operate initially in a novel environment (because males of high sexual fitness are unlikely to be of high genetic quality). However, as discussed above, population-specific environmental effects appear to have been large in our productivity measures. Consistent with this, the effect on mean relative productivity of these 10 mutations actually increased in the novel environment, although non-significantly ($t = -0.9628$, *d.f.* = 9, $P = 0.361$), despite the fact that this environment was more stressful, reducing the mean absolute productivity of the stock population by 78.8% on average. It is generally thought that stress reduces an organism's ability to compensate for the effects of deleterious mutations; therefore individuals carrying deleterious mutations are likely to incur larger fitness declines in a stressful environment. Empirical evidence supports this prediction and shows that the average mutation effect is either aggravated or unchanged in response to environmental stress (Kondrashov & Houle 1994; Vassilieva et al 2000; Korona 1999; Fry et al 1996; Szafraniec et al 2001). However, in some cases environmental stress has been shown to alleviate the effects of mutations. Kishony & Leibler (2003) found that environmental stress, despite reducing wildtype growth in *Escherichia coli* actually lessened the average effect of deleterious mutations. It remains unclear whether our result represents the rare occasion of an environmental stress alleviating mutational effects or if it is a consequence of large within-block effects on productivity measures. Further work is necessary to determine the effects of genotype × environment interactions on a good genes process during an environmental shift.

In conclusion, I found little evidence for indirect benefits of mate choice in an ancestral or a novel environment, suggesting that female mate preferences are not being maintained in this population by a good genes process. However, population-specific block effects on productivity appear to have been substantial and provide an alternative explanation for the results. Genotype × environment interactions for male mating success, a major component of their sexual fitness, appeared weak. Given the apparent ubiquity of such interactions for non-sexual fitness components (Lynch & Walsh 1998; Hunt et al 2004; Vieira et al 2000; Fry et al 2006), this suggests that good genes mate choice is unlikely to promote adaptation initially following a change in environment. Such benefits may eventually be restored, however, if female preferences also evolve in response to the novel environment (Rundle et al. 2009). How environmental changes affects the operation of good genes remains a fundamental topic for future research.

Table 2.1. Blocking of male mating success and productivity measures by environment for each mutation.

Mutant	Ancestral	Novel
bw	1	7
cn	3	10
cl	2	8
ey	4	11
F	3	7
px	6	11
se	4	7
sp	6	10
W	5	9
Y	5	9

Table 2.2. Effect of 10 separate mutations on productivity and male mating in the ancestral (cornmeal) environment. Productivity (w_{prod}) is measured relative to that of the stock (i.e. (w_{prod} = 1 denotes equal performance of the mutant and stock populations), with significance (P) of each mutation determined by a non-parametric Kruskal-Wallis rank sum test (H) treating individuals as replicates (*d.f.* = 1 in all cases). Male mating success is the average proportion (p_{ms}) of matings achieved by mutant males. Significance (P) was evaluated using a one-sample *t*-test (t) to determine whether p_{ms} differed from 0.5 (i.e., equal mating success of mutant and stock males), treating cages as replicates (*d.f.* = 19 in all cases). Mutations in bold denote those for which the effects on productivity and mating success are concordant.

Mutant	w_{prod}	H	P	p_{ms}	t	P
bw	0.880	9.28	0.002*	0.335	-6.77	<0.001*
cn	1.266	13.78	<0.001*	0.385	-2.98	0.008*
ca	0.887	12.46	<0.001*	0.425	-3.47	0.003*
ey	0.661	35.61	<0.001*	0.285	-8.46	<0.001*
f	1.218	14.09	<0.001*	0.440	-1.58	0.131
px	1.148	3.82	0.051	0.480	-0.54	0.599
se	0.436	88.88	<0.001*	0.435	-2.16	0.044*
sp	0.695	23.70	<0.001*	0.540	1.09	0.288
w	0.941	0.15	0.699	0.140	-16.19	<0.001*
y	0.588	19.40	<0.001*	0.365	-3.56	0.002*

Table 2.3. Effect of 10 separate mutations on productivity and male mating in the novel (corn-flour) environment. Productivity (w_{prod}) is measured relative to that of the stock (i.e. ($w_{prod} = 1$ denotes equal performance of the mutant and stock populations), with significance (*P*) of each mutation determined by a non-parametric Kruskal-Wallis rank sum test (*H*) treating individuals as replicates (*d.f.* = 1 in all cases). Male mating success is the average proportion (p_{ms}) of matings achieved by mutant males. Significance (*P*) was evaluated using a one-sample *t*-test (*t*) to determine whether p_{ms} differed from 0.5 (i.e., equal mating success of mutant and stock males), treating cages as replicates (*d.f.* = 19 in all cases). Mutations in bold denote those for which the effects on productivity and mating success are concordant.

Mutant	p_{prod}	*H*	*P*	p_{ms}	*t*	*P*
bw	0.381	23.45	<0.001*	0.360	-4.50	<0.001*
cn	1.161	2.74	0.098	0.340	-5.14	<0.001*
ca	1.008	0.003	0.959	0.465	-0.91	0.376
ey	1.641	11.20	<0.001*	0.215	-9.45	<0.001*
f	0.773	2.16	0.142	0.380	-3.85	0.001*
px	1.729	17.44	<0.001*	0.530	0.92	0.368
se	0.617	3.92	0.048*	0.470	-0.88	0.390
sp	0.741	3.32	0.068	0.555	1.56	0.134
w	1.077	0.02	0.901	0.105	-23.27	<0.001*
y	0.941	0.63	0.427	0.440	-1.58	0.131

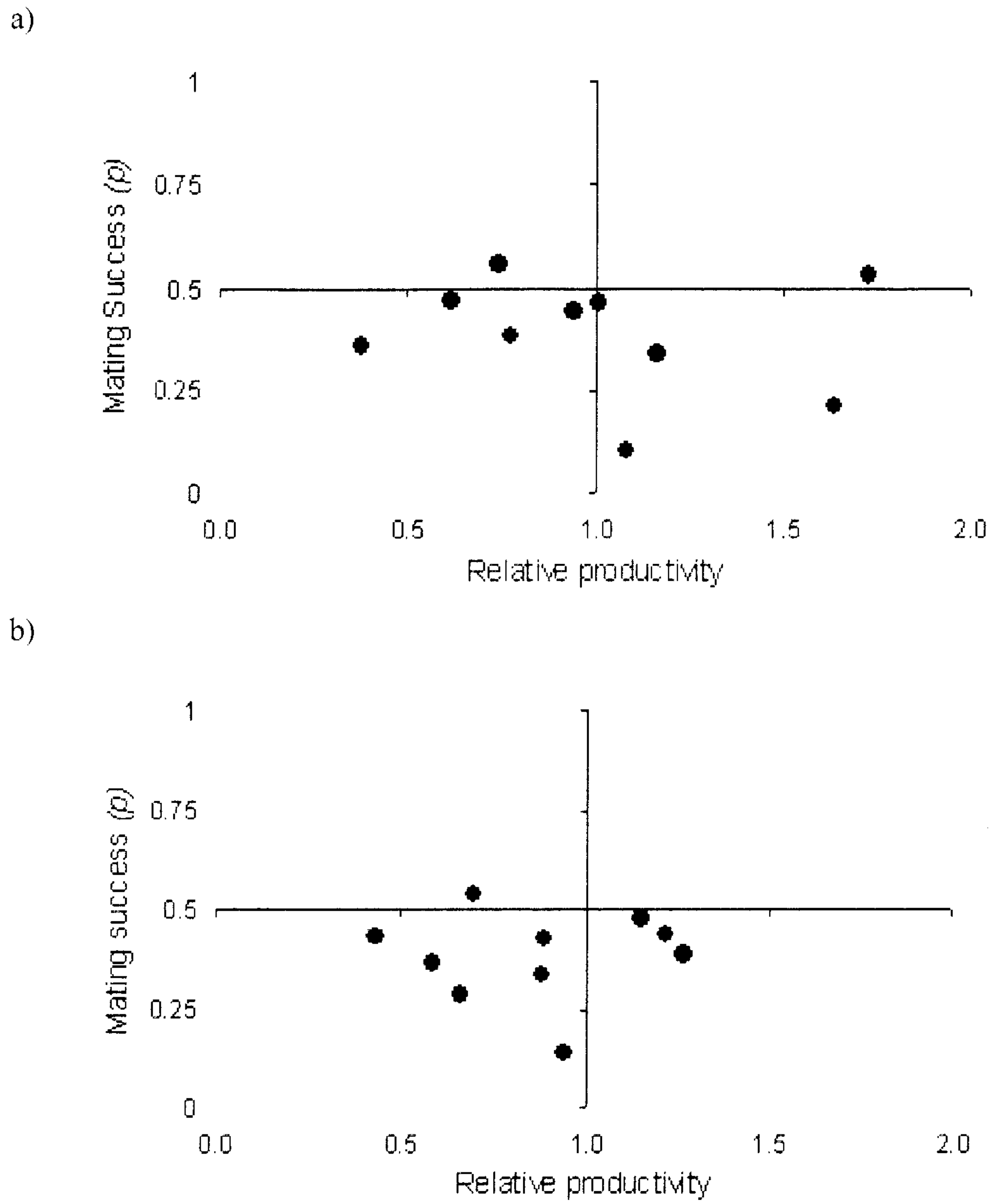

Figure 2.1. Effects of the 10 mutations on mating success and productivity relative to the stock population in a) the ancestral (cornmeal) environment and b) the novel (corn-flour) environment. A productivity of 1 denotes equal performance of the mutant and stock populations, and a mating success value of 0.5 indicates equal success of mutant and stock males.

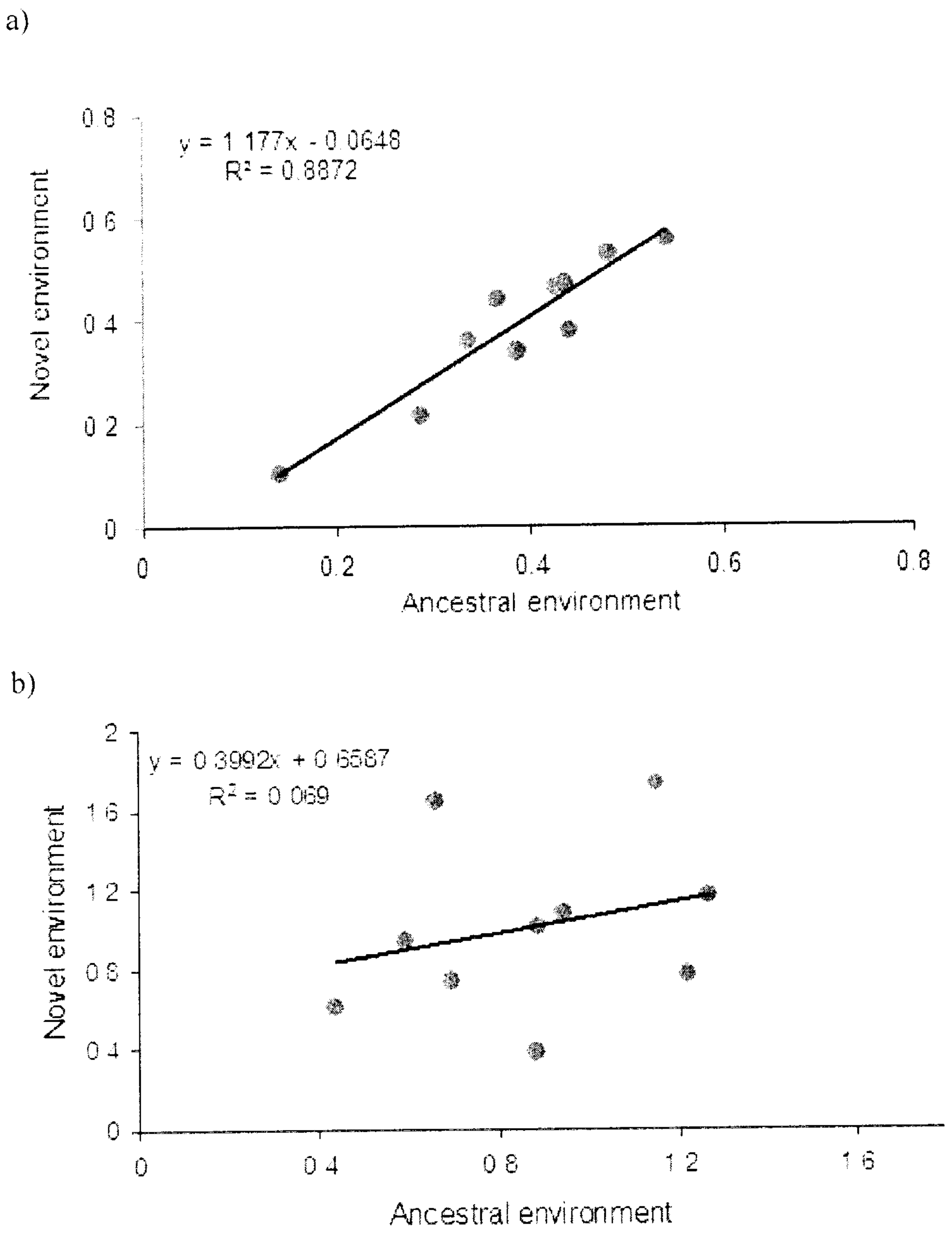

Figure 2.2. Correlations between the ancestral (cornmeal) environment and the novel (corn-flour) environment for the effects of 10 mutations on a) male mating success *(p)* and b) relative productivity.

CHAPTER 3

Sexual Selection via Variable Male Search Success

INTRODUCTION

Sexual selection is a process that occurs when a non-random relationship exists between trait (or genetic) variation and an individual's reproductive success. In most species males compete, either directly or indirectly (Murphy 1998), for access to females and females discriminate among potential males via pre- and/or post-copulatory processes. Successful reproduction may therefore require a male to succeed at several stages (Andersson 1994; Whitlock & Agrawal 2009). First, he must search for and locate (or attract) a female, potentially competing indirectly with other males in his ability to do this (i.e. scramble competition). He may then have to compete directly for access to her (i.e. contest competition among males), as well as court or otherwise coerce her into mating. Finally, after mating he may have to guard her against rival suitors or have his sperm compete with that of other males.

Although Darwin's (1859) original description of sexual selection in the *Origin of Species* recognized that it may often act in concert with natural selection, favouring the most vigorous and best adapted males, his focus quickly narrowed to that subset of characters whose evolution could not be explained by natural selection alone. Darwin (1871) paid particular attention to the evolution of the elaborate sexual display traits and weaponry found in the males of many species. Since then, female preferences and male-male competition have been the components of sexual selection that have received the most attention (Andersson 1994; Whitlock & Agrawal 2009). Although consideration has been given to the effects on trait evolution of other components of sexual fitness, such as sperm competition (e.g., Harcourt *et al.* 1981) and search effort (e.g., Able 1999; Follmer & Fairbairn 2005; Kelly *et al.* 2008), the population genetic consequences of these components in terms of population mean fitness remain largely overlooked.

It has been suggested by several authors that, if mutations harmful to population mean fitness also tend to reduce male reproductive success, then sexual selection may have an important consequence by reducing the mutation load (Whitlock 2000; Siller 2001; Agrawal 2001; reviewed in Whitlock & Agrawal 2009). Provided that females mate with other males, deleterious mutations may be purged from a population via sexual selection on males without a corresponding reduction in population mean fitness. Evaluating the consequences of sexual selection on the frequency of deleterious mutations requires determining whether total selection against such mutations is stronger in males than in females (Whitlock & Agrawal 2009). Here we are interested in a potentially important contributor to variance in male reproductive success that has been overlooked in this regard: searching for and locating a mate. Failure to locate a female precludes the possibility of any reproductive success, so by strengthening sexual selection on males, search effort may have important population genetic consequences in terms of purging deleterious mutations.

Males often invest substantial time and energy in searching for potential mates and such investment is costly (Kokko and Wong 2007). Search effort is therefore expected to be condition-dependent: males of lower quality will have fewer resources overall and allocation to search effort is likely to suffer (Whitlock & Agrawal 2009). Because most genes in the genome are likely to contribute to an individual's overall condition (Rowe and Houle 1996), variable search effort is likely to reinforce natural selection, favouring the same alleles responsible for high condition and hence fitness.

Here we quantify the contribution of search effort to sexual selection against ten independent, presumed deleterious, mutations with visible phenotypic effects in a laboratory population of *Drosophila melanogaster*. Specifically, we ask whether including a component of search effort lowers the reproductive success of mutant relative to wild-type males in replicate binomial mating trials, as would be expected if search effort is an important component of sexual selection against these mutations. We directly manipulate the opportunity for search effort by conducting the mating trials in two different sized arenas that vary approximately 600-fold in volume. If search effort is an important condition-dependent component of male sexual fitness, then mutant males should suffer disproportionately relative to wild-type males in the larger arena in which it is more difficult and/or more costly for them to locate the female.

MATERIAL & METHODS

Study populations

A stock population of *Drosophila melanogaster* was created from a sample of approximately 200 flies collected from a number of localities within a 5km radius of Dundas, ON, Canada in fall 2005, and supplemented by a second collection of similar size from the same area in fall 2006. This stock was maintained at a large population size in two cages under constant conditions (25°C, 70% relative humidity, 12L:12D photoperiod) with overlapping generations in the laboratory of R. Dukas at McMaster University, Canada. In February 2007, a large sample was transferred to the University of Ottawa, Canada, and was maintained in 16 half-pint bottles under constant conditions (25°C. 50% relative humidity, 12L:12D photoperiod) with discreet non-overlapping generations on a standard cornmeal based food for 27 generations prior to the start of the experiment.

Ten mutant lines were obtained from the Bloomington stock centre. Three of these, yellow (*y*), forked (*f*), and white (*w*), were fixed for different X-linked visible recessive mutations, and seven of them, brown (*bw*), claret (*ca*), cinnabar (*cn*), eyeless (*ey*), plexus (*px*), sepia (*se*), and speck (*sp*), were fixed for different autosomal visible recessive mutations. Six of these mutations affect eye phenotype (*bw, w, cn, ca, se, ey*), two affect wing phenotype (*sp* and *px*), and one each affect bristles and body colour. Each mutation was introgressed separately into a stock population (see the electronic supplementary material for a description of this population) via five rounds of backcrossing, thereby yielding ten populations that shared a similar wild-type stock genetic background but was each fixed for a different mutation. At least one round of backcrossing was initiated by a cross in each direction (i.e. stock female × mutant male and mutant female × stock male) to ensure that the final introgressed mutant populations carried mtDNA and Y chromosomes from the stock. After backcrossing, mutant populations were maintained under the same conditions as the stock.

Mating assays

Flies were collected as virgins upon emergence using light CO_2 anaesthesia and held separately by sex in vials with 5ml of standard medium for 4-5 days prior to their use in the mating trials. For each population, 98-100 replicate binomial mating trials were performed simultaneously under each of two experimental treatments that differed in arena size. Each trial consisted of a single mutant female from one of the populations together with a mutant male from the same population and a stock (wild-type) male. In the small mating arenas, trials were conducted in standard glass *Drosophila* rearing vials of approximately 30 ml volume with 10 ml of standard medium, whereas in the large mating arena, trials were conducted in rectangular cages (40.9 × 28.2 × 23.1 cm) of approximately 19L in volume. Cages were made of translucent plastic with clear household plastic wrap (Glad Cling Wrap ®) in place of a lid and contained a petri dish with 10 ml of standard medium. A small hole in the side of the cage, plugged with a foam stopper, provided access to the cage for adding or removing flies. Individuals remained in both mating treatments for 24 hours, after which the males were discarded and females were removed, individually transferred to separate vials containing 10ml standard food for 24h of egg-laying, and finally discarded. All adult offspring produced by each female were subsequently counted and scored by phenotype (mutant vs. wild-type).

Statistical analyses

The reproductive success of mutant relative to stock males was calculated for each mutation as a proportion (p) by dividing the total number of mutant offspring produced by all females of that mutation divided by the total number of offspring produced by the same females (mutant + wild-type). Calculating relative mating success in this way weights each female by her total fecundity, as would occur in nature.

Because we are primarily interested in the contribution of search effort to male sexual fitness in general, we conducted a single overall test of whether sexual selection against theses mutations was stronger in the large as compared to the small arena. A one-sample *t*-test, treating the ten mutations as replicates, was used to determine whether the difference in mean proportion differed from zero (i.e., $\bar{p}_{vial} - \bar{p}_{cage} = 0$; sexual fitness of mutant relative to stock males is the same in the small vs. large arena). To test for heterogeneity among mutations in the effect of the mating arena treatment, individual *p* values were calculated for every female. These values were bimodally distributed, however, because most females produced a majority of offspring of one type or the other (i.e. mutant or wild-type). We therefore assigned a binomial score to each replicate female in which a value of "1" was given to all females for which $p \geq 0.5$ and a value of "0" when $p < 0.5$ (results do not depend on the assignment of females for which $p \equiv 0.5$). To test for heterogeneity among mutations in the effect of the mating arenas, these binomial score data were modeled as a linear function of the fixed effects of treatment (vial vs. cage), mutation, and their interaction. The model was fit using maximum likelihood and a logistic link function, with significance of the terms determined using likelihood ratio tests. The effect of search effort on each individual mutation was tested using a simplified version of this model that was fit separately for each mutation after removal of the mutant and mutant × treatment terms.

RESULTS

Average reproductive success of mutant relative to stock males, as measured by the proportion of mutant offspring (p) produced by females, was lower in the large cages as compared to the small vials for nine of the ten mutations (Fig. 2.1). This difference is significant overall ($t_9 = 2.70$, $P = 0.025$) and represents a 6.6% ± 2.4 (mean ± SE) reduction in the proportion of mutant offspring in cages than in vials. Significant heterogeneity was detected among the mutations in the effect of the mating arena ($\chi^2 = 17.03$, $df = 9$, $P = 0.048$). Within the individual mutations, the effect of mating arena was significant in three cases, in all of which mutant males suffered disproportionately in the large as compared to the small arenas (Fig. 2.1).

DISCUSSION

For males of many species, the initial step in reproducing is to successfully locate a female. Although not previously considered in this respect, here we have shown that sexual selection against deleterious mutations in males tends to be stronger overall when an opportunity for search effort is included than when it is excluded. Significant heterogeneity existed among mutations in the effects of search effort. Such heterogeneity is expected because, along with their indirect effects on sexual fitness mediated via reductions in male health or condition, individual mutations may have separate direct effects on sexual fitness. Such effects may be particularly likely given that the mutations we used had large and visible effects that may impinge on a male's search ability (e.g., eye and wing mutations). An important goal of future research will be to determine the generality of these results with respect to mutations of small effects segregating within natural populations.

Cages reduced female remating rates as compared to vials, as indicated by a greater number of individual females producing both mutant and wild-type offspring in the latter as compared to the former (results not shown). Differential sperm competition may therefore have existed between our treatments, although to explain our results mutant males would have to consistently outperform wild-type males in this respect. Preliminary data suggest the opposite, however, with stock males outperforming mutant males in sperm offensive ability for four of five mutations tested (unpublished results).

Variable search effort may be of particular importance because it provides a potential mechanism by which sexual selection can reinforce natural selection that does not rely on the evolution or maintenance of costly female preferences as required by a good genes process. In this scenario, females mate with higher condition males not because of any innate preference for exaggerated displays exhibited by these males, but because low condition males are simply not present, or they arrive too late.

In species in which population mean fitness is determined by the number and fecundity of females, mutation load is reduced whenever total selection against deleterious mutations is stronger in males than in females (Whitlock & Agrawal 2009). By strengthening sexual selection on males, search effort is potentially an important component of sexual fitness that needs to be considered. During mate search, males may experience an increase in various risks (e.g., predation) or heightened exposure to certain stresses (e.g., starvation, desiccation) relative to females. Although blurring the distinction between natural and sexual selection, such processes may further aid in reducing mutation load. How search effort covaries with other components of sexual and nonsexual fitness is an important topic for future research.

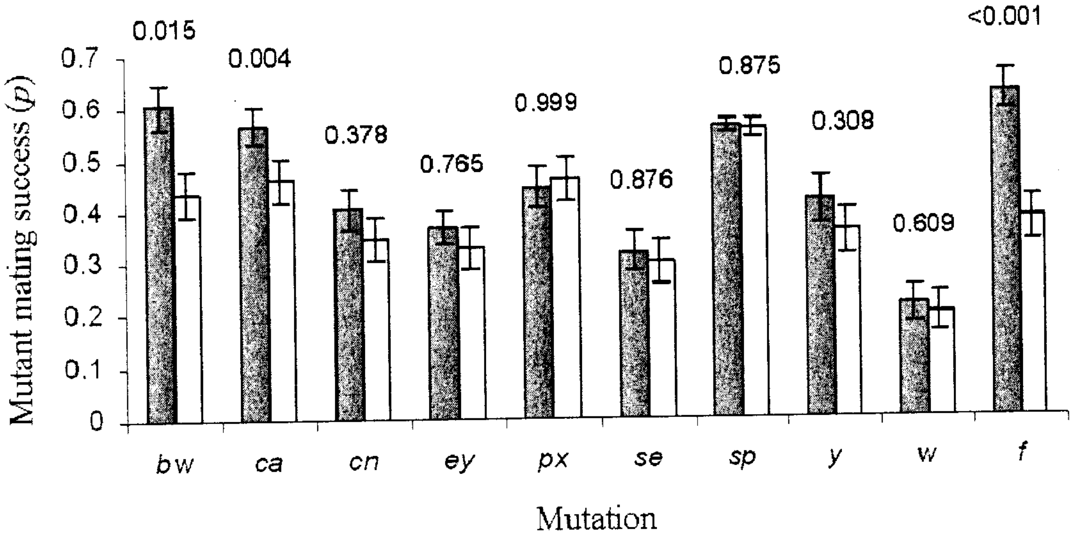

Figure 3.1. Reproductive success of mutant relative to stock males (proportion of mutant offspring, p ± SE) for 100 replicate mating trials conducted in small (closed bars) and large (open bars) mating arenas for each of ten mutations. P-values across the top are from separate tests comparing small vs. large arenas, treating individuals as replicates.

LITERATURE CITED

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