3-31-2015 12:00 AM

Reproductive Success and Sexual Selection in Drosophila melanogaster (Diptera: Drosophilidae)

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Graduate Program in Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy

REPRODUCTIVE SUCCESS AND SEXUAL SELECTION IN DROSOPHILA MELANOGASTER (DIPTERA: DROSOPHILIDAE)

(Thesis format: Integrated Article)

by

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A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

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Abstract

Sexual selection is a branch of natural selection which acts upon variation in reproductive success. Sexual selection is a complex field of study in biology as each species have their own mating system and strategies. Models of sexual selection theory and female mate choice are not mutually exclusive, and often times there are multiple layers of selection within a given mating system. For instance, both direct and indirect benefits of sexual selection can occur simultaneously, and selection can act both before and after mating occurs. Postmating sexual selection, which is not as well understood, can be comprised of both the male-male interaction of sperm competition and the male-female interaction of cryptic female choice. Although there are many studies which show the existence of postmating sexual selection, there is limited knowledge of its underlying mechanisms or genetic basis. Although we know of the physical male traits that females prefer, the relationships among male trait, female preference, and postmating sexual selection are unknown. Here I show accurate alternative measurements for female lifetime reproductive success (Chapter 2) and the genetic architecture underlying lifetime reproductive success (Chapter 3). I found that the short term measure of 5 days can accurately predict the lifetime reproductive success of females, and that this reproductive success is a result of additive genetic variation. In Chapter 4, I compared lifetime reproductive success to mating success in a multi-generational study and found that males who were more successful at mating produced sons with lower fitness. I then examined mechanisms of sperm competition, specifically the role of Acps (accessory gland proteins) in sperm competition in Chapter 5. I discovered that Acps from the first male to mate are beneficial to the second mated male, contributing to second male advantage. Lastly, in Chapter 6, I assessed male quality based on five fitness measures and determined

male performance in both pre- and postcopulatory sexual selection. I concluded that a combined fitness measure most accurately predicted male offspring production. This thesis characterizes the various factors that contribute to variation in lifetime reproductive success, specifically from a sexual selection perspective.

Keywords

Sexual selection, *Drosophila melanogaster*, fitness, mating success, sperm competition, cryptic female choice, second male advantage, precopulatory and postcopulatory, genetic quality, evolution

Acknowledgments

This thesis could not have been completed without the supervision of Amanda Moehring, and the guidance and advice of my committee members Beth MacDougall-Shackleton and Bryan Neff. I thank Beth for always being available and willing to fit me into her busy schedule whenever I needed guidance. I appreciate Bryan's input in proof reading this thesis. I would like to thank Amanda for taking a risk and accepting me as her first PhD graduate student in January 2011. Although they were big shoes to fill, I am very grateful that Amanda took a chance on me, even though I had no prior knowledge of the topics in her lab, as my background was in another field. It turns out we are very compatible in the student-supervisor dynamic relationship and our work ethics parallel. Although her standards are very high, she balances it well with her compassion and understanding. Amanda has definitely established herself as being a strong female role model to myself, as well as in the department.

One of the most important people I've met in the Moehring lab, as well as my time here as Western University, is Christopher Austin. I'd like to thank Chris for being there for me, through everything. The many experiences we had both in the lab and in life are invaluable. He is an amazing person and will forever remain a lifelong friend. I'd like to thank Amy Saba for being my emotional support, Luis Alvarez for housing me for a while, and Bradley Howell for his statistical assistance, especially in time of need (ie comprehensive exam).

I would not have been able to complete my thesis without the assistance of my team of work study undergraduates. A special thanks to Pria M., Anges K., Hannah G., Anqi J.,

Rebecca K., Agnes K., Amanda M., Chaewon J., David J., Hassan S., Hemani P., James L., Jongwook K., Josh S., Josh T., Matthew M., Patrick Z., Collin R., Stephan L., Yoni B., Sarah K., Rigya., Michael H., Sam L., Injun S., Alice L., Harpreet G., Monica, and Andy S. I thank them for putting up with my high standards and the demanding work load. They taught me just as much about being a supervisor as I taught them about being a researcher. I'd like to especially mention Eric D. and Shawn M. for meeting my insane demand of fly food without complaint. A particular undergraduate student to note is Amanda Tong. Not only is she one of my brightest and hard working assistants, she has become an important friend. I now think of her as my little sister who I have become very fond and protective of.

Lastly, I would like to thank my family, who has always and will always be there for me. I appreciate my parents, especially my mom, for feeding me when I was too busy to feed myself, especially during the crazy experiment times which I call "the dark days". I am grateful for my uncle, who is such a kind and generous man, and my sister, who showed me the value of family.

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List of Abbreviations

Acps: Accessory gland proteins

ANOVA: analysis of variance

CHCs: cuticular hydrocarbons

GLIMMIX: generalized linear mixed model (command for SAS)

GLM: generalized linear model

GLMM: generalized linear mixed model

kni: *knirps*

LM: linear model

LMM: linear mixed model

LRS: lifetime reproductive success

MHC: major histocompatibility complex

NSERC: Natural Sciences and Engineering Research Council

SAS: Statistical Analysis System

SP: sex peptide

vs.: versus

Chapter 1

1 Introduction

Sexual selection is an expanding field in evolutionary biology, initially proposed by Darwin to explain traits that do not appear to be adaptive via natural selection (Darwin 1871). Exaggerated traits such as the peacock's tail are detrimental to the male's survival in that they are energetically costly to produce and maintain, makes them conspicuous to predators, and is a hindrance for flight and predatory escape. Darwin proposed that these traits which are maladaptive to survival instead help the individual to successfully obtain mates. This mating advantage will increase an individual's fitness: securing a mate will ensure the production of offspring. These offspring inherit the genes for the sexually-selected trait, which can increase their success in producing their own offspring.

The observation of the recurring phenomenon of promiscuous females (polyandry) across many species has expanded the focus for studies of sexual selection. When a female mates with more than one male, there is the opportunity for selection to continue to act after copulation has occurred. Being polyandrous comes at a very high cost for females in terms of time, energy, increased vulnerability to predation, transmission of sexual diseases and decreased female longevity (Turner and Anderson 1983; Fowler and Partridge 1989; Magurran and Nowak 1991; Rowe 1994; Chapman et al. 1995). In order for polyandry to persist, polyandrous females must acquire benefits to counteract the severe costs of mating. The benefits females receive can be direct or indirect, and female mate choice is often a complex assessment of the quality of the benefits that a potential mate can confer.

1.1 Precopulatory sexual selection: evolution of female mate choice

1.1.1 Direct benefits

Direct benefits occur when a female mates with a male to increase her direct fitness, which could result in acquiring a higher immediate fecundity or fertility. These direct benefits include increased paternal care (reducing the cost of parental care for the female), better quality of resources through territory, nuptial gifts, and male protection from other harassing males or predators (Wagner et al. 2001). In resource-based mating systems, females that are polyandrous can receive more resources than females that mate only once. For example, females can obtain additional nutritional benefits through nuptial gifts or by absorbing male ejaculates. In the bushcricket *Requena verticalis* (Orthoptera: Tettigoniidae), females who consumed more spermatophylax (a male nutrient contribution) produced more and heavier eggs (Gwynne 1984). A direct benefit of polyandry can be increased fertility through maintaining sufficient sperm supply (Fjerdingstad and Boomsma 1998). Mating can also reduce male harassment of females from other males, as seen in the water strider *Aquarius remigis* (Hemiptera: Gerridae); this protection allows females to enjoy increased feeding rates (Rowe et al. 1994). Females can also mate with males to gain access to higher quality territory. In the pied flycatcher *Ficedula hypoleuca* (Passeriformes: Muscicapidae), females prefer to mate with males that possessed a higher quality territory of low birch density, thick-trunked trees, and high nest sites (Alatalo et al. 1986). Side-blotched lizard females *Uta stansburiana* (Squamata: Phrynosomatidae) who mated with males on high quality

territories enjoyed the direct benefits of earlier egg laying and produced larger eggs (Calsbeek and Sinervo 2002).

1.1.2 Indirect benefits

In non-resource based mating systems, polyandrous females can acquire indirect benefits which increase the fitness of her offspring. Indirect benefits of polyandry are difficult to study since they involve an interplay between the genetic basis of female mate choice, male attractiveness, and other fitness components (Kokko et al. 2003). Polyandrous females mating with higher genetic quality males should benefit from an increased in fitness through their offspring. Therefore, selection should favor females who can identify males that are of higher genetic quality. There are several models that examine indirect benefits of sexual selection that are not mutually exclusive, and more than one can occur in a given mating system.

1.1.2.1 Fisherian model

In the Fisherian model of sexual selection, females prefer to mate with males that are more attractive (Fisher 1930). The genes for the attractive trait and the preference for it will be coupled and passed down in subsequent generations, causing a linkage disequilibrium. Although it is unclear how the initial attraction for this trait arose, the exaggeration of this trait by sexual selection, to the point where it can be detrimental in natural selection, is called the Fisherian runaway process (Fisher 1930). In the Fisherian model, males have a higher fitness due to being more attractive, which will result in a higher mating success. However, the benefit of attractiveness can come at a high cost of viability, and therefore decrease their lifespan (Kokko 2001). An extension of the Fisherian model is the sexy sons hypothesis where females will gain indirect benefits by mating with attractive males since they will

produce sons with the same traits that allow for superior mating success (Weatherhead and Robertson 1979).

1.1.2.2 Indicator traits

In contrast to the Fisherian model, where the attractive trait of a male is arbitrary, the "good genes" model proposes that a female can enhance her offspring's survival by preferentially mating with males that advertise their good genetic quality. A positive association between the male trait a female is selecting on, male genetic quality, and offspring quality allows females to preferentially mate with males that provide superior growth, fecundity or survival to their offspring. A related theory, the handicap hypothesis, states that attractive sexually selected traits are very costly (Zahavi 1975). Therefore, only high quality males can afford to bear the cost of displaying the attractive trait and survive; the trait becomes an honest indicator of overall male quality. The indicator trait of attractive males can also be condition-dependent and indicate the male's current condition. For example, Hamilton and Zuk propose that indicator traits can reveal a male's parasite and disease resistant status (Hamilton and Zuk 1982). As with the good genes scenario, females who mate with these more attractive males will acquire indirect benefits of increased fitness to their offspring.

Studies that test theories of indicator traits often examine plumage and song in birds. In the house finch *Carpodacus mexicanus* (Passeriformse: Fringillidae), males can vary in their plumage colour as a result of their diet quality (Hill 1991). Furthermore, male plumage is heritable as brightly coloured fathers produce brightly coloured sons. Brightly coloured males also fed their mates and offspring at a higher rate than males with dull plumage and had a higher survival rate. Males who were artificially made brighter mated earlier and had a

higher mating success, indicating that females select male bright plumage colour as an honest indicator of male quality (Hill 1991).

1.1.2.3 Compatible genes

Genetic quality can also be assessed as genetic compatibility, where a female preferentially mates with a male whose genome is compatible to her own. This can occur as inbreeding avoidance: inbreeding depression results in a decrease in fitness due to increasing homozygosity and the expression of deleterious recessive alleles, a decrease in the heterozygote advantage, or overdominance. Females of *Mus musculus* (Rodentia: Muridae) prefer the scent of outbred males, and this preference was enhanced when the females were inbred themselves, suggesting that inbred females may gain a greater fitness benefit than outbred females when mating to heterozygous males (Ilmonen et al. 2009). A very well-studied instance of genetic compatibility involves the major histocompatibility complex (MHC) genes, which are highly polymorphic loci that influence immune function by promoting immune response and resistance to infections and diseases (Penn and Potts 1999; Penn 2002). Females of several species have a preference for males that have dissimilar MHC alleles (Wedekind et al. 1995; Penn and Potts 1999; Penn 2002). In house mice, females preferentially mate with males carrying dissimilar MHC alleles by using MHC odours of their natal nest mates as a reference to avoid, a mechanism called negative familial imprinting (Penn and Potts 1998). This MHC disassortative mating allows females to acquire MHC heterozygosity in their offspring, increasing their fitness as they are more resistant to diseases due to the increased diversity at this locus. Similarly, wild-caught Atlantic salmon, *Salmo salar* (Salmoniformes: Salmonidae), had more dissimilar MHC alleles than expected by

random chance, indicating that their parents likely exhibited dissassortative mating for this locus (Consuegra and Leaniz 2008). Wild-caught salmon with dissimilar MHC alleles were less likely to be infected by a marine nematode parasite, *Anisakis* (Ascaridida: Anisakidae), than those with similar MHC alleles. Additionally, wild-caught salmon on average were less likely to be infected compared to artificially-spawned salmon, and had a lower parasite intensity when infected (Consuegra and Leaniz 2008). These studies emphasizes the importance of sexual selection and mate choice on offspring fitness through indirect genetic benefits.

1.1.3 Sensory bias

Sensory bias is a theory in mate choice evolution proposed by Ryan and Rand where female preference for mate choice evolved for reasons other than sexual selection (Ryan and Rand 1990). For instance, females may be biased towards a certain colour to allow them to detect food more easily. Males then exploit this sensory bias in females in order to be more attractive to them. Cases demonstrating sensory bias are further supported by phylogenetic analyses which show that females sometimes prefer a trait which conspecific males do not posses, but which are present in heterospecific males of closely-related species (Smith et al. 2004). A popular example of sensory bias occurs in the three-spined stickleback *Gasterosteus aculeatus* (Gasterosteiformes: Gasterosteidae), where females prefer to mate with red coloured males. In the stickleback family, both the three-spined and the nine-spined sticklebacks *Pungitius pungitius* (Gasterosteiformes: Gasterosteidae) have a feeding preference for red colouration, regardless of sex and age (Smith et al. 2004). However, males of nine-spined sticklebacks do not exhibit red colouration, indicating that the evolution for the preference of red colouration occurred before the female preference for red

colouration in a mating context. Therefore, a female's preference for red colouration in a foraging context is likely exploited by males who use the sensory bias in a mating context to increase their mating success.

1.2 Prezygotic postcopulatory sexual selection

Sexual selection can take place at different levels, from pre-mating to post-mating, and sometimes even post-fertilization in differential parental investment (Price et al. 1999; Wagner et al. 2001; Gowaty et al. 2007). While the initial level of selection is behavioural (who the female decides to mate with), selection can also take place after mating through postmating sexual selection. Postmating sexual selection can act at the cellular level, such as at the interaction site between the sperm and egg (gametic selection), between sperm that compete for fertilization within the female reproductive tract (sperm competition), and between sperm and the female's reproductive tract (cryptic female choice). These mechanisms can lead to deviations from Mendelian ratios and unexpected prevalence of particular offspring genotypes. For instance, in the stalk-eyed fly, *Cyrtodiopsis dalmanni* and *C. whitei* have X chromosome meiotic drive, which results in fewer male offspring. Females prefer to mate with males with long stalk eyes, which have a drive resistant Y-chromosome, and which results in more male offspring (Wilkinson et al. 1998). Understanding the mechanisms of postmating sexual selection can aid in explaining what causes non-random fertilization and the selection pressures driving changes in species traits causing evolution.

The ability for gametic recognition is vital for successful fertilization, and is especially apparent in open marine fertilization systems. Species-specific surface proteins on sperm and eggs allow for gamete recognition and fusion (Aketa 1967, 1973; Aketa and Onitake 1969). In the sea urchin *Echinometra mathaei*, the sperm's acrosome contains the protein bindin,

which binds to species-specific sperm receptor glycoproteins on the vitelline layer of the egg (Schmell et al. 1977; Vacquier and Moy 1977; Palumbi 1999). The eggs of *E. mathaei* also demonstrate assortative fertilization: eggs are fertilized with sperm carrying bindin alleles that are the same as their own 80% of the time (Palumbi 1999). This demonstrates that gametic selection can act at a molecular level on the site of sperm and egg recognition. However, our knowledge of the molecular basis of gametic recognition is limited to only a few species. Only very recently, within the past year, has the mammalian egg receptor protein (Juno) to the sperm cell surface protein Izumo been identified (Bianchi et al. 2014).

If the Fisherian model is a precopulatory mechanism of female mate choice involving male-male competition where females choose to mate with more attractive males, a postcopulatory equivalent would be the 'sexy sperm hypothesis' involving sperm-sperm competition (Andersson and Simmons 2006). Here, females mate with males that have a high fertilization success and produce sons who inherit the traits conferring high fertilization success. Similarly, the 'good sperm hypothesis' is a postcopulatory parallel of the indicator mechanisms of female mate choice, where high quality sperm gain the majority of fertilization successes and will confer indirect fitness benefits through high quality offspring (Andersson and Simmons 2006).

Sexual selection and fertilization success is often a combination of both male and female interactions. As a result, it is often difficult to tease apart the components of sperm competition from males and the influence of females through cryptic female choice.

1.2.1 Sperm competition

To increase their reproductive success, males have evolved strategies to limit their exposure to sperm competition that include mate-guarding, copulatory plugs, prolonged copulation after insemination, and mechanical removal of residing sperm (Parker 1970, 1984; Waage 1979; Alcock 1994). However, in the event that these initial strategies fail and females successfully mate with multiple males, sperm-sperm interactions can occur and induce postmating sexual selection via sperm competition, where two or more male ejaculates compete for fertilization (Parker 1970). As sperm number is an important factor in successful fertilization during competition (Parker et al. 1997; Hosken et al. 2001), a simple strategy that males employ is to vary the number of sperm released per ejaculate (Pizzari et al. 2003, 2004). Not only can males vary the sperm number in their ejaculates, they can also vary the quality of sperm. When compared to females who had previously mated only once, male field crickets (*Teleogryllus oceanicus*) transfer less viable sperm when mated to females who were virgins as there is minimal risk of sperm competition. (Thomas and Simmons 2007). *Drosophila melanogaster* is one species that displays the common phenomenon of second male sperm precedence, wherein the second male to which a female is mated fathers 50 – 100% of the offspring (Clark et al. 1995). This trend is partially attributed to accessory gland proteins in the seminal fluid, which physically displace and incapacitate sperm already present in a female (Harshman and Prout 1994; Price et al. 1999). First-mated males counteract second male sperm precedence through the release of their own accessory gland proteins in seminal fluid that increase oviposition rates in females and decreases their receptivity to remating (Chen et al. 1988). In the polyandrous deer mice *Peromyscus maniculatus*, sperm from the same male aggregate together more often than to

sperm of another conspecific male, even if that other male was a sibling, demonstrated that sperm have the capability of recognizing sperm from different males (Fisher and Hoekstra 2010). Sperm competition has likely led to the evolution of *P. maniculatus* sperm's ability to recognize relatedness since aggregation of self sperm results in faster swimming (Fisher and Hoekstra 2010) and therefore increased fertilization and fitness (Casselman et al. 2006). If eggs can recognize sperm genotype via the same mechanism that sperm recognize relatedness, preferential fertilization of sperm with compatible or good genes can occur.

1.2.2 Cryptic female choice

To increase their fitness, females have evolved strategies in response to male tactics. In order to minimize forced copulation, female genitalia has undergone anatomical changes in some species. The reproductive tract of female waterfowls (Anseriformes: Anatidae) contain anatomical barriers such as "dead end" pouches and spirals in a counter-clockwise direction to the cork-screw shape of the male phallus in order to control mating (Brennan et al. 2007). When mating does occur, the female reproductive tract can be a hostile environment for sperm. For example, females of *Drosophila* have accessory reproductive glands that excrete proteins into the reproductive tract that are toxic to sperm; these glands are larger (and thus more toxic) in polyandrous species than monogamous species (Hosken et al. 2001).

Females do not necessarily play a passive role in postmating sexual selection, and can use cryptic female choice to bias the paternity of their offspring and influence which sperm will fertilize their eggs (Eberhard 1996). Cryptic female choice can occur at the same time as sperm competition or be confounded by differential abortion (mortality) and/or genetic incompatibility. Therefore, it is often difficult to tease apart the contributions of sperm

competition, cryptic female choice, and male (sperm) and female (egg) interaction towards fertilization success.

Eberhard's definition of cryptic female choice is inclusive, in that it can involve behavioural events under female control that are non cryptic (Eberhard 1996). The most direct method for a female to control fertilization is to remate. Inbreeding causes deleterious recessive disorders to be expressed and eliminates heterozygous advantages, causing a reduction in fitness (Charlesworth and Charlesworth 1987). Michalczyk *et al.* (2011) showed that inbred red flour beetles become more polyandrous: females were quicker to mate, mated for longer periods of time, and had an increased rate of remating (Michalczyk et al. 2011). Several studies have also demonstrated that females were more likely to remate to a male of higher genetic quality (Gabor and Halliday 1997; Pitcher et al. 2003), thus increasing her future offspring's fitness over what it would have been if she continued to use only the first male's sperm for fertilization.

Cryptic female choice can also be seen in the process of sperm storage. In field crickets, females are able to preferentially store sperm of unrelated males to father their offspring, resulting in increased egg hatching success (Bretman et al. 2009). Similarly, female red junglefowl *Gallus gallus* have significantly decreased sperm storage when they are mated with a related male (Pizzari et al. 2004). *Gallus gallus* females receive direct and indirect fitness benefits by mating with dominant males and, therefore, are less likely to eject sperm of dominant males (Pizzari and Birkhead 2000). In a more complex demonstration of cryptic female choice, females of some species exhibit non random sperm use in the presence of sperm competition. Cryptic female choice in these instances can be shown with significant male x female interaction effects on P_2 values (the proportion of offspring sired by

the second mated male) (Pitnick and Brown 2000). The variation of P_2 attributed to male x female interaction would indicate that the non random use of sperm by females depends on the identity of the male and of the female.

1.3 Postzygotic sexual selection

Sexual selection can occur at and even after fertilization. The differential allocation hypothesis suggests that since mating is costly, preferential allocation in investment and resources should be given to offspring from attractive high quality males (Sheldon 2000). In the house crickets, *Acheta domesticus* (Orthoptera: Gryllidae), females invested more in reproductive effort when mated with attractive males by laying larger eggs (Head et al. 2005). However, this could be a result of attractive males manipulating the behaviour of females. Side-blotched lizard females *Uta stansburiana* who mated with multiple males produced sons with sperm from the larger male while they produced daughters with sperm from the smaller males (Calsbeek and Sinervo 2002). Offspring sired by the larger males were also larger and in better condition. In this species, males have a greater fitness when they are larger and females when they are smaller. Therefore, females of this species mate with both large and small males, and control the sex of the resulting offspring, to maximize fitness. On the opposite end of the spectrum with respect to the differential allocation hypothesis, the compensation hypothesis predicts that females should preferentially increase investment and resources to offspring when mated to low quality males in order to offset the harmful effects of poor mate quality (Gowaty et al. 2007). In the pronghorn *Antilocapra americana*, females who mate with unattractive males produce offspring with higher mortality (Byers and Waits 2006). However, they compensate by increasing the amount of milk production for their offspring (Byers and Waits 2006). In a

comprehensive study, Gowaty et al. (2007) tested the compensation hypothesis in a wide range of species: wild mallards *Anas platyrhynchos* (Anseriformes: Anatidae), Tanzanian cockroaches *Nauphoeta cinera* (Blattodea: Blaberidae), fruit flies *D. pseudoobscura* (Diptera: Drosophilidae), pipefish *Syngnathus typhle* (Syngnathiformes: Syngnathidae) and feral house mice *Mus musculus* (Rodentia: Muridae). In all species tested, they found that non-preferred mating pairs produced offspring with lower viability. However, females in non-preferred mating pairs increased their fecundity in order to compensate for lower offspring quality. The compensation hypothesis is not limited to females: *D. pseudoobscura* males produced more sperm in their ejaculates when mated to non-preferred females.

1.4 *Drosophila melanogaster* as a model system

Drosophila melanogaster is a widely-used model species in studies of evolutionary biology due to its small body size, simple rearing requirements and fast generation time. It is especially useful in sexual selection studies since the species exhibits sexually dimorphic traits and the mating behaviour of *D. melanogaster* is well-documented (reviewed in Spieth 1974; O'Dell 2003). *Drosophila* males can perform a variety of courtship behaviours that include tapping, leg rubbing, licking, circling, and produce a species-specific courtship song from the vibrations produced with his wings. Furthermore, *D. melanogaster* females are polyandrous and possess two types of sperm storage organs, the seminal receptacle and a pair of spermathecae, allowing for postmating sexual selection to occur (Lefevre 1962). Males produce Acps (accessory gland proteins) in their ejaculates, of which some have been characterized and play a significant role in sperm competition (Ram and Wolfner 2007). *Drosophila melanogaster* is also genetically well-characterized and a vast array of molecular tools

are readily available. Although one of the limitations of using *D. melanogaster* as a model species in lab experiments relates to whether the results are valid in terms of what happens in nature, the findings can nonetheless provide a foundation for future studies.

1.5 Thesis structure

Although there has been an increased interest in sexual selection within the past several decades, many questions still remain unknown. In order to address quality of individuals, accurate measures of fitness need to be determined, as well as which fitness traits are an accurate representation of an individual's overall quality. Furthermore, the genetic architecture of fitness traits in the context of sexual selection is rarely identified. An inclusive view of sexual selection that incorporates both survivorship and mating success and how both components contribute to an individual's overall fitness allows for an accurate assessment of an individual's quality and its relationship to sexual selection. In my PhD thesis, I use *D. melanogaster* to address these questions in order to advance our knowledge of sexual selection.

This thesis is presented as an integrated article where the five data chapters (Chapters 2-6) are independent units for publication. The goal of this research is to expand our knowledge of reproductive success, and specifically how sexual selection contributes to variation in reproductive success. One of the chapters has been published (Chapter 2), and one is currently under review (Chapter 3). The remaining three chapters are in preparation for submission (Chapter 4-5-6).

1.5.1 Lifetime reproductive success

Accurate measures of fitness allow us to assess the quality of individuals. The first two data chapters (Chapter 2-3) focus on the fitness measurement of lifetime reproductive success (the total number of offspring produced in an individual's lifetime) as lifetime reproductive success is an important measure of fitness. Chapter 2 ("Accurate Alternative Measurements for Female Lifetime Reproductive Success in *Drosophila melanogaster*"; Nguyen and Moehring 2015) focuses on accurate proxies for lifetime reproductive success, as measuring lifetime reproductive success is often a very time consuming process or non-feasible. I hypothesize short term reproductive success measures of 1-2 days are not accurate indicators of lifetime reproductive success since reproductive success measures, particularly at the onset of reproduction, contain a high amount of variation. In Chapter 3, I further analyze the lifetime reproductive success fitness measure by identifying the genetic architecture of lifetime reproductive success in a multi-generational study using the Cockerham and Weir Biomodel to disentangle the genetic components responsible for variation in this phenotype. I hypothesize that the fitness trait of lifetime reproductive success will be result from significant genetic components as fitness traits are often heritable.

1.5.2 Mating success in a competitive environment

An inclusive view of sexual selection that incorporates both male mating success and male quality allows for a comprehensive assessment of male fitness. In Chapter 4, I compare male mating success to the direct and indirect benefits females may receive. I used a multi-generational study measuring female lifetime reproductive success and the lifetime reproductive success of F_1 individuals (daughters and sons). Male mating

success was measured in a novel mating arena that allowed both male-male competition and female-female competition. I hypothesize that high quality males who have a high lifetime reproductive success will also have a high mating success.

1.5.3 Male reproductive success

An important aspect of sexual selection involves the female's assessment of male quality. This raises the question of which traits accurately represent a male's overall fitness. Chapters 5-6 focus on the various aspects of male reproductive success. I identify how both sperm itself and the proteins found in seminal fluid each contribute to sperm competition and, by extension, offspring production. To measure this, males were competed with sterile mutant males which produce no sperm, but still produced Acps (accessory gland proteins) (Chapter 5). I then assessed male quality using five fitness measures (1- productivity, 2- productivity of F_1 sons, 3- productivity of F_1 daughters, 4- mating success in competition, and 5- combined fitness traits) and measured male performance in both precopulatory (using mating assays) and postcopulatory (using various treatments of competition) sexual selection (Chapter 6). I hypothesize high quality males will perform better than low quality males in both pre- and postcopulatory sexual selection.

1.5.4 Summary

To conclude, in Chapter 7, I discuss the limitations of reproductive success and sexual selection. I present how my research contributes to our knowledge of sexual selection and how it incorporates an inclusive view of reproductive success. Lastly, I consider the direction that sexual selection research is taking and future studies that can expand our understanding of sexual selection.

1.6 References

Aketa, K. 1967. On the sperm-egg bonding as the initial step of fertilization in the sea urchin. Embryological (Nagoya) 9:238–245.

Aketa, K. 1973. Physiological studies on the sperm surface component responsible for sperm-egg bonding in sea urchin fertilization: I. Effect of sperm-binding protein on the fertilizing capacity of sperm. Exp. Cell Res. 80:439–441.

Aketa, K., and K. Onitake. 1969. Effect on fertilization of antiserum against sperm-binding protein from homo- and heterologous sea urchin egg surfaces. Exp. Cell Res. 56:84–86.

Alatalo, R. V., A. Lundberg, and C. Glynn. 1986. Female pied flycatchers choose territory quality and not male characteristics. Nature 323:152–153.

Alcock, J. 1994. Postinsemination associations between males and females in insects: the mate-guarding hypothesis. Annu. Rev. Entomol. 39:1–21.

Andersson, M., and L. W. Simmons. 2006. Sexual selection and mate choice. Trends Ecol. Evol. 21:296–302.

Bianchi, E., B. Doe, D. Goulding, and G. J. Wright. 2014. Juno is the egg Izumo receptor and is essential for mammalian fertilization. Nature 508:483–487.

Brennan, P. L. R., R. O. Prum, K. G. McCracken, M. D. Sorenson, R. E. Wilson, and T. R. Birkhead. 2007. Coevolution of the male and female genital morphology in waterfowl. PLoS ONE 2:e418. doi:10.1371/journal.pone.0000418.

Bretman, A., D. Newcombe, and T. Tregenza. 2009. Promiscuous females avoid inbreeding by controlling sperm storage. Mol. Ecol. 18:3340–3345.

Byers, J. A., and L. Waits. 2006. Good genes sexual selection in nature. Proc. Natl. Acad. Sci. 103:16343–16345.

Calsbeek, R., and B. Sinervo. 2002. Uncoupling direct and indirect components of female choice in the wild. Proc. Natl. Acad. Sci. 99:14897–14902.

Casselman, S. J., A. I. Schulte-Hostedde, and R. Montgomerie. 2006. Sperm quality influences male fertilization success in walleye (*Sander vitreus*). Can. J. Fish. Aquat. Sci. 63:2119–2125.

Chapman, T., L. F. Liddle, J. M. Kalb, M. F. Wolfner, and L. Partridge. 1995. Cost of mating in *Drosophila malanogaster* females is mediated by male accessory-gland products. Nature 373:241–244.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. Annu. Rev. Ecol. Syst. 18:237–268.

Chen, P. S., E. Stumm-Zollinger, T. Aigaki, J. Balmer, M. Bienz, and P. Böhlen. 1988. A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. Cell 54:291–298.

Clark, A. G., M. Aguadé, T. Prout, L. G. Harshman, and C. H. Langley. 1995. Variation in sperm displacement and its association with accessory gland protein loci in *Drosophila melanogaster*. Genetics 139:189–201.

Consuegra, S., and C. G. de Leaniz. 2008. MHC-mediated mate choice increases parasite resistance in salmon. Proc. R. Soc. Lond. B Biol. Sci. 275:1397–1403.

Darwin, C. 1871. The descent of man and selection in relation to sex. Murray, London.

Eberhard, W. G. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton, New Jersey.

Fisher, H. S., and H. E. Hoekstra. 2010. Competition drives cooperation among closely related sperm of deer mice. Nature 463:801–803.

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

Fjerdingstad, E. J., and J. J. Boomsma. 1998. Multiple mating increases the sperm stores of Atta colombica leafcutter ant queens. Behav. Ecol. Sociobiol. 42:257–261.

Fowler, K., and L. Partridge. 1989. A cost of mating in female fruitflies. Nature 338:760–761.

Gabor, C. R., and T. R. Halliday. 1997. Sequential mate choice by multiply mating smooth newts: females become more choosy. Behav. Ecol. 8:162–166.

Gowaty, P. A., W. W. Anderson, C. K. Bluhm, L. C. Drickamer, Y.-K. Kim, and A. J. Moore. 2007. The hypothesis of reproductive compensation and its assumptions about mate preferences and offspring viability. Proc. Natl. Acad. Sci. 104:15023–15027.

Gwynne, D. T. 1984. Courtship feeding increases female reproductive success in bushcrickets. Nature 307:361–363.

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

Harshman, L. G., and T. Prout. 1994. Sperm displacement without sperm transfer in *Drosophila melanogaster*. Evolution 48:758–766.

Head, M. L., J. Hunt, M. D. Jennions, and R. Brooks. 2005. The indirect benefits of mating with attractive males outweigh the direct costs. PLoS Biol 3:e33.

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

Hosken, D. J., T. W. J. Garner, and P. I. Ward. 2001. Sexual conflict selects for male and female reproductive characters. Curr. Biol. 11:489–493.

Ilmonen, P., G. Stundner, M. ThoSZ, and D. J. Penn. 2009. Females prefer the scent of outbred males: good-genes-as-heterozygosity? BMC Evol. Biol. 9:104.

Kokko, H. 2001. Fisherian and “good genes” benefits of mate choice: how (not) to distinguish between them. Ecol. Lett. 4:322–326.

Kokko, H., R. Brooks, M. D. Jennions, and J. Morley. 2003. The evolution of mate choice and mating biases. Proc. R. Soc. Lond. B Biol. Sci. 270:653–664.

Lefevre, G. J., and U. B. Jonsson. 1962. Sperm transfer, storage, displacement, and utilization in *Drosophila melanogaster*. Genetics 47:1719–1736.

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

Michalczyk, Ł., A. L. Millard, O. Y. Martin, A. J. Lumley, B. C. Emerson, T. Chapman, and M. J. G. Gage. 2011. Inbreeding promotes female promiscuity. Science 333:1739–1742.

O'Dell, K. M. C. 2003. The voyeurs' guide to *Drosophila melanogaster* courtship. Behav. Processes 64:211–223.

Palumbi, S. R. 1999. All males are not created equal: Fertility differences depend on gamete recognition polymorphisms in sea urchins. Proc. Natl. Acad. Sci. 96:12632–12637.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45:525–567.

Parker, G. A. 1984. Sperm competition and the evolution of animal mating systems. Academic Press, Inc. (London) LTD., London.

Parker, G. A., M. A. Ball, P. Stockley, and M. J. G. Gage. 1997. Sperm competition games: a prospective analysis of risk assessment. Proc. R. Soc. Lond. B Biol. Sci. 264:1793–1802.

Penn, D. J. 2002. The scent of genetic compatibility: sexual selection and the Major Histocompatibility Complex. Ethology 108:1–21.

Penn, D. J., and W. K. Potts. 1999. The evolution of mating preferences and Major Histocompatibility Complex genes. Am. Nat. 153:145–164.

Penn, D., and W. Potts. 1998. MHC–disassortative mating preferences reversed by cross–fostering. Proc. R. Soc. Lond. B Biol. Sci. 265:1299–1306.

Pitcher, T. E., B. D. Neff, F. H. Rodd, and L. Rowe. 2003. Multiple mating and sequential mate choice in guppies: females trade up. Proc. R. Soc. Lond. B Biol. Sci. 270:1623–1629.

Pitnick, S., and W. D. Brown. 2000. Criteria for demonstrating female sperm choice. Evolution 54:1052–1056.

Pizzari, T., and T. R. Birkhead. 2000. Female feral fowl eject sperm of subdominant males. Nature 405:787–789.

Pizzari, T., C. K. Cornwallis, H. Løvlie, S. Jakobsson, and T. R. Birkhead. 2003. Sophisticated sperm allocation in male fowl. Nature 426:70–74.

Pizzari, T., H. Lø, and C. K. Cornwallis. 2004. Sex–specific, counteracting responses to inbreeding in a bird. Proc. R. Soc. Lond. B Biol. Sci. 271:2115–2121.

Price, C. S. C., K. A. Dyer, and J. A. Coyne. 1999. Sperm competition between *Drosophila* males involves both displacement and incapacitation. Nature 400:449–452.

Ram, K. R., and M. F. Wolfner. 2007. Seminal influences: *Drosophila* Acps and the molecular interplay between males and females during reproduction. Integr. Comp. Biol. 47:427–445.

Rowe, L. 1994. The costs of mating and mate choice in water striders. Anim. Behav. 48:1049–1056.

Rowe, L., G. Arnqvist, A. Sih, and J. J.Krupa. 1994. Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. Trends Ecol. Evol. 9:289–293.

Ryan, M. J., and A. S. Rand. 1990. The sensory basis of sexual selection for complex calls in the tungara frog, *Physalaemus pustulosus* (sexual selection for sensory exploitation). Evolution 44:305–314.

Schmell, E., B. J. Earles, C. Breaux, and W. J. Lennarz. 1977. Identification of a sperm receptor on the surface of the eggs of the sea urchin *Arbacia punctulata*. J. Cell Biol. 72:35–46.

Sheldon, B. C. 2000. Differential allocation: tests, mechanisms and implications. Trends Ecol. Evol. 15:397–402.

Smith, C., I. Barber, R. J. Wootton, and C. Lars. 2004. A receiver bias in the origin of three–spined stickleback mate choice. Proc. R. Soc. Lond. B Biol. Sci. 271:949–955.

Spieth, H. T. 1974. Courtship behavior in *Drosophila*. Annu. Rev. Entomol. 19:385–405.

Thomas, M. L., and L. W. Simmons. 2007. Male crickets adjust the viability of their sperm in response to female mating status. Am. Nat. 170:190–195.

Turner, M. E., and W. W. Anderson. 1983. Multiple mating and female fitness in *Drosophila pseudoobscura*. Evolution. 37: 714-723.

Vacquier, V. D., and G. W. Moy. 1977. Isolation of bindin: the protein responsible for adhesion of sperm to sea urchin eggs. Proc. Natl. Acad. Sci. 74:2456–2460.

Waage, J. K. 1979. Dual function of the damselfly penis: sperm removal and transfer. Science 203:916–918.

Wagner, W. E., R. J. Kelley, K. R. Tucker, and C. J. Harper. 2001. Females receive a life-span benefit from male ejaculates in a field cricket. Evolution 55:994–1001.

Weatherhead, P. J., and R. J. Robertson. 1979. Offspring quality and the polygyny threshold: “the sexy son hypothesis.” Am. Nat. 113:201–208.

Wedekind, C., T. Seebeck, F. Bettens, and A. J. Paepke. 1995. MHC-dependent mate preferences in humans. Proc. R. Soc. Lond. B Biol. Sci. 260:245–249.

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

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

Chapter 2

2 Accurate Alternative Measurements for Female Lifetime Reproductive Success in *Drosophila melanogaster*

Fitness reflects an individual's ability to survive and reproduce, and is an important concept in evolutionary biology. However, accurately measuring fitness is often difficult, and appropriate fitness surrogates need to be identified. Lifetime reproductive success, the total progeny an organism can produce in its lifetime, is thought to be a suitable proxy for fitness, but the measure of an organism's reproductive output across a lifetime can be difficult or impossible to obtain. Here I demonstrate that the short-term measure of reproductive success across five days provides a reasonable prediction of an individual's total lifetime reproductive success in *Drosophila melanogaster*. However, the lifetime reproductive success of a female that has only mated once is not correlated to the lifetime reproductive success of a female that is allowed to mate multiple times, demonstrating that these measures should not serve as surrogates nor be used to make inferences about one another.

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Citation: Nguyen, T. T. X., A. J. Moehring. (In press) Accurate Alternate Measurements for Female Lifetime Reproductive Success in *Drosophila melanogaster*. PLoS ONE.

2.1 Introduction

An organism's success in the presence of selection is defined by its fitness (Endler, John A. 1986; Stearns 1992; Falconer and Mackay 1996; Smith 1998). While the idea of fitness as the production of offspring, who are in turn successful in producing offspring, is conceptually easy to understand, there has been debate as to the appropriate way to measure fitness within a laboratory setting (Rosenberg 1982; Orr 2009; Hunt and Hodgson 2010). These measurements must be of a phenotype that is able to be scored in a reasonable manner, yet accurately capture the essence of an organism's fitness. In an attempt to measure fitness, studies often measure more tractable surrogates of fitness such as body size, survivability, viability, growth rate, mating success, longevity, fecundity, and fertility (Reid et al. 2004; Anderson et al. 2007; Hosokawa et al. 2007). Of these alternative measurements, the number of offspring an individual produces over its lifetime (lifetime reproductive success) is generally considered an acceptable estimate of fitness (Stearns 1992; Brommer et al. 2004; Hunt and Hodgson, D. 2010). However, for species with multiple reproductive cycles, long generation times, or large numbers of offspring, lifetime reproductive success is often difficult and time-consuming to measure. Studies therefore often measure reproductive success over only a subset of an organism's lifespan as an approximation of lifetime reproductive success (Turner and Anderson 1983; Singh and Singh 2001; Fleming 2008; Marshall and Sinclair 2010; Kudupali and Shivanna 2013; Parkash et al. 2013; Vijendravarma et al. 2013). However, using short-term reproductive success as a measure of fitness can potentially be inaccurate if organisms vary in their rates of offspring production, such as through a trade-off in quantity of early *vs.* late lifetime reproductive output.

Drosophila melanogaster is a model organism that is often used in studies with a fitness component (Wigby et al. 2009; Billeter et al. 2012; Klepsatel et al. 2013; Carazo et al. 2014). Under unlimited conditions of food and access to mates, a female will produce an average total of 615 offspring throughout her lifetime (Clutton-Brock, T. H. 1988), which is approximately 90 days at 21 degrees Celsius for wild-type *D. melanogaster* (Miquel et al. 1976). The long life expectancy and high productivity of *D. melanogaster* make it time-consuming to measure the total lifetime reproductive success, particularly when sample sizes are large, and thus surrogate measures of fitness are usually used in this species. Measuring reproductive output over a much shorter time span or after only a single mating could potentially serve as accurate proxies for lifetime reproductive success, but a direct test of the relationship between these alternative measures and lifetime reproductive success has not been conducted for this widely-used model species. Here, I used multiply-mated females from ten isofemale lines of *D. melanogaster* to determine if a female's short-term reproductive output (after one day and/or seven days) can accurately predict lifetime offspring production. I also determined the optimal number of days to measure reproductive output in order to achieve the strongest correlation with lifetime reproductive success using the fewest number of measurements. I then compared lifetime reproductive success of multiply-mated females to that of singly-mated females to assess whether a female's reproductive output from a single mating, which is less cumbersome to measure, is indicative of her output after multiple matings, which is more representative of a female's mating status in the wild.

2.2 Methods

2.2.1 Experimental procedures

Ten isofemale lines of *D. melanogaster*, collected from the wild in Sudbury, Ontario Canada, in 2011, were generously provided by T. Merritt. Flies were maintained in the laboratory on standard cornmeal agar media (Bloomington *Drosophila* Stock Center, Indiana) in 8-dram vials on a 14:10 light-dark cycle, at 24ºC and approximately 75% relative humidity. Males and females were separated upon eclosion (to ensure virginity), aged four to six days, and then placed in single mating pairs within a vial. Additional males were collected at the same time but left unmated; these aged males were used as replacements for similarly-aged males who died.

For multiply-mated females, pairs were kept together throughout the female's lifetime, allowing for remating. The ten isofemale lines were mated in a full-factorial diallel cross, resulting in 100 mating pairs, each with four replicates. Mated pairs were checked daily and dead males were replaced with a male of similar age. Mating pairs were transferred into a new vial after one day, transferred again after an additional six days (seven days after initial mating), and then every seven days thereafter. The measure of offspring from the initial vial is the reproductive output from one day (the number of offspring that eclose from the total eggs laid in one day), the measure of offspring from the first vial plus the second vial is the reproductive output after seven days (the number of offspring that eclose from the total eggs laid in 7 days), and the measure of the offspring produced from all of the vials in a female's lifetime is the lifetime reproductive success. The number of offspring eclosing from each vial was scored daily, up until 16-17 days after the last egg was laid or the female died, ensuring enough time for all larvae to emerge

and that all offspring that were produced were scored. Since offspring eclosion was recorded daily, the total daily eclosion and the total daily cumulative eclosion measures were analyzed. The total daily eclosion measures consist of the total number of eclosions that occurred each day after the first eclosion, regardless of when the eggs were laid. The total daily eclosion measures may differ from the eclosion measures from the one day and 7 day block (previously stated) since these were scored based on the day the eggs were laid rather than the day of eclosion, and variation in larval developmental times could cause these values to differ. Any female that did not produce any larvae, indicating that mating did not occur or that individuals were sterile, was removed from the data set. I note that the lifetime reproductive success of females measured here may not be representative of the values that may occur in nature, as these laboratory females are supplied with unlimited food and mating opportunities, and are not subjected to predation or competition.

For singly mated females, mating assays were performed with a single male and female in each vial and males were removed after mating; unmated flies were discarded. Isofemale line combinations that were mated are shown in Figure 2.1 for a total of 47 mating pairs, each with 20 replicates. Females were transferred into a new vial every seven days and the number of offspring eclosing from each vial was scored in a similar manner as above.

2.2.2 Statistical analysis

To determine whether early short term reproductive success (one day and seven days) could be used to predict lifetime reproductive success, a linear model (LM) was performed using lifetime reproductive success as the response variable and short term

Isofemale line producing the ♂	Isofemale line producing the ♀									
	1	2	3	4	5	6	7	8	9	10
1	■	■	■		■	■	■	■		
2		■	■			■	■		■	■
3		■	■					■		■
4										
5			■		■		■		■	
6			■			■			■	
7	■	■			■		■	■		
8	■		■		■	■	■	■	■	■
9	■				■	■	■			■
10	■	■	■		■			■		

Figure 2.1 Isofemale line combinations that were assayed. Combinations that were mated in singly-mated crosses are shaded (see Methods). All combinations (shaded and unshaded) were used in the multiply-mated crosses.

reproductive success (one day or seven days) as the predictors. A similar LM was used to determine whether early reproductive success could be used to predict late reproductive success. Late reproductive success was calculated by excluding early reproductive success measures from lifetime reproductive success. For comparison to a previous study (Pekkala et al. 2011), a LM with quasipoisson distribution was performed using a short term reproductive success window of 7 days after approximately 30 days of offspring emergence. The between line and within line variation in isofemale lines for lifetime reproductive success of singly mated females was analyzed in a two-way ANOVA with a Tukey's post hoc using female line and male line as factors. To compare singly and multiply mated isofemale line crosses, a linear mixed model (LMM) was performed using the average multiply mated lifetime reproductive success for each isofemale line combination as the response variable and the corresponding isofemale line combination average of singly mated lifetime reproductive success as the predictor variable, along with female line and male line as random factors. All analyses were performed in R 3.0.3 (2013)

2.3 Results

Early, one-day reproductive success can predict lifetime reproductive success (Figure 2.2A; Estimate = 3.8386 ± 0.8717 S.E., $F_{(1, 267)} = 19.39$, $P < 0.0001$, $R^2 = 0.0642$). Similarly, one-day reproductive success can predict late (older than 1 day) reproductive success (Figure 2.2B; Estimate = 2.8386 ± 0.8717 S.E., $F_{(1, 267)} = 10.60$, $P = 0.0012$. $R^2 = 0.0346$). While these measures are predictive, they only explain 6.4% of the variation in lifetime measurement. This is likely because pairs of flies were not scored for the timing of mating, and were simply removed 24 hours after being paired. Fly pairs therefore

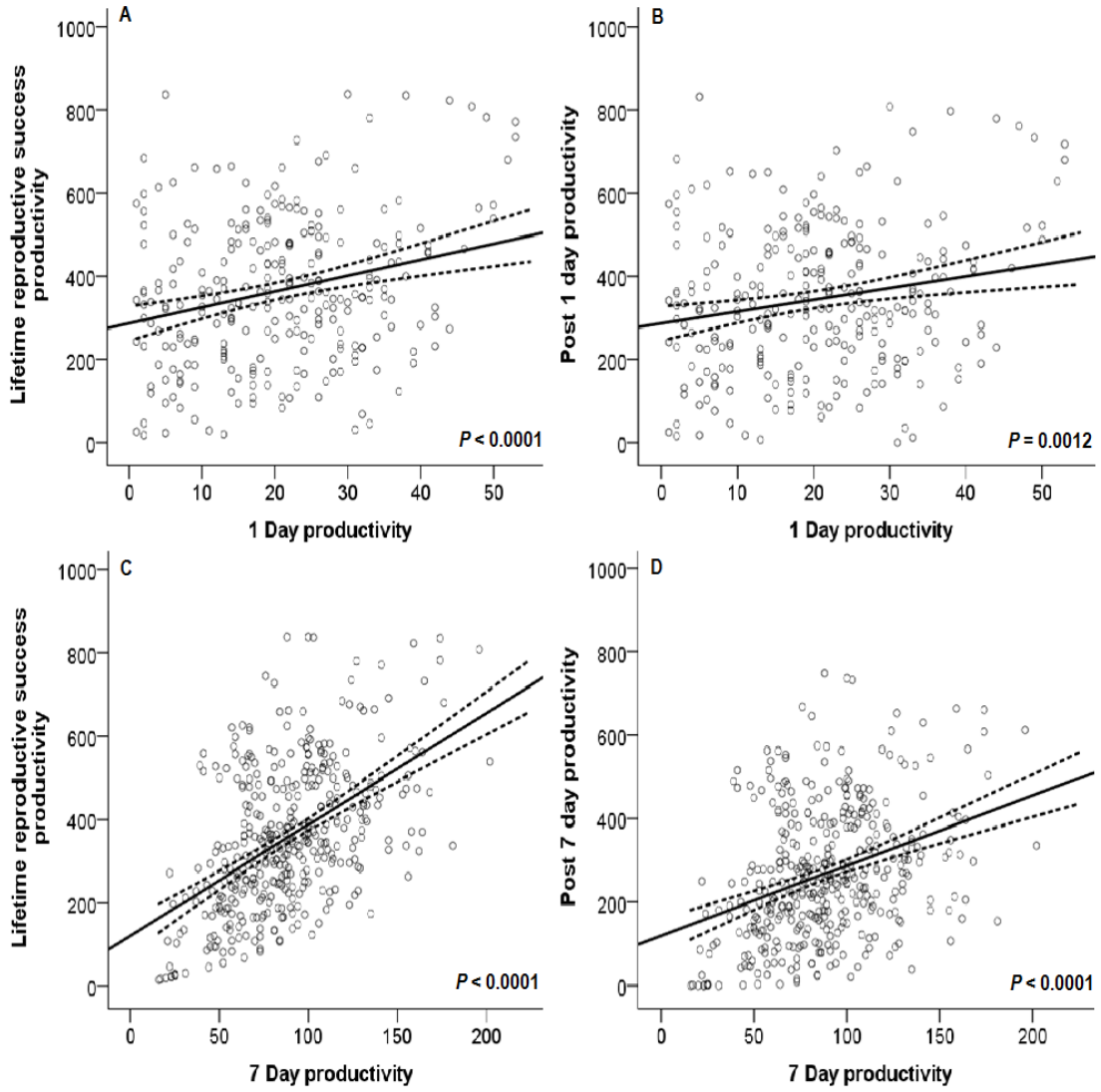

Figure 2.2 Regression of early short-term reproductive outputs on lifetime reproductive success. Early reproductive success is defined by the number of offspring that eclosed from eggs laid in the first day (A, B) or the first seven days (C, D). These values were compared to a total lifetime reproductive success response variable that either included values of short-term reproductive success (A, C) or that excluded the short-term reproductive success values of one day (B) or seven days (D). Dashed lines represent the 95% CI.

could have mated at any time within the 24 hours, and females who mated at the end of this time period would have laid very few fertilized eggs.

Similarly, early seven-day reproductive success is a strong predictor for lifetime reproductive success (Figure 2.2C; Estimate = 2.6790 ± 0.2250 S.E., $F_{(1, 398)} = 141.8$, $P < 0.0001$, $R^2 = 0.2608$) and can predict late reproductive success (older than 7 days) (Figure 2.2D; Estimate = 1.6790 ± 0.2250 S.E, $F_{(1, 398)} = 55.68$, $P < 0.0001$, $R^2 = 0.1205$). The mean one-day reproductive output is 20.72 (19.28-22.17 95% CI, values ranging from 1- 53), mean seven-day reproductive output is 84.38 (80.68-88.07 95% CI, values ranging from 16 - 165), and mean lifetime reproductive output is 345.63 (325.72-365.54 95% CI, values ranging from 16-838).

There is a consistently high rate of offspring eclosion up until approximately day 25 after the first offspring ecloses, with peak eclosion at approximately day 10 (Figure 2.3). Interestingly, there are fluctuations in eclosion rates on an approximately 7 day cycle (Figure 2.3A). This may correspond with the timing of tipping the females to new vials, but since the correspondence of fly tipping with eclosion was not scored I am unable to assess this directly. However, this is unlikely to be due to food limitation since I see the cycle even when the peak number of offspring eclosing is relatively low (e.g. days 29-36 and 37-43, Figure 2.3A), suggesting that the cycle may be due to inducing increased egg laying upon transfer to a new food source. When evaluating the minimum window of early reproduction that could be measured as a proxy for lifetime reproductive success (LRS), even the first day of eclosion has a significant correlation with LRS (Table 2.1).

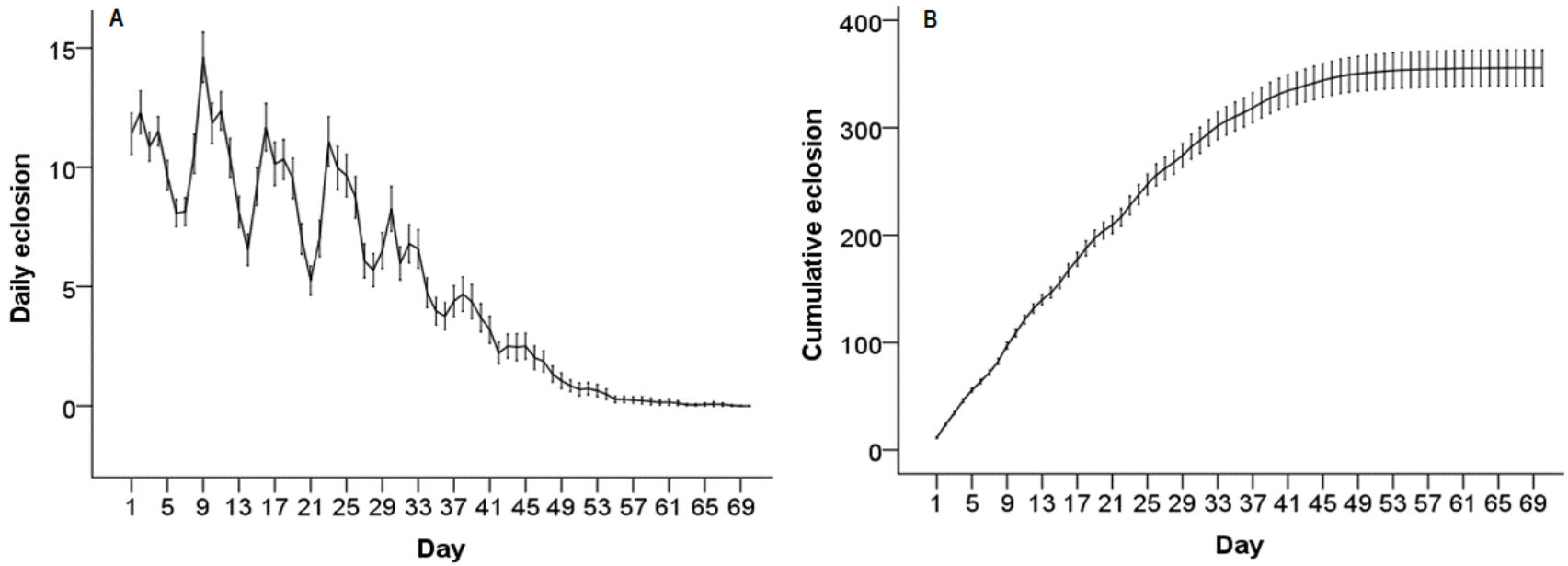

Figure 2.3 Daily eclosion rates. (A) Mean daily eclosion, measured as the total number of offspring eclosing on each day. (B) Mean cumulative eclosion per day. 'Day 1' is the first day that offspring eclosed. Error bars represent the 95% CI.

Table 2.1 Predicting total lifetime reproductive success from daily cumulative eclosion in *D. melanogaster*

Parameter[1]	**Estimate (SE)**[2]	$\mathbf{F_{(1, 398)}}$	***P* - value**	$\mathbf{R^2}$
1 Day	3.8686 (0.9579)	16.31	6.45e-05	0.0369
2 Day	3.5868 (0.6783)	27.96	2.045e-07	0.0633
3 Day	3.9042 (0.5549)	49.50	8.704e-12	0.1084
4 Day	3.7284 (0.4545)	67.30	3.259e-15	0.1425
5 Day	3.7665 (0.3953)	90.80	<2.2e-16	0.1837
6 Day	3.3235 (0.3534)	88.46	<2.2e-16	0.1798
7 Day	3.3636 (0.3129)	115.50	<2.2e-16	0.2230
8 Day	3.2656 (0.2654)	151.40	<2.2e-16	0.2737
9 Day	2.8440 (0.2106)	182.30	<2.2e-16	0.3124
10 Day	2.6479 (0.1869)	200.70	<2.2e-16	0.3335

[1] The number of cumulative days after the day of first eclosion

[2] Estimated via a linear model.

However, as expected, correlation values increase as more days are scored, with the greatest gains in R^2 occurring up to day 5 (Table 2.1).

A seven-day reproductive success window for older females (after approximately 30 days of offspring emergence) is a strong predictor for total lifetime reproductive success (Figure 2.4; Estimate = 0.0072 ± 0.0004 S.E., $t_{(211)} = 14.88$, $P < 0.0001$, pseudo $R^2 = 0.5083$). The two-way ANOVA revealed a significant female line effect (Figure 2.5A; $F_{(8, 866)} = 8.2960$, $P < 0.0001$) and significant male line effect ($F_{(8, 866)} = 7.7590$, $P < 0.0001$) for the lifetime reproductive success of singly-mated females. No significant interaction was detected ($F_{(30, 836)} = 0.7170$, $P = 0.8680$). Of note, the productivity from singly-mated flies was not a significant variable in determining productivity from multiply-mated flies (Figure 2.5B; $\chi^2_{(1)} = 0.0228$, $P = 0.8801$).

2.4 Discussion

Early, short-term reproductive success measures of one or seven days can accurately predict both lifetime reproductive success and late reproductive success in *D. melanogaster* (Figure 2.2). However, seven days of reproductive success is more accurate as an indicator and can explain more of the variation in lifetime reproductive success than the very short-term measure of one day. Similarly, a short term reproductive success measurement of a seven day window in older females is highly significant ($P<0.0001$) in predicting their lifetime reproductive success (Figure 2.4). These results concur with those of Pekkala *et al*. (2011) who showed low but significant correlations of short-term measures (2 day, 4 day, and 10 day windows) of offspring production and lifetime reproductive success for young females in *Drosophila littoralis*

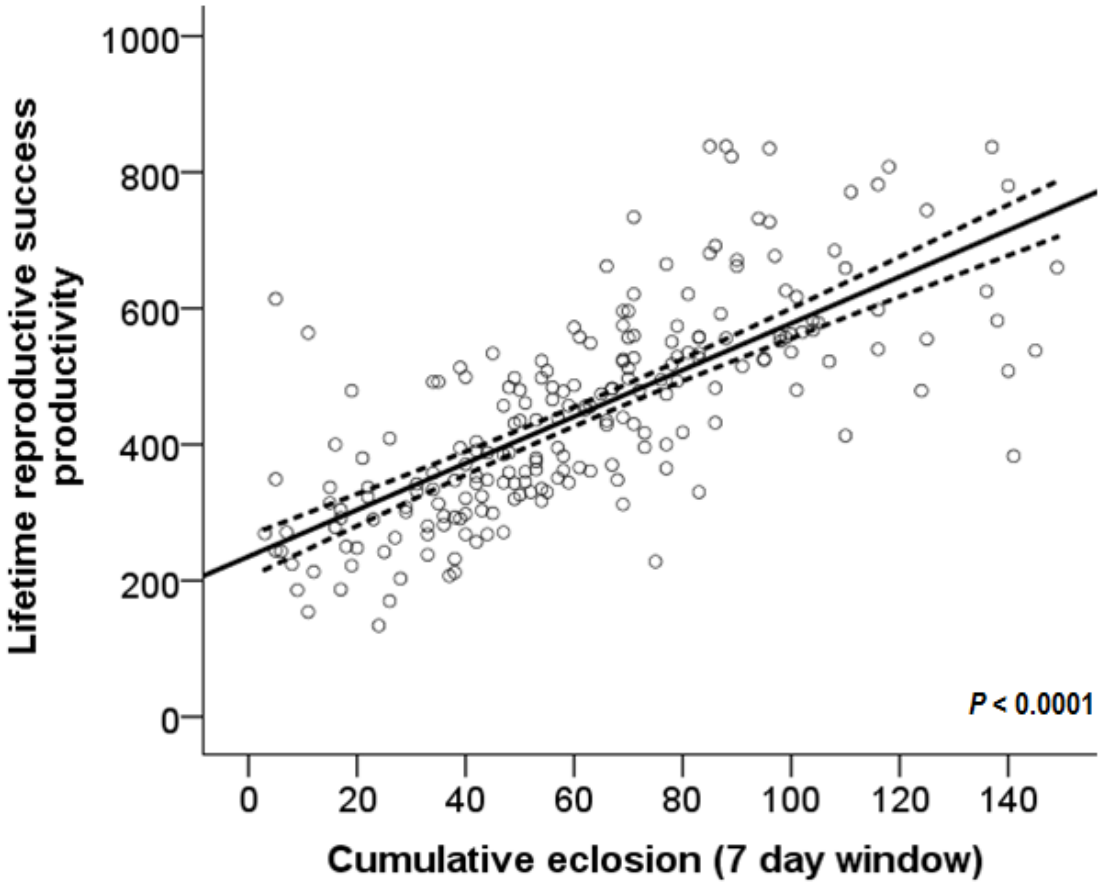

Figure 2.4 Regression of late short term reproductive output on lifetime reproductive success. Late short-term reproductive success was measured as the total number of offspring eclosing during a seven day window after females were approximately 30 days old. Dashed lines represent the 95% CI.

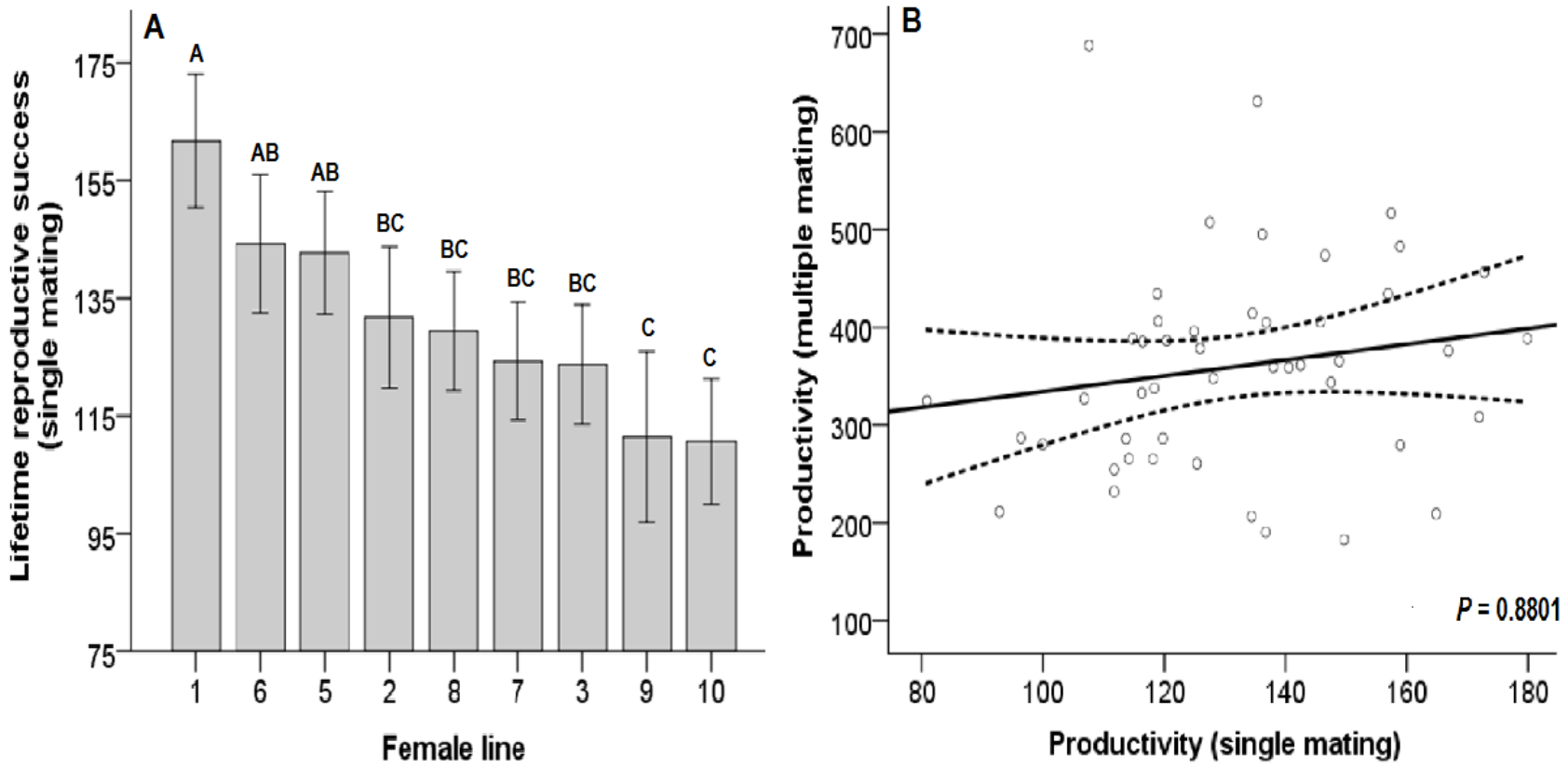

Figure 2.5 Reproductive success by line and by mating level. (A) Variation of lifetime reproductive success of singly mated females separated by female line. Columns with the same letters are not significantly different. Error bars represent the 95% CI. (B) Regression of mean productivity of females with multiple matings on productivity of singly mated females. Dashed lines represent the 95% CI.

(Pekkala et al. 2011). These results also concur with their findings in older females, where there is a high correlation between offspring production measured during a brief window later in life and lifetime reproductive success (correlation up to 0.83). This comparison of similar studies in different species demonstrates that some aspects of reproductive success may show a consistent trend across *Drosophila*; however caution should still be used in applying these results to other species of *Drosophila*.

Although it is evident the longer the initial measures of reproductive success, the more accurately it can predict lifetime reproductive success, the question remains is how many days in early life is optimum to predict lifetime reproductive success. Although a measure of one day of eclosion is statistically significant, it only explains 6.4% of the variation in lifetime reproductive success. According to these results, it appears the cumulative eclosion measure of the initial 5 days in early life is optimal, explaining 18.37% of the variation in lifetime reproductive success, with minimal increase in predictive power at day 6 (Table 2.1). Therefore, studies involving lifetime reproductive success measures may obtain an optimal balance of accuracy *vs.* labor by measuring the initial reproductive success of the first 5 days of offspring eclosion.

The regression of early short term reproductive success (1 day or 7 days) on later reproductive success (>1 day or >7 days) shows a positive correlation (Figure 2.2B, 2.2D). Therefore, having an initially high reproductive output does not come with a reproductive trade-off cost later in life, counter to what would be expected if antagonistic pleiotropy was occurring (Sgrò and Partridge 1999; Maklakov et al. 2005). Similar positive pleiotroic effects are seen in the bedbug, *Cimex lectularius*, where higher

ejaculate doses both increase reproductive rates and delays female reproductive senescence (Reinhardt et al. 2009). Interestingly, the peak daily eclosion does not occur from eggs laid in very early life, counter to expected. Instead, peak eclosion numbers occur from eggs laid later in life, approximately on day 10 of eclosion (eggs laid when females are approximately 14-16 days old), which is shortly after females would be expected to regain receptivity towards a courting male and accept a second mating (at ~8-9 days old; (Manning 1962)). This suggests that peak female fecundity may not occur until females have mated a second time.

Although very short term reproductive success values from one day are not strongly predictive of lifetime reproductive success in the laboratory, they may be an accurate fitness measure in natural environments, although this likely depends on the species being examined. The average life expectancy in the wild is approximately three days for domesticate species of *Drosophila* (e.g. *D. melanogaster*, *D. simulans*, *D. immigrans*, etc; (Rosewell and Shorrocks 1987)), approximately 6 days for *D. serrata* (Robson et al. 2006), and approximately seven days for *D. mercatorum* (Templeton et al. 1993). Hence, the reproductive output from a shorter time span may more accurately reflect the biological fitness of an organism, even if it does not reflect the total reproductive output possible in the laboratory, if that longer lifespan is not realized in the wild.

Significant female line effects for the lifetime reproductive success of singly mated females indicate that the fecundity of a singly mated female can predict the fecundity of another singly mated female from the same isofemale line, regardless of who the female mates with. Therefore, a similar relationship could be expected with singly and multiply mated females. However, contrary to this, the productivity of from a single mating does

not predict lifetime productivity when allowing for remating in *D. melanogaster*. The relationship (or lack thereof) between the reproductive output of single and multiple matings is not universal across species: for example, in the Bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae), there was no difference in fecundity between singly mated females and females who were confined to a single male during her lifetime, which allowed for remating (Fox 1993). In *D. melanogaster*, the lack of a relationship between single and multiply-mated females is likely due to sperm limitation (the male's contribution) in the former case and egg production limitation (the female's contribution) in the latter case. Similar to these results, multiply-mated *D. pseudoobscura* females had a higher productivity than singly-mated females, suggesting that singly-mated females are sperm limited (Turner and Anderson 1983). However, this sperm limitation has only a moderate effect on productivity in this study since singly mated females had 82% of the productivity of multiply mated females (Templeton et al. 1993).

These results, together with Pekkala *et al*. (2011) suggest that one or two day reproductive measurements are appropriate indicators of an individual's total lifetime reproductive success in *Drosophila*. Short-term measurements of the initial seven days of offspring production in young females can, however, explain more variation (26%) in total lifetime reproductive success in *D. melanogaster*. It is important to note that this significant short term measure of reproductive success applies to multiply-mated females. There was no correlation between singly and multiply mated females, and thus these measures should not be used to make inferences about each other. However, within both *D. melanogaster* (presented here) and *D. littoralis* (Pekkala et al. 2011), it appears that a

well-timed window measurement of seven days in older females is significantly correlated to lifetime reproductive success, and thus this measure may also potentially serve as an accurate proxy across the *Drosophila* genus in laboratory controlled conditions (Pekkala et al. 2011).

2.5 Chapter acknowledgements

I thank Thomas Merritt for generously providing us with the isofemale lines used in this study. This work was funded by an NSERC Discovery Grant to Amanda J. Moehring.

2.6 References

Anderson, W. W., Y.-K. Kim, and P. A. Gowaty. 2007. Experimental constraints on mate preferences in *Drosophila pseudoobscura* decrease offspring viability and fitness of mated pairs. Proc. Natl. Acad. Sci. 104:4484–4488.

Billeter, J.-C., S. Jagadeesh, N. Stepek, R. Azanchi, and J. D. Levine. 2012. *Drosophila melanogaster* females change mating behaviour and offspring production based on social context. Proc. R. Soc. B Biol. Sci. 279:2417–2425.

Brommer, J. E., L. Gustafsson, H. Pietiäinen, and J. Merilä. 2004. Single-generation estimates of individual fitness as proxies for long-term genetic contribution. Am. Nat. 163:505–517.

Carazo, P., C. K. W. Tan, F. Allen, S. Wigby, and T. Pizzari. 2014. Within-group male relatedness reduces harm to females in *Drosophila*. Nature 505:672–675.

Clutton-Brock, T. H. 1988. Reproductive success: studies of individual variation in contrasting breeding systems. The University of Chicago Press, Chicago.

Endler, John A. 1986. Natural selection in the wild. Princeton University Press, Princeton, New Jersey.

Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longmans Green, United Kingdom.

Fleming, D. A. 2008. The influence of photoperiod upon the productivity of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). J. Stored Prod. Res. 44:213–218.

Fox, C. W. 1993. Multiple mating, lifetime fecundity and female mortality of the bruchid beetle, *Callosobruchus maculatus* (Coleoptera: Bruchidae). Funct. Ecol. 7:203–208.

Hosokawa, T., Y. Kikuchi, M. Shimada, and T. Fukatsu. 2007. Obligate symbiont involved in pest status of host insect. Proc. R. Soc. B Biol. Sci. 274:1979–1984.

Hunt, J., and Hodgson, D. 2010. What is fitness, and how do we measure it? In: Evolutionary Behavioral Ecology. Oxford University Press, New York, New York.

Klepsatel, P., M. Gáliková, N. De Maio, S. Ricci, C. Schlötterer, and T. Flatt. 2013. Reproductive and post-reproductive life history of wild-caught *Drosophila melanogaster* under laboratory conditions. J. Evol. Biol. 26:1508–1520.

Kudupali, S. L., and N. Shivanna. 2013. Comparison of fitness parameters in different species of *Drosophila*. Am. J. Biosci. Bioeng. 1:1–6.

Maklakov, A. A., N. Kremer, and G. Arnqvist. 2005. Adaptive male effects on female ageing in seed beetles. Proc. R. Soc. B Biol. Sci. 272:2485–2489.

Manning, A. 1962. A sperm factor affecting the receptivity of *Drosophila melanogaster* females. Nature 194:252–253.

Marshall, K. E., and B. J. Sinclair. 2010. Repeated stress exposure results in a survival–reproduction trade-off in *Drosophila melanogaster*. Proc. R. Soc. B Biol. Sci. 277:963–969.

Miquel, J., P. R. Lundgren, K. G. Bensch, and H. Atlan. 1976. Effects of temperature on the life span, vitality and fine structure of *Drosophila melanogaster*. Mech. Ageing Dev. 5:347–370.

Orr, H. A. 2009. Fitness and its role in evolutionary genetics. Nat. Rev. Genet. 10:531–539.

Parkash, R., S. Ramniwas, and B. Kajla. 2013. Climate warming mediates range shift of two differentially adapted stenothermal *Drosophila* species in the Western Himalayas. J. Asia-Pac. Entomol. 16:147–153.

Pekkala, N., J. S. Kotiaho, and M. Puurtinen. 2011. Laboratory relationships between adult lifetime reproductive success and fitness surrogates in a *Drosophila littoralis* population. PLoS ONE 6:e24560.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Reid, J. M., P. Arcese, A. L. E. V. Cassidy, S. M. Hiebert, J. N. M. Smith, P. K. Stoddard, A. B. Marr, and L. F. Keller. 2004. Song repertoire size predicts initial mating success in male song sparrows, *Melospiza melodia*. Anim. Behav. 68:1055–1063.

Reinhardt, K., R. A. Naylor, and M. T. Siva-Jothy. 2009. Ejaculate components delay reproductive senescence while elevating female reproductive rate in an insect. Proc. Natl. Acad. Sci. 106:21743–21747.

Robson, S. K. A., M. Vickers, M. W. Blows, and R. H. Crozier. 2006. Age determination in individual wild-caught *Drosophila serrata* using pteridine concentration. J. Exp. Biol. 209:3155–3163.

Rosenberg, A. 1982. On the propensity definition of fitness. Philos. Sci. 49:268–273.

Rosewell, J., and B. Shorrocks. 1987. The implication of survival rates in natural populations of *Drosophila*: capture-recapture experiments on domestic species. Biol. J. Linn. Soc. 32:373–384.

Sgrò, C. M., and L. Partridge. 1999. A delayed wave of death from reproduction in *Drosophila*. Science 286:2521–2524.

Singh, B. N., and S. R. Singh. 2001. Female remating in *Drosophila ananassae*: evidence for sperm displacement and greater productivity after remating. Zoolog. Sci. 18:181–185.

Smith, J. M. 1998. Evolutionary genetics. Oxford University Press, New York.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, New York.

Templeton, A. R., H. Hollocher, and J. S. Johnston. 1993. The molecular through ecological genetics of abnormal abdomen in *Drosophila mercatorum*. V. Female phenotypic expression on natural genetic backgrounds and in natural environments. Genetics 134:475–485.

Turner, M. E., and W. W. Anderson. 1983. Multiple mating and female fitness in *Drosophila pseudoobscura*. Evolution. 37: 74-723.

Vijendravarma, R. K., S. Narasimha, and T. J. Kawecki. 2013. Predatory cannibalism in *Drosophila melanogaster* larvae. Nat. Commun. 4:1789.

Wigby, S., L. K. Sirot, J. R. Linklater, N. Buehner, F. C. F. Calboli, A. Bretman, M. F. Wolfner, and T. Chapman. 2009. Seminal fluid protein allocation and male reproductive success. Curr. Biol. 19:751–757.

Chapter 3

3 Daughters affected most strongly by good genes and inbreeding

Males and females often have opposing strategies for increasing fitness, which can cause sexual conflict. Male-male competition creates variation in lifetime reproductive success: males that out-compete others will benefit by acquiring more mating opportunities and thus producing a higher number of offspring. Females benefit from mating with a high quality male that possesses good genes or genes that are more compatible with her genotype, receiving either direct benefits through acquisition of additional resources or indirect benefits through the increased fitness of offspring. The genetic basis of lifetime reproductive success may also be in conflict, causing alleles that are beneficial for one sex to have detrimental effects in the opposite sex. Here we attempt to tease apart the genetic architecture of lifetime reproductive success in a multigenerational study in *Drosophila melanogaster*. I found significant additive, maternal and paternal effects for lifetime reproductive success of offspring, with a much stronger effect for daughters than sons. Interestingly, inbreeding depression also had a

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[2] A version of this chapter has been submitted to Evolution and is currently under review.

Citation: Nguyen, T. T. X., A. J. Moehring. (submitted) Daughters affected most strongly by good genes and inbreeding. Evolution

significant effect on the lifetime reproductive success of daughters, but did not have a significant effect on the productivity of sons or parents. I found no evidence of intersexual conflict in the lifetime reproductive success of daughters and sons.

3.1 Introduction

One of the most important aspects in evolution is an animal's ability to reproduce, making lifetime reproductive success (LRS) a vital measure of fitness. Males and females often have differing reproductive strategies to increase their lifetime reproductive success (Andersson 1994). Males typically increase their fitness by competing and acquiring as many mating opportunities as possible. Variation in reproductive success is thus usually larger for males than it is for females, since some males may not achieve any matings while others achieve multiple matings. In contrast, females are usually mated, resulting in low variation in reproductive success in females compared to males. While there may be some advantages to females for repeatedly mating, there are also costs (Turner and Anderson 1983; Fowler and Partridge 1989; Magurran and Nowak 1991; Rowe 1994; Chapman et al. 1995), and thus females may instead increase their fitness by mating selectively. Polyandrous females can receive indirect benefits of multiple mating through their offspring. Indirect benefits are only acquired through mating with multiple males, and not merely multiple mating events with the same male (Zeh 1997; Tregenza and Wedell 1998; Ivy and Sakaluk 2005), indicating that these benefits are genetic. Indirect benefits can be obtained by mating with individuals with good genes through additive genetic variation in the offspring or by mating with individuals with compatible genes and acquiring non-additive genetic benefits (Neff and Pitcher 2005).

In non-resource based mating systems, females may evolve and maintain mate preferences in order to gain indirect additive and non-additive genetic benefits to increase the fitness of their offspring. Females can obtain additive genetic benefits by mating with males that signal higher genetic quality, thus acquiring his good genes in the resulting offspring (Andersson 1994), which can result in their superior growth, fecundity, or survival (Møller and Alatalo 1999). A number of studies have provided evidence that females of some species choose mates based on good genes, and when they do so, the offspring have higher fitness. A meta-analysis showed a significant correlation between male trait and offspring survival and found that male characteristics explain 1.5% of the variability in offspring survival (Møller and Alatalo 1999). In the pronghorn *Antilocapra americana*, (Artiodactyla: Antilocapridae), dominant males who acquired the most matings produced offspring with higher survival (Byers and Waits 2006). Attractive males produce offspring with faster growth rates, possibly allowing the evasion of predators and increasing survival rates (Byers and Waits 2006). Female poison frogs *Dendrobates leucomelas* (Anura: Dendrobatidae) and *Epipedobates tricolor* (Anura: Dendrobatidae) preferred to mate with males with higher calling rates and chirp duration, an indicator of good genes (Forsman and Hagman 2006). These males with higher calling performance produced offspring with higher fitness, measured as higher hatching success and lower mortality in several life-history stages (Forsman and Hagman 2006). These studies indicate that females preferentially mate with males who signal honest indicators of good genes in order to confer a fitness advantage to their offspring.

In addition, females can acquire non-additive genetic benefits by mating with males to increase their genetic compatibility (Trivers 1972). Females can have a preference for

outbred males to avoid inbreeding, as inbreeding can result in decreased offspring fitness due to increased homozygosity and accumulation of deleterious mutations, and a decrease in heterozygote advantage or overdominance (Ilmonen et al. 2009). A well documented system of genetic compatibility involves the major histocompatibility complex (MHC) genes, where females of many organisms have a preference for males with dissimilar MHC alleles (Wedekind et al. 1995; Penn and Potts 1999; Penn 2002). MHC genes are highly polymorphic loci that influence immune function by promoting immune response and resistance to infections and diseases (Penn and Potts 1999; Penn 2002). Therefore, females who mate with males that have dissimilar MHC genes will produce offspring with an increase in fitness as they have a better immune response by recognizing more pathogens. These studies emphasize the importance of sexual selection and mate choice on offspring fitness through indirect genetic benefits.

There may also be sex-specific differences in the fitness of the resulting male and female offspring due to differential investment or sexual conflict (Arnqvist and Rowe 2005). The unequal cost of mating produces different selection pressures in the two sexes. Since most genes are expressed in both sexes, there can be intersexual genetic conflict whereby alleles can be beneficial in one sex but harmful to the other (Chippindale et al. 2001). In some cases, sexual conflict is extreme enough to cause a decrease in lifespan and even death (Chapman et al. 1993, 1995; Pitnick and García–González 2002). When the female sex was prevented from selectively contributing to the gene pool, causing 99% of the haploid genome was transferred from father to son in *Drosophila melanogaster* creating a synthetic Y chromosome, males rapidly increased in fitness, most likely a result from the elimination of counterselection by females (Rice 1998). Males containing

the synthetic Y chromosome had a higher mating rate, higher remating rate, and a higher offence paternity in competition. When this synthetic Y chromosome was expressed in females, they suffered a reduced fitness through a slower developmental time.

These studies provide extensive evidence for the ability of females to mate selectively based on a male's genetic quality in order to increase offspring fitness. However, they also show the existence of potential genetic conflict between the sexes, which could cause fitness to instead be reduced in offspring of a particular sex. To date, very few studies have examined the relationship between parental fitness and the fitness of each sex of resulting offspring (Kokko 2001).

In this study, my first aim was to identify the genetic relationship between parental and offspring fitness. I obtained lifetime reproductive success (LRS) measurements (the number of offspring an individual can produce throughout its lifetime) in *D. melanogaster* for parentals and all F_1 individuals (both sons and daughters) from a full factorial diallel cross. I used multiple simple regressions to analyze additive, paternal and maternal effects. We then used the more complex Cockerham and Weir biomodel (Cockerham and Weir 1977) to tease apart the genetic and parental effects contributing to variation in reproductive success. These models revealed significant additive, maternal and paternal effects for the reproductive success of offspring, with a stronger effect for daughters than sons. My second aim was to identify the effects of inbreeding across generations and between males and females to determine if there were sex-specific effects of inbreeding on lifetime reproductive success. I found that inbreeding did not affect the reproductive output of parental crosses or their sons, but had a significant effect on daughter fitness. Lastly, I looked for a negative relationship between the fitness of

daughters and sons, which would indicate sexual conflict between loci contributing to reproductive output. I did not find any evidence of sexual conflict for this trait, indicating that the differential offspring fitness I observed is caused by factors other than intersexual conflict.

3.2 Methods

3.2.1 Inbred lines

Isofemale lines of *Drosophila melanogaster* were started from individual females collected from the wild in Sudbury, Ontario Canada in 2011, generously provided by T. Merritt. Rearing methods are similar to that of (Nguyen and Moehring, in press). Isofemale populations are reared in the lab on standard cornmeal agar and maintained at 24°C and 75% RH on a 14 h light: 10 h dark cycle. A total of 10 isofemale lines were used in this experiment. Each line was kept with non-overlapping generations as a population of approximately 500 flies distributed among vials that were intermittently intermixed.

3.2.2 Diallel cross - LRS fitness measured

Diallel crossing methods are similar to those of Nguyen and Moehring (in press). Ten isofemale lines were used in a full diallel cross, mating females and males in all combinations to create 100 mating pairs. Male and female virgins were collected upon eclosion, aged 4-6 days, and mated. Mated pairs were kept together throughout the female's lifetime, allowing for remating. Mated pairs were checked daily and dead males were replaced with a male of similar age and strain. Mating pairs were transferred into a new vial every 7 days. Vials were checked daily and counted for number of eclosing

adult offspring. Vials were counted for 16-17 days after the last egg was laid or the female died, ensuring enough time for all larvae to emerge, providing a measure of total lifetime reproductive success. A total of 4 replicates of the complete 10x10 diallel cross were performed (400 pairings total).

To measure the F_1 productivity (lifetime reproductive success), four F_1 males (sons) and four F_1 females (daughters) were taken from the first 10 days of offspring production for each of the four replicates of the 100 diallel crosses (for a total of 1600 F_1 males and 1600 F_1 females). Each F_1 focal son was paired in a vial with a single standard female, and each F_1 female was paired with a single standard male, allowing for remating. Standard females and males used in F_1 mating pairs are from an outbred (synthetic) population made from 19 isofemale lines. A synthetic population line was started from two virgin males and two virgin females from each of the 19 isofemale lines. It was then maintained in a population cage. Lifetime reproductive success of F_1's were measured in a similar manner as above. F_1 daughter's productivity was measured for the entire lifespan of the female. F_1 son's productivity was measured as the number of offspring an F_1 male can produce with a single standard female when paired with her for seven days. After seven days the parents were discarded and all offspring that eclosed were counted. This productivity measure of 7 days is an accurate measure of lifetime productivity in *D. melanogaster* (Nguyen and Moehring, in press).

3.2.3 Data analysis: Multiple regressions

Additive effects can be detected by regressing offspring values on parental values (Falconer 1989). To detect sexual conflict, mean productivity of sons were regressed on mean productivity of daughters. To detect paternal and maternal effects, crosses by sire

line (across different dam lines) and dam line (across different sire lines) were grouped and regressed on values of paternal and maternal lines (Buzatto et al. 2012). The model for paternal effects of productivity on daughters had a non-normal distribution and so a quasipoisson distribution was used; all other comparisons were normally distributed. Analysis was performed in R 3.0.3 (2013).

3.2.4 Data analysis: Cockerham and Weir Biomodel

Reproductive success measures were analyzed by the Cockerham and Weir Biomodel (Cockerham and Weir 1977; Lynch and Walsh 1988) which allows for an estimation of genetic, maternal and paternal variance components for reproductive success (Table 3.1). Data for inbred crosses (crosses using dam and sires from the same isofemale line) were discarded for analysis in the model as recommended. The equation of the model was

$$Y_{ijkl} = \mu + N_i + N_j + T_{ij} + M_j + P_i + K_{ij} + R_{k(ij)} + W_{l(k(ij))}$$

where Y_{ijkl} is the reproductive success of the *l*'th individual from the *k*'th replicate of cross between male line *i* and female line *j*, μ is the mean reproductive success of the population. N_i and N_j are the haploid nuclear additive effects of lines *i* and *j*, independent of sex. T_{ij} is the haploid nuclear nonadditive interaction (including dominance and epistatic effects). M_j and P_i are the maternal and paternal genetic and environmental effects of line *j* when used as dams and line *i* when used as sires. K_{ij} is the interaction between maternal and paternal effects. $R_{k(ij)}$ is the effect of *k*'th replicate cross within dam x sire line combinations. $W_{l(k(ij))}$ is the within replicate cross (the residual) effect of individual *l* (Fry 2004; Bilde et al. 2008; Dowling et al. 2010; Buzatto et al. 2012). Note

Table 3.1 Variance parameters. Table adapted from (Bilde et al. 2008; Dowling et al. 2010; Buzatto et al. 2012).

Observational variance	**Causal variance** *	**Description**
σ^2_N	$V_A = 2\,\sigma^2_N / F$	Nuclear additive variance
σ^2_T	$V_D = \sigma^2_T / F^2$	Nuclear interaction variance (dominance, if epistatic is small)
σ^2_M	$V_M = \sigma^2_M$	Maternal effects variance (both genotype and environmental effects)
σ^2_P	$V_P = \sigma^2_P$	Paternal effects variance (both genotype and environmental effects)
σ^2_K	$V_K = \sigma^2_K$	Interaction variance (of maternal and paternal effects and of nuclear and extra-nuclear effects)
σ^2_R	$V_E = \sigma^2_R + \sigma^2_W$ †	Among replicate crosses variance

σ^2_W	$V_E = (V_{TOT} - V_A - V_D - V_M - V_P - V_K)$	Within replicate crosses variance
	$V_{TOT} = (\sigma^2_N + \sigma^2_T + \sigma^2_M + \sigma^2_P + \sigma^2_K + \sigma^2_R + \sigma^2_W)$	

* F is the inbreeding coefficient.

† Only used if F = 1

that the analysis for parental's lifetime reproductive success does not contain the term $W_{l(k(ij))}$ as there is no within-replicate cross (residual) effect of individuals.

The Cockerham and Weir Biomodel was fitted using the GLIMMIX procedure in SAS 9.3 (SAS Institute Inc. SAS/STAT 9.2 User's Guide, Second Edition 2009). The EFFECT command was used to define the nuclear parental contributions as a multimember effect (SAS/STAT 9.2 User's Guide, Second Edition; Example 38.16, pg 2412). The COVTEST command was used to provide a likelihood ratio test to compare a reduced model, where a given covariance parameter is set to zero, to a full model where all parameters were allowed to have positive values.

Observational variance parameters (Table 3.1) were used to calculate causal variance parameters using F, the inbreeding coefficient (Bilde et al. 2008). Isofemale lines are estimated to have a total inbreeding coefficient of F = 0.4375. This inbreeding coefficient is estimated from $F_{IT} = F_{ST} + F_{IS}(1-F_{ST})$ (Wright 1969), assuming: (1) a population bottleneck of 2 individuals and that the individual female caught from the wild used to start the isofemale line was mated to a single male or that there is strong second-male sperm precedence (drift inbreeding) and (2) a full brother and sister sibling mating in the population (pedigree inbreeding). This level of inbreeding is slightly less than that of previous studies that have used the Cockerham and Weir Biomodel, which have inbreeding coefficients of approximately 0.67-0.89 (Bilde et al. 2008; Dowling et al. 2010; Buzatto et al. 2012).

3.2.5 Data analysis: Inbred *vs.* Outbred

The productivity of inbred *vs.* outbred crosses were compared within each isofemale line for productivity, productivity of F_1 sons and daughters using three separate Linear Mixed Model (LMM). A nested LMM was used with inbred or outbred as a fixed factor and female line as the random factor. To analyze the F_1 productivity of inbred *vs.* outbred crosses of sons and daughters, the ratio of inbred to outbred productivity of sons and daughters were compared. Inbred and outbred values were analyzed using Welch's test. Analyses were performed in R 3.0.3 (2013).

3.3 Results

3.3.1 Generational comparisons of productivity

The regression of productivity values of sons (Figure 3.1A; $R^2 = 0.096$, d.f. = 98, $P = 0.002$) and daughters (Figure 3.1B; $R^2 = 0.083$, d.f. = 98, $P = 0.004$) on parental productivity detected significant additive effects. The slope of the regression gives the heritability values of productivity of sons and daughters (Falconer 1989). The heritability of productivity for sons is 0.035 ± 0.011 (mean ± SE) and for daughters is 0.236 ± 0.079 (mean ± SE). Regression of productivity of F_1 sons on productivity of F_1 daughters was not significant and did not detect any sexual conflict (Figure 3.1C; $R^2 = 0.002$, d.f. = 98, $P = 0.665$). Regressions detected significant paternal (Figure 3.2A; $R^2 = 0.698$, d.f. = 8, $P = 0.003$), but no significant maternal (Figure 3.2B; $R^2 = 0.0380$, d.f. = 8, $P = 0.589$) effect for productivity of sons and significant paternal (Figure 3.3A; pseudo $R^2 = 0.499$, d.f. = 8, $P = 0.021$) and maternal (Figure 3.3B; $R^2 = 0.701$, d.f. = 8, $P = 0.002$) effects for productivity of daughters.

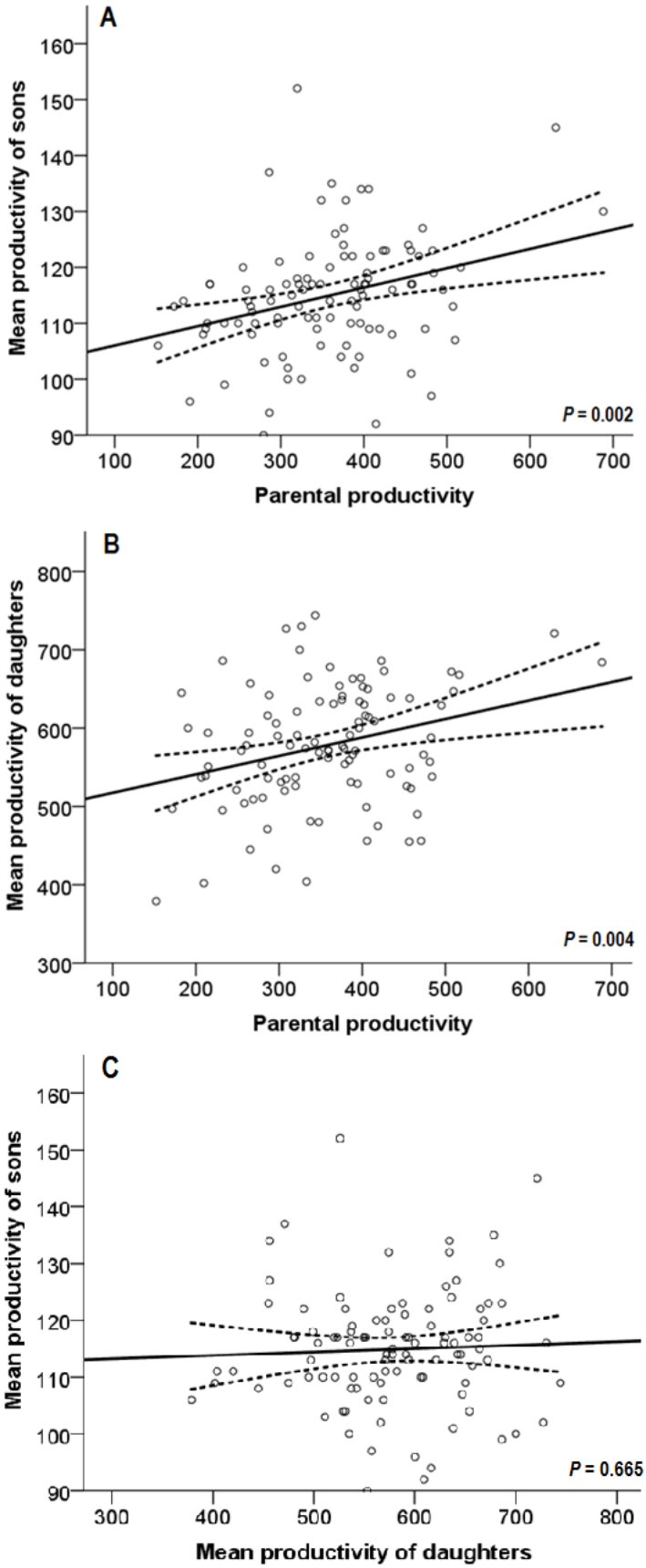


Figure 3.1 Regression of productivity of (A) F_1 sons and (B) F_1 daughters on parental productivity identified significant additive genetic effects. Regression of productivity of F_1 sons on productivity of F_1 daughters (C) detected no sexual conflict. Dashed lines represent 95% CI.

3.3.2 Partitioning the productivity variance

The Cockerham and Weir Biomodel partitions the productivity variance into genetic and parental effects. The model detected no significant additive or non additive genetic effects, maternal, paternal or interaction effects for productivity of parentals or productivity of F_1 sons (Table 3.2). The productivity of F_1 daughters is a result of significant nuclear additive genetic effects ($P = 0.0079$), but no nonadditive, maternal, paternal or interaction effects (Table 3.2). This significant nuclear additive genetic effects accounts for only 0.03% of the variation in productivity (Table 3.3); this is not surprising since lifetime reproductive success (productivity) is an extremely variable polygenic complex trait. The majority of the variation for productivity of parentals and F_1 sons and daughters was accounted for by replicate variance (explaining 99% of the variation) (Table 3.3).

3.3.3 Comparison of inbred vs. outbred productivity

There is no significant difference between inbred and outbred crosses for productivity in female lines of parentals (Figure 4 A ; Figure 5; $\chi^2_{(1)} = 0$, $P = 1.0$) and productivity of F_1 sons (Figure 4 B; Figure 5; $\chi^2_{(1)} = 0$, $P = 1.0$). However, inbred crosses of F_1 daughters have significantly lower productivity than outbred crosses (Figure 4 C; Figure 5; $\chi^2_{(1)} = 10.862$, $P = 0.0001$). Paired t-tests show that inbreeding affects the productivity of F_1 daughters significantly more than it affects the productivity of F_1 sons, whereby inbreeding decreases the productivity of F_1 daughters ($t = 5.2836$, d.f. = 3, $P = 0.01322$).

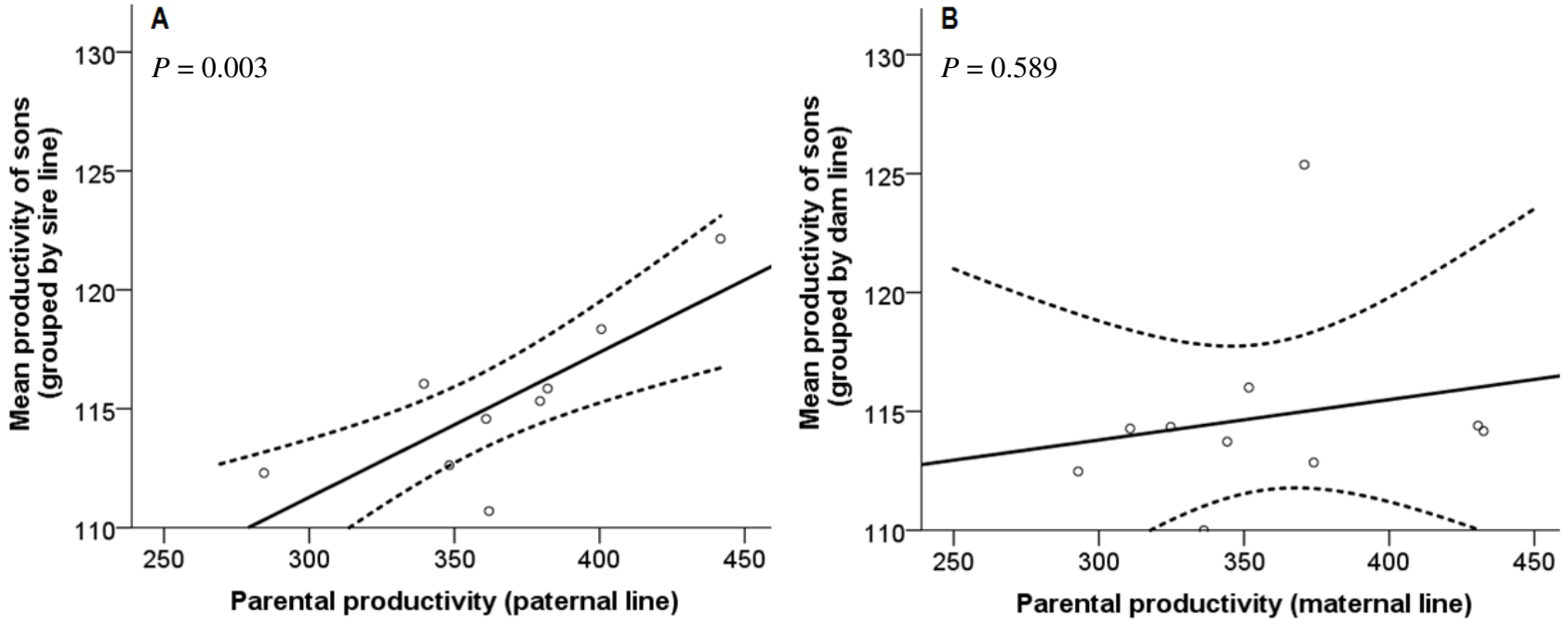


Figure 3.2 Regression of productivity of F_1daughters, grouped by (A) sire lines or (B) dam lines on parental productivity detected significant paternal and maternal effects. Dashed lines represent 95% CI.

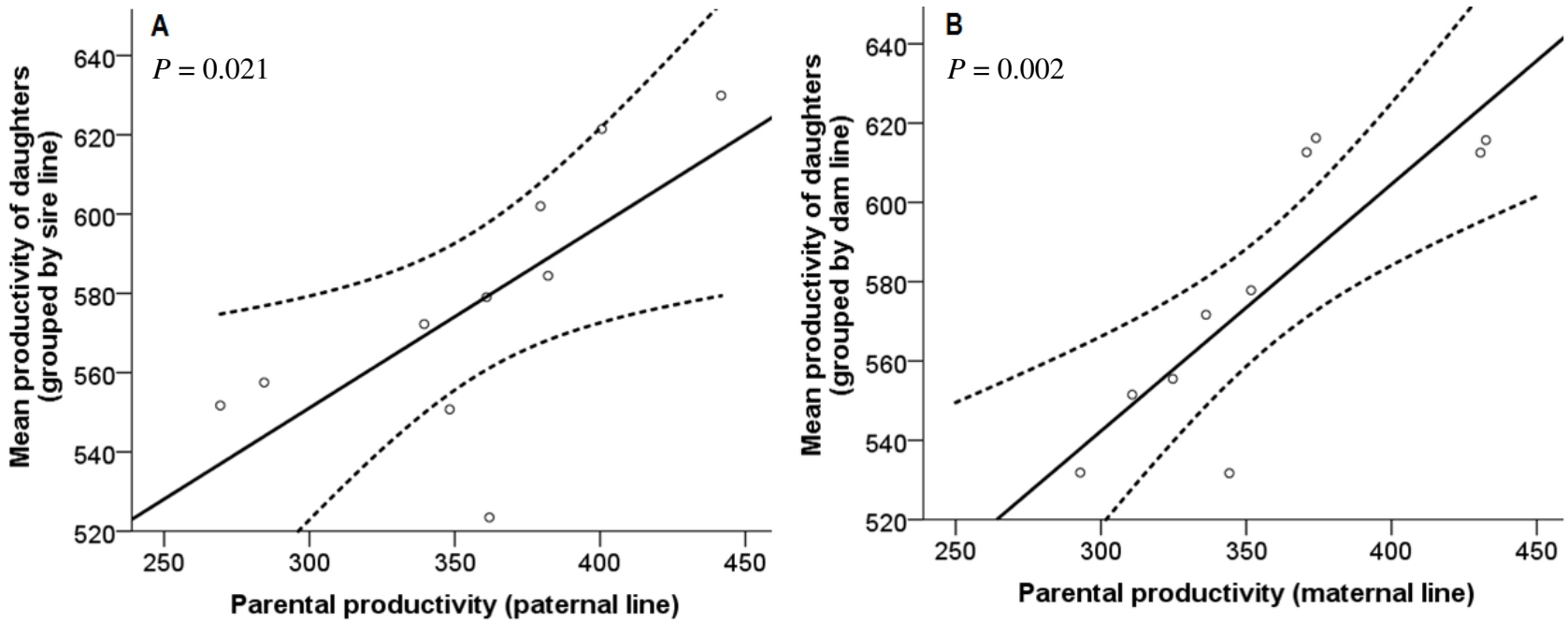


Figure 3.3 Regression of productivity of F_1daughters, grouped by (A) sire lines or (B) dam lines on parental productivity detected significant paternal and maternal effects. Dashed lines represent 95% CI.

Table 3.2 Observational variance component estimates from the Cockerham and Weir Biomodel to estimate the genetic architecture of lifetime reproductive succes measures in isofemale lines of *D. melanogaster* and their F_1 offspring.

	Lifetime reproductive success		**F_1 sons productivity**		**F_1 daughters productivity**	
Variance component	Estimate (SE)	*P* - value	Estimate (SE)	*P* - value	Estimate (SE)	*P* - value
σ^2_N	0.0105 (0.0078)	0.0932	0.0002 (0.0003)	0.5273	0.0025 (0.0015)	0.0079
σ^2_T	0	-	0	-	0.0007 (0.0013)	0.5499
σ^2_M	0.0019 (0.0066)	0.7530	0	-	0	-
σ^2_P	0.0068 (0.0082)	0.2955	0.0001 (0.0005)	0.8040	0	-
σ^2_K	0	-	0	-	0	-
σ^2_R	73.4872 (5.6284)		0.0188 (0.0023)	<0.0001	0.0181 (0.0031)	<0.0001
σ^2_W			5.1874 (0.2254)		43.5622 (1.9144)	

Table 3.3 Causal variance component estimates from the Cockerham and Weir Biomodel to estimate the genetic architecture of lifetime reproductive succes measures in isofemale lines of *D. melanogaster* and their F_1 offspring.

	Lifetime reproductive success		**F_1 sons productivity**		**F_1 daughters productivity**	
Variance component	Estimate	Percent	Estimate	Percent	Estimate	Percent
V_A	0.0480	0.06	0.0009	0.02	0.0114	0.03
V_D	0	0	0	0	0.0036	0
V_M	0.0019	0	0	0	0	0
V_P	0.0068	0	0.0001	0	0	0
V_K	0	0	0	0	0	0
V_E	73.4497	99.94	5.2055	99.98	43.5690	99.96
V_{TOT}	73.5064		5.2065		43.5840	

3.4 Discussion

There is a significant positive correlation for both F_1 sons' and daughters' productivity when regressed over parental productivity, but not a significant correlation between sons and daughters (Figure 3.1). Thus, some parental combinations produce high quality sons and some produce high quality daughters, but these offspring values have no relationship (either positive or negative) to one another, indicating that there is no intersexual conflict or intersexual cohesiveness for this trait. This is counter to the findings of a negative correlation in *D. melanogaster* adult reproductive success between males (male fertilization success) and females (female fecundity) (Chippindale et al. 2001). They suggested that good genes are sex specific; high quality males produce high quality sons, but low quality daughters. Sexual conflict was also evident in *Tribolium castaneum* (Coleoptera: Tenebrionidae) where polyandrous females produced fit sons, but not fit daughters (Pai and Yan 2002). I found good genes for lifetime reproductive success are expressed in both sexes. Similar positive pleiotropic effects are found in *Teleogryllus commodus* (Orthoptera: Gryllidae). A study using a full-sib/half-sib breeding design found a positive genetic correlation between male calling effort and female fecundity, indicating no intra-locus sexual conflict and a positive correlation in reproductive efforts between males and females (Zajitschek et al. 2007).

I found significant additive genetic effects for the productivity of F_1 daughters, but no other genetic or parental effects. The Cockerham and Weir Biomodel did not detect any genetic genetic or parental effects for productivity of parentals or productivity of F_1 sons. Thus, lifetime reproductive success of daughters is more strongly affected by good genes than is the reproductive success of sons. Additional regression analysis detected

significant additive genetic, paternal and maternal effects for the productivity of F_1 sons and F_1 daughters. This difference in results is likely due to the Cockerham and Weir Biomodel partitioning all of the phenotypic variation into the replicate variance. Similar results were found in Buzatto *et al.* (2012), where additional regression analysis detected effects not found using the Cockerham and Weir Biomodel (Buzatto et al. 2012). They suggested that the Cockerham and Weir Biomodel is a conservative model that underestimates the variance components (Buzatto et al. 2012). This effect is likely enhanced by the strains that I used in my experiment since isofemale lines are not fully inbred. The detection of an effect in F_1 offspring but not parentals could also be due to the larger number of replicates for this group (16 *vs*. 4), and the effect in daughters but not sons could be due to productivity differences resulting from our different measures (ranges of 10-1220 and 3-306 offspring, respectively). Alternatively, it is possible that the lack of a significant additive effect of offspring production in sons resulted from a reduced variation in spermatogenesis and the resulting sperm (compared to egg production in daughters) due a lack of recombination in *D. melanogaster* male gametes (Morgan 1914). Furthermore, in non-resource based mating systems, females acquire indirect benefits in the form of increased fitness in their offspring. This could possibly explain why there is more phenotypic variation in the F_1 generation (i.e. daughters) than in the parentals.

Although 99% of the productivity variation lies in the replicate variance, there are distinct differences among the mean productivity of parentals and F_1 sons versus F_1 daughters when comparing between inbred *vs.* outbred crosses (Figure 3.4). Inbreeding depression can be caused by an increase in homozygosity and result in an accumulation

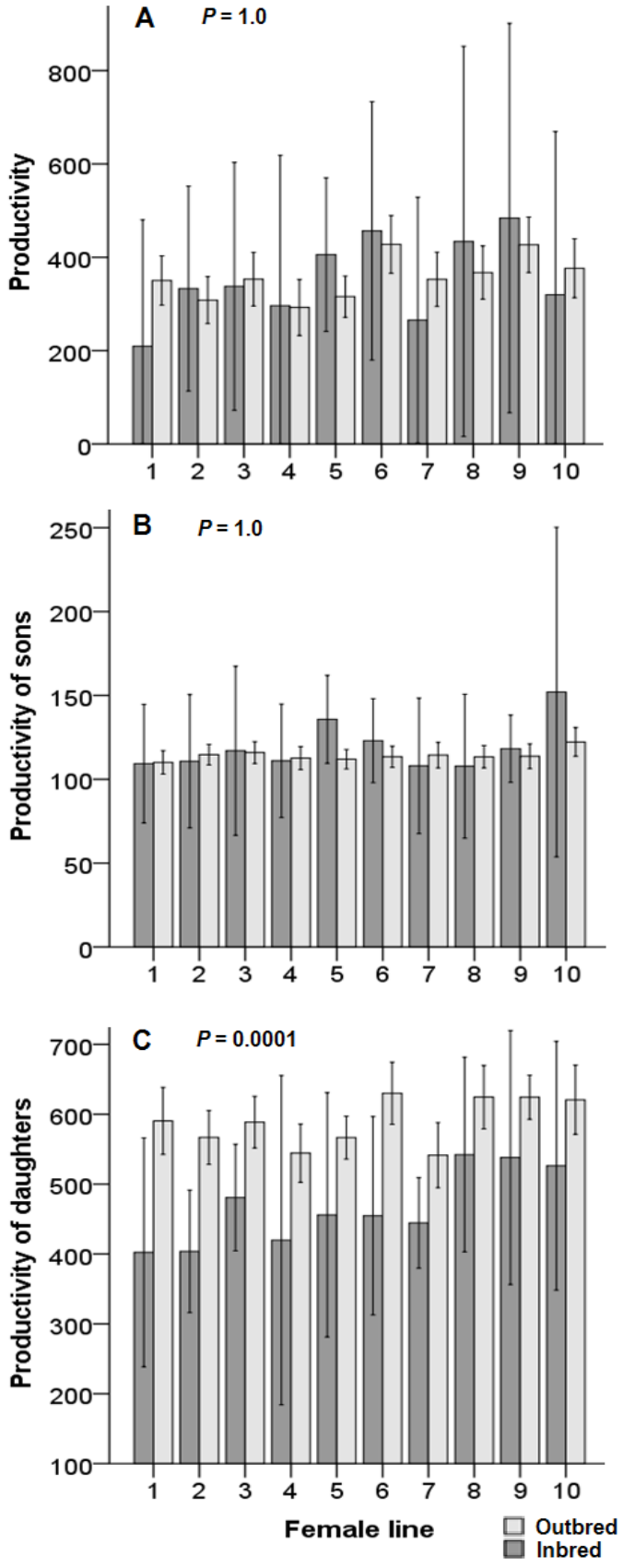


Figure 3.4 Productivity of outbred *vs*. inbred crosses for (A) parentals, (B) F_1 sons and (C) F_1 daughters.

of recessive deleterious alleles, or by decreasing heterozygote advantage, also known as overdominance. I found that female offspring (F_1 daughters) from inbred crosses produce significantly fewer offspring than those from outbred crosses. Surprisingly, this inbreeding depression is only present in the productivity of F_1 daughters, and not F_1 sons or parentals. This is counter to what was seen in *Bicyclus anynana* (Lepidoptera: Nymphalidae), where inbreeding depression was detected in both parents and offspring (Saccheri et al. 2005). For example, outbred parental crosses had a higher hatching rate of 44% compared to inbred lines with a hatching rate of 37% (Saccheri et al. 2005). Also opposite to my findings, inbred males of *B. anynana* suffer a greater loss of fertility than inbred females. Inbred males suffered a 40% loss of fertility, measured as percent of egg hatching, whereas inbred females had no measurable inbreeding depression (Saccheri et al. 2005). The contrasting results between that study and mine may potentially be explained by the different chromosomal complement in the sexes of the two species: males of *B. anynana* are homogametic and females are heterogametic, while males are the heterogametic sex in *D. melanogaster*. In both studies, it is the homogametic sex that suffers the greatest inbreeding depression. Indeed, a study in seed beetles *Callosobruchus maculatus* (Coleoptera: Chrysomelidae), where the females are the homogametic sex, females suffer a significant reduction in lifespan due to inbreeding, while males actually had an increased lifespan when inbred (Bilde et al. 2009). Likewise, inbred females of *C. maculatus* had a reduced lifespan, 9-13% shorter than outbred females while inbred males suffer no cost of inbreeding depression (Fox et al. 2006). In the endangered New Zealand bird, *Notiomystis cincta* (Passeriformes: Notiomystidae), the homogametic male sex was found to be more inbred than females (Brekke et al.

2010). Furthermore, these males were more sensitive to inbreeding depression as inbred males suffer a higher embryo and nestling mortality than inbred females (Brekke et al. 2010). This male-biased sensitivity to inbreeding was not a result of males being more inbred, as this relationship was still significant when highly inbred males were removed. These studies may indicate a trend where the homogametic sex is more sensitive to inbreeding depression. However, I found exceptions in some birds where the heterogametic inbred females of *Porphyrio hochstetteri* (Gruiformes: Rallidae) and *Melospiza melodia* (Passeriformes: Emberizidae) show a significantly lower fledging success (Jamieson et al. 2003) and lifetime reproductive success due to egg mortality (Keller 1998) respectively, while inbred males suffer no inbreeding cost.

These findings where the homogametic sex suffers a greater inbreeding depression are counter to expectation since the heterogametic sex is hemizygous for their sex chromosomes and will express all sex-linked alleles. Any negative epistatic interactions with the autosomes would be expected to surface within the heterogametic sex, as seen with the reduction of fertility in the heterogametic sex of interspecies hybrids (Haldane 1922) and the increased reduction in lifespan due to inbreeding within heterogametic individuals (reviewed in Tower and Arbeitman 2009). Deleterious effects of recessive X-linked alleles can be masked in heterozygous individuals who are homogametic, even when they are inbred, as long as there is some residual genetic variation. There are numerous studies that show that the X chromosome evolves faster than the autosomes (reviewed in Meisel and Connallon 2013), and that the X chromosome more rapidly accumulates changes affecting male sterility (reviewed in Presgraves 2008). Additionally, mating and reproducing is usually more costly for females, whereas males

have fairly little investment by comparison, and thus there should be stronger selection for reproductive robustness in females. However, my results indicate that sensitivity to inbreeding depression for lifetime reproductive success is sex-dependent and appears to be biased towards the homogametic sex, which is female in *D. melanogaster*.

It has previously been suggested that sexual size dimorphism can result in biased sensitivities in inbreeding depression (Brekke et al. 2010). The (homogametic) males of *N. cincta*, who suffer a greater inbreeding depression than females, are significantly larger than females with respect to weight, tarsus length and head-bill length (Brekke et al. 2010). Likewise, (homogametic) *D. melanogaster* females are often larger than their male counterparts. The energetic requirements for increased growth and maintenance of larger individuals can potentially cause them to be more sensitive to inbreeding depression. Additionally, mating and egg laying are energetically costly for *D. melanogaster* females, decreasing lifespan. Inbred females can thus be more sensitive to these energetic demands, resulting in a decreased lifespan and lower productivity. In contrast, inbred males could have an increased lifespan if they do not perform energetically costly reproductive behaviours (Bilde et al. 2009). Several studies have shown a decrease in reproductive behaviour and performance in inbred males. Inbred males of *Mus domesticus* (Rodentia: Muridae) have a lower mating and reproductive success because they could not obtain quality territory and were less aggressive (Eklund 1996; Meagher et al. 2000). *Bicyclus anynana* males produce less sex pheromones when inbred, resulting in reduced mating success, and had an 18% reduction in flight time compared to outbred males (Bergen et al. 2013). Although inbred males of *Teleogryllus commodus* have a 30% reduced calling effort compared to outbred males, their call is just

as attractive to females compared to calls from outbred males, indicating that call structure in inbred males have not been compromised (Drayton et al. 2010). Unlike female *D. melanogaster*, inbred males avoiding energetically costly behaviours could allocate their resources to maintaining their lifespan and productivity, as suggested in my study. Whether the differential effect on fitness is caused by differences in genetic structure (homogamy) or differences in energetic investment between the two sexes requires further study.

3.5 Chapter acknowledgements

I thank Thomas Merritt for providing us with the isofemale lines used in this study, and Bruno Buzatto for his patience and kindness in statistical assistance. I also thank Amanda Tong, Anqi Jiang, Rebecca Kovacs, Agnes Kwan, Eric Dolinar, and Shaun McNiven for assistance with data collection. This work was supported by an NSERC Discovery Grant to AJM.

3.6 References

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

Arnqvist, G., and L. Rowe. 2005. Sexual conflict. Princeston University Press, Princeton, New Jersey.

Bergen, E. van, P. M. Brakefield, S. Heuskin, B. J. Zwaan, and C. M. Nieberding. 2013. The scent of inbreeding: a male sex pheromone betrays inbred males. Proc. R. Soc. B Biol. Sci. 280:20130102.

Bilde, T., U. Friberg, A. Maklakov, J. Fry, and G. Arnqvist. 2008. The genetic architecture of fitness in a seed beetle: assessing the potential for indirect genetic benefits of female choice. BMC Evol. Biol. 8:295.

Bilde, T., A. A. Maklakov, K. Meisner, L. la Guardia, and U. Friberg. 2009. Sex differences in the genetic architecture of lifespan in a seed beetle: extreme inbreeding extends male lifespan. BMC Evol. Biol. 9:33.

Brekke, P., P. M. Bennett, J. Wang, N. Pettorelli, and J. G. Ewen. 2010. Sensitive males: inbreeding depression in an endangered bird. Proc. R. Soc. B Biol. Sci. rspb20101144.

Buzatto, B. A., L. W. Simmons, and J. L. Tomkins. 2012. Paternal effects on the expression of a male polyphenism. Evolution 66:3167–3178.

Byers, J. A., and L. Waits. 2006. Good genes sexual selection in nature. Proc. Natl. Acad. Sci. 103:16343–16345.

Chapman, T., J. Hutchings, and L. Partridge. 1993. No reduction in the cost of mating for *Drosophila melanogaster* females mating with spermless males. Proc. R. Soc. Lond. B Biol. Sci. 253:211–217.

Chapman, T., L. F. Liddle, J. M. Kalb, M. F. Wolfner, and L. Partridge. 1995. Cost of mating in *Drosophila melanogaster* females is mediated by male accessory-gland products. Nature 373:241–244.

Chippindale, A. K., J. R. Gibson, and W. R. Rice. 2001. Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. Proc. Natl. Acad. Sci. 98:1671–1675.

Cockerham, C. C., and B. S. Weir. 1977. Quadratic analyses of reciprocal crosses. Biometrics 33:187–203.

Consuegra, S., and C. G. de Leaniz. 2008. MHC-mediated mate choice increases parasite resistance in salmon. Proc. R. Soc. B Biol. Sci. 275:1397–1403.

Dowling, D. K., M. Nystrand, and L. W. Simmons. 2010. Maternal effects, but no good or compatible genes for sperm competitiveness in Australian crickets. Evolution 64:1257–1266.

Drayton, J. M., R. N. C. Milner, J. Hunt, and M. D. Jennions. 2010. Inbreeding and advertisement calling in the cricket *Teleogryllus commodus*: laboratory and field experiments. Evolution 64:3069–3083.

Eklund, A. 1996. The effects of inbreeding on aggression in wild male house mice (*Mus domesticus*). behaviour 133:883–901.

Falconer, D. S. 1989. Introduction to quantitative genetics. John Wiley & Sons, New York, New York.

Forsman, A., and M. Hagman. 2006. Calling is an honest indicator of paternal genetic quality in poison frogs. Evolution 60:2148–2157.

Fowler, K., and L. Partridge. 1989. A cost of mating in female fruitflies. Nature 338:760–761.

Fox, C. W., K. L. Scheibly, W. G. Wallin, L. J. Hitchcock, R. C. Stillwell, and B. P. Smith. 2006. The genetic architecture of life span and mortality rates: gender and species differences in inbreeding load of two seed-feeding beetles. Genetics 174:763–773.

Fry, J. 2004. Genetic analysis of complex traits using SAS. A. Saxton. SAS Institute Inc., Cary, NC.

Haldane, J. B. S. 1922. Sex ratio and unisexual sterility in hybrid animals. J. Genet. 12:101–109.

Ilmonen, P., G. Stundner, M. Thob, and D. J. Penn. 2009. Females prefer the scent of outbred males: good-genes-as-heterozygosity? BMC Evol. Biol. 9:104.

Ivy, T. M., and S. K. Sakaluk. 2005. Polyandry promotes enhanced offspring survival in decorated crickets. Evolution 59:152–159.

Jamieson, I. G., M. S. Roy, and M. Lettink. 2003. Sex-Specific Consequences of recent inbreeding in an ancestrally inbred population of New Zealand Takahe. Conserv. Biol. 17:708–716.

Keller, L. F. 1998. Inbreeding and its fitness effects in an insular population of song sparrows (Melospiza melodia). Evolution 52:240–250.

Kokko, H. 2001. Fisherian and "good genes" benefits of mate choice: how (not) to distinguish between them. Ecol. Lett. 4:322–326.

Lynch, M., and B. Walsh. 1988. Genetics and analysis of quantitative traits. Sinauer Associates Inc., Sunderland, MA.

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

Meagher, S., D. J. Penn, and W. K. Potts. 2000. Male–male competition magnifies inbreeding depression in wild house mice. Proc. Natl. Acad. Sci. 97:3324–3329.

Meisel, R. P., and T. Connallon. 2013. The faster-X effect: integrating theory and data. Trends Genet. 29:537–544.

Møller, A. P., and R. V. Alatalo. 1999. Good-genes effects in sexual selection. Proc. R. Soc. Lond. B Biol. Sci. 266:85–91.

Morgan, T. H. 1914. No crossing over in the male of *Drosophila* of genes in the second and third pairs of chromosomes. Biol. Bull. XXVI.

Neff, B. D., and T. E. Pitcher. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Mol. Ecol. 14:19–38.

Nguyen, T., and A. Moehring. (in press). Accurate alternative measurements for female lifetime reproductive success in *Drosophila melanogaster*. PLoS ONE.

Pai, A., and G. Yan. 2002. Polyandry produces sexy sons at the cost of daughters in red flour beetles. Proc. R. Soc. Lond. B Biol. Sci. 269:361–368.

Penn, D. J. 2002. The scent of genetic compatibility: sexual selection and the Major Histocompatibility Complex. Ethology 108:1–21.

Penn, D. J., and W. K. Potts. 1999. The evolution of mating preferences and Major Histocompatibility Complex genes. Am. Nat. 153:145–164.

Penn, D., and W. Potts. 1998. MHC–disassortative mating preferences reversed by cross–fostering. Proc. R. Soc. Lond. B Biol. Sci. 265:1299–1306.

Pitnick, S., and F. García–González. 2002. Harm to females increases with male body size in *Drosophila melanogaster*. Proc. R. Soc. Lond. B Biol. Sci. 269:1821–1828.

Presgraves, D. C. 2008. Sex chromosomes and speciation in *Drosophila*. Trends Genet. 24:336–343.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rice, W. R. 1998. Male fitness increases when females are eliminated from gene pool: Implications for the Y chromosome. Proc. Natl. Acad. Sci. 95:6217–6221.

Rowe, L. 1994. The costs of mating and mate choice in water striders. Anim. Behav. 48:1049–1056.

Saccheri, I. J., H. D. Lloyd, S. J. Helyar, and P. M. Brakefield. 2005. Inbreeding uncovers fundamental differences in the genetic load affecting male and female fertility in a butterfly. Proc. R. Soc. B Biol. Sci. 272:39–46.

Tower, J., and M. Arbeitman. 2009. The genetics of gender and life span. J. Biol. 8:38.

Tregenza, T., and N. Wedell. 1998. Benefits of multiple mates in the cricket *Gryllus bimaculatus*. Evolution 52:1726–1730.

Trivers, R. L. 1972. Parental investment and sexual selection. Pp. 136–179 *in* Sexual selection and the descent of man 1871-1971. Campbell, B. G., ed., Adine, Chicago.

Turner, M. E., and W. W. Anderson. 1983. Multiple mating and female fitness in *Drosophila pseudoobscura*. Evolution 37: 714-723.

Wedekind, C., T. Seebeck, F. Bettens, and A. J. Paepke. 1995. MHC-dependent mate preferences in humans. Proc. R. Soc. Lond. B Biol. Sci. 260:245–249.

Wright, S. 1969. Evolution and the genetics of populations. Volume 2. University of Chicago Press, Chicago.

Zajitschek, F., J. Hunt, S. R. K. Zajitschek, M. D. Jennions, and R. Brooks. 2007. No intra-Locus sexual conflict over reproductive fitness or ageing in field crickets. PLoS ONE 2:e155.

Zeh, J. A. 1997. Polyandry and enhanced reproductive success in the harlequin-beetle-riding pseudoscorpion. Behav. Ecol. Sociobiol. 40:111–118.

2009. SAS Institute Inc. SAS/STAT 9.2 User's Guide, Second Edition. SAS Institute I nc., Cary, NC.

Chapter 4

4 Males with higher mating success produce sons with lower fitness

Female mate choice can result in direct benefits to the female or indirect benefits to her offspring. Females can increase their fitness by mating with males of higher genetic quality, where genetic quality consists of both survivorship and reproductive output. Attractive males will gain more copulations, and thus have a higher fitness due to increased mating success. However, male mating success is not only dependent upon a female's receptivity towards a courting male, and in nature can involve complex interactions between individuals of both sexes in the time preceding copulation. Here I used a novel approach to measure male mating success in a mating arena that allows for male-male, male-female, and female-female interactions using 10 isofemale lines of *D. melanogaster*. I then correlated mating success with direct and indirect benefits females may receive. Surprisingly, I found that males with higher mating success reduce female fitness as they produce sons with lower lifetime reproductive success (productivity).

4.1 Introduction

Female mate choice can occur when there is variation in male phenotypes. Male variation in fitness traits can be evaluated by females as important indicators of male quality. Females will preferentially mate with males that will provide them with increased fitness benefits. Direct benefits are those that females gain to increase her fitness; they are acquired in the current generation (Andersson 1994). Often, these direct benefits are obtained in resource-based mating systems, such as when a male provides a

female with a nuptial gift, and can enhance the female's immediate fecundity or fertility (Gwynne 1984).

In many mating systems, the female does not gain any apparent direct benefit, yet females still demonstrate mate choice. For example, Taylor *et al*. (2007) examined the fitness effects of female *Drosophila simulans* (Diptera: Drosophilidae) mating to preferred and non-preferred males (Taylor et al. 2008). They found no significant correlation between female preference and female lifetime productivity (Taylor et al. 2008). For mating systems in which there is a non-positive correlation between male attractiveness and direct female fitness, females may be choosing a male on the basis of the indirect benefits he provides, such as in the form of higher quality offspring. These 'good genes' within the male will allow him to successfully survive, and the preference of females for these genes will allow him to out-compete rival males in sexual selection. An individual's total fitness, or lifetime reproductive success, includes an individual's survivorship (viability), and mating success (Stearns 1992). However, an important aspect of reproductive success that can often be overlooked is a male's ability to obtain mates. Both components of fitness viability and attractiveness contributes to a male's genetic quality (Kokko et al. 2002; Neff and Pitcher 2005). Ideally, studies examining the benefits of mate choice should consist of both components; whether a male contains good genes due to survivorship or good genes due to an increase in mating success are equally significant (Zahavi 1975; Eshel et al. 2000; Kokko 2001).

The Fisherian hypothesis predicts that females will mate with males that advertise an arbitrarily attractive trait (Fisher 1930). Attractive males will enjoy an increase in fitness by attaining copulations and having a higher mating success. Females who mate with

attractive males can gain indirect fitness benefits by producing sons who are more successful at mating (sexy sons hypothesis) (Weatherhead and Robertson 1979). The sexy sons hypothesis is supported by several studies. Males of crickets *Allonemobius socius* (Orthoptera: Gryllidae) who were successful at mating in the field produced sons who were also more successful at mating (Fedorka and Mousseau 2004). Similarly, attractive males of *D. simulans* provide indirect benefits to females by siring attractive sons (Taylor et al. 2007). These studies demonstrate that the ability to obtain mates and mating are important heritable measures of total fitness.

The mating behaviour and courtship of *Drosophila melanogaster* have been well characterized (reviewed in Spieth 1974; O'Dell 2003). After a *Drosophila* male orients and approaches a potential mate, he first engages in a variety of behaviours that include tapping, leg rubbing, licking, and circling. He then produces a species-specific courtship song by vibrating one of his wings before attempting to mount and copulate. A female signals acceptance by standing still and spreading her wings, removing a physical barrier, to allow a male to successfully mount. Forceful coercion of mating by males is almost always unsuccessful. Unreceptive females display rejection behaviour by kicking, decamping, and abdomen elevation or depression. If males are successful at copulation, it is possible that they provide a material resource to females through nutrients in their ejaculate as ejaculate traces can often be found in somatic and ovarian tissues of females (Pitnick et al. 1997). Since *D. melanogaster* usually aggregate at food sources (Tinette et al. 2004), which are also where copulation is most likely to occur (Spieth 1974), more complex male-male interactions have the potential to significantly affect a male's reproductive success in the wild.

The majority of studies examining mating success involve focusing on a single male-female interaction in order to dissect male courtship and female receptive behaviour (e.g., (Reynolds and Gross 1992; Head et al. 2005; Taylor et al. 2007)). While multiple-choice mating assays have sometimes been used, and allow for male-male competition, a single female is typically presented with a choice of only two males (Fedorka and Mousseau 2004; Taylor et al. 2008). In another study, a mating chamber was used that allowed for female-female interactions with multiple females and male-male interactions with multiple males; however there were only two isofemale lines involved in the male-male competition (Taylor et al. 1987). In the lab, male-male aggression has been documented where larger males chase smaller males away (Partridge and Farquhar 1983), and the outcome of these interactions affects a male's future aggressive behaviour (Yurkovic et al. 2006), making it likely that these interactions are also present in natural environments. Group composition and social life also affect male-female interactions. *Drosophila* males court virgin females more aggressively than mated females (Siegel and Hall 1979). However, male courtship can be modified by experience as males that are exposed to mated females do not court virgin females as forcefully (Siegel and Hall 1979). Females also display learning behaviour in a mating context. *Drosophila melanogaster* females that were exposed to the courtship from small males (but not mating) were more likely to mate to small and large males compared to females who were only exposed to large males (Dukas 2005). Furthermore, female-female interactions can also affect mating behaviour, as seen in mate-choice copying where a female's choice in mate is influenced by another female's mate choice (Vakirtzis 2011). In *D. melanogaster*, females preferentially mate with males who they had observed successfully copulating with

another female (Mery et al. 2009). It is also possible for males and females to have opposing selection pressures on mating. In the cockroach *Nauphoeta cinerea* (Blattodea: Blaberidae), the pheromones that make males more dominant and successful in male-male competition also make them less attractive to females; the chemicals females prefer makes males subordinate (Moore and Moore 1999). These studies indicate that mating behaviour is multifaceted and involves male-male, male-female, and female-female interactions.

Competitive male mating encounters are complex, and involve male-male, male-female and female-female interactions. Due to this complexity, studies examining mating success of males are often indirect and use proxies such as male size (Pitnick and García–González 2002; Friberg and Arnqvist 2003) or pheromone composition (Boake 1985) for determining attractiveness to females in a no-choice mating assay (Boake 1985; Pitnick and García–González 2002; Friberg and Arnqvist 2003; Head et al. 2005). These no-choice assays do not include possible male-male competition, which could play a significant role in mating success in the wild. Fitness is defined as not only the success of an individual in reproducing, but also the subsequent reproductive success of the offspring that are produced. In studying the adaptive value of mate choice, therefore, it is critical to assess both the reproductive output of the individual and the output from their sons and daughters (Kokko et al. 2003; Fedorka and Mousseau 2004; Hunt et al. 2004). Since examining fitness in a multi-generational study is labour-intensive, especially for measures such as lifetime reproductive success that reflect an individual's fitness throughout their entire lifetime, these measures are historically rarely done (Pitnick and García–González 2002; Friberg and Arnqvist 2003; Hunt et al. 2004). Several studies

have examined the effect of male attractiveness on the number of grandchildren produced (Boake 1985; Reynolds and Gross 1992; Fedorka and Mousseau 2004; Head et al. 2005; Rundle et al. 2007; Gilbert et al. 2011). In general these studies found that there is no relationship between a male's attractiveness and the resulting fitness of the offspring (Boake 1985; Reynolds and Gross 1992; Head et al. 2005; Rundle et al. 2007). However, the study by Gilbert et al. (2001) did find a positive relationship between male attractiveness and number of offspring produced. In that study, they altered individual male attractiveness in order to disconnect the perceived male attractiveness from the male's actual fitness (Gilbert et al. 2001). While these studies advance our understanding of the association between male attractiveness and reproductive output, there are some limitations to these inferences. Male attractiveness in these studies was usually measured in a no-choice mating assay (Boake 1985; Reynolds and Gross 1992; Head et al. 2005; Rundle et al. 2007), and the number of grandchildren was examined in only one sex (Reynolds and Gross 1992; Fedorka and Mousseau 2004) or indirectly measured as an estimate calculation (Head et al. 2005).

Here I examine mating success in *D. melanogaster* in a 'semi-natural' context that allows for complex female-female, male-female, and male-male interactions. I placed males and females from ten isofemale lines within a mating arena that allowed for male-male competition, learning behaviour, and female choice and scored which individuals copulated. I then compared the male's mating success to the number of offspring produced by each line combination, and the subsequent sons' and daughters' offspring production. I then compared male attractiveness (mating success) with direct fitness (offspring production) and indirect fitness (offspring production by sons and daughters).

This provides the first study to compare male mating success within a complex social environment to multi-generational lifetime reproductive success. This multi-generational study allows for testing of both direct and indirect fitness benefits of male attractiveness.

4.2 Methods

4.2.1 *Drosophila* strains and maintenance

Ten isofemale lines of *D. melanogaster* were collected from the wild in Sudbury, Ontario, Canada in 2011 by T. Merritt and maintained in the laboratory in 8-dram vials with standard cornmeal agar media (Bloomington *Drosophila* Stock Center, Indiana). Flies were reared in a 14:10 light-dark cycle, at 24ºC and approximately 75% relative humidity.

4.2.2 Mating success

To measure male mating success for the 10 isofemale lines, males and females were placed together in a mating arena that allows for male-male, male-female, and female-female interactions. Density-controlled vials were set up by crossing 10 females and 15 males from the focal isofemale line. This ensures that the offspring were of similar size since high density can reduce the resulting developmental size, and male size is often correlated with fitness and mating success (Partridge et al. 1987a,b). Virgin males and females were collected from the density-controlled vials, separated by sex, and aged 4-6 days prior to use in mating assays. Males were colour-coded by their isofemale line using coloured nail polish marks on the dorsal side of their thorax approximately 24 hours prior to when the assays began. A latin square design was performed for the colour code used to identify male line in order to randomize any effects due to the markings.

For each focal female line, 10 virgin females (all from the same isofemale line) and 2 marked males from each isofemale line (20 males total) were placed in a 500 ml jar (for a total density of 30 flies). Mating assays commenced in the morning (9-11 am, which is 0-3 hours after 'lights on') at room temperature (21-23ºC). Females from isofemale line 4 were not assayed in this study due experimental difficulties; males from this line were still used in the mating arena. The mating arena was observed and mating pairs were removed with aspiration. The mating male's progenitor line was identified by the colour on his thorax. The female and a male from the appropriate line were replaced so that the mating arena density remained constant. The experiment continued until a total of 17-20 matings occurred (approximately 5-8 hours). Mating arenas that did not contain at least 17 mated pairs after ~8 hours were discarded and repeated the next day. A total of 20 replicates were performed for each of the 9 focal isofemale female lines, for a total of 3478 observed matings.

4.2.3 Statistical analysis

The data collected here on male mating success is being compared and analyzed with previously reported data on the lifetime reproductive success (productivity) of the same lines for parental crosses, F_1 sons, and F_1 daughters (Chapter 3). Mating success was analyzed using a nested Linear Mixed Model (LMM) with female and male lines as random factors. Mating success was also analyzed in three separate Linear Models (LM), using productivity of parentals, F_1 sons, or F_1 daughters as the response variable and the corresponding mating success of the cross as the predictor variable in three separate regression analyses. All analyses were performed in R 3.0.3 (2013).

4.3 Results

There were significant male line effects for male mating success (Figure 4.1; $\chi^2_{(1)} = 31.451$, $P < 0.0001$): males from some lines consistently achieved a high mating success, while males from other lines had a consistently low mating success across the female lines that they were assayed with. For each female line that they were paired with, males were ranked based on the percentage of successful matings. A correlation matrix of male mating success ranking for each female isofemale line shows an average correlation value of 0.56 (Ranges from 0.18-0.83) across female lines (Figure 4.2), indicating that the ranking of male mating success was fairly consistent across female lines and further supporting the fact that male mating success was strongly correlated across female lines. Regressions of productivity of parentals, F_1 sons and F_1 daughters on male mating success (the proportion of matings the sire line achieved) all showed a negative slope, however it was only statistically significant for the productivity of F_1 sons (Figure 4.3B; $R^2 = 0.07$, d.f. = 88, $P = 0.0099$), and not the productivity of parentals (Figure 4.3A; $R^2 = 0.032$, d.f. = 88, $P = 0.0874$) or F_1 daughters (Figure 4.3C; $R^2 = 0.0054$, d.f. = 88, $P = 0.491$). Thus, males that have a high mating success within a competitive arena produce fewer offspring, produce daughters that have fewer offspring, and produce sons that have significantly fewer offspring.

4.4 Discussion

Using a mating assay that allowed for complex social interactions, I showed that attractive males did not provide a direct benefit to females. In fact, there was a negative correlation (although not significant) which showed that males that are more successful at

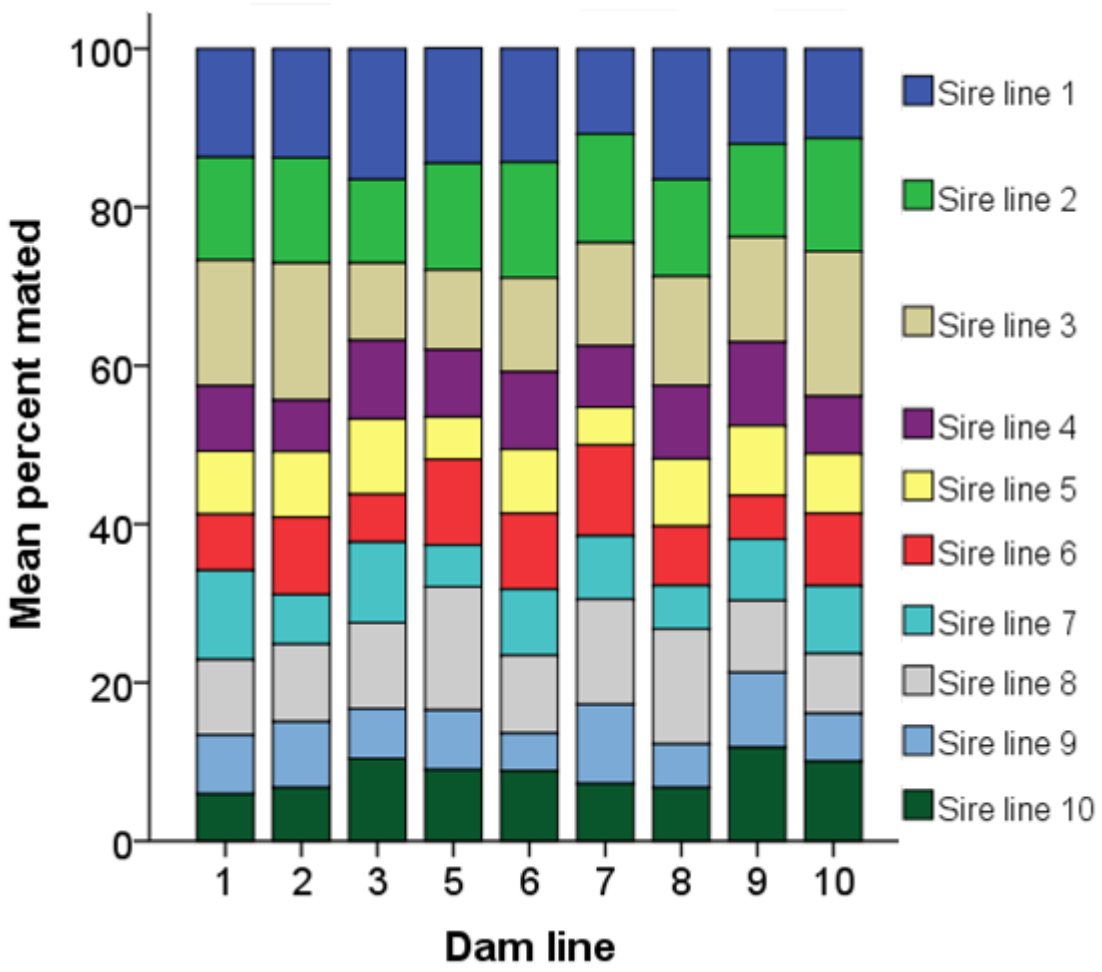


Figure 4.1 Percent of males that mated in a mating arena for each isofemale line. Percents are shown as stacked.

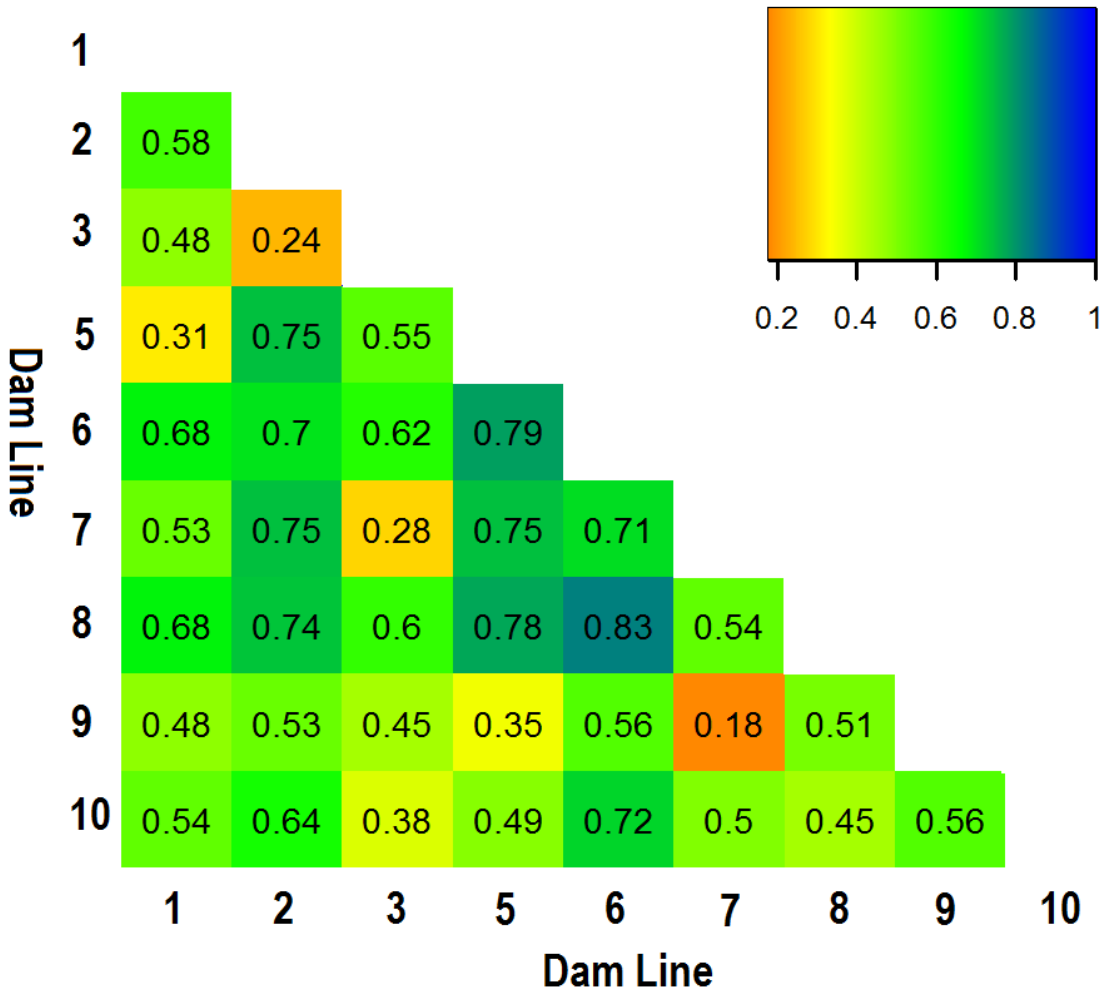


Figure 4.2 Comparison of mate preferences among females. The correlation matrix compares average male mating success percentages across the isofemale lines. Female lines that have identical mate preferences are shown in blue, while those that have dissimilar preferences are shown in orange, with scaled colours in between.

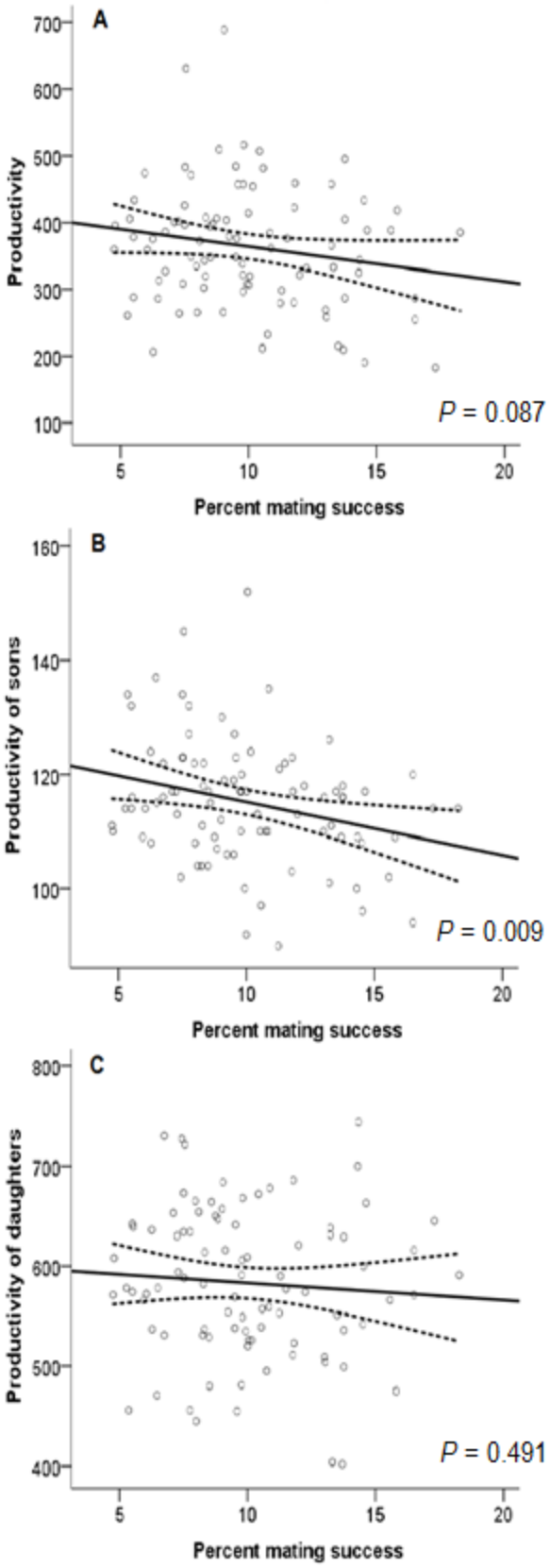


Figure 4.3 Regression of (A) parental productivity, (B) productivity of F_1 sons, and (C) productivity of F_1 daughters on percent mating success. Productivity was assessed using females continually housed with males, allowing for remating. Dashed lines represent 95% CI.

mating have a lower productivity. This may be because high mating success can come at a high cost, causing a trade-off between mating success and fitness or survival, resulting in an antagonistic relationship (Kokko 2001). For example, a higher level of mating frequency can decrease the lifespan of males (Partridge and Farquhar 1983), and females mated to more attractive males suffer a reduced longevity and increased mortality (Rice 1998; Taylor et al. 2008). Similar to these results, several studies have shown a negative correlation between male mating success and productivity: females who mated with larger males, used as a proxy for attractiveness, had a lower fecundity and egg-adult survival as a result of reduced lifespan (Pitnick and García–González 2002; Friberg and Arnqvist 2003). Similarly, female house crickets, *Acheta domesticus* (Orthoptera: Gryllidae), who mate with more attractive males suffer a direct cost of survival (Head et al. 2005). These results show a sexually antagonistic relationship between mating success at the cost of direct fitness for females. Antagonistic coevolution of female mate choice and/or mating in general can cause a reduced direct fitness in females, and thus it is possible that the more attractive males in this study infer a greater cost of mating to females. Rice (1998) suggests that these more attractive males had an increased seminal fluid toxicity, a pleiotropic effect, causing the females that mated with them to suffer a greater direct fitness cost. Similarly, the males that are more successful at mating in this study could have a lower productivity due to an increased mortality rate inferred on females, which was not measured in this study. Taylor et al. (1987) attempted to correlate male mating success in *D. melanogaster* with various fitness traits. Male mating success did not correlate with male survival rate or developmental time, but did

significantly correlate with offspring fitness in competitiveness, indicating an indirect benefit for females that mate with an attractive male (Taylor et al. 1987).

In non-resource based mating systems, such as in *D. melanogaster*, females are thought to remain choosy to acquire an indirect benefit in the form of increased fitness in their offspring. However, these results show that males that achieve the most matings in a competitive environment do not provide females with indirect benefits: attractive males do not produce higher quality offspring as daughters are not more fecund and sons produce fewer offspring. This was surprising as the mating arena had high potential for female choice. Why would females prefer males that confer lower fecundity? My measurement of mating success does not necessarily reflect female preference, as the end act of copulation could arise due to other factors. Forced copulations are extremely rare in this species (reviewed in Spieth 1974; O'Dell 2003) but it is possible that females were only courted by the males that were most aggressive in chasing off competitor males, and thus the male-male interactions, rather than female preference, drives this outcome. If so, this indicates a strong sexual antagonism in mating, where males who are more successful at mating are not those that confer the highest fitness to females.

Although there are many studies that examined male attractiveness and its indirect fitness benefit to offspring, these studies did not include generational measurements of lifetime reproductive success (i.e., number of grandchildren) (Brooks 2000; Friberg and Arnqvist 2003). Of the few studies that measured the number of grandchildren produced, the majority found no significant effect of male attractiveness on the production of grandchildren. Both attractive and unattractive males produced the same number of grandchildren by F_1 sons or F_1 daughters of the house cricket, *Acheta domesticus* (Head

et al. 2005). However, in this study, male attractiveness was measured indirectly by the latency of mounting by females in a no-choice mating assay. Similarly, there was no significant correlation between attractive males and the number of progeny produced by F_1 males or F_1 females in the red flour beetle, *Tribolium castaneum* (Coleoptera: Tenebrionidae) (Boake 1985). In that study, male attractiveness was indirectly measured using a two-choice pitfall trap apparatus where females were presented with pheromones from a focal male and a blank control. In the guppy, *Poecilia reticulata* (Cyprinodontiformes: Poeciliidae), male attractiveness was scored as a measure of female preference, the amount of orienting and gliding displays performed towards the male, when they were presented with a single focal male (Reynolds and Gross 1992). As with the cricket and flour beetle studies, male attractiveness in the guppy did not affect offspring (daughters, in this case) fecundity.

However, there is one study that reported a significant effect of male attractiveness on offspring fecundity. In another species of cricket, *Allonemobius socius* (Orthoptera: Gryllidae), there was a significant negative correlation between male attractiveness and daughters' fecundity (Fedorka and Mousseau 2004). In this study, male attractiveness was measured in a two-choice mating assay, allowing for a single male competitor. Thus, the primary similarity between this study and my own, which both found a negative correlation between male attractiveness and offspring fecundity, was an assessment of male mating success that allowed for female choice and male-male interactions. This significant negative correlation demonstrates the importance male-male competition in determining male mating success, and the cost that this success has on the quality of the resulting offspring

A significant negative correlation between male mating success and the lifetime reproductive success of F_1 sons can potentially be explained by the sexy sons hypothesis. Males that are more successful at mating can persist in spite of a cost of lower productivity if they produce sons that are also attractive (Weatherhead and Robertson 1979; Brooks 2000). Mothers that mate with attractive males can compensate for the initial loss of productivity by producing sons that have a higher mating success, producing an overall greater number of grandchildren (Kokko et al. 2002). While very few studies have examined the relationship between male mating success and the mating success of their sons, and this was not measured in this study, the heritability of male attractiveness has been demonstrated in other species. In *Drosophila simulans*, cuticular hydrocarbons (CHCs) protect from desiccation and act as pheromones, and are reflective of male attractiveness. The CHC profile is heritable across varying temperatures and diet; sire attractiveness (measured as CHCs) can accurately predict attractiveness of sons even in environmental heterogeneity (Ingleby et al. 2013). In *Gryllus bimaculatus* (Orthoptera: Gryllidae), attractive males produced sons that were more successful in mating, but these sons suffer the cost of an increased developmental time, indicating a trade-off between mating success and fitness (Wedell and Tregenza 1999). Attractive males of house crickets, *Acheta domesticus* (Orthoptera: Gryllidae), produce sons who are more attractive, but females who mated with attractive males suffered a direct fitness cost of reduced survival (Head et al. 2005).

Furthermore, evidence of sexual conflict was not found as there was a negative correlation between male attractiveness and the lifetime reproductive success of both sons and daughters. This result is counter to a previously reported study that examined

male attractiveness in crickets, *A. socius.* These authors found that attractive males produced sexy sons, but had daughters with reduced fecundity, indicating the presence of sexual conflict (Fedorka and Mousseau 2004). However, this result is consistent with previous data in *D. melanogaster* that showed no sexual conflict when comparing parental productivity to the productivity of F_1 sons and F_1 daughters (Chapter 3). For both sexes, there was a significant positive correlation, indicating that individuals with a high lifetime reproductive success produced sons and daughters with a high lifetime reproductive success. These results thus show when females mate with males that have a high mating success, females suffer from both direct and indirect fitness cost. Females who mate with attractive males have a lower lifetime reproductive success and produce sons and daughters with a lower lifetime reproductive success.

4.5 Chapter acknowledgements

I'd like to thank my research assistants Pria Mahbir, Sam Lee, and Colin Robertson for their assistance on the mating arena assays, especially for the delicate microscopic painting of the fruit flies. This work was supported by an NSERC Discovery Grant to Amanda J. Moehring.

4.6 References

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

Boake, C. R. B. 1985. Genetic consequences of mate choice: a quantitative genetic method for testing sexual selection theory. Science 227:1061–1063.

Brooks, R. 2000. Negative genetic correlation between male sexual attractiveness and survival. Nature 406:67–70.

Dukas, R. 2005. Learning affects mate choice in female fruit flies. Behav. Ecol. 16:800–804.

Eshel, I., I. Volovik, and S. Emilia. 2000. On Fisher-Zahavi's handicapped sexy son. Evol. Ecol. Res. 509–523.

Fedorka, K. M., and T. A. Mousseau. 2004. Female mating bias results in conflicting sex-specific offspring fitness. Nature 429:65–67.

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

Friberg, U., and G. Arnqvist. 2003. Fitness effects of female mate choice: preferred males are detrimental for *Drosophila melanogaster* females. J. Evol. Biol. 16:797–811.

Gilbert, L., K. A. Williamson, and J. A. Graves. 2011. Male attractiveness regulates daughter fecundity non-genetically via maternal investment. Proc. R. Soc. Lond. B Biol. Sci. 279: 523-528.

Gwynne, D. T. 1984. Courtship feeding increases female reproductive success in bushcrickets. Nature 307:361–363.

Head, M. L., J. Hunt, M. D. Jennions, and R. Brooks. 2005. The indirect benefits of mating with attractive males outweigh the direct costs. PLoS Biol 3:e33.

Hunt, J., L. F. Bussière, M. D. Jennions, and R. Brooks. 2004. What is genetic quality? Trends Ecol. Evol. 19:329–333.

Ingleby, F. C., J. Hunt, and D. J. Hosken. 2013. Heritability of male attractiveness persists despite evidence for unreliable sexual signals in *Drosophila simulans*. J. Evol. Biol. 26:311–324.

Kokko, H. 2001. Fisherian and "good genes" benefits of mate choice: how (not) to distinguish between them. Ecol. Lett. 4:322–326.

Kokko, H., R. Brooks, M. D. Jennions, and J. Morley. 2003. The evolution of mate choice and mating biases. Proc. R. Soc. B Biol. Sci. 270:653–664.

Kokko, H., R. Brooks, J. M. McNamara, and A. I. Houston. 2002. The sexual selection continuum. Proc. R. Soc. Lond. B Biol. Sci. 269:1331–1340.

Mery, F., S. A. M. Varela, É. Danchin, S. Blanchet, D. Parejo, I. Coolen, and R. H. Wagner. 2009. Public versus personal information for mate copying in an invertebrate. Curr. Biol. 19:730–734.

Moore, A. J., and P. J. Moore. 1999. Balancing sexual selection through opposing mate choice and male competition. Proc. R. Soc. Lond. B Biol. Sci. 266:711–716.

Neff, B. D., and T. E. Pitcher. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Mol. Ecol. 14:19–38.

O'Dell, K. M. C. 2003. The voyeurs' guide to *Drosophila melanogaster* courtship. Behav. Processes 64:211–223.

Partridge, L., A. Ewing, and A. Chandler. 1987a. Male size and mating success in *Drosophila melanogaster*: the roles of male and female behaviour. Anim. Behav. 35:555–562.

Partridge, L., and M. Farquhar. 1983. Lifetime mating success of male fruitflies (*Drosophila melanogaster*) is related to their size. Anim. Behav. 31:871–877.

Partridge, L., A. Hoffmann, and J. S. Jones. 1987b. Male size and mating success in *Drosophila melanogaster* and *D. pseudoobscura* under field conditions. Anim. Behav. 35:468–476.

Pitnick, S., and F. García–González. 2002. Harm to females increases with male body size in *Drosophila melanogaster*. Proc. R. Soc. Lond. B Biol. Sci. 269:1821–1828.

Pitnick, S., G. S. Spicer, and T. Markow. 1997. Phylogenetic examination of female incorporation of ejaculate in *Drosophila*. Evolution 51:833–845.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

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

Rice, W. R. 1998. Male fitness increases when females are eliminated from gene pool: implications for the Y chromosome. Proc. Natl. Acad. Sci. 95:6217–6221.

Rundle, H. D., A. Odeen, and A. O. Mooers. 2007. An experimental test for indirect benefits in *Drosophila melanogaster*. BMC Evol. Biol. 7:36.

Siegel, R. W., and J. C. Hall. 1979. Conditioned responses in courtship behavior of normal and mutant *Drosophila*. Proc. Natl. Acad. Sci. 76:3430–3434.

Spieth, H. T. 1974. Courtship behavior in *Drosophila*. Annu. Rev. Entomol. 19:385–405.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, New York.

Taylor, C. E., A. D. Pereda, and J. A. Ferrari. 1987. On the correlation between mating success and offspring quality in *Drosophila melanogaster*. Am. Nat. 129:721–729.

Taylor, M. L., N. Wedell, and D. J. Hosken. 2008. Sexual selection and female fitness in *Drosophila simulans*. Behav. Ecol. Sociobiol. 62:721–728.

Taylor, M. L., N. Wedell, and D. J. Hosken. 2007. The heritability of attractiveness. Curr. Biol. 17:R959–R960.

Tinette, S., L. Zhang, and A. Robichon. 2004. Cooperation between *Drosophila* flies in searching behavior. Genes Brain Behav. 3:39–50.

Vakirtzis, A. 2011. Mate choice copying and nonindependent mate choice: a critical review. Ann. Zool. Fenn. 48:91–107.

Weatherhead, P. J., and R. J. Robertson. 1979. Offspring quality and the polygyny threshold: “the sexy son hypothesis.” Am. Nat. 113:201–208.

Wedell, N., and T. Tregenza. 1999. Successful fathers sire successful sons. Evolution 53:620–625.

Yurkovic, A., O. Wang, A. C. Basu, and E. A. Kravitz. 2006. Learning and memory associated with aggression in *Drosophila melanogaster*. Proc. Natl. Acad. Sci. 103:17519–17524.

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

Chapter 5

5 The first male's seminal proteins contribute to the second male advantage

Polyandrous females allow for sexual selection to persist after mating. In the event that females successfully mate with more than one male, sperm competition can occur. One outcome of this competition is the common phenomenon of second male advantage, whereby the second male to mate sires the majority of offspring. In response to sexual selection, males have evolved strategies that reduce postcopulatory competition. Male ejaculates consist of both sperm and seminal fluid. Accessory gland proteins (Acps) found in seminal fluid play a significant role in sexual selection as they can both alter the behaviour of females and directly influence sperm competition. Acps provided by the second mated male can incapacitate and displace residing sperm. However, not much is known about the role the ejaculate of first mated males plays in sperm competition. Here I show that Acps provided by first mated males, in absence of first males' sperm, contribute to second male advantage by increasing the lifetime reproductive success (productivity) of second mated males. These competing Acps provide a "protective effect," where the Acps from the first mated male protects the sperm from the second mated male, increasing its longevity and extending the female's egg laying duration.

5.1 Introduction

Successful reproduction is a primary component of fitness: selection favours both males and females that have maximized their reproductive success. Females are polyandrous, which allows selection to persist after mating. In many species, this selection acts after copulation but prior to fertilization (postcopulatory prezygotic selection). Some

organisms can exhibit cryptic female choice during this time, whereby females bias the paternity of their offspring to a particular male (Eberhard 1996). Sperm competition between males can occur when there are ejaculates from two or more males within the female reproductive tract (Parker 1970). Males can evolve strategies to try and reduce the exposure to sperm competition that include mate-guarding, insertion of a copulatory plug, prolonged copulation after insemination, and mechanical removal of residing sperm (Parker 1970, 1984; Waage 1979; Alcock 1994). However, in the event that sperm competition occurs, it can drive the evolution of sperm number and size (Parker 1993).

There is a growing body of evidence that suggest sperm are costly to produce (Pitnick and Markow 1994; Pitnick et al. 1995; Snook 2005), and sperm competition can sometimes result in different sperm phenotypes to offset this cost. White butterfly *Pieris napi* (Lepidoptera: Pieridae) males use a non-fertile enucleated 'apyrene' sperm to delay the female's remating by filling her sperm storage organ with sperm that are less costly to produce (Cook and Wedell 1999). In many ways, males must balance resources between surviving and acquiring mates, as well as being successful in fertilization and sperm competition (Parker 1990). Males benefit if they can strategically allocate resources to sperm production, altering their ejaculates in the presence of competition. For example, males of the dung fly *Sepsis cynipsea* (Diptera: Sepsidae) can increase their ejaculate transfer size in the presence of competition (Martin and Hosken 2002). The fair raffle theory suggests that sperm from two competing males are equal and have an equal chance of being used for fertilization (Parker et al. 1990). In this instance, males would have a reproductive advantage by simply increasing their ejaculate size and sperm number. In contrast, the loaded raffle suggests that sperm from different males are not equal and

therefore, some have a better probability of being used for fertilization than others (Parker et al. 1990). Males not only have to allocate their resources to ejaculate size and sperm number, but sperm quality is also of significance as it can influence fertilization efficiency (Pattarini et al. 2006). Sperm quality consists of traits such as velocity, viability, longevity and size (reviewed in Snook 2005). Of the two sexes, postcopulatory sexual selection generally has a greater impact on a male's fitness, as not only do they have to compete for mating events, but also have to compete after mating for fertilization.

Species with internal fertilization have varying mechanisms of sperm competition. Males of most species with internal fertilization transfer both sperm and proteins in the seminal fluid. Sperm competition is usually intense in insects, where females of many species have a sperm storage organ, allowing for overlap of ejaculates from multiple males (Lefevre and Jonsson 1962). Unlike mammals, whose sperm survive 5-6 days, and birds who have a 12-13 day sperm survival time, sperm in insects can survive up to several years (Parker 1984). In *Drosophila melanogaster*, a species whose ejaculate has been extensively studied, males transfer at least 112 different proteins (called accessory gland proteins, or Acps) (Ram and Wolfner 2007), of which a few are characterized. One of the first identified and most well characterized Acps is known as sex peptide (SP, encoded by the gene *Acp70A*). When SP is injected into a female it reduces her receptivity to remating and increases egg laying behaviour (Chen et al. 1988). This is beneficial to a male as it decreases the opportunity for sperm competition and increases his rate of fertilization. Similarly, *Acp26Aa* also increases ovulation (Herndon and Wolfner 1995). These behavioural changes can have detrimental effects to females. Females mated to Acp-producing spermless males have a decreased lifespan similar to

that caused by mating to males that transfer both sperm and Acps (Chapman et al. 1993), indicating that the harmful effects of mating for females are not a result of stored sperm, but rather seminal fluid. While the co-evolution of male and female reproductive proteins can be antagonistic, there is also evidence that some male and female reproductive proteins have evolved to interact cooperatively (LaFlamme et al. 2014). Additionally, Acps are involved in efficient sperm transfer and are required for sperm storage. Mutant males that produced a reduced amount of Acps transferred more variable amounts of sperm and had a reduced number of sperm stored within the female's sperm storage organs (Tram and Wolfner 1999). In the presence of perceived competition, males can vary the amount of Acps transferred, increasing the amount of sex peptide and ovulin (Wigby et al. 2009).

A well known phenomenon that occurs in many species involving sperm competition is the 'second male advantage,' where the second male to mate fertilizes the majority of offspring. Several mechanisms of second male advantage have been identified, and Acps are thought to play a critical role. Studies focused on sperm competition most often examine the effects of the second male to mate (offensive traits). For this male, Acps can physically displace residing sperm stored by females (Harshman and Prout 1994), and can act to incapacitate any remaining sperm and thus prevent their use in fertilization (Price et al. 1999). Several Acps have been associated with offensive traits responsible for second male advantage and P_2 values (*Acp29AB, Acp33A*, *CG17331, Acp26Aa, CG6168, Acp62F*; Fiumera et al. 2005, 2007). For the first male that mates (defensive traits) a partially overlapping suite of Acp proteins are significantly correlated with the ability for sperm to resist being physically displaced (*CG8137*, *CG6168, Acp33A,*

Acp26Aa/Ab, Acp29B, Acp36DE and *Acp53E*; Clark et al. 1995; Fiumera et al. 2005). These Acps can have pleiotropic effects, as they can be seen to be associated with both a male's offspring production when he is the first male to mate (P_1) and when he is the second male to mate (P_2). Aside from the physical resistance to displacement, very little is known about the role that the first mated male's Acps have on sperm competition. Here, I test the effects of Acps from the first mated male on sperm competition via their effect on the productivity of the second male to mate. Surprisingly, I found that Acps from the first male have a protective effect on the second male's sperm, and thus contribute to the second male advantage.

5.2 Methods

5.2.1 *Drosophila* strains and maintenance

Ten isofemale lines were provided by T. Merritt, who started them from individual females collected in Sudbury, Ontario Canada in 2011. Isofemale lines were maintained on standard cornmeal agar in 8-dram vials at 24ºC, 75% RH and a 14 h light: 10 h dark cycle.

In order to assign paternity in the P_2 assays (below), $th^1\ st^1\ cp^1\ in^1\ kni^{ri\text{-}1}\ p^p$ stock line was used to cross in a homozygous recessive phenotypic marker, *knirps* (*kni*), into all ten isofemale lines. The *kni* mutation is a 252 bp deletion located at 3L:20,700,201...20,700,452 that results in a shortened L2 wing vein phenotype when homozygous (Lunde et al. 2003) The *kni* line was first crossed to each isofemale line, then backcrossed to each isofemale line for five generations in order to retain most of the isofemale line genome, selecting each generation for individuals bearing the recessive *kni*

mutation. Genotyping was performed to identify individuals harboring *kni* by using forward 5' GCTGGCCTTTGCCTTTTAG and reverse 5' AATGATGAGGCGATGGATGT primers flanking the deletion and a touchdown PCR at 1 cycle 95º 5', 3 cycles 94º 1' / 58º 30" / 72 º 1', 3 cycles 94º 1' / 55º 30" / 72º 1', 30 cycles 94º 1' / 52º 30" / 72º 1', 1 cycle 72º 10'.

5.2.2 Sperm competition assays

Ten isofemale lines of *D. melanogaster* were used in this study (Nguyen and Moehring, in press). Rearing methods are described in Nguyen and Moehring (in press). Individual virgin males and females were collected from density-controlled vials to control for size (as in Chapter 4) and aged 4-6 days. A total of 47 isofemale line combinations of mating pairs were used in this study (as in Nguyen and Moehring, in press: Figure 1). There were a total of three treatments: 1) productivity after a single mating (without competition) (data used from Nguyen and Moehring, in press), 2) productivity in a double mating with an initial male that produces both Acps and sperm (Acps + sperm), followed by a wild type male and 3) productivity in a double mating with an initial male that produces Acps but no sperm (only Acps), followed by a wild type male.

For the control treatment (without competition), a single mating pair was placed in an 8-dram vial containing standard cornmeal agar media (Bloomington *Drosophila* Stock Center, Indiana) and observed until mating occurred, or approximately 4 hours passed, at which point the vial was discarded if mating had not occurred. After mating occurred, males were removed and females were allowed to oviposit. Females were transferred into a new vial every seven days and the number of offspring eclosing from each vial was scored daily (as in Nguyen and Moehring, in press) . Experiment continued until a

female no longer produced fertile eggs or the female died. A total of 20 replicates for each isofemale line combination was performed.

The second treatment with competition serves as a second control (the first male has Acps + sperm). Males from isofemale line mating combinations were tested in both mating orders with an alternating recessive marker against another male from a different line: (1) focal male^{kni}, second male, (2) focal male, second male^{kni}, (3) first male^{kni}, focal male and (4) first male, focal male^{kni}. Females used contained the homozygous *kni* phenotypic marker. Mating assays were performed in the same manner described above. The total number of offspring from the first male to mate (P_1) and the second male to mate was counted (P_2). Ten replicates for each of the 4 orders were performed for a total of 40 replicates for each of the 47 isofemale line combinations. Females were initially mated to a first male and remated to a second male after 24 hours. Females who did not remate were allowed to remate again the following day. Females who did not remate in 24-48 hours after initial mating were discarded. Mating was scored in a no choice mating assay where males were removed immediately after mating was completed. The total number of offspring produced was scored in a similar manner as Nguyen and Moehring, (in press), with offspring paternity assigned based on the presence / absence of the *kni* phenotype. Only offspring produced after the second male mated were scored and counted.

In the third treatment considered the experimental treatment with competition (the first male has only Acps), females were initially mated to sons of *tudor* mothers; these sons are standard sterile males that produce Acps but no sperm (Boswell and Mahowald 1985; Chapman et al. 1993). These sterile males were obtained by crossing stock genotypes

vas^{1} *cn*^{1} *tud*^{1} *bw*^{1} *sp*^{1}*/CyO* and *BicD*^{2} *cn*^{1} *tud*^{1} *bw*^{1}*/CyO* to create homozygous *tudor* mutant females. Homozygous *tudor* mothers were mated to a male from a separate but similar isofemale line that was not one of the ten isofemale lines used in this study. Spermless male offspring of homozygous *tudor* mothers were collected and aged 4-6 days. Females and males were paired in a mating assay as above. After successful copulation, spermless males were aspirated and removed, and mated females remained housed with food singly in the vial. After females were initially mated to sons of *tudor* mothers, females were mated secondly to isofemale line males approximately 24 hours later. Females who did not remate were placed again in a mating assay 48 hours after the initial mating. Females who did not remate in the 24-48 hour window were discarded. The number of offspring produced (productivity) was scored in a similar manner as above (and as in Nguyen and Moehring, in press) since the first male was sterile, all offspring that were produced were sired by the second male.

5.2.3 Statistical analysis

A Linear Mixed Model (LMM) was performed to determine the components that affect sperm competition. Lifetime reproductive success (productivity) was used as the response variable while the female and male lines were used as random factors and treatment as a fixed factor. Treatment factor consists of the control (without competition) or the experimental competition treatment where females were initially mated to a spermless, Acp-producing male. A three way interaction of female line - male line - treatment was used as the predictor variable, including their lower order components, in the full model. Predictor variables that were not significant from the log likelihood test

were removed in the reduced model unless they were significant in a higher order interaction.

An analysis of variance (ANOVA) with a post hoc Bonferroni correction was performed to compare the lifetime reproductive success (productivity) of all three treatments: control treatment (without competition), competition (the first male has sperm + Acps), and experimental treatment (competition with spermless, Acp-producing males). A t-test was also performed in the treatment with competition (involving both sperm and Acps) to compare the number of offspring sired by the second male and the number of offspring sired by the first male.

A Linear Model (LM) was used to perform a linear regression of productivity when in competition (competition with spermless, Acp-producing males) regressed on productivity when not in competition. LM was also used to regress the increase in productivity due to competition on productivity without competition. All analyses were performed in R 3.0.3 (2013).

5.3 Results

The three way interaction of female line - male line - and treatment was not a significant predictor of productivity (Table 5.1; $\chi^2_{(1)} = 0$, $P = 1$). However, both two way interactions of female line * treatment, and male line * treatment were significant (Table 5.1: $\chi^2_{(1)} = 10.093$, $P = 0.0014$ and $\chi^2_{(1)} = 7.185$, $P <= 0.0073$ respectively).

Regression of productivity with competition (competition with spermless, Acp-producing males) on productivity without competition was statistically significant (Figure 5.1; $R^2 =$

Table 5.1 Treatment and line effects, determined by a Linear Mixed Model regression. Treatment is either the control (without competition) or the experimental competition treatment where females were initially mated to a spermless, Acp-producing male.

Variance component	$\chi^2_{(1)}$	***P*-value**
Female line * Male line * Treatment	0	1
Male line * Treatment	7.185	0.0073
Female line * Treatment	10.093	0.0014
Female line * Male line	0.329	0.5657
Male line	5.095	0.0239
Female line	5.303	0.0212
Treatment	1.761	0.1844

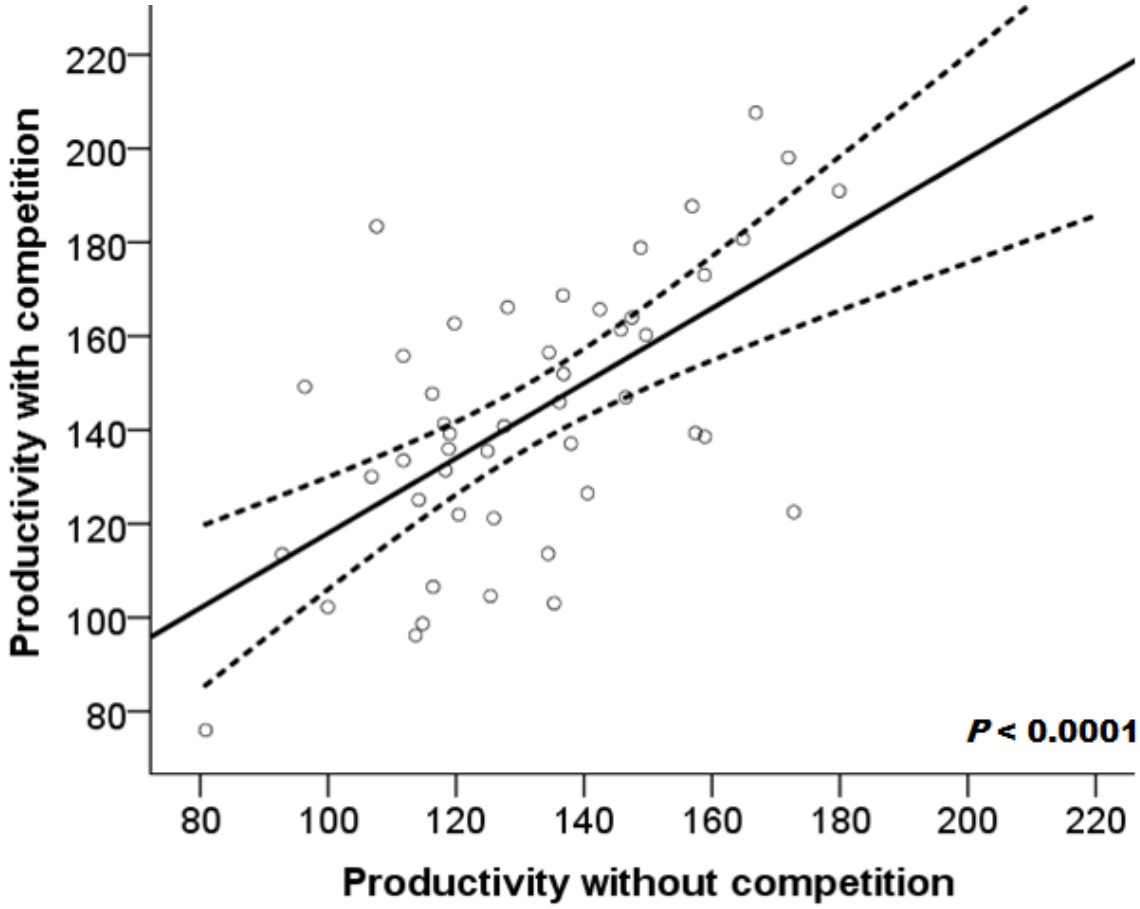


Figure 5.1 Regression of each isofemale line combination's productivity when in competition (competition with spermless, Acp-producing males) on productivity without competition (control). Dashed lines represent 95% CI.

0.375, d.f. = 45, $P < 0.0001$). Regression of the increase in productivity due to the presence of competition (the difference between productivity with and without competition) on productivity without competition was not statistically significant (Supplementary Figure B.3; $R^2 = 0.036$, d.f. = 45, $P = 0.196$). As expected, second male sperm precedence was confirmed in *D. melanogaster* isofemale lines. The second male to mate fathered the majority of the offspring ($P_2 = 0.76$) when females were doubly mated to wildtype males that had both sperm and Acps (Figure 5.2; t = -38.035, d.f. = 2001, $P < 0.0001$). There was a significant difference between treatment of control treatment (without competition), the experimental competition treatment (the first male has only Acps), and the competition treatment (the first male has both sperm + Acps) (Figure 5.2; F = 51.247, d.f. = 2, 3063, $P < 0.0001$). Unexpectedly, the females from the experimental competition treatment (the first male has only Acps) produced more offspring than the control treatment (without competition) (Figure 5.2; $P < 0.0001$). Similarly, females from the competition treatment (the first male has both sperm + Acps) produced more offspring than the experimental competition treatment (the first male has only Acps) (Figure 5.2; $P < 0.0001$). Females from the competition treatment (the first male has both sperm + Acps) also produce more offspring than females from the control treatment (without competition) (Figure 5.2; $P < 0.0001$). The average total number of offspring produced from the control treatment (without competition) is 131.98 ± 1.93 (mean ± SE), experimental competition treatment (the first male has only Acps) is 143.06 ± 2.06 and when females were doubly mated to wildtype males that had both Acps and sperm is 152.88 ± 1.75. Therefore, adding another set of Acps has a greater effect on increasing the female's productivity (a 9% increase) than the effect due to sperm itself

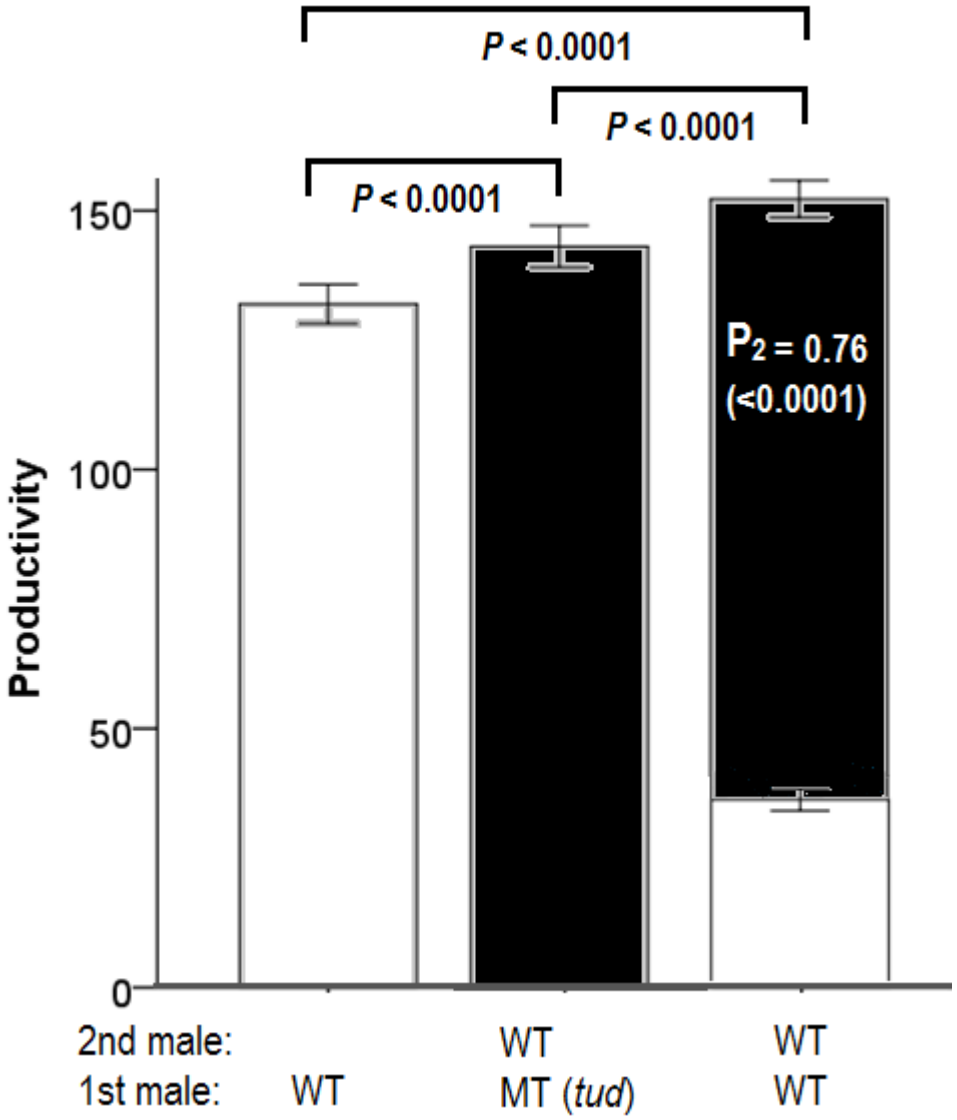


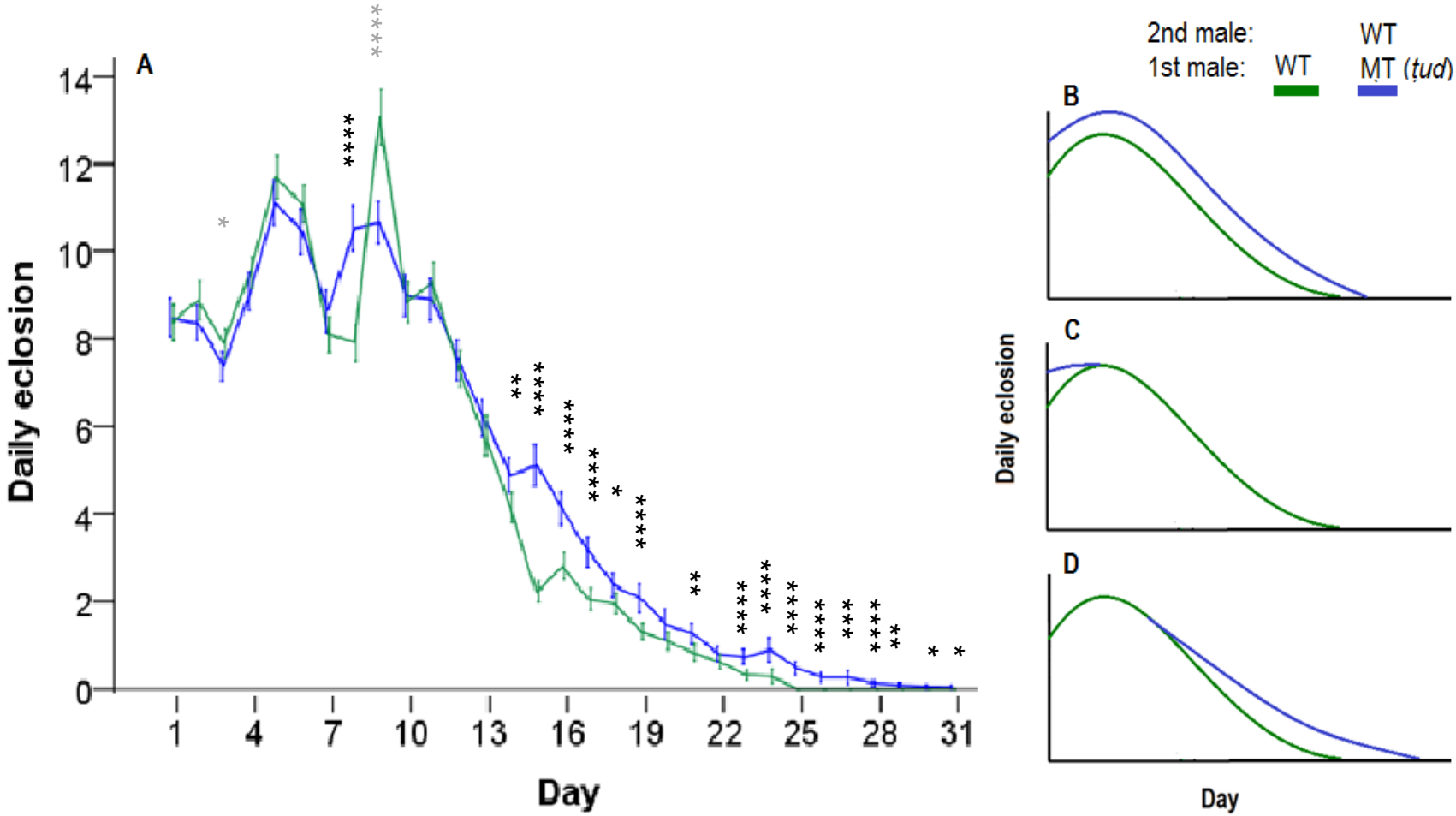
Figure 5.2 Number of offspring produced from the first male (white) and the second male (black) when a female is mated with a single male (without competition), mated first to a mutant (MT *tud*) male producing only Acps, or mated first to a wildtype (WT) male producing Acps and sperm. P_2 represents the proportion of offspring sired by the second male. Error bars represent 95% CI.

(adding sperm and Acps increases the productivity by another 7%; this increase is the sperm effect). To examine why there was increased productivity in the presence of additional Acps, the daily eclosion for the control (without competition) was compared to the experimental treatment (competition where the first male has only Acps) (Figure 5.3A; Supplementary Table B.1).

5.4 Discussion

I found that males that mated in competition with spermless, Acp-producing males had a greater fitness (higher productivity) than when they were the only male to mate with a female (Figure 5.2). In other words, males sired more offspring when mated to a female that already contains Acps (but no sperm) from a previous male. To explain how secondly mated males can have a higher productivity in the presence of competing Acps, I offer three possible mechanisms that could occur. Scenario 1 (Figure 5.3B) I consider an "additive effect", where the increased concentration of Acps increases the female's egg laying rate throughout her reproductive life. Scenario 2 (Figure 5.3C) would result if a "priming effect" occurred. The Acps from the initial male would increase the female's egg-laying rate, causing it to be at a higher standing level at the time she mates with the second male, increasing his initial productivity. A "protective effect" would result in scenario 3 (Figure 5.3D) where the Acps from the first male protects the sperm from the second mated male, increasing its longevity and extending the female's egg laying duration. A comparison of daily eclosion between the control (without competition) and experimental treatment (competition with spermless, Acp-producing males) (Figure 5.3A), shows that the third scenario of Acp protection is most probable. Therefore, secondly mated males have a higher productivity in the presence of residing competing

Figure 5.3 Daily eclosion rates of offspring. (A) The control treatment (green; females mated to one male; N=862 females) compared to the experimental treatment (blue; females first mated to a sterile male that produces only Acps; N=932 females). Error bars represent 95% CI. Vertical asterisks indicate significant differences between the two groups via a t-test: * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$; the grey asterisks indicate a significant effect in the opposite direction (controls with higher eclosion rate than treatment groups). Three possible mechanisms that could benefit the second mated male: (A) additive effect, (B) priming effect, (C) protective effect (see Discussion).


A
Daily eclosion
Day
2nd male:
1st male:
WT
WT
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B
C
D
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Acps due to an extension of the time that offspring are produced. This is most likely due to the competing Acps increasing the survival of the second male's sperm, allowing thefemale to continue laying fertilized eggs, and increasing total productivity by about 9%. This is possible if some Acps have a generally protective effect that is not male specific -- that is, if their function does not act specifically to benefit the male they came from. This type of across-male benefit has previously been shown for sterile Acp-less males (who produce sperm but no Acps), whose fertility is partially restored if a female first mates with a male that provides Acps (Xue and Noll 2000). However, this benefit was previously thought to only apply to the severe case of sterile Acp-less males, who gain minimal fertility from the presence of a competitor's Acps, a benefit that presumably would not apply to males with functioning Acps of their own. Here, I show that this is not the case, and an intact male benefits from having a competitor's Acps present.

I found no significant three-way interaction of female line, male line, and treatment effects in the LMM, indicating that male success (productivity) does not depend on the genotype of the female that he mates with. Instead, significant male line and treatment interaction effects indicate that certain male genotypes perform better than others when in sperm competition (competition with spermless, Acp-producing males). This performance in the presence of competition has a strong positive association with how males perform without competition (Figure 5.1). Additionally, the increase in productivity due to the presence of additional Acps equally affects low-producing and high-producing males (Supplementary Figure B.3). This indicates that the increase in average offspring production due to the presence of additional Acps is not merely due to a 'rescue' of the productivity of poorly-performing males (that may have less effective

Acps). Instead, males that perform poorly when singly mated perform slightly better in the presence of additional Acps, and males who perform well also have a slight increase in offspring production.

One possible explanation for the results is simply that second male advantage is a result of males preferentially allocating their resources to increase ejaculate size. *Drosophila melanogaster* males transfer 15% more sperm to mated females (835 ± 29) than virgin females (728 ± 31) (Lüpold et al. 2010). *Drosophila melanogaster* males are able to assess the mating status of females due to changes in her cuticular hydrocarbon profile after mating (Everaerts et al. 2010). However, this is very unlikely to explain the "protective effects" observed for the first male's Acps. *Drosophila melanogaster* females store only about 1/5 of the sperm that males transfer (Lefevre and Jonsson 1962) and a maximum of ~530 sperm can reside in the sperm storage organs (Manier et al. 2010). Thus, the increased transfer of sperm to a mated female does not have a corresponding increase in sperm storage. Therefore, the increased productivity of second males in the presence of competing Acps is unlikely to be due to female's initial exposure to more sperm from that male. An ideal test of this hypothesis would involve repeating these experiments by first mating females to males that do not produce Acps or sperm, but are otherwise wildtype. Unfortunately, all available mutations of this type have some leaky expression of Acps, precluding this test (M. Wolfner, pers. comm.).

These finding appears to contradict previous findings that have demonstrated that Acps are harmful for a competitor's sperm. Remated females store less sperm from the first mated male in their sperm storage organs compared to singly mated females (Price et al. 1999). The increase of second male sperm in the sperm storage organs occurs

simultaneously as the first male's residing sperm decreases; the second male's sperm is thought to physically displace the residing first male's sperm (Manier et al. 2010). Additionally, the presence of the residing first male's sperm can be seen in the bursa (the female organ where male ejaculates are initially expelled into) during the second mating, either before sperm transfer or after sperm transfer but before sperm storage (Manier et al. 2010). This leads the authors to conclude that either the mechanical act of copulation itself or the second male's Acps can trigger the female to eject the residing first male's sperm (Manier et al. 2010). Acps from the second male are also reported to be capable of incapacitating the residing first male's sperm. When females were initially mated to a wildtype male and remated to a spermless, Acp-producing mutant male (the opposite mating order to what I report here), females produced a lower number of offspring than when they were singly mated to the wildtype male (Harshman and Prout 1994; Price et al. 1999). This loss of productivity is not a result of sperm availability as there was no significant difference in the number of sperm stored in the sperm storage organs. Since first male sperm numbers do not decrease when the second male only deposit Acps, but do decrease when he deposits sperm and Acps (Manier et al. 2010), this is further support that the first male's sperm is physically displaced by the second male's sperm, rather than Acps. Therefore, the second mated male's sperm physically displaces the first male's sperm, and second male's Acps incapacitate the remaining residing first male's sperm. These mechanisms reveal the offensive traits of the second male and how they contribute to second male advantage. These offensive mechanisms (Figure 5.4; shown in red) are harmful to the first male.

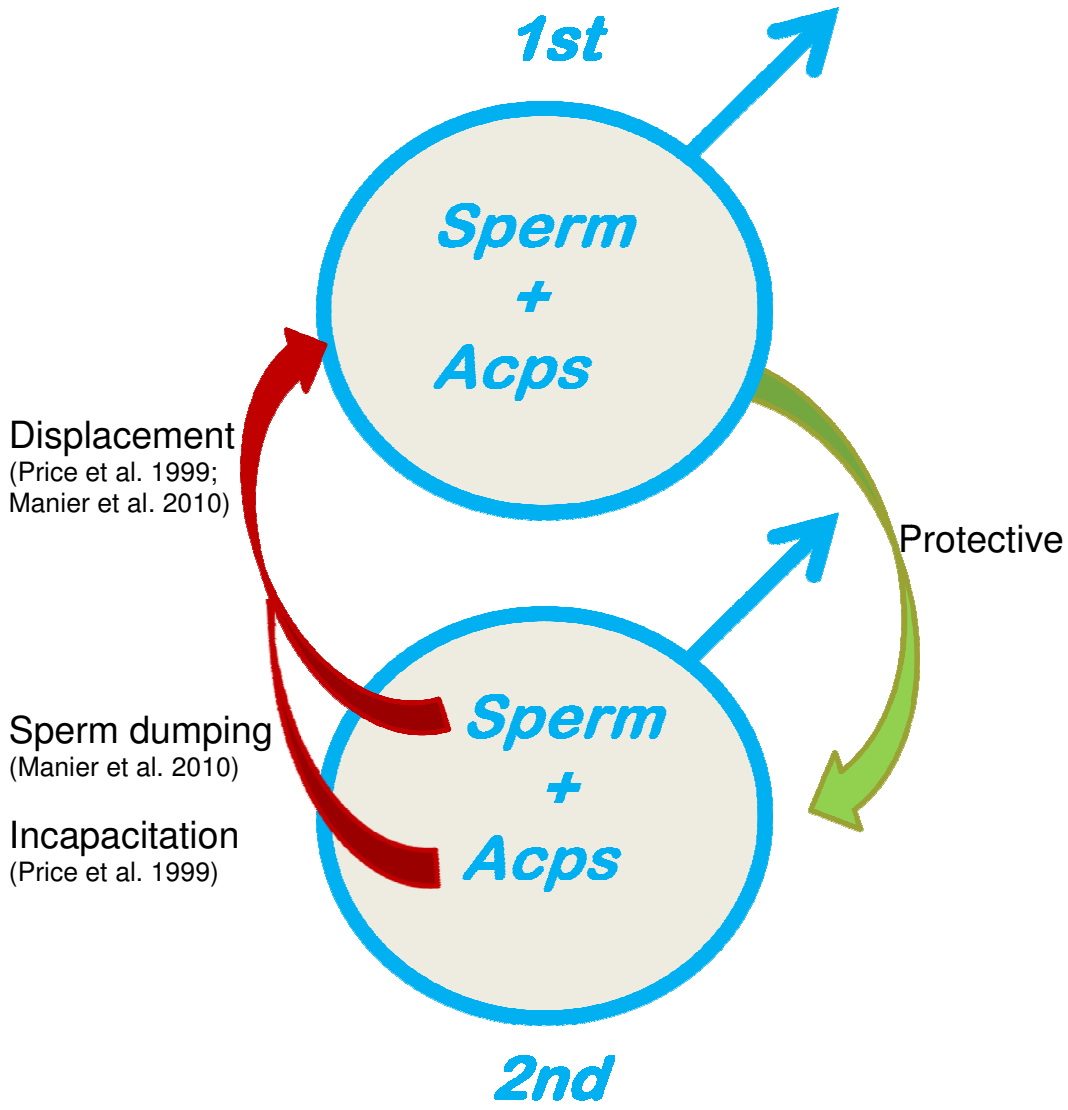


Figure 5.4 Mechanisms underlying sperm competition and second male advantage. Arrow heads represent the target male, arrow ends represent the component responsible for the mechanism. Red arrows represent harmful mechanisms, green arrows represent beneficial mechanisms.

It should be noted however, that while some studies have found instances of sperm incapacitation (Harshman and Prout 1994; Price et al. 1999), others have not detected this phenomenon (Snook and Hosken 2004; Manier et al. 2010). Snook and Hosken (2004) argued that the incapacitation phenomonon could be better explained by sperm death caused by aging, sperm-storage effects, or that the effect is female mediated. They measured the proportion of dead sperm found in the seminal receptacle of females and found no significant difference in the proportion of dead sperm between singly mated females and females remated to spermless Acps producing males, indicating that competing seminal fluid had no effect on resident sperm death (Snook and Hosken 2004). However, this finding does not eliminate the possibility of sperm incapacitation through mechanisms that do not cause death, such as through reducing competitor sperm motility.

Here I present for the first time an effect of the first mated male's ejaculate acting on the second male's ejaculate. In this scenario, the Acps from the first male are beneficial to his competitor (Figure 5.4; shown in green) by a protective mechanism that increases the longevity of their sperm survival. Why are Acps protective when provided by the first male, but detrimental when provided by the second male? One option is that the males tailor their ejaculates to contain harmful proteins only when they know that a female has previously mated. While this is possible, I think that a more likely explanation is that the age of the ejaculate impacts the effectiveness of any harmful components. When a second male deposits his ejaculate, the first male's sperm immediately comes into contact with it, and any harmful components can be at full efficacy. In contrast, the first male's ejaculate was at least one day old before the second male mated, and it is likely that some harmful proteins within the ejaculate lost their potency in this time, and only the

protective proteins remain. Regardless of the mechanism, this beneficial protective mechanism by the first mated male further contributes to and reinforces the second male advantage.

5.5 Chapter acknowledgements

I thank Mariana Wolfner for providing the *tudor* flies. This work would not be completed without the assistance of Amanda Tong, Pria Mahabir, Anes Kwon, Hannah Guiang, Amanda Morgan, Chaewon Jung, David Jo, Hassan Shahbaz, Hemani Patel, James Lim, Jonwook Kim, Josh Skapinker, Josh Tordjman, Mathew Mathew, Patrick Zhang, Stephen Lu, Yoni Balboul, Sarah Kim, Injun Seo, and Alice Lee This work was supported by an NSERC Discovery Grant and a Canada Research Chair to Amanda J. Moehring.

5.6 References

Alcock, J. 1994. Postinsemination associations between males and females in insects: the mate-guarding hypothesis. Annu. Rev. Entomol. 39:1–21.

Boswell, R. E., and A. P. Mahowald. 1985. tudor, a gene required for assembly of the germ plasm in *Drosophila melanogaster*. Cell 43:97–104.

Chapman, T., J. Hutchings, and L. Partridge. 1993. No reduction in the cost of mating for *Drosophila melanogaster* females mating with spermless males. Proc. R. Soc. Lond. B Biol. Sci. 253:211–217.

Chen, P. S., E. Stumm-Zollinger, T. Aigaki, J. Balmer, M. Bienz, and P. Böhlen. 1988. A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. Cell 54:291–298.

Clark, A. G., M. Aguadé, T. Prout, L. G. Harshman, and C. H. Langley. 1995. Variation in sperm displacement and its association with accessory gland protein loci in *Drosophila melanogaster*. Genetics 139:189–201.

Cook, P. A., and N. Wedell. 1999. Non-fertile sperm delay female remating. Nature 397:486–486.

Eberhard, W. G. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton, New Jersey.

Everaerts, C., J.-P. Farine, M. Cobb, and J.-F. Ferveur. 2010. *Drosophila* cuticular hydrocarbons revisited: mating status alters cuticular profiles. PLoS ONE 5:e9607.

Fiumera, A. C., B. L. Dumont, and A. G. Clark. 2007. Associations between sperm competition and natural variation in male reproductive genes on the third chromosome of *Drosophila melanogaster*. Genetics 176:1245–1260.

Fiumera, A. C., B. L. Dumont, and A. G. Clark. 2005. Sperm competitive ability in *Drosophila melanogaster* associated with variation in male reproductive proteins. Genetics 169:243–257.

Harshman, L. G., and T. Prout. 1994. Sperm displacement without sperm transfer in *Drosophila melanogaster*. Evolution 48:758–766.

Herndon, L. A., and M. F. Wolfner. 1995. A *Drosophila* seminal fluid protein, Acp26Aa, stimulates egg laying in females for 1 day after mating. Proc. Natl. Acad. Sci. 92:10114–10118.

LaFlamme, B. A., F. W. Avila, K. Michalski, and M. F. Wolfner. 2014. A *Drosophila* protease cascade member, seminal metalloprotease-1, is activated stepwise by male factors and requires female factors for full activity. Genetics 196:1117–1129.

Lefevre, G. J., and U. B. Jonsson. 1962. Sperm transfer, storage, displacement, and utilization in *Drosophila melanogaster*. Genetics 47:1719.

Lunde, K., J. L. Trimble, A. Guichard, K. A. Guss, U. Nauber, and E. Bier. 2003. Activation of the knirps locus links patterning to morphogenesis of the second wing vein in *Drosophila*. Development 130:235–248.

Lüpold, S., M. K. Manier, O. Ala-Honkola, J. M. Belote, and S. Pitnick. 2010. Male *Drosophila melanogaster* adjust ejaculate size based on female mating status, fecundity, and age. Behav. Ecol. 22:184–191.

Manier, M. K., J. M. Belote, K. S. Berben, D. Novikov, W. T. Stuart, and S. Pitnick. 2010. Resolving mechanisms of competitive fertilization success in *Drosophila melanogaster.* Science 328:354–357.

Martin, O. Y., and D. J. Hosken. 2002. Strategic ejaculation in the common dung fly *Sepsis cynipsea*. Anim. Behav. 63:541–546.

Nguyen, T., and A. Moehring. (*in press*). Accurate alternative measurements for female lifetime reproductive success in *Drosophila melanogaster*. PLoS ONE.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45:525–567.

Parker, G. A. 1984. Sperm competition and the evolution of animal mating systems. Academic press, Inc. (London) LTD., London.

Parker, G. A. 1990. Sperm competition games: raffles and roles. Proc. R. Soc. Lond. B Biol. Sci. 242:120–126.

Parker, G. A. 1993. Sperm competition games: sperm size and sperm number under adult control. Proc. R. Soc. Lond. B Biol. Sci. 253:245–254.

Parker, G. A., L. W. Simmons, and H. Kirk. 1990. Analysing sperm competition data: simple models for predicting mechanisms. Behav. Ecol. Sociobiol. 27:55–65.

Pattarini, J. M., W. T. Starmer, A. Bjork, and S. Pitnick. 2006. Mechanisms underlying the sperm quality advantage in *Drosophila melanogaster*. Evolution 60:2064–2080.

Pitnick, S., and T. A. Markow. 1994. Large-male advantages associated with costs of sperm production in *Drosophila hydei*, a species with giant sperm. Proc. Natl. Acad. Sci. 91:9277–9281.

Pitnick, S., T. A. Markow, and G. S. Spicer. 1995. Delayed male maturity is a cost of producing large sperm in *Drosophila*. Proc. Natl. Acad. Sci. 92:10614–10618.

Price, C. S. C., K. A. Dyer, and J. A. Coyne. 1999. Sperm competition between *Drosophila* males involves both displacement and incapacitation. Nature 400:449–452.

Ram, K. R., and M. F. Wolfner. 2007. Seminal influences: *Drosophila* Acps and the molecular interplay between males and females during reproduction. Integr. Comp. Biol. 47:427–445.

R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Snook, R. R. 2005. Sperm in competition: not playing by the numbers. Trends Ecol. Evol. 20:46–53.

Snook, R. R., and D. J. Hosken. 2004. Sperm death and dumping in *Drosophila*. Nature 428: 939-941.

Tram, U., and M. F. Wolfner. 1999. Male Seminal fluid proteins are essential for sperm storage in *Drosophila melanogaster*. Genetics 153:837–844.

Waage, J. K. 1979. Dual function of the damselfly penis: sperm removal and transfer. Science 203:916–918.

Wigby, S., L. K. Sirot, J. R. Linklater, N. Buehner, F. C. F. Calboli, A. Bretman, M. F. Wolfner, and T. Chapman. 2009. Seminal fluid protein allocation and male reproductive success. Curr. Biol. 19:751–757.

Xue, L., and M. Noll. 2000. *Drosophila* female sexual behavior induced by sterile males showing copulation complementation. Proc. Natl. Acad. Sci. 97:3272–3275.

Chapter 6

6 Assessing male quality in precopulatory and postcopulatory sexual selection

Although males and females have different reproductive strategies to increase their reproductive success, the variation in reproductive success is usually larger for males than it is for females. Not only do males have to compete for matings, but they also have to compete after mating for fertilization. Due to intense competition, males have evolved reproductive strategies to increase their reproductive success. To assess male reproductive strategies, I used 10 isofemale lines of *Drosophila melanogaster* to determine male quality based on five fitness measures: (1) productivity, (2) productivity of F_1 sons, (3) productivity of F_1 daughters, (4) mating success in competition, and (5) combined fitness traits. I then measured high quality and low quality male performance in both pre- and postcopulatory sexual selection. The most consistent results across treatments are for males ranked using a combined fitness measure (fitness measure 5), emphasizing the importance of using a composite measurement in determining fitness. High quality males were not more successful at acquiring matings as females did not accept high quality male courtship more readily. However, high quality males courted earlier and more often than low quality males and copulated for a longer period of time. High quality males also outcompeted low quality males in sperm competition, whether they were competing with Acps (Accessory gland proteins) alone or with sperm and Acps.

6.1 Introduction

Sexual selection is a branch of natural selection that explains evolution through differential reproductive success. The first barrier of sexual selection is being able to successfully acquire mates. The conventional view of sexual selection involves female mate choice, and males have evolved reproductive strategies that increase their chances of reproductive success through either increasing the likelihood of being chosen by the female or by circumventing her ability to choose. For instance, to increase their chances of being chosen by a female, males can provide direct benefits to females. Direct benefits increase the direct fitness of the female, such as through increased paternal care (reducing the cost of parental care for the female), enhanced fertility and fecundity, and better quality of resources through territory or nuptial gifts (Moller and Jennions 2001; Wagner et al. 2001; Wedell and Ritchie 2004). While males usually provide direct benefits to females in resource-based mating systems, these benefits may also be present in non-resource-based mating systems. For example, there is significant variation in lifetime reproductive success for female *Drosophila melanogaster* due to male line effects (Nguyen and Moehring, in press), indicating that females in this non-resource-based mating system could potentially gain direct benefits in increased fecundity through mate choice.

While it is known that males are usually less discriminating in mating than females, the occurrence of adaptive male mate choice is plausible since there is mounting evidence that mating is also costly for males (Pitnick and Markow 1994; Pitnick et al. 1995; Snook 2005). In the fruit fly, *D. melanogaster* males preferentially mate and remate with larger females, where size is positively correlated with fecundity (Byrne and Rice 2006). This

preference was intensified in resource-depleted males, where the cost of mating for males was higher (Byrne and Rice 2006). Not only are males interested in obtaining high quality mates, but they can also alter their copulation behaviour to increase their fertilization success. *Drosophila melanogaster* males copulate with females longer when males perceive them to be non-virgin and therefore a high sperm competition risk (Friberg 2006). This longer copulation duration reduced the female's remating frequency and increased the male's fitness by siring more offspring (Friberg 2006).

Males can also possess traits that are indirectly linked to fitness benefits for offspring. For example, in *D. melanogaster*, male body size is an important indicator of fitness and also a predictor of mating success (Partridge and Farquhar 1983). It is possible for this male phenotype (size) to be correlated with indirect benefits if this increased size is inherited by the offspring. In the collared flycatcher *Ficedula albicollis* (Passeriformes: Muscicapidae), males with a larger white forehead patch, a secondary sexually selected trait, produce offspring of better condition (Sheldon et al. 1997). Genetic models of indirect fitness benefits include good genes obtained through additive genetic variation and compatible genes through non-additive genetic variation (Neff and Pitcher 2005). In the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* (Coleoptera: Chrysomelidae), fast-stroking males, who stroke females using their antennae, are more successful at transferring sperm than slow-stroking males (Tallamy et al. 2003). These males provide indirect good gene benefits by producing sons who are also fast-stroking, and therefore more successful at acquiring mates and gaining fertilizations. Direct and indirect benefits can occur simultaneously in a given mating system. For example, in the striped ground cricket *Allonemobius socius* (Orthoptera: Gryllidae), females who mated

multiply acquired direct benefits through an increase in nutritional resources via more nuptial gifts, and indirect genetic benefits through increased hatching success of offspring (Fedorka and Mousseau 2002).

In the event where female mate choice does not occur or fails due to forced copulation, and in cases where females gain benefits by having additional control over fertilization, both direct and indirect fitness benefits can be acquired through postcopulatory sexual selection. Postcopulatory sexual selection allows selection to persist after mating. This has a particularly strong impact on males, who not only have to compete to acquire matings, but also have to compete for fertilization after successfully mating. Similarly to how males have reproductive tactics to increase their probability of mating, they also have strategies to increase their probability of successful fertilization after mating. When two or more ejaculates reside in a female reproductive tract, sperm competition can occur (Parker 1970). In many species, second male sperm precedence occurs where the second male to mate fertilizes the majority of eggs (P_2). However, this value can vary from 2% to 100% of P_2 fertilization between species (Ridley 1989) and 0% to 100% within species (Lewis and Austad 1990), emphasizing the complex nature of sperm competition. Therefore, it is important to tease apart the mechanisms of sperm competition and how they contribute to the variation of P_2 values.

Sperm competition most commonly takes the form of sperm displacement, where a male removes a rival male's sperm, or sperm incapacitation, where the use of rival sperm for fertilization is inhibited. Sperm competition involves both the sperm itself and the seminal fluid, which contains accessory gland proteins (Acps). In *D. melanogaster*, Acps can have a wide range of effects that can significantly influence fertilization (Ram and

Wolfner 2007). Some of these effects can alter female behaviour. Examples include increasing female egg laying rate and therefore producing more eggs fertilized by the given male or delay female remating in order to reduce the exposure to competition. These effects can also be context dependent. For example, Acps' effect on fertilization depends on the order of mating, and may involve separate mechanisms from those induced by the sperm themselves (Nguyen and Moehring, Chapter 5). In addition, males can also adjust their ejaculates to be better competitors. Male crickets *Gryllus veletis* (Orthoptera: Gryllidae) transferred more sperm when in competition with a single male in order to increase his fertilization success, in comparison to when there was no competition (Schaus and Sakaluk 2001). Not only can males vary their sperm quantity, but can also vary their quality. In the Australian field cricket *Teleogryllus oceanicus* (Orthoptera: Gryllidae), males mated to virgin or singly mated females transfer the same amount of sperm but with more viable sperm than when mated to multiply mated females, as the cost of producing high quality sperm in the letter case would outweigh the benefits in the presence of intense competition (Thomas and Simmons 2007).

The interaction between female and male genotypes is important to take into consideration when examining sexual selection mechanisms. For example, both male (Clark et al. 1995) and female (Clark and Begun 1998) genotypes can affect variation in sperm displacement in *D. melanogaster*. Females are not passive vessels in this process and can play an active role in postcopulatory sexual selection since it takes place within the female reproductive tract. When a female can bias the paternity of her offspring or influence the preferential use of sperm after copulation has occurred, cryptic female choice is exhibited (Eberhard 1996). Perhaps in an evolutionary response to cryptic

female choice, males have evolved counter adaptations to manipulate females to preferentially use their sperm for fertilization. In the red flour beetle *Tribolium castaneum* (Coleoptera, Tenebrionidae), a male uses the tarsi of his legs to rub the lateral edge of the female's elytra. Males who were manipulated by having their legs truncated, and therefore could not rub the female's elytra, had a lower fertilization success and P_2 values compared to unmanipulated males even though both transfer the same amount of sperm (Edvardsson and Arnqvist 2000). Furthermore, the intensity at which rubbing occurred by unmanipulated males was positively correlated to his fertilization success. This demonstrates the ability of males to increase their fertilization success by manipulating female behaviour.

There may be a link between precopulatory and postcopulatory sexual selection since there is some evidence that males who are more successful during precopulatory selection are also more likely to succeed during postcopulatory selection. In *D. simulans*, males who had a lower copulation latency, and therefore were more attractive and preferred by females, were also more competitive in sperm competition as they had a higher paternity share (Hosken et al. 2008). However, the mechanism for this is often unknown; attractive males could be better competitors in sperm competition due to superior ejaculate and/or females could bias paternity towards attractive males through cryptic female choice. It is often difficult to disentangle the reproductive success of males as the nature of mating systems are complex, where both male traits and female influence can occur simultaneously and cooperate or conflict. The goal of this paper is to assess the reproductive success of males at both pre- and postcopulatory stages and to determine

possible connections between them using males with varying fitness measures in *D. melanogaster*.

6.2 Methods

6.2.1 *Drosophila* strains and maintenance

Ten isofemale lines of *D. melanogaster* were collected from the wild in Sudbury, Ontario Canada, in 2011 by T. Merritt. Flies were maintained in the laboratory on standard cornmeal agar media (Bloomington *Drosophila* Stock Center, Indiana) in 8-dram vials on a 14:10 light-dark cycle, at 24ºC and approximately 75% relative humidity.

6.2.2 Measures of fitness

To rank the quality of males, five fitness measures were used: (1) productivity, (2) productivity of F_1 sons, (3) productivity of F_1 daughters, (4) mating success in competition, and (5) combined fitness traits. The ten isofemale lines of *Drosophila melanogaster* (Nguyen and Moehring, in press) were previously measured in a full factorial breeding design for fitness measures 1-3 ((Nguyen and Moehring, in press; Nguyen and Moehring, submitted). Male mating success (fitness measure #4) on these same lines was previously measured in a mating arena that allowed for competition (Nguyen and Moehring, Chapter 4). Males were ranked for their combined (overall) fitness measure (#5) by using an average ranking score of the first four measures of fitness. A high quality male and a low quality male were identified for each of the five measures of fitness for each isofemale line. Therefore, the high and low quality males are fitness measure specific and isofemale line specific (Supplementary Table C.1). Isofemale line 4 was unable to be used here due to loss of the line. Therefore, if males

from line 4 were determined to be the highest or lowest ranking male for a specific female line, the second highest or lowest male line was chosen instead.

6.2.3 No-choice mating assay

To determine how high and low quality males perform in mating without competition, a no choice mating assay was performed. Individual virgin males and females were collected upon eclosion from density controlled vials to control for size (as in Nguyen and Moehring, Chapter 4) and aged 4-6 days. A single female was placed in an 8-dram vial without food with a single male that was the corresponding high or low quality male for that isofemale line as determined by each of the five fitness measures (Supplementary Table C.1). Measurements that were recorded are: (1) time it took until the male started courting, (2) time it took to start copulation, and (3) time it took for copulation to end. From these measurements, courtship duration can be calculated to determine female preference. If copulation was not initiated within 1 hour, the experiment was terminated and repeated the following day with new mating pairs. Mated experiments continued until there was 20 replicates of successful copulation for each mating pair combination. Males were taken and thorax was measured as a control for male size.

6.2.4 Postcopulatory performance assay

To assess male quality on postcopulatory performance, high and low quality males were exposed to two types of competition: (1) Acps competition, and (2) Sperm and Acps competition. To determine how males perform in Acps competition, virgin isofemale line male and females used were collected upon eclosion from density controlled vials to control for size (as in Nguyen and Moehring, Chapter 4) and aged 4-6 days. Females from each line were initially mated to sons of *tudor* mothers, aged 4-6 days, who produce

no sperm and are therefore sterile, but produce Acps (See detailed methods in Nguyen and Moehring, Chapter 5). Mating was scored in an assay with a single male and female in a vial. Males who mated were removed by aspiration. Mated females were remated to either a corresponding high or low quality male, aged 4-6 days, the following day. The total number of offspring produced from the double mating event were counted in a similar manner as Nguyen and Moehring (in press). A total of 20 replicates were performed for each isofemale line combination.

To determine how males perform in sperm and Acps competition, high and low quality males were competed against each other. A female from each isofemale line containing a recessive visible marker (*kni*; see detailed methods in Chapter 5) was crossed to a high and low male, alternating which male also contained the recessive marker : (1) high^{kni}, low, (2) high, low^{kni}, (3) low^{kni}, high and (2) low, high^{kni}. Mating was scored in the same manner as above. The total number of offspring from the second male to mate (P_2) was counted using the homozygous recessive marker as an indicator of paternity. Ten replicates for each order were performed for a total of 40 replicates for each isofemale line combination. Virgin males and females were collected from isofemale density controlled vials and aged 4-6 days, as above. Mated females were remated after 24 hours. Females who did not remate were paired again the following day to allow for remating. Females who did not remate in 24-48 hours after the initial mating were discarded. Mating was scored in a no-choice mating assay where males were removed immediately after mating was completed. The total number of offspring produced from the single pair mating combination was scored in a similar manner as Nguyen and

Moehring, (in press). Only offspring produced after females were remated to a second male were scored and counted.

6.2.5 Statistical analysis

Differences in high quality vs. low quality males

To determine significant differences between high quality and low quality male phenotypes, a one-way ANOVA for each female line for four fitness measures were performed: (1) productivity, (2) productivity of F_1 sons, (3) productivity of F_1 daughters, (4) mating success in competition. For those that did not fit the assumptions for the parametric test, a Kruskal-Wallis was performed.

High quality vs. low quality males' performance in mating

To analyze high quality and low quality males' performance in mating, the percent of males that courted, the percent of males that mated out of those that courted and out of the total number of replicates was analyzed using three separate Generalized Linear Mixed Models (GLMMs) with a binomial distribution for all five measures of fitness. The terms were male quality (high or low) as fixed factors, female line and male thorax size as random factors. The interactions between male quality and female line and male quality and thorax size were also included. Terms that were not significant using a likelihood ratio test were removed in the final reduced model. The time taken for males to start courting, courtship duration, and copulation duration were analyzed using three separate GLMMs in a similar manner as above, but with a negative binomial distribution, for all five measures of fitness.

The percent of males that successfully mated out of the total number of replicates performed is the male mating success without competition. The male mating success without competition for the single mating pair isofemale line combinations performed here were compared to the corresponding male mating success combinations with competition data presented in Nguyen and Moehring, Chapter 4. A Generalized Linear Model (GLM) with a quasipoisson distribution was used to analyze mating success with competition as a response variable and male mating success without competition as the predictor variable.

High quality vs. low quality males' performance in post-copulatory selection

To analyze how these high and low quality males perform in postcopulatory sexual selection (e.g., cryptic female choice or sperm competition), a Linear Mixed Model (LMM) was performed to analyze the lifetime reproductive success of males competing with a spermless but Acps producing male for all five measures of fitness. The response variable is the total number of offspring produced, male quality (high or low) was used as a fixed factor, female line and male quality and female line interaction was used as random factors. To determine how high quality and low quality males performed against each other, a GLMM with a binomial distribution was performed for all five measures of fitness to analyze the fertilization success of the second male. Each replicate is weighted by the total number of offspring to control for brood size. The total number of offspring was used as the binomial denominator. Male quality (high or low) was used as the fixed factor, female line and male quality and female line interaction was used as the random factor variable. Due to overdispersion, individual observations were included as a random effect (Bolker et al. 2009).

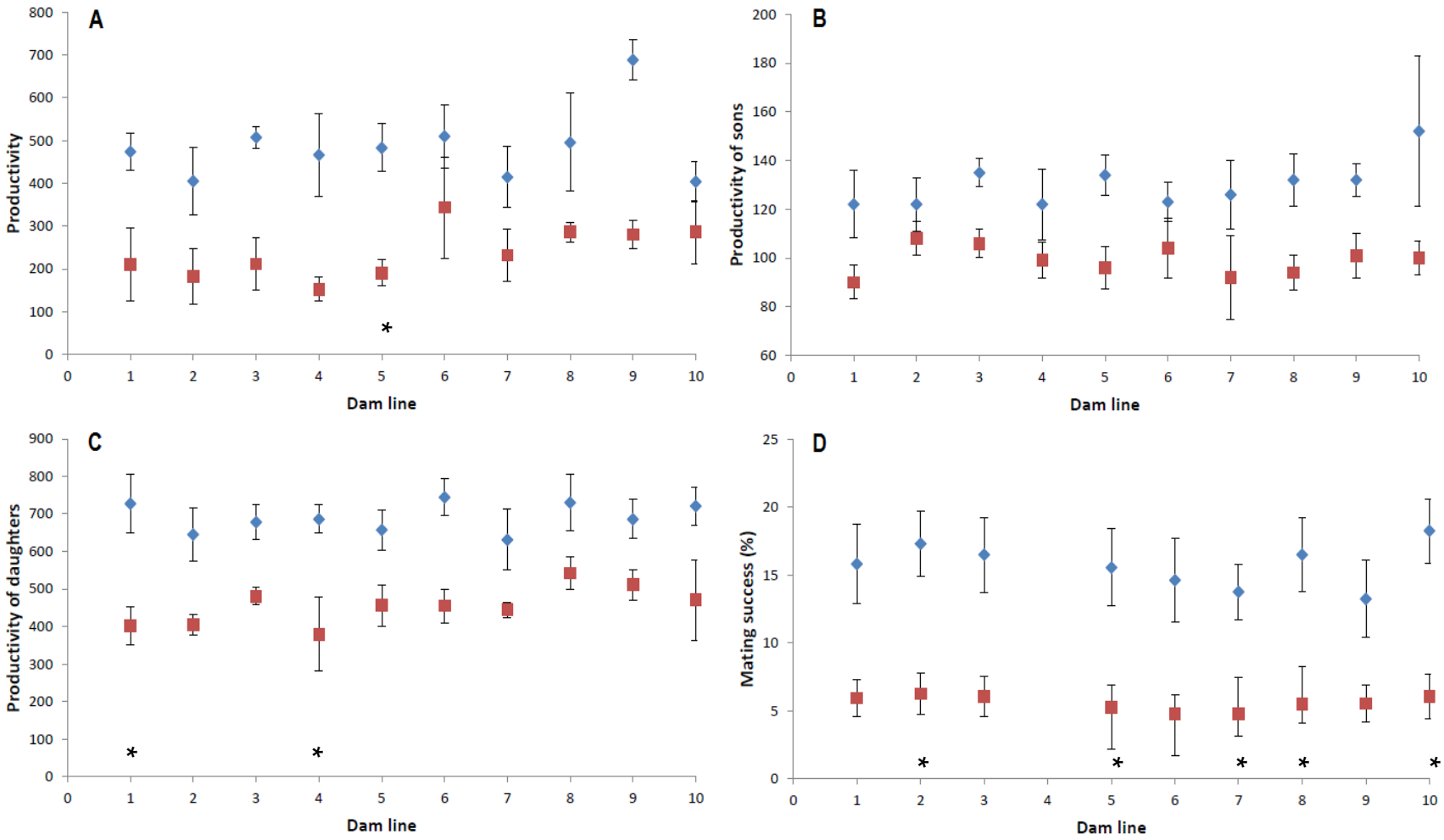
To determine how low quality males perform in relation to high quality males, the ratio of low quality and high quality male performance was compared across postcopulatory treatments of Acps competition and sperm and Acps competition for all five measures of fitness. The ratio performance was compared to two previously-reported measures of control where the performance of low quality and high quality males were individually measured without competition (Nguyen and Moehring, in press): (1) lifetime reproductive success when a female was mated to a focal high quality or low quality male in a single mating and (2) lifetime reproductive success when a female was mated to a focal high quality or low quality male when allowing for multiple matings. Ratio performance across treatments was compared in a One-way ANOVA followed by a Tukey's multiple comparisons post hoc or Kruskal-Wallis if parametric assumptions were not met.

6.3 Results

Determining high quality vs. low quality males

Male quality was measured using five fitness measures: (1) productivity, (2) productivity of F_1 sons, (3) productivity of F_1 daughters, (4) mating success in competition, and (5) combined fitness traits. For fitness measure (1) productivity, the only statistically significant difference in high quality and low quality male performance were for female line 5 (Figure 6.1A; $F_{(9, 30)} = 2.972$, $P = 0.0119$). Productivity of F_1 sons (2) had no statistically significant differences between high quality and low quality males for any of the female lines. For fitness measure (3) productivity of F_1 daughters, there were significant differences between a high quality and low quality male for female line 1

Figure 6.1 Performance of high quality (diamonds) and low quality (squares) male lines for four fitness measures: (A) productivity of parentals cross, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, and (D) mating success. Error bars represent SE. Asterisks represent significant differences between high and low quality males.


A
Productivity
Dam line
B
Productivity of sons
Dam line
C
Productivity of daughters
Dam line
D
Mating success (%)
Dam line

(Figure 6.1C; $F_{(9, 30)} = 2.354$, $P = 0.0380$) and female line 4 (Figure 6.1C; $F_{(9, 30)} = 2.719$, $P = 0.0191$). The fitness measure of (4) mating success in competition had significant differences in high and low quality males for female line 2, 5, 7, 8, and 10 (Figure 6.1D; 2: $F_{(9, 190)} = 3.282$, $P = 0.0009$, 5: $F_{(9, 190)} = 2.812$, $P = 0.0040$, 7: $F_{(9, 190)} = 1.925$, $P = 0.0507$, 8: $F_{(9, 190)} = 3.417$, $P = 0.0006$, 10: $F_{(9, 190)} = 3.625$, $P = 0.0003$).

High quality vs. low quality males' performance in mating

High quality males defined by (1) productivity, (2) productivity of F_1 sons, and (5) combined fitness traits initiated courtship significantly more often (Figure 6.2A; $\chi^2_{(1)} = 19.1070$, $P < 0.0001$, Figure 6.2B; $\chi^2_{(1)} = 12.8720$, $P = 0.0003$, Figure 6.2E; $\chi^2_{(1)} = 11.5140$, $P = 0.0006$), and had a faster initiation of courtship (Figure 6.3A; $\chi^2_{(1)} = 4.2276$, $P = 0.0397$, Figure 6.3B; $\chi^2_{(1)} = 9.5710$, $P = 0.0019$, Figure 6.3E; $\chi^2_{(1)} = 14.2680$, $P = 0.0007$) than low quality males. However, there is no significant difference in how long males courted (courtship duration) between high quality and low quality males for any measures of fitness (Figure 6.4). The proportion of males that copulated, when only considering those males that courted, there are no significant differences between high quality and low quality males for any of the five measures of fitness used to determine male quality (Figure 6.5). However, when all males are considered (whether they courted first or not), high quality males defined by (2) productivity of F_1 sons mated significantly more often than low quality males (Figure 6.6B; $\chi^2_{(1)} = 4.4465$, $P = 0.0349$). The copulation duration of high quality males defined by (2) productivity of F_1 sons and (5) combined fitness traits was significantly longer than the copulation duration of low quality males (Figure 6.7B; $\chi^2_{(1)} = 7.9129$, $P = 0.0049$, Figure 6.7E; $\chi^2_{(1)} = 7.2763$, $P =$

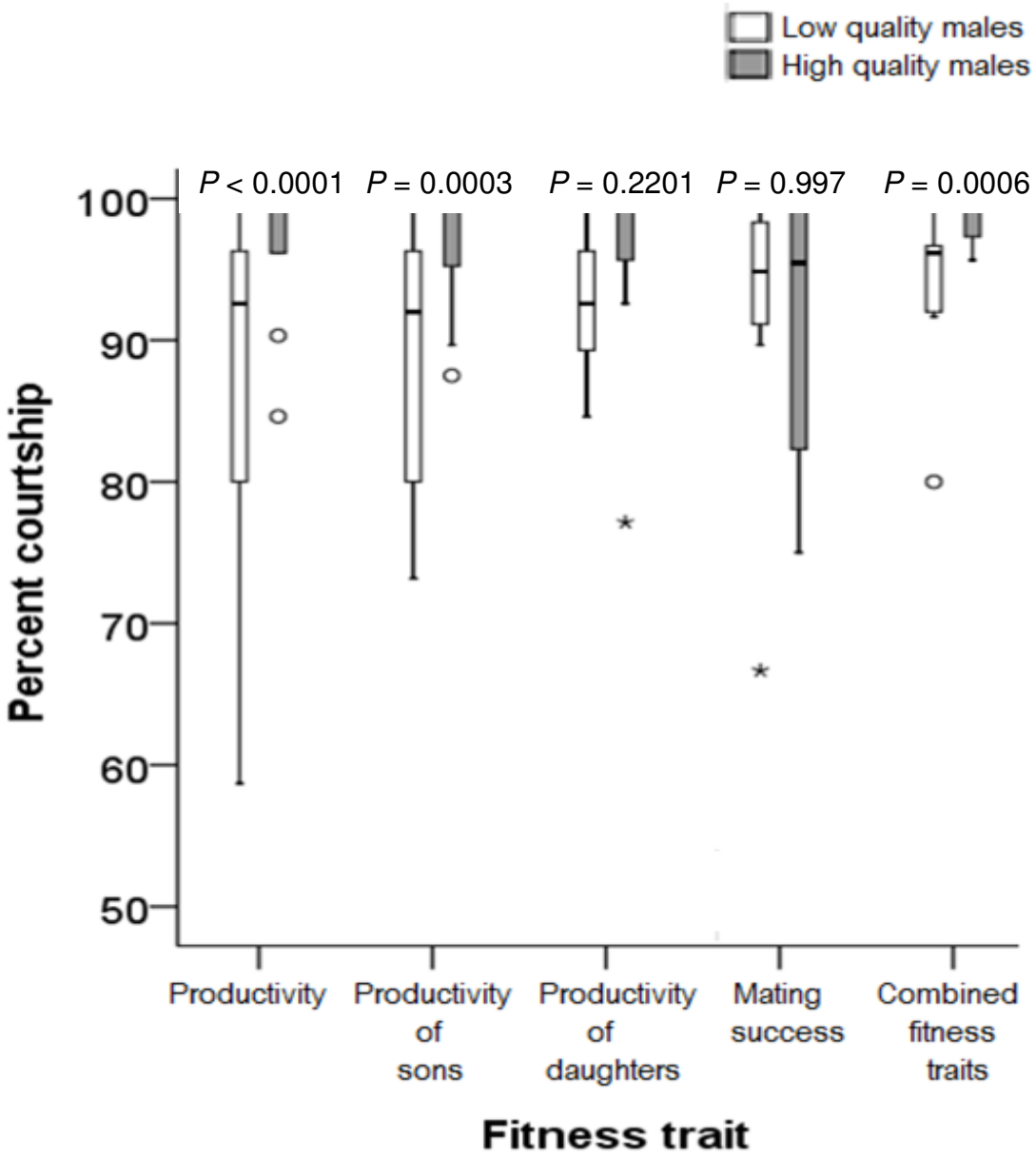


Figure 6.2 Box plots for percent of high quality and low quality males that courted for all five fitness measures: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Boxes represent the upper (third) quartile and lower (first) quartile range. The thick horizontal line represents the median. Whiskers represent minimum and maximum values. Circles represent minor outliers (1.5 × Interquartile Range) and stars represent major outliers (3.0 × Interquartile Range).

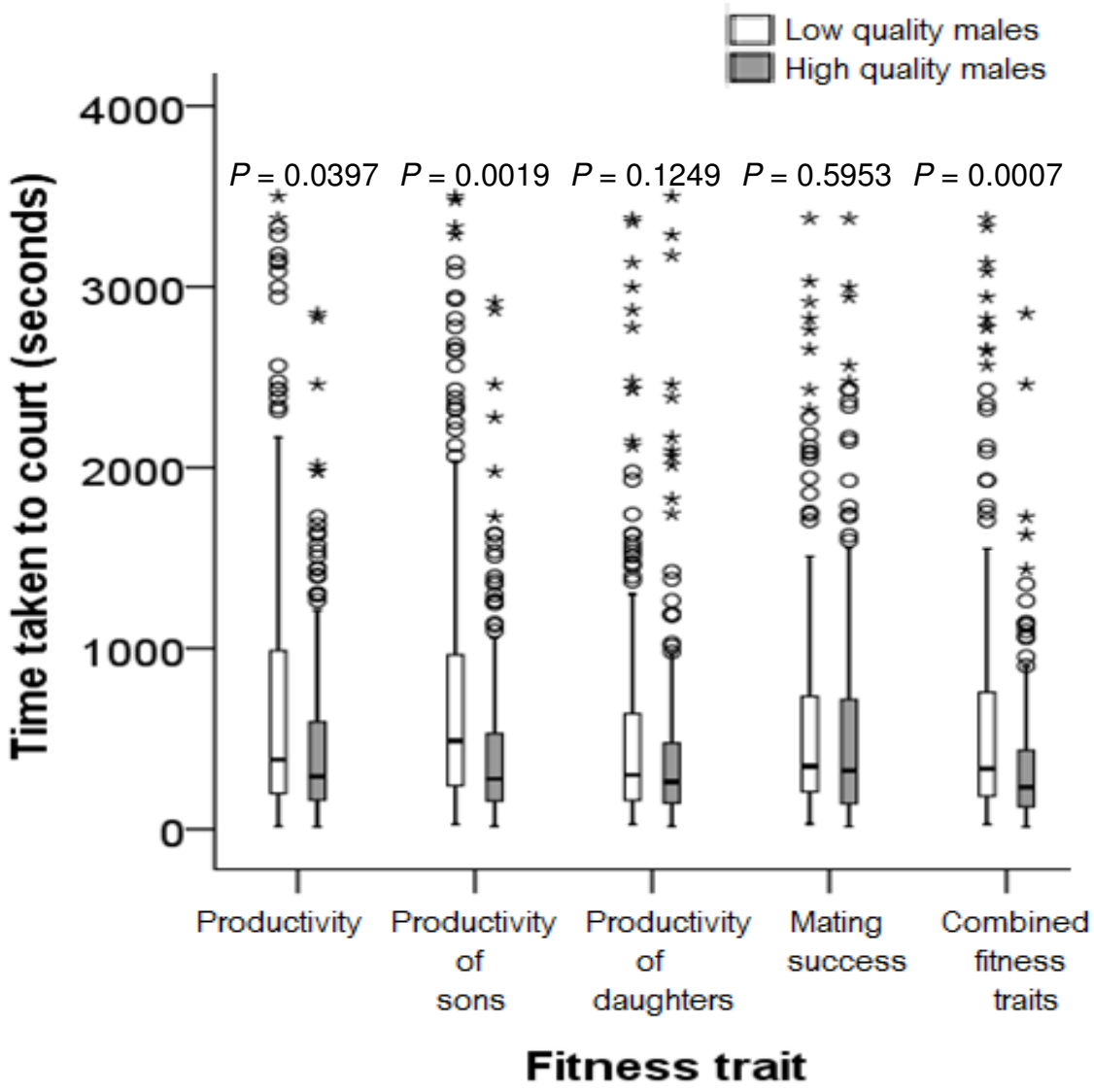


Figure 6.3 Box plots for time taken for high quality and low quality males to start courting for all five fitness measures: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Boxes represent the upper (third) quartile and lower (first) quartile range. The thick horizontal line represents the median. Whiskers represent minimum and maximum values. Circles represent minor outliers (1.5 × Interquartile Range) and stars represent major outliers (3.0 × Interquartile Range).

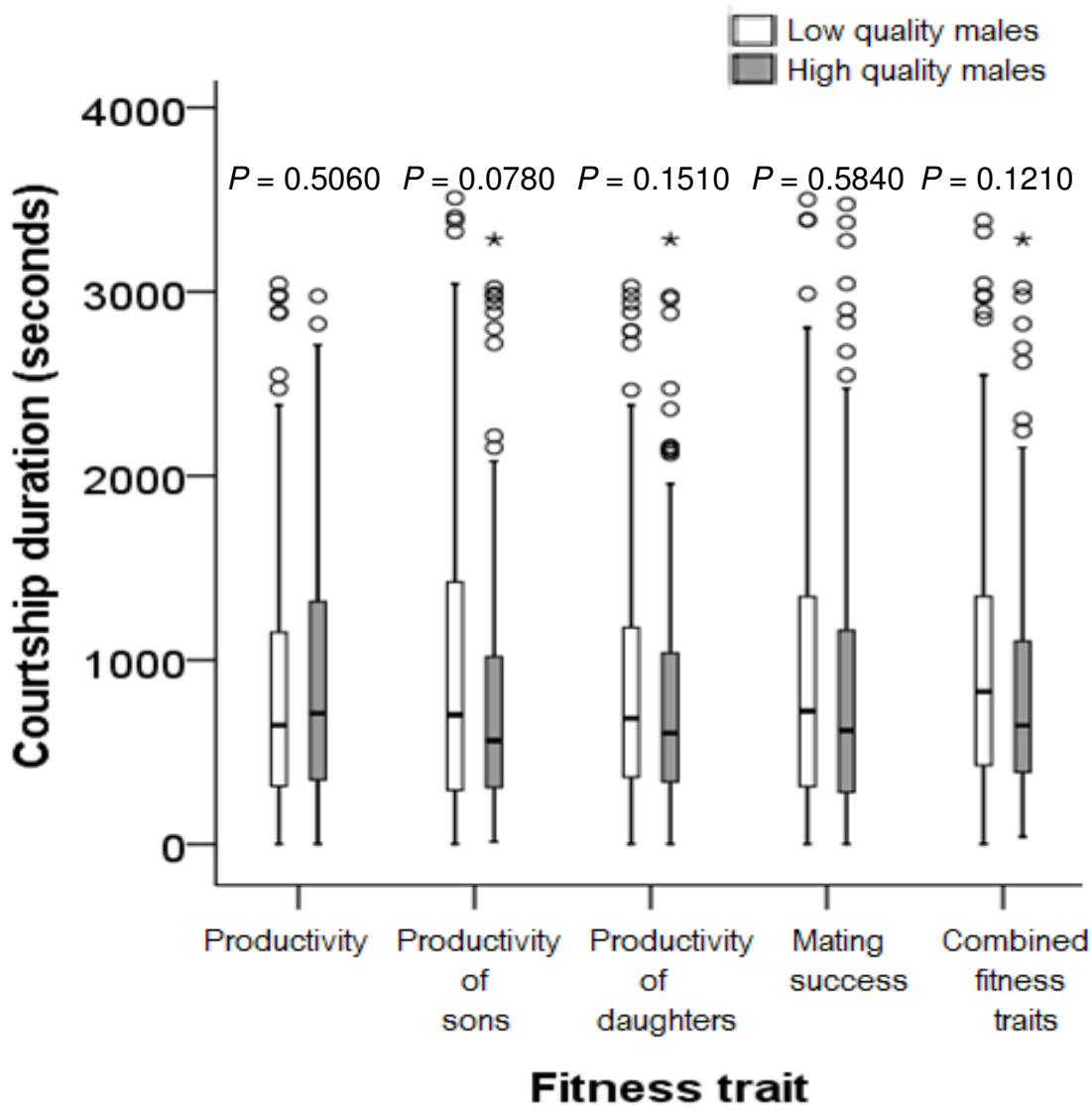


Figure 6.4 Box plots for a measure of female preference: courtship duration. When males started to court, the time taken for females to accept the male's courtship and start mating. Performance of high and low quality males are shown for all five fitness measures: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Boxes represent the upper (third) quartile and lower (first) quartile range. The thick horizontal line represents the median. Whiskers represent minimum and maximum values. Circles represent minor outliers (1.5 × Interquartile Range) and stars represent major outliers (3.0 × Interquartile Range).

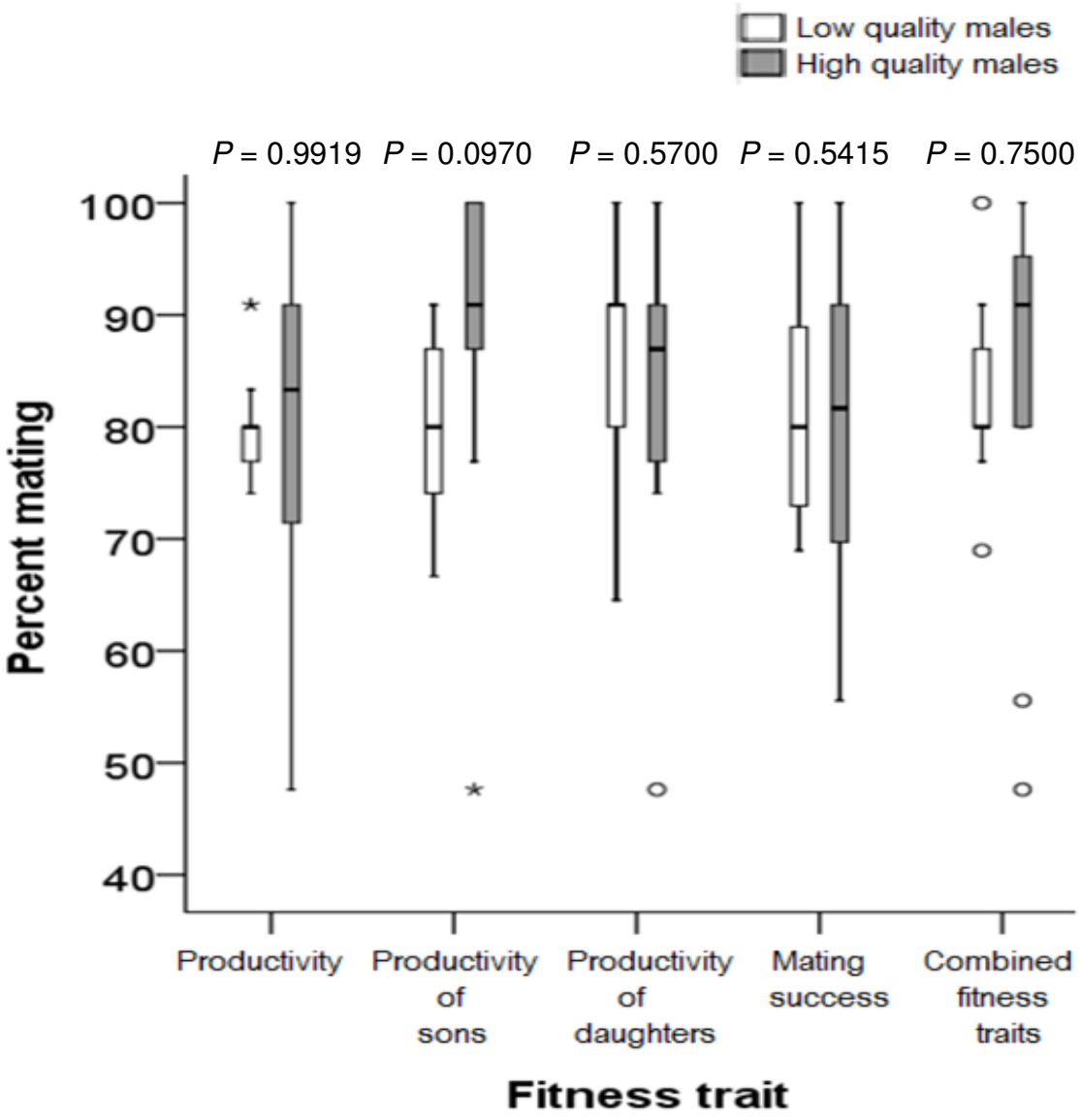


Figure 6.5 Box plots for a measure of male success: percent of high and low quality males that mated out of those that courted. High quality and low quality male performance for all five fitness measures are shown: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Boxes represent the upper (third) quartile and lower (first) quartile range. The thick horizontal line represents the median. Whiskers represent minimum and maximum values. Circles represent minor outliers (1.5 × Interquartile Range) and stars represent major outliers (3.0 × Interquartile Range).

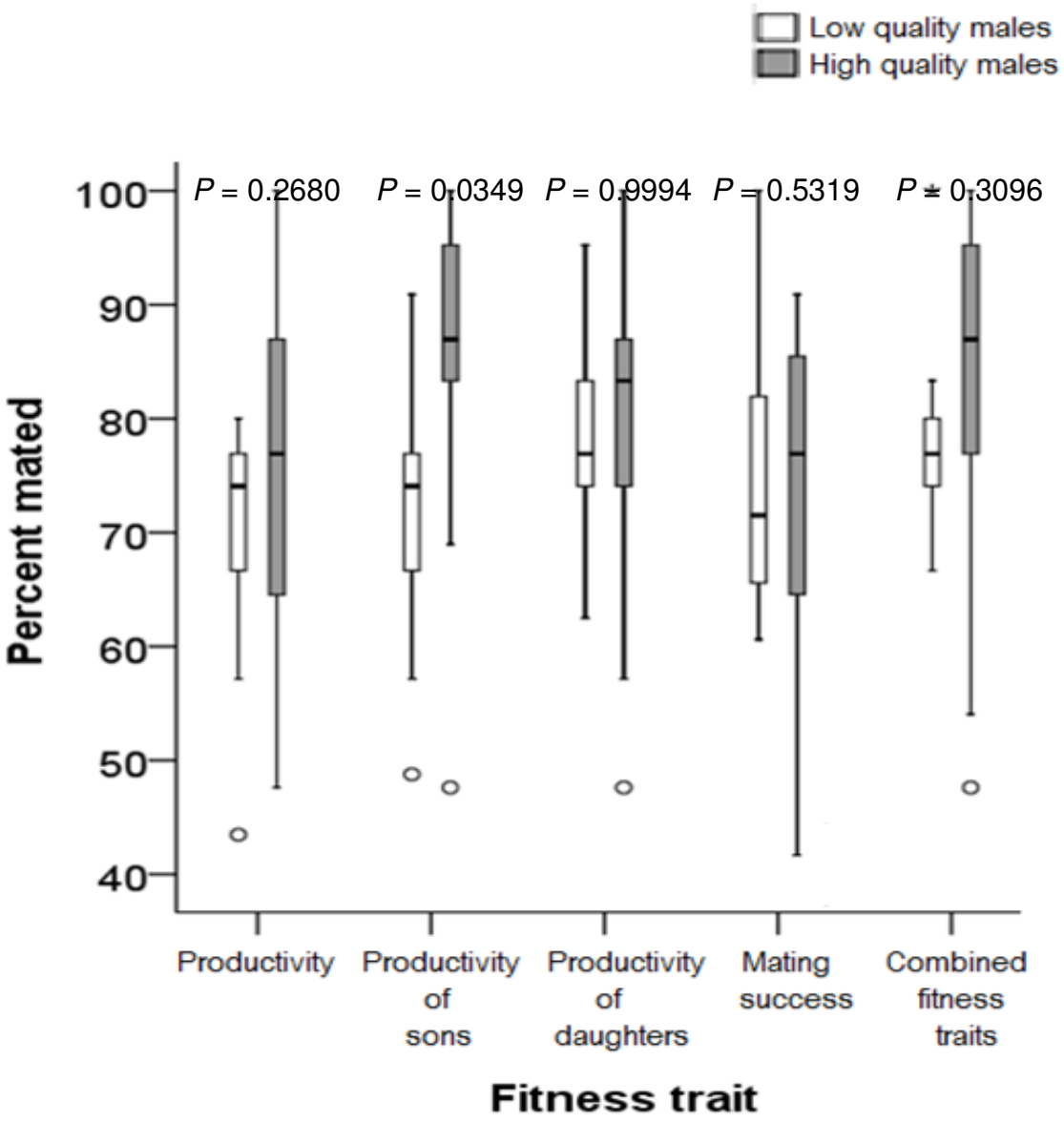


Figure 6.6 Box plots for a measure of male success: percent of high and low quality males that mated out of the total number of replicates performed. High quality and low quality male performance for all five fitness measures are shown: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Boxes represent the upper (third) quartile and lower (first) quartile range. The thick horizontal line represents the median. Whiskers represent minimum and maximum values. Circles represent minor outliers (1.5 × Interquartile Range) and stars represent major outliers (3.0 × Interquartile Range).

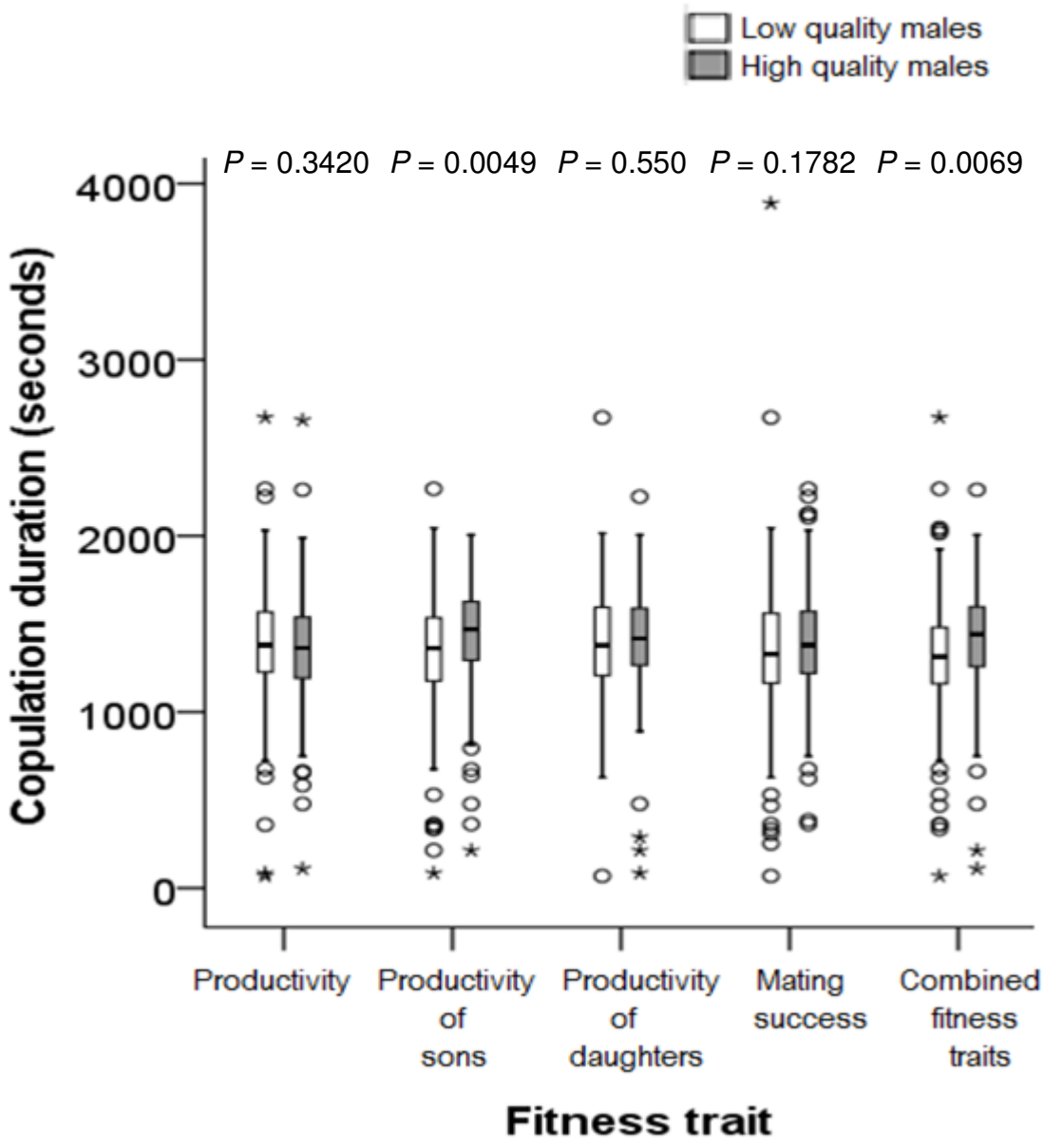


Figure 6.7 Box plots for the length of copulation duration for high and low quality males for all five fitness measures: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Boxes represent the upper (third) quartile and lower (first) quartile range. The thick horizontal line represents the median. Whiskers represent minimum and maximum values. Circles represent minor outliers (1.5 × Interquartile Range) and stars represent major outliers (3.0 × Interquartile Range).

0.0069). Individual female line effects for high quality and low quality males' performance in mating are shown in supplementary Figures C.1-C.6. Individual results of each parameter for each model are summarized in a supplementary Table C.2. Male mating success without competition was not significantly correlated with male mating success with competition (Figure 6.8; pseudo $R^2 = 0.0114$, d.f. = 45, $P = 0.4700$)

High quality vs. low quality males' performance in postcopulatory selection

High quality males based on (5) combined fitness traits produce significantly more offspring than low quality males when competing against a spermless Acp producing male (Figure 6.9E; $\chi^2_{(1)} = 4.6018$, $P = 0.0319$). There were no significant differences in high quality and low quality male performance based on any other fitness measure. Similarly, when high quality and low quality males were in competition with each other (both sperm and Acp competition), high quality males fertilized more offspring as the second male to mate in comparison to low quality males when high and low quality males were defined using (5) combined fitness traits (Figure 6.10E; $\chi^2_{(1)} = 17.5640$, $P < 0.0001$). Individual female line effects for high quality and low quality male performance in postcopulatory selection are shown in supplementary Figure C.7 and Figure C.8. Individual results of each parameter for each model are summarized in supplementary Table C.3.

The difference between high vs. low male productivity (low/high) was compared across treatments to allow for an assessment of how the degree of difference between the

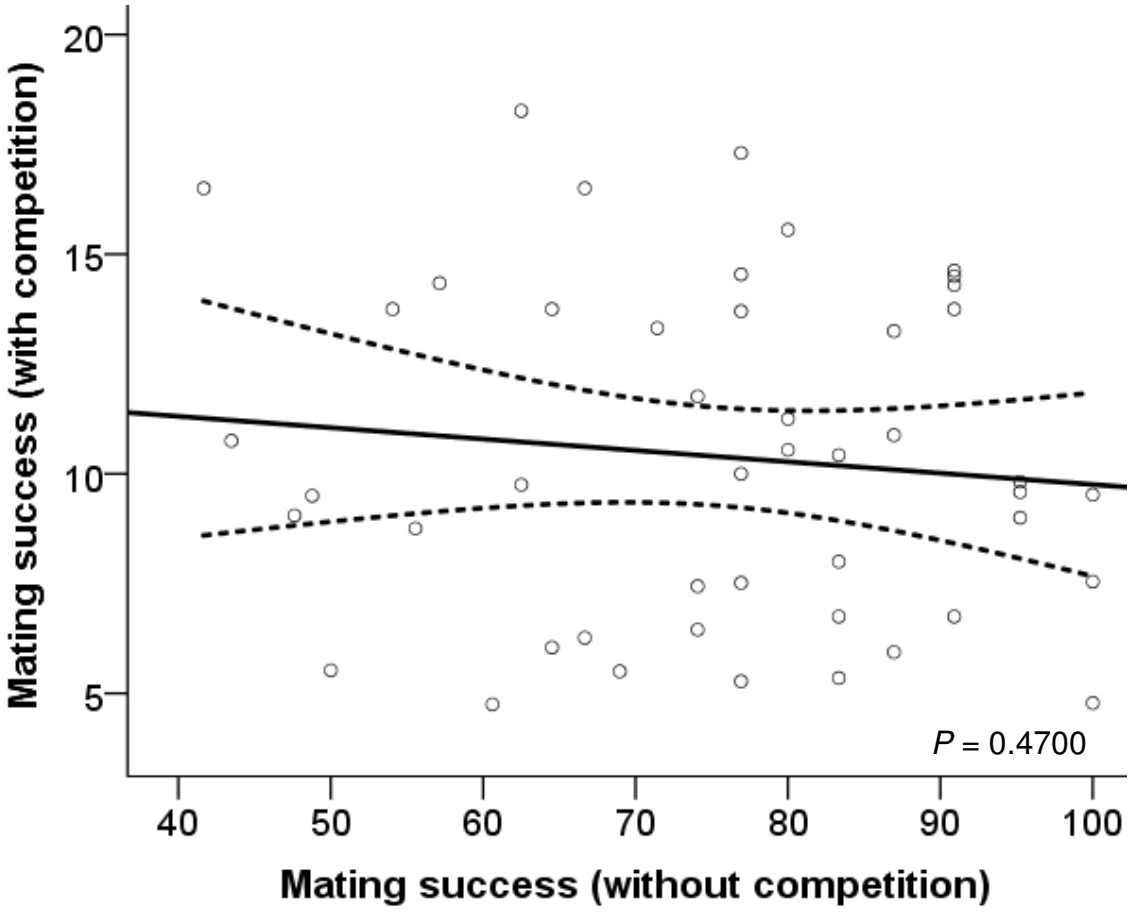


Figure 6.8 Comparison of male mating success without competition to male mating success with competition. Dashed lines represent 95% CI.

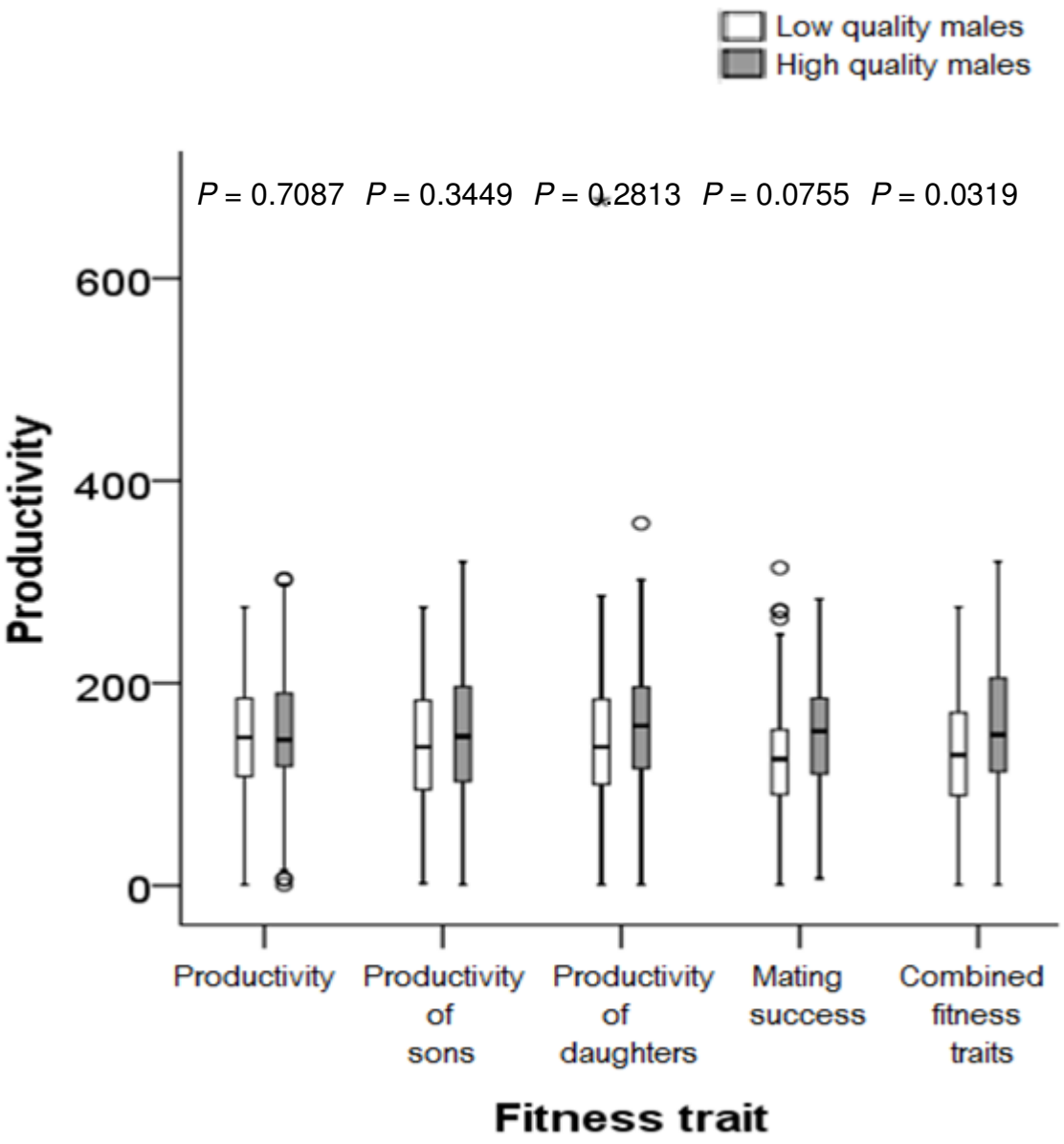


Figure 6.9 Box plots for lifetime reproductive success for high and low quality males when in competition with a spermless Acps producing male for all five fitness measures: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Boxes represent the upper (third) quartile and lower (first) quartile range. The thick horizontal line represents the median. Whiskers represent minimum and maximum values. Circles represent minor outliers (1.5 × Interquartile Range) and stars represent major outliers (3.0 × Interquartile Range).

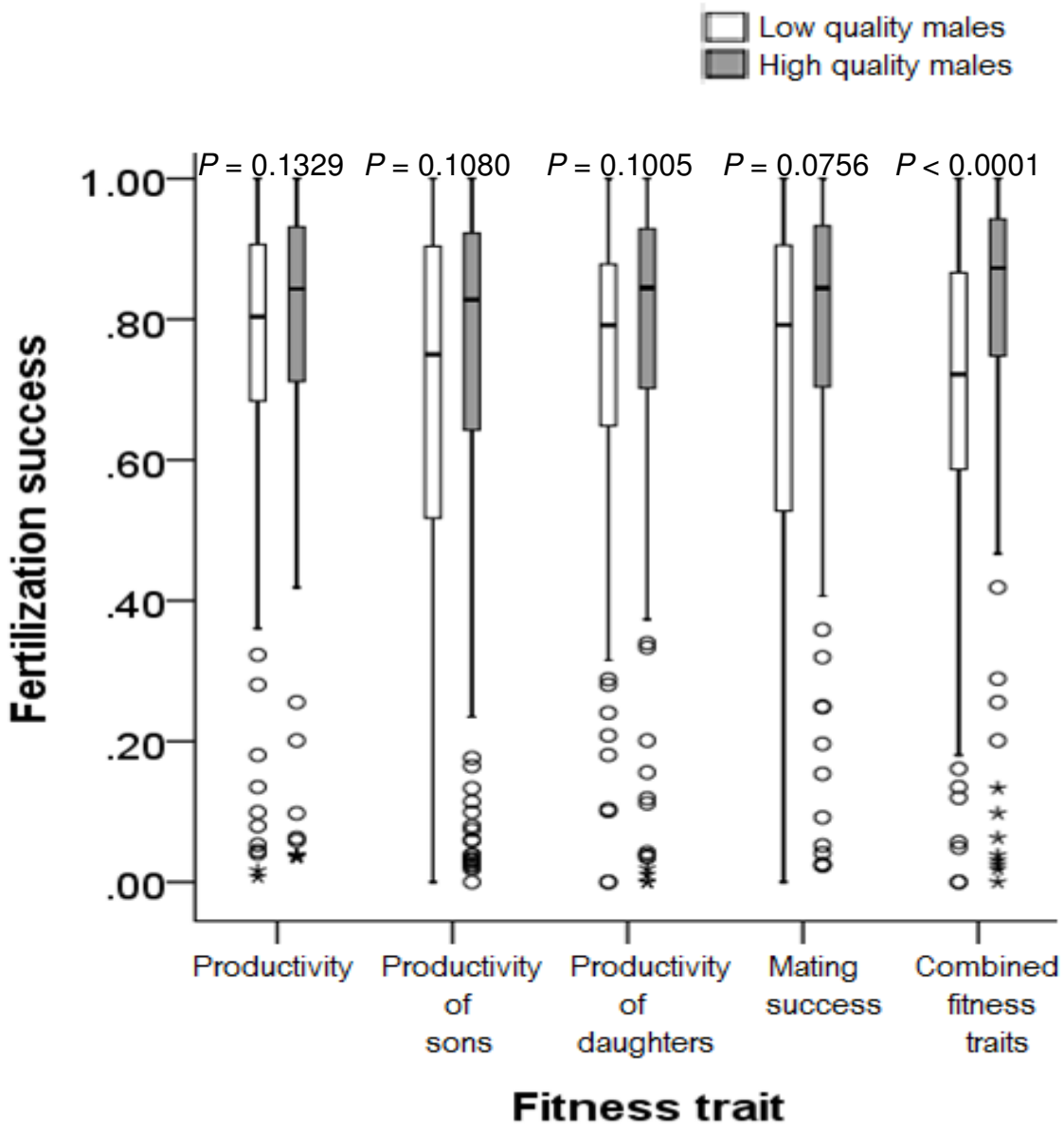


Figure 6.10 Box plots for the proportion of offspring sired by the second male as high or low quality males when in competition with each other for all five fitness measures: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Boxes represent the upper (third) quartile and lower (first) quartile range. The thick horizontal line represents the median. Whiskers represent minimum and maximum values. Circles represent minor outliers (1.5 × Interquartile Range) and stars represent major outliers (3.0 × Interquartile Range).

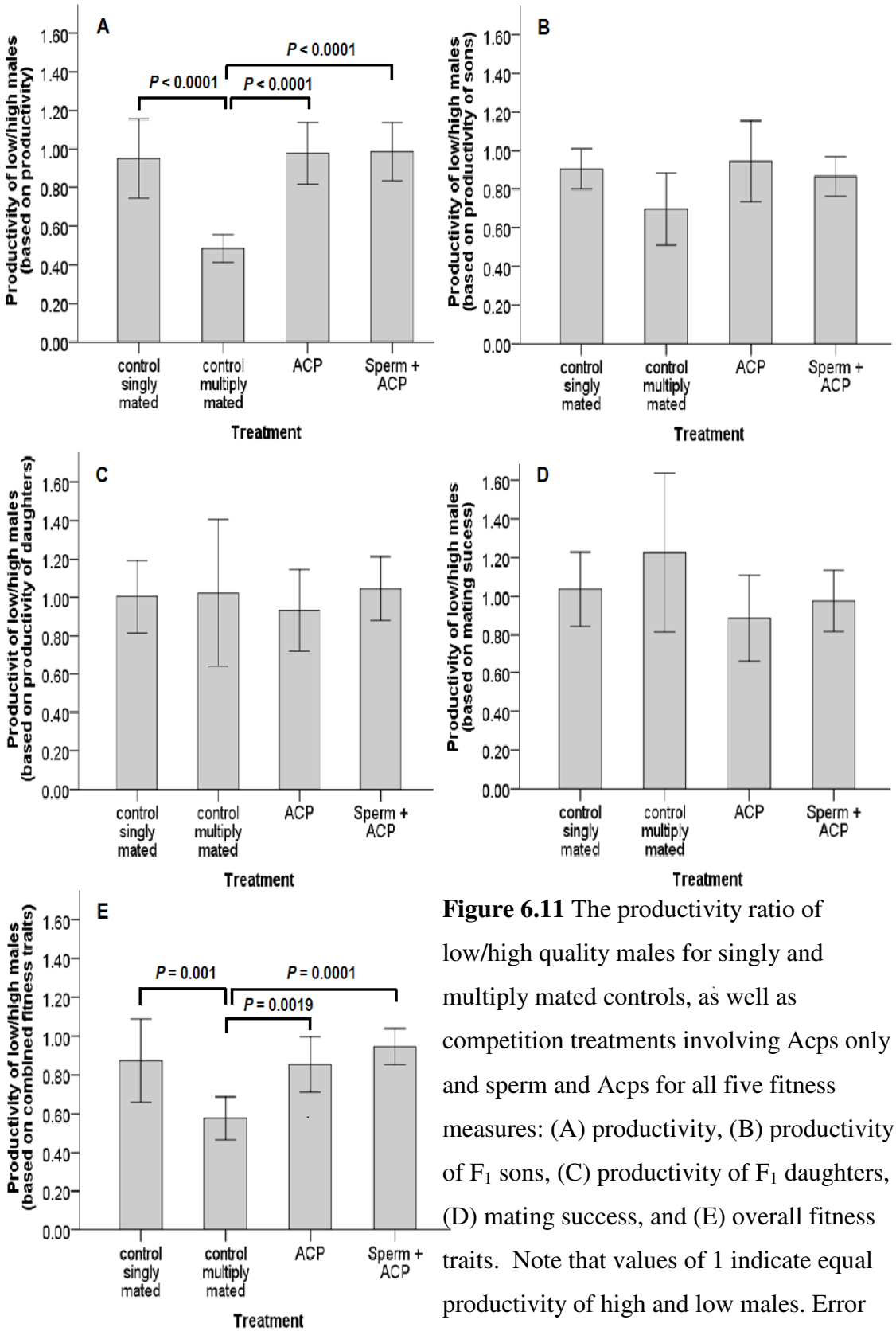


Figure 6.11 The productivity ratio of low/high quality males for singly and multiply mated controls, as well as competition treatments involving Acps only and sperm and Acps for all five fitness measures: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Note that values of 1 indicate equal productivity of high and low males. Error bars represent 95% CI.

two categories may increase or decrease based on the assay being performed. In comparing the ratio performance of low quality and high quality males across treatments, there was a significant effect of treatment for high quality and low quality males defined by (1) productivity (Figure 6.11A; $F_{3,32} = 19.17$, $P < 0.0001$) and (5) overall fitness measure (Figure 6.11E; $F_{3,32} = 10.85$, $P < 0.0001$). This effect is caused by a significant increase in the difference between low and high male productivity when those males are paired alone with a female but allowed to multiply mate (Figure 6.12). The benefits from multiply mating are apparent even at day 1 of eclosion, where high quality males paired alone with a female but allowed to multiply mate produced more offspring than females who singly mated to high quality males (Figure 6.12 A-C; t = 3.056, d.f. = 39.510, P = 0.004). Similarly, females multiply mated to high and low quality males have significantly different productivity beginning immediately at day 1 of eclosion (Figure 6.12 A-B; t = 2.626, d.f. = 57.392, P = 0.011), whereas females singly mated to high and low quality males only reveal significant differences of productivity after day 10 of eclosion (Figure 6.12 C-D; t = 2.111, d.f. = 318.167, P = 0.036).

6.4 Discussion

A male's reproductive success can be measured in a variety of ways, but the most accurate measurement is thought to be one that includes male mating success, the amount of offspring that are produced, and the quality of the offspring that are produced. When examining the data between high and low quality males for all five fitness measures (1: productivity, 2: productivity of F_1 sons; 3: productivity of F_1 daughters, 4: mating success in competition, and 5: overall fitness), the most consistent results that show significant differences between high and low quality males was indeed that of the combined fitness

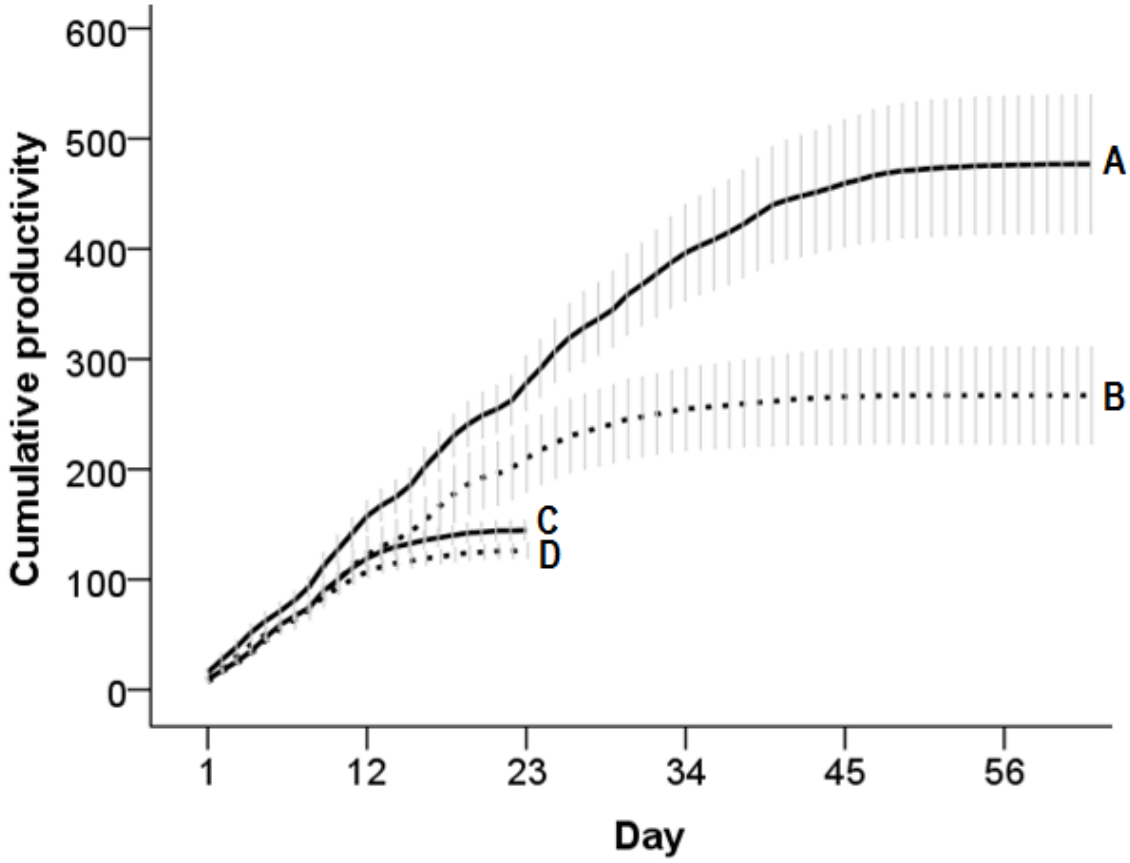


Figure 6.12 Average cumulative daily number of offspring for singly mated (C,D) and multiply mated (A, B) female controls. Solid lines represent high quality males, dashed lines represent low quality males based on combined fitness traits measure. Error bars represent 95% CI.

traits measure. This demonstrates, as expected, that both productivity and mating success contribute to an individual's total overall fitness (Stearns 1992), and that neither measure independently demonstrates a male's true fitness level. Thus, whether 'good genes' result in an increase in productivity or an increase in mating success is irrelevant (Zahavi 1975) as both contribute to an individual's reproductive success. This stresses the importance of measuring an inclusive and comprehensive set of fitness components as possible, including multi-generational fitness measurements of F_1 sons and daughters. Similarly to these results, significant differences when females were mated to attractive *vs.* unattractive males were not seen using individual fitness components, but were only revealed when the combined effects of son's attractiveness and daughters' fecundity were included in the model, further emphasizing the importance of using a multi-generational comprehensive set of fitness components (Head et al. 2005). The focus of the discussion will be on high and low quality male performances based on the overall combined fitness traits measure.

High quality males court faster and more often than low quality males. Although high quality males court earlier and more often, they were not more successful and females did not prefer them. This is surprising as there was a significant male quality and thorax size interaction; high quality males were larger than low quality males (Table C.2). Male thorax size is known to significantly correlate with an increase in fitness and mating success (Partridge and Farquhar 1983). Significant differences in thorax size would have given females a male trait to select upon. These results conflict with Partridge and Farquhar (1983) who showed larger males have faster mating speeds (time from courtship to copulation) (Partridge and Farquhar 1983). A lack of female preference

could be explained by using virgin female. It is possible that virgin females should not be choosy since they currently have no sperm storage and mating with any male should be beneficial and result in an increased fitness. The trade-up hypothesis states that females should only remate with higher quality males when she has already mated and therefore can afford to be choosy (Jennions and Petrie 2000). Furthermore, there was significant female and male line interaction for mating success (Table C.2), indicating the mating success of males depends on the female that he's courting. It is interesting to note that the mating success of males in this study did not correlate to their mating success when they were in a high density environment resulting in intense competition (Figure 6.8). Typical laboratory-based studies measuring mating success of *D. melanogaster* may not be indicative of what occurs in natural populations.

High quality males have a higher fertilization success as P_2 than low quality males (Figure 6.2E). Although there was no significant interaction between male quality and female line ($P = 0.1908$), P_2 values vary across female line even when using the same high or low quality male line (supplementary Figure C.8). Since sperm competition and postcopulatory sexual selection occurs in the female reproductive tract, this variation in P_2 success is likely influenced by the female environment. In *Callosobruchus maculatus* (Coleoptera: Bruchidae), male mating pairs that were mated to genetically similar females (full-siblings) had more repeatable P_2 values than if they were mated to unrelated females (Wilson et al. 1997); there was more variation in P_2 values when females were more variable. This suggests that females are capable of influencing male fertilization success. Cryptic female choice is more likely to occur when female mate choice is too costly (Birkhead and Pizzari 2002). Females can still rely on cryptic female choice for

preferential fertilization. For instance, in the feral fowl, *Gallus gallus domesticus* (Galliformes: Phasianidae), dominant and subordinate males had no difference in their mating success (Pizzari and Birkhead 2000). However, females who copulations with subordinate males were more likely to expel their ejaculates (Pizzari and Birkhead 2000). In this study, I did not detect any female mate choice as both low quality and high quality males had equal mating success, even though high quality males initiated courtship more often and earlier. In perceived competition when focal males were competed with a sterile spermless--Acp producing male, similar results were observed where high quality males fertilized more offspring than low quality males. Furthermore, there was significant female and male line interaction under perceived competition. This significant interaction indicates female influence in male success depending on the male, evidence for cryptic female choice (Pitnick and Brown 2000). However, this result could also be due to sperm competition in the instance of Acps. The presence of cryptic female choice under perceived competition (Acps competition) and the absence of cryptic female choice under direct sperm competition (sperm and Acps competition) indicates that the sperm itself may have a stronger influence than cryptic female choice on the outcome of sperm competition than.

These results do not agree with Bilde et al. (2009), whose study is very comparable. The authors found that high quality males based on productivity and productivity of daughters (comparable to my fitness measurements 1 and 3) performed worse when in competition with low quality males as they had a lower fertilization success (Bilde et al. 2009). I show no significant difference in fertilization success when male quality was based on productivity (fitness measure 1) and productivity of daughters (fitness measure 3). These

conflicting results may be due to the different species that were measured as their study was performed in the seed beetle *Callosobruchus maculatus* (Coleoptera: Chrysomelidae); different species may have different sexual selection strategies. However, when male quality was measured using a combined fitness trait, high quality males had higher fertilization success than low quality males. This highlights the importance of how the fitness measure used can impact the perception of high and low quality males.

Although high quality males started courting faster and courted longer, they were not more successful at mating compared to low quality males; females did not choose to mate with high quality males. This is surprising as high quality males outcompeted low quality males in both instances of sperm competition, indicating their superior quality. One would expect females to be able to recognize superior quality males and preferentially mate with them and/or a positive relationship between attractiveness and sperm quality. It is possible for postcopulatory sexual selection to reinforce precopulatory choice. The interaction between pre- and post-copulatory sexual selection has been examined in the guppy, *Poecilia reticulata* (Cyprinodontiformes: Poecilidae). Females prefer to mate with the more attractive colourful orange males who also court more readily. In an artificially inseminated experiment using equal amounts of ejaculates from two males, the more colourful male had the greatest share of paternity (Evans et al. 2003). These results demonstrate that when female mate choice is prevented, postcopulatory sexual selection can compensate, biasing traits that females desire since colourful males also have superior ejaculates. In contrast to the findings by Evans et al. (2003), my results may indicate a tradeoff between postcopulatory and precopulatory

performance; perhaps being superior in postcopulatory selection is expensive, and therefore little resources are left to allocate to precopulatory advantage.

A possible explanation for why high quality males may perform better in postcopulatory competition but do not excel in mating success may be due to females storing more sperm from males that are higher quality. In an experiment where the relative attractiveness of males was manipulated, female guppies contain 68% more sperm from males when they were perceived to be more attractive (Pilastro et al. 2004). Therefore, either females retain more sperm from attractive males or are able to manipulate the amount of sperm transferred from males. This is another example where postcopulatory sexual selection, in this case cryptic female choice, reinforces female mate choice. Another explanation would be that the ejaculates of high quality males contain more sperm than that of low quality males in this study. In the phenotype-linked fertility hypothesis, there is a positive correlation between male phenotype and functional fertility (Sheldon 1994). For instance, male guppies that are more colourful and therefore more attractive transfer more sperm to females, even though there was no significant difference between sperm stores of attractive and unattractive males at rest (Pilastro et al. 2002). However, I do not believe this to be the case as I did not detect any differences in male attractiveness in this study. Furthermore, the productivity for a high quality male line for a particular female line would be a low quality male line for a different female line (see supplementary Table C.1), indicating a female line interaction and that male quality is dependent on female line. It is possible that males can vary the amount of sperm transferred to a female. In the cricket *Acheta domesticus* and *Gryllodes supplicans* (Orthoptera: Gryllidae), males transferred more sperm to females when there was a

presence of increasing competition (Gage and Barnard 1996). *A. domesticus* males can also vary their sperm transfer with respect to female quality; they transferred more sperm when mating to larger females who are likely more fecund (Gage and Barnard 1996). In the guppy, males transfer different ejaculate sizes in solicited vs. forced copulations, indicating female control in ejaculate size for solicited copulations as there was a significant negative correlation between ejaculate size and mating speed (Pilastro et al. 2002). In this study, although high quality males did not have a greater mating success, they did have a longer copulation duration. This longer copulation duration may allow them to transfer more sperm, which would result in high quality males having a higher productivity when in sperm competition, perceived competition, and even no competition controls than compared to their low quality male counterparts (Figure 6.10, Figure 6.9, Figure 6.12 respectively). High quality males may be able to maintain a longer copulation due to their higher general fitness, or the increased copulation duration could potentially be a mechanism of cryptic female choice where females allow more attractive high quality males to mate longer. Similar results are seen in the damselfly, *Ceriagrion tenellum* (Odonata: Coenagrionidae) where males who copulated longer had a greater fertilization success (Andrés and Cordero Rivera 2000). In double mating experiments, smaller male orb-web spiders, *Argiope keyserlingi* (Araneae: Araneidae) had a higher fertilization success (P_2) with higher copulation duration -- a female controlled trait since females wrap the males in silk before cannibalizing them, ending copulation (Elgar et al. 2000). These studies demonstrate female manipulation of paternity through control of copulation duration.

Although low quality males had a lower performance than high quality males in all treatments, the performance of low quality males in relation to high quality males remained the same in the singly mated control and both treatments of competition (Figure 6.11E). This indicates low quality males do not suffer a loss in performance and high quality males do not perform better when in competition. However, low quality males will perform significantly worse when males are allowed to multiply mate. A cumulative curve (Figure 6.12) illustrates that high quality males have a longer period of productivity (a longer time until productivity plateaus) than low quality males when singly mated. When multiply mated, high quality males have both a greater initial productivity and longer productivity than low quality males. Therefore the greater effect on productivity when multiply mated (compared to singly mated) appears to be driven by both increased initial offspring production and increased duration of offspring production.

These results emphasize the importance of measuring an inclusive and comprehensive set of fitness components when assessing male quality as the fitness measure used can impact the perception of high and low quality males. When high quality males were defined using a combined fitness measure which incorporated both mating success and offspring fitness, high quality males performed consistently better than low quality males in both pre- and postcopulatory sexual selection.

6.5 Chapter acknowledgements

I thank Ben Rubin for his statistical assistance. This work would not be completed without the assistance of Amanda Tong, Pria Mahabir, Anes Kwon, Hannah Guiang, Amanda Morgan, Chaewon Jung, David Jo, Hassan Shahbaz, Hemani Patel, James Lim,

Jonwook Kim, Josh Skapinker, Josh Tordjman, Mathew Mathew, Patrick Zhang, Stephen Lu, Yoni Balboul, Sarah Kim, Injun Seo, and Alice Lee This work was supported by an NSERC Discovery Grant and a Canada Research Chair to Amanda J. Moehring.

6.6 References

Andrés, J. A., and A. Cordero Rivera. 2000. Copulation duration and fertilization success in a damselfly: an example of cryptic female choice? Anim. Behav. 59:695–703.

Bilde, T., A. Foged, N. Schilling, and G. Arnqvist. 2009. Postmating sexual selection favors males that sire offspring with low fitness. Science 324:1705–1706.

Birkhead, T. R., and T. Pizzari. 2002. Postcopulatory sexual selection. Nat. Rev. Genet. 3:262–273.

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J.-S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. Trends Ecol. Evol. 24:127–135.

Byrne, P. G., and W. R. Rice. 2006. Evidence for adaptive male mate choice in the fruit fly *Drosophila melanogaster*. Proc. R. Soc. Lond. B Biol. Sci. 273:917–922.

Clark, A. G., M. Aguadé, T. Prout, L. G. Harshman, and C. H. Langley. 1995. Variation in sperm displacement and its association with accessory gland protein loci in *Drosophila melanogaster*. Genetics 139:189–201.

Clark, A. G., and D. J. Begun. 1998. Female genotypes affect sperm displacement in *Drosophila*. Genetics 149:1487–1493.

Eberhard, W. G. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton, New Jersey.

Edvardsson, M., and G. Arnqvist. 2000. Copulatory courtship and cryptic female choice in red flour beetles *Tribolium castaneum*. Proc. R. Soc. Lond. B Biol. Sci. 267:559–563.

Elgar, M. A., J. M. Schneider, and M. E. Herberstein. 2000. Female control of paternity in the sexually cannibalistic spider *Argiope keyserlingi*. Proc. R. Soc. Lond. B Biol. Sci. 267:2439–2443.

Evans, J. P., L. Zane, S. Francescato, and A. Pilastro. 2003. Directional postcopulatory sexual selection revealed by artificial insemination. Nature 421:360–363.

Fedorka, K. M., and T. A. Mousseau. 2002. Material and genetic benefits of female multiple mating and polyandry. Anim. Behav. 64:361–367.

Friberg, U. 2006. Male perception of female mating status: its effect on copulation duration, sperm defence and female fitness. Anim. Behav. 72:1259–1268.

Gage, A. R., and C. J. Barnard. 1996. Male crickets increase sperm number in relation to competition and female size. Behav. Ecol. Sociobiol. 38:349–353.

Head, M. L., J. Hunt, M. D. Jennions, and R. Brooks. 2005. The indirect benefits of mating with attractive males outweigh the direct costs. PLoS Biol 3:e33.

Hosken, D. J., M. L. Taylor, K. Hoyle, S. Higgins, and N. Wedell. 2008. Attractive males have greater success in sperm competition. Curr. Biol. 18:R553–R554.

Jennions, M. D., and M. Petrie. 2000. Why do females mate multiply? A review of the genetic benefits. Biol. Rev. 75:21–64.

Lewis, S. M., and S. N. Austad. 1990. Sources of intraspecific variation in sperm precedence in red flour beetles. Am. Nat. 135:351–359.

Moller, A. P., and M. D. Jennions. 2001. How important are direct fitness benefits of sexual selection. Naturwissenschaften 88:401–415.

Neff, B. D., and T. E. Pitcher. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Mol. Ecol. 14:19–38.

Nguyen, T., and A. Moehring. (in press). Accurate alternative measurements for female lifetime reproductive success in *Drosophila melanogaster*. PLoS ONE.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biol. Rev. 45:525–567.

Partridge, L., and M. Farquhar. 1983. Lifetime mating success of male fruitflies (*Drosophila melanogaster*) is related to their size. Anim. Behav. 31:871–877.

Pilastro, A., J. P. Evans, S. Sartorelli, and A. Bisazza. 2002. Male phenotype predicts insemination success in guppies. Proc. R. Soc. Lond. B Biol. Sci. 269:1325–1330.

Pilastro, A., M. Simonato, A. Bisazza, and J. P. Evans. 2004. Cryptic female preference for colorful males in guppies. Evolution 58:665–669.

Pitnick, S., and W. D. Brown. 2000. Criteria for demonstrating female sperm choice. Evolution 54:1052–1056.

Pitnick, S., and T. A. Markow. 1994. Large-male advantages associated with costs of sperm production in *Drosophila hydei*, a species with giant sperm. Proc. Natl. Acad. Sci. 91:9277–9281.

Pitnick, S., T. A. Markow, and G. S. Spicer. 1995. Delayed male maturity is a cost of producing large sperm in *Drosophila*. Proc. Natl. Acad. Sci. 92:10614–10618.

Pizzari, T., and T. R. Birkhead. 2000. Female feral fowl eject sperm of subdominant males. Nature 405:787–789.

Ram, K. R., and M. F. Wolfner. 2007. Seminal influences: *Drosophila* Acps and the molecular interplay between males and females during reproduction. Integr. Comp. Biol. 47:427–445.

Ridley, M. 1989. The incidence of sperm displacement in insects: four conjectures, one corroboration. Biol. J. Linn. Soc. 38:349–367.

Schaus, J. M., and S. K. Sakaluk. 2001. Ejaculate expenditures of male crickets in response to varying risk and intensity of sperm competition: not all species play games. Behav. Ecol. 12:740–745.

Sheldon, B. C. 1994. Male phenotype, fertility, and the pursuit of extra-pair copulations by Female Birds. Proc. R. Soc. Lond. B Biol. Sci. 257:25–30.

Sheldon, B. C., J. Merilö, A. Qvarnström, L. Gustafsson, and H. Ellegren. 1997. Paternal genetic contribution to offspring condition predicted by size of male secondary sexual character. Proc. R. Soc. Lond. B Biol. Sci. 264:297–302.

Snook, R. R. 2005. Sperm in competition: not playing by the numbers. Trends Ecol. Evol. 20:46–53.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, New York.

Tallamy, D. W., M. B. Darlington, J. D. Pesek, and B. E. Powell. 2003. Copulatory courtship signals male genetic quality in cucumber beetles. Proc. R. Soc. Lond. B Biol. Sci. 270:77–82.

Thomas, M. L., and L. W. Simmons. 2007. Male crickets adjust the viability of their sperm in response to female mating status. Am. Nat. 170:190–195.

Wagner, W. E., R. J. Kelley, K. R. Tucker, and C. J. Harper. 2001. Females receive a life-span benefit from male ejaculates in a field cricket. Evolution 55:994– 1001.

Wedell, N., and M. G. Ritchie. 2004. Male age, mating status and nuptial gift quality in a bushcricket. Anim. Behav. 67:1059–1065.

Wilson, N., S. C. Tubman, P. E. Eady, and G. W. Robertson. 1997. Female genotype affects male success in sperm competition. Proc. R. Soc. Lond. B Biol. Sci. 264:1491–1495.

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

Chapter 7

7 Overview

Sexual selection results in differential reproductive success, where reproductive success can be defined as the number of offspring an individual produces over its lifetime. Various factors can affect lifetime reproductive success including genetic quality, mating success, and postcopulatory sexual selection. Here I determined how to accurately measure lifetime reproductive success and its genetic architecture in a multi-generational study. I quantified mating success using a novel approach and correlated it to lifetime reproductive success to determine the direct and indirect benefits females may receive. To further incorporate an inclusive view of sexual selection, I assessed male quality using various fitness traits and measured male performance in both pre- and postcopulatory sexual selection. I also teased apart the mechanisms of sperm competition and the role that sperm and proteins found in the seminal fluid (Accessory gland proteins, Acps) play in the second male advantage in gaining fertilizations.

7.1 Lifetime reproductive success

Sexual selection studies often measure phenotypic variation of a fitness trait. Studies attempting to measure fitness often measure more tractable surrogates of fitness such as body size, survivability, viability, growth rate, mating success, longevity, fecundity, or fertility (Reid et al. 2004; Anderson et al. 2007; Hosokawa et al. 2007). Of these alternative measurements, the number of offspring an individual produces over its lifetime (lifetime reproductive success) is generally considered to be an acceptable estimate of fitness (Stearns 1992; Brommer et al. 2004; Hunt and Hodgson, D. 2010).

However, these studies are very rarely multi-generational as measuring fitness traits such as lifetime reproductive success can be very time consuming and often not feasible. To make the fitness measure of lifetime reproductive success more feasible within a commonly-used model system, I examined alternative measurements of lifetime reproductive success in *Drosophila melanogaster*. I determined that measuring the short term cumulative productivity of a singly mated female for five days can accurately predict her total lifetime reproductive success (Nguyen and Moehring, in press). However, it is important to note that using this short term measure of five days as a surrogate for total lifetime reproductive success applies to singly mated females only, as no correlation between singly and multiply mated females was found (Nguyen and Moehring, in press).

Since reproductive success involves both the reproductive output of both the parents and their offspring, to obtain accurate measurements of reproductive success it is important to examine breeding values and the phenotypic variation in the grandchildren. An initial decline in fitness can often be compensated for in future generations, and this would not be reflected in single-generation studies (Kokko et al. 2003). Therefore, the lifetime reproductive success of F_1 sons and F_1 daughters was obtained and quantitative genetic analysis was performed using the Cockerham and Weir Biomodel (Cockerham and Weir 1977; Lynch and Walsh 1988). Results show that although there was no genetic variation in lifetime reproductive success in the parental generation, there is significant variation in the F_1 generation which would not have been detected in a single generation study (Nguyen and Moehring, submitted).

7.2 Genetics of sexual selection

As genetic tools are becoming more available, studies of sexual selection are focusing more on the underlying genetic causes of selection and phenotypic traits. Quantitative genetic analysis of phenotypic traits in sexual selection is often not feasible as they involve lifetime reproductive success measurements (the number of offspring an individual produces throughout its lifetime). However, the genetic architecture of phenotypic traits in sexual selection can be estimated by partitioning the variance into additive, non-additive, and parental affects (Cockerham and Weir 1977; Lynch and Walsh 1988).

Mating is costly for both sexes, although usually more for females (Turner and Anderson 1983; Fowler and Partridge 1989; Magurran and Nowak 1991; Pitnick and Markow 1994; Rowe 1994; Chapman et al. 1995; Pitnick et al. 1995; Snook 2005). Females can benefit by selectively mating to provide indirect genetic benefits to offspring. Models of genetic benefits come in the form of good genes through additive genetic variation or in compatible genes through non-additive genetic variation (Neff and Pitcher 2005). Very few studies have examined the relationship between parental fitness and the fitness of each sex of resulting offspring (Kokko 2001). When assessing this relationship, I found that lifetime reproductive success was a result of additive genetic variation (good genes) for F_1 daughters (Nguyen and Moehring, submitted). Furthermore, F_1 females were also more sensitive to inbreeding depression (Nguyen and Moehring, submitted). These results indicate a sex specific effect as F_1 daughters were most strongly influenced by good genes and inbreeding depression.

Due to the increasing focus of quantitative genetic analysis, several studies have identified the genetic basis for phenotypes involving fitness (Bilde et al. 2008), mating (Lawniczak and Begun 2005; Hughes and Leips 2006; Lew et al. 2006), and sperm competition (Civetta and Clark 2000). Although these studies have identified genomic regions that contribute to some of the phenotypic traits involved in sexual selection, few individual candidate genes have been identified. Identifying polymorphic genes in natural populations causing phenotypic variation in sexually selected traits would be a significant contribution to the field of sexual selection. For instance, although we know that sperm precedence is a result of non-additive genetic variation (Hughes 1997), very little is known about the molecular basis of this variation. Genetic variation in sperm precedence likely involves seminal fluid proteins (Accessory gland proteins, or Acps), as Acps are known to be involved in sperm transfer and are required for sperm storage (Tram and Wolfner 1999), but a direct link between variation in Acps and variation in sperm precedence has not yet been shown.

7.3 Accessory gland proteins in sperm competition

Sperm competition consists of not only sperm itself but also of the proteins found in the seminal fluid, Acps (Accessory gland proteins). Acps are known to have a variety of affects on female behaviour, and consequently increase the male's reproductive success (Ram and Wolfner 2007). Second male advantage is a widespread phenomenon where the second male to mate fathers the majority of offspring (P_2). Several mechanisms have been identified to explain second male advantage, of which both sperm and Acps are thought to play a significant role (Price et al. 1999; Manier et al. 2010). Acps from the second mated male can cause females to eject the first male's sperm (Manier et al. 2010)

and can cause incapacitation of residing sperm from the first male (Price et al. 1999). In Chapter 5, I identified an additional mechanism that contributes to second male advantage. Acps from the first mated male have a "protective effect" on the sperm from the second mated male, increasing sperm longevity and extending the female's egg laying duration (Nguyen and Moehring, Chapter 5). However, it is unclear how this is achieved on a molecular level. There is very little knowledge on the function of Acps. Out of 112 Acps identified in *D. melanogaster*, only a handful of them are characterized (Ram and Wolfner 2007).

Association tests can be used to identify polymorphic regions or candidate genes in the variation of natural populations and link them to a phenotype. Using these tests, several Acps have been identified to associate with sperm competition. P_2 values and offensive traits in sperm competition are associated with *Acp29AB, Acp33A, CG17331*, *CG6168, Acp26Aa* and *Acp62F*, (Fiumera et al. 2005, 2007), while P_1 values and defensive traits are associated with *CG8137, CG6168, Acp33A, Acp26Aa/Ab, Acp29B*, *Acp36DE* and *Acp53E* (Clark et al. 1995; Fiumera et al. 2005). However, association studies merely provide a correlation for identifying candidate genes. To prove the causality of these candidate genes, transgenics involving targeted mutation, knock-down/knock-out, and genetic rescue experiments need to be performed.

The detailed mechanism by which Acps achieve their function is unknown. Although it is clear Acps play an important role in sperm competition through incapacitation, displacing, dumping, and causing behavioural changes in females, very little is known about the molecular mechanisms and pathways of Acps. Furthermore, the majority of our knowledge on Acps are limited to *D. melanogaster,* with the exception of Acps being

identified in a few other insects (Ram and Wolfner 2007). The first step after gene identification would therefore be to characterize how these proteins function in sperm competition within a model system. The tests of candidate genes and their functions would then need to be repeated in other species in order to determine whether these functions are conserved across taxa. With improved genetic tools and increased interest in sexual selection, the identification and characterization of Acps and their roles in sexual selection will expand, increasing our knowledge of the molecular interplay between males and females.

7.4 Inclusive view of sexual selection

The Fisherian and good genes models have often been pitted against each other in the field of sexual selection. In the Fisherian model, the attractive trait is arbitrary and only increases a male's mating success, whereas in the good genes model, the attractive trait is an honest indicator of male quality and condition (Fisher 1930; Zahavi 1975; Hamilton and Zuk 1982). If attractive males produce sons of higher fitness and vitality, it is often assumed to be a result that aligns with the good genes theory. A lack of this relationship or a negative correlation would indicate that the Fisherian model is more likely to be correct. However, it is possible that females still gain a fitness benefit by mating with these males with lower survival. Males possessing 'good genes' can invest more heavily in mating success than other fitness traits, causing a reduction in survival and lifespan (Kokko 2001). However, it is irrelevant whether a male is of high quality due to survivorship or an increased mating success as both are indicators of high breeding value and total fitness (Kokko et al. 2003). A more inclusive approach to understanding sexual selection and female mate choice should therefore be adopted that defines good genes as

a combination of both mating success and the success of the offspring that are produced (sexy sons hypothesis) (Kokko 2001; Kokko et al. 2003). In Chapter 4, I quantified an inclusive measure of sexual selection by correlating male mating success to male quality. To measure male mating success, I used a novel experimental design representative of what occurs in nature that allowed for intense male-male and female-female interactions within a mating arena. Furthermore, I incorporated the indirect fitness benefits to offspring as a part of my measure of male quality. This is one of the few studies that has measured male attractiveness and the direct fitness effects on females as well as the indirect benefits females may gain in their offspring. Surprisingly, I found that males with a high mating success produced low quality sons (Nguyen and Moehring, Chapter 4). This is most likely due to differential allocation of resources, as it is costly for males to possess both a mating advantage and be of high quality.

As previously stated, both productivity and mating success contribute to an individual's total overall fitness (Stearns 1992). An inclusive and comprehensive set of fitness components, including the multi-generational fitness of F_1 sons and daughters, also contribute to overall reproductive success. Likewise, the environment (i.e., male and female condition, presence of competition, mating order, etc.) can alter reproductive strategies (Byrne and Rice 2006; Wigby et al. 2009; Nguyen and Moehring, Chapter 5). To study sexual selection in varying context and environments, in Chapter 6 I identified high and low quality males using the five fitness measures across 10 female lines: (1) productivity, (2) productivity of F_1 sons, (3) productivity of F_1 daughters, (4) mating success in competition, and (5) combined fitness traits. Therefore, high and low quality males used in experimentation are fitness measure specific and female line specific. The

five fitness measures incorporated an inclusive view of male quality and the use of multiple isofemale lines accounted for genotypic variation.

In assessing high and low quality male performance in both pre- and postcopulatory sexual selection, I noticed the most consistent results across treatments for the combined fitness measure (measure 5) (Nguyen and Moehring, Chapter 6). I determined that high quality males courted earlier and more often, but not longer than low quality males; females did not accept high quality male courtship more readily. However, high quality males did copulate longer. Furthermore, high quality males produced more offspring when in sperm competition (competing with Acps alone, or sperm and Acps) than low quality males. Females do not play a passive role in postcopulatory sexual selection. Male (Clark et al. 1995) and female (Clark and Begun 1998) genotypes, and their interactions (Clark et al. 1999) can affect postcopulatory sexual selection and sperm displacement. I found significant female x male interactions when focal males were competing with Acps, indicating the presence of cryptic female choice. However, no significant female x male interactions were detected when focal males were competing with both sperm and Acps. These outcomes suggest the possibility of sperm competition interactions having a stronger influence on fertilization success than cryptic female choice. The results in Chapter 6 emphasize the importance of measuring an inclusive and comprehensive set of fitness components when assessing male quality and the significance of studying sexual selection across varying genotypes, specifically female genotypes since female x male interactions can significantly affect reproductive success.

7.5 Concluding remarks

Sexual selection, an important branch of natural selection, results in variation of reproductive success. Both males and females have evolved reproductive strategies to increase their fitness. Inclusive views of sexual selection that incorporate components of male fitness, female fitness, and their interactions are likely the most accurate. Comprehensive sets of fitness components should be measured in a multi-generational study, where genetic quality incorporates mating success, survivorship, and the reproductive success of the offspring.

7.6 References

Anderson, W. W., Y.-K. Kim, and P. A. Gowaty. 2007. Experimental constraints on mate preferences in *Drosophila pseudoobscura* decrease offspring viability and fitness of mated pairs. Proc. Natl. Acad. Sci. 104:4484–4488.

Bilde, T., U. Friberg, A. Maklakov, J. Fry, and G. Arnqvist. 2008. The genetic architecture of fitness in a seed beetle: assessing the potential for indirect genetic benefits of female choice. BMC Evol. Biol. 8:295.

Brommer, J. E., L. Gustafsson, H. Pietiäinen, and J. Merilä. 2004. Single generation estimates of individual fitness as proxies for long term genetic contribution. Am. Nat. 163:505–517.

Byrne, P. G., and W. R. Rice. 2006. Evidence for adaptive male mate choice in the fruit fly *Drosophila melanogaster*. Proc. R. Soc. Lond. B Biol. Sci. 273:917–922.

Chapman, T., L. F. Liddle, J. M. Kalb, M. F. Wolfner, and L. Partridge. 1995. Cost of mating in *Drosophila malanogaster* females is mediated by male accessory-gland products. Nature 373:241–244.

Civetta, A., and A. G. Clark. 2000. Chromosomal effects on male and female components of sperm precedence in *Drosophila*. Genet. Res. 75:143–151.

Clark, A. G., M. Aguadé, T. Prout, L. G. Harshman, and C. H. Langley. 1995. Variation in sperm displacement and its association with accessory gland protein loci in *Drosophila melanogaster*. Genetics 139:189–201.

Clark, A. G., and D. J. Begun. 1998. Female genotypes affect sperm displacement in *Drosophila*. Genetics 149:1487–1493.

Clark, A. G., D. J. Begun, and T. Prout. 1999. Female × male interactions in *Drosophila* sperm competition. Science 283:217–220.

Cockerham, C. C., and B. S. Weir. 1977. Quadratic analyses of reciprocal crosses. biometrics 33:187–203.

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

Fiumera, A. C., B. L. Dumont, and A. G. Clark. 2007. Associations between sperm competition and natural variation in male reproductive genes on the third chromosome of *Drosophila melanogaster*. Genetics 176:1245–1260.

Fiumera, A. C., B. L. Dumont, and A. G. Clark. 2005. Sperm competitive ability in *Drosophila melanogaster* associated with variation in male reproductive proteins. Genetics 169:243–257.

Fowler, K., and L. Partridge. 1989. A cost of mating in female fruitflies. Nature 338:760–761.

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

Hosokawa, T., Y. Kikuchi, M. Shimada, and T. Fukatsu. 2007. Obligate symbiont involved in pest status of host insect. Proc. R. Soc. B Biol. Sci. 274:1979–1984.

Hughes, K. A. 1997. Quantitative genetics of sperm precedence in *Drosophila melanogaster*. Genetics 145:139–151.

Hughes, K. A., and J. Leips. 2006. Quantitative trait locus analysis of male mating success and sperm competition in *Drosophila melanogaster*. Evolution 60:1427–1434.

Hunt, J., and Hodgson, D. 2010. What is fitness, and how do we measure it? In: Evolutionary Behavioral Ecology. Oxford University Press, New York, New York.

Kokko, H. 2001. Fisherian and "good genes" benefits of mate choice: how (not) to distinguish between them. Ecol. Lett. 4:322–326.

Kokko, H., R. Brooks, M. D. Jennions, and J. Morley. 2003. The evolution of mate choice and mating biases. Proc. R. Soc. B Biol. Sci. 270:653–664.

Lawniczak, M. K. N., and D. J. Begun. 2005. A QTL analysis of female variation contributing to refractoriness and sperm competition in *Drosophila melanogaster*. Genet. Res. 86:107–114.

Lew, T. A., E. H. Morrow, and W. R. Rice. 2006. Standing genetic variance for female resistance to harm from males and its relationship to intralocus sexual conflict. Evolution 60:97–105.

Lynch, M., and B. Walsh. 1988. Genetics and analysis of quantitative traits. Sinauer Associates Inc., Sunderland, MA.

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

Manier, M. K., J. M. Belote, K. S. Berben, D. Novikov, W. T. Stuart, and S. Pitnick. 2010. Resolving mechanisms of competitive fertilization success in *Drosophila melanogaster*. Science 328:354–357.

Neff, B. D., and T. E. Pitcher. 2005. Genetic quality and sexual selection: an integrated framework for good genes and compatible genes. Mol. Ecol. 14:19–38.

Nguyen, T., and A. Moehring. (in press). Accurate alternative measurements for female lifetime reproductive success in *Drosophila melanogaster*. PLoS ONE.

Nguyen, T., and A. Moehring. (submitted). Daughters affected most strongly by good genes and inbreeding depression. Evolution.

Pitnick, S., and T. A. Markow. 1994. Large-male advantages associated with costs of sperm production in *Drosophila hydei*, a species with giant sperm. Proc. Natl. Acad. Sci. 91:9277–9281.

Pitnick, S., T. A. Markow, and G. S. Spicer. 1995. Delayed male maturity is a cost of producing large sperm in *Drosophila*. Proc. Natl. Acad. Sci. 92:10614–10618.

Price, C. S. C., K. A. Dyer, and J. A. Coyne. 1999. Sperm competition between *Drosophila* males involves both displacement and incapacitation. Nature 400:449–452.

Ram, K. R., and M. F. Wolfner. 2007. Seminal influences: *Drosophila* Acps and the molecular interplay between males and females during reproduction. Integr. Comp. Biol. 47:427–445.

Reid, J. M., P. Arcese, A. L. E. V. Cassidy, S. M. Hiebert, J. N. M. Smith, P. K. Stoddard, A. B. Marr, and L. F. Keller. 2004. Song repertoire size predicts initial mating success in male song sparrows, *Melospiza melodia*. Anim. Behav. 68:1055–1063.

Rowe, L. 1994. The costs of mating and mate choice in water striders. Anim. Behav. 48:1049–1056.

Snook, R. R. 2005. Sperm in competition: not playing by the numbers. Trends Ecol. Evol. 20:46–53.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, New York.

Tram, U., and M. F. Wolfner. 1999. Male seminal fluid proteins are essential for sperm storage in *Drosophila melanogaster*. Genetics 153:837–844.

Turner, M. E., and W. W. Anderson. 1983. Multiple mating and female fitness in *Drosophila pseudoobscura*. Evolution. 37: 74-723

Wigby, S., L. K. Sirot, J. R. Linklater, N. Buehner, F. C. F. Calboli, A. Bretman, M. F. Wolfner, and T. Chapman. 2009. Seminal fluid protein allocation and male reproductive success. Curr. Biol. 19:751–757.

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

Appendices

Appendix A: Chapter 3 supplemental material

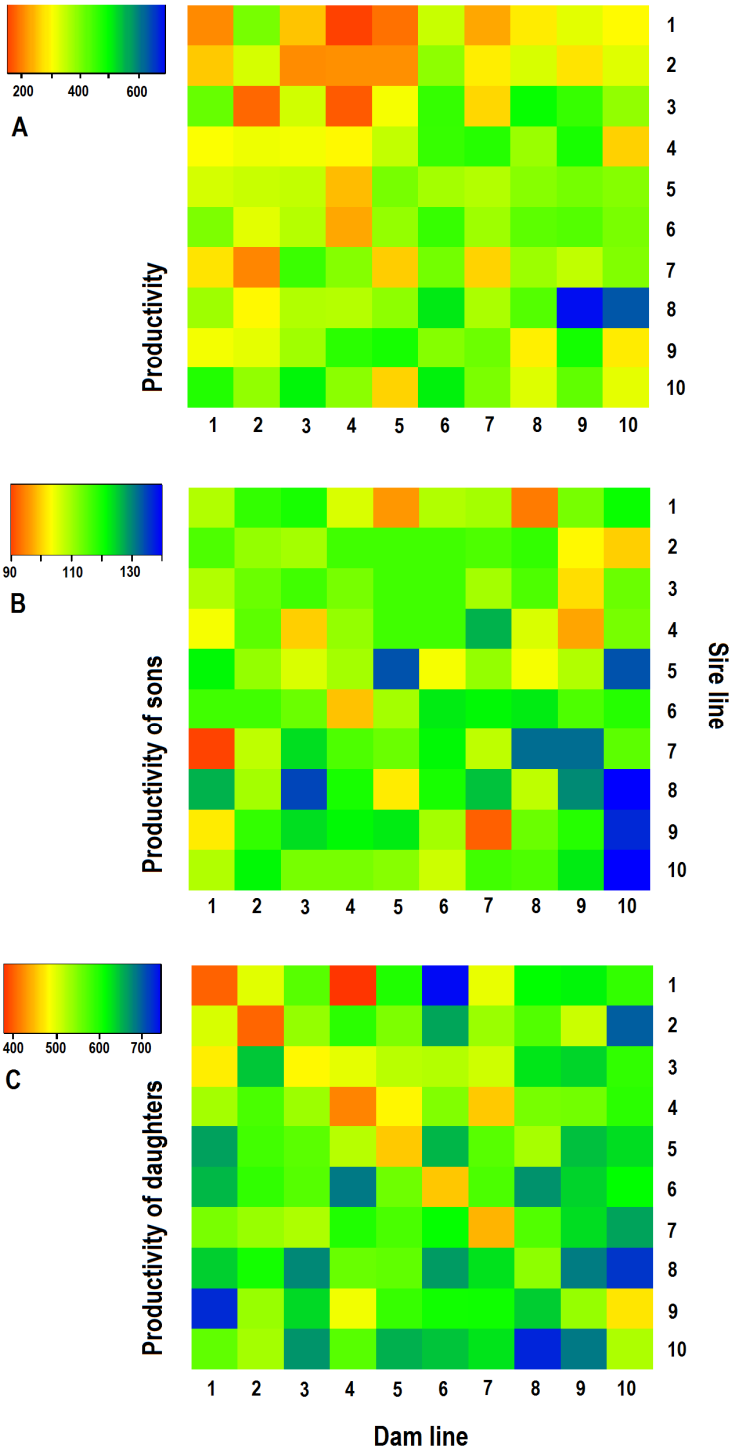


Figure A.1 Heat map of mean productivity for the dialell cross of (A) parentals, (B) F_1 sons and (C) F_1 daughters. The numbers on the X and Y axis represent the ten isofemale lines; the heat map values represent the number of offspring that were produced.

Appendix B: Chapter 5 supplementary material

Table B.1 The daily eclosion for the control (without competition) was compared to the experimental treatment (competition where the first male has only Acps). Day 1-24 was performed with independent 2-group t-tests, while day 25-31 was performed with a one-sample one sided t-test.

Eclosion day	**t**	**d.f.**	***P***
1	-0.362	1791.956	0.717
2	1.714	1762.417	0.087
3	2.102	1789.072	0.036
4	1.551	1745.321	0.121
5	1.681	1791.709	0.093
6	1.861	1751.826	0.063
7	-1.605	1759.321	0.109
8	-7.587	1741.396	<0.0001
9	5.883	1654.000	<0.0001
10	-0.460	1791.452	0.646
11	1.126	1787.319	0.260
12	-0.598	1787.063	0.550
13	-1.200	1739.345	0.230
14	-2.757	1774.737	0.006

15	-10.614	1346.436	<0.0001
16	-5.244	1743.795	<0.0001
17	-4.922	1678.305	<0.0001
18	-2.232	1788.604	0.026
19	-3.836	1545.065	<0.0001
20	-1.819	1490.661	0.069
21	-2.706	1688.127	0.007
22	-1.381	1789.793	0.167
23	-3.925	1643.22	<0.0001
24	-3.665	1474.442	<0.0001
25	5.8316	931	<0.0001
26	4.9018	931	<0.0001
27	3.2931	931	0.0005
28	3.8316	931	<0.0001
29	2.9707	931	0.0015
30	1.9468	931	0.0259
31	1.8638	931	0.0313

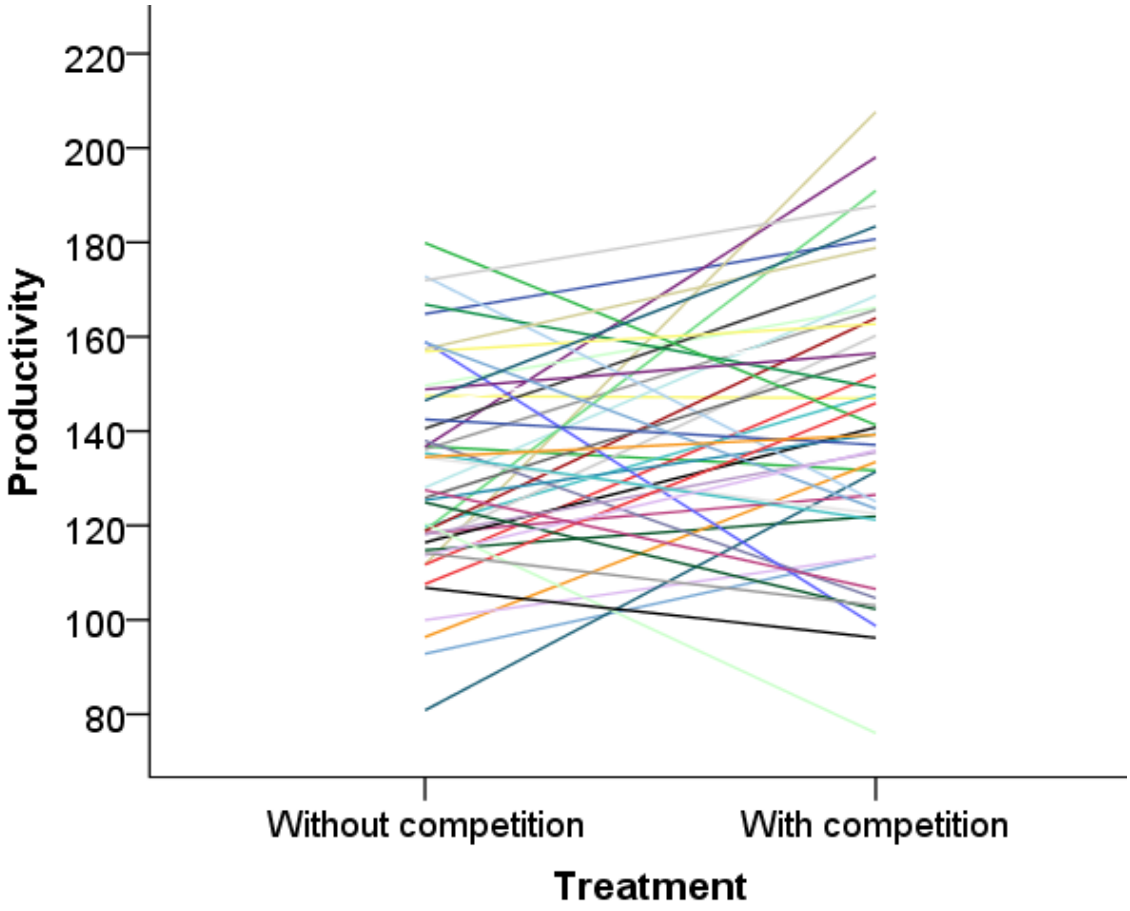


Figure B.1 Colored lines link the average productivity (number of offspring produced) of females from an isofemale line when mated to a single male (without competition) to the same isofemale line when mated first to a spermless, Acp-producing male and then an isofemale line male (with competition). LMM reveals non significant three way interaction of male line, female line, and treatment effects.

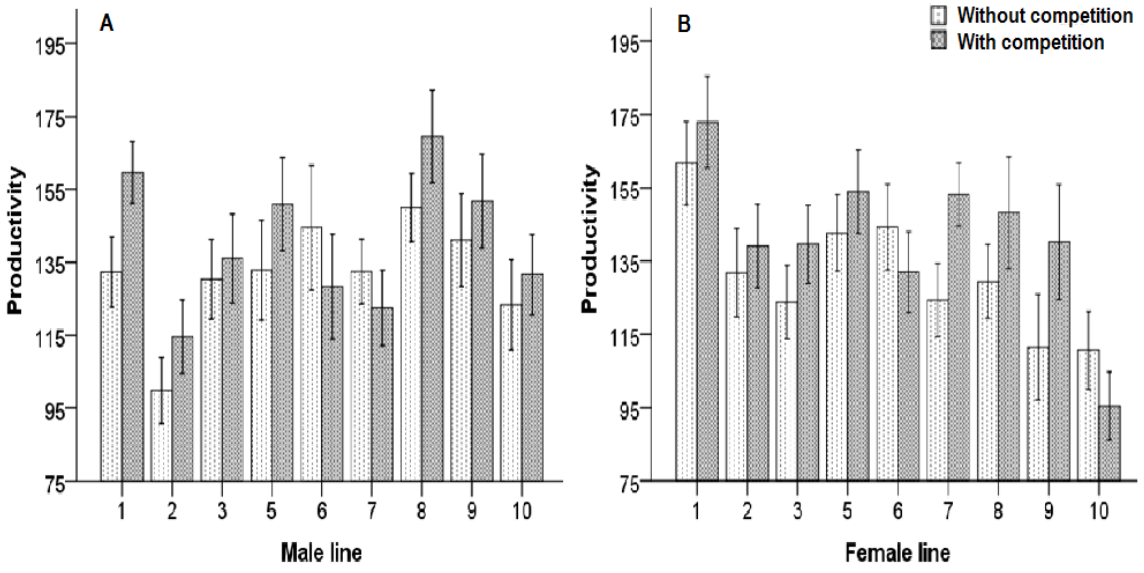


Figure B.2 Productivity (number of offspring produced) when sorted by sex and line. Individuals were either singly mated (without competition, light bars) or were mated in competition (dark bars) where females were initially mated to a spermless, Acp-producing male and then the isofemale line male. LMM reveals significant male line and treatment interaction effects as well as female line and treatment interaction effects.

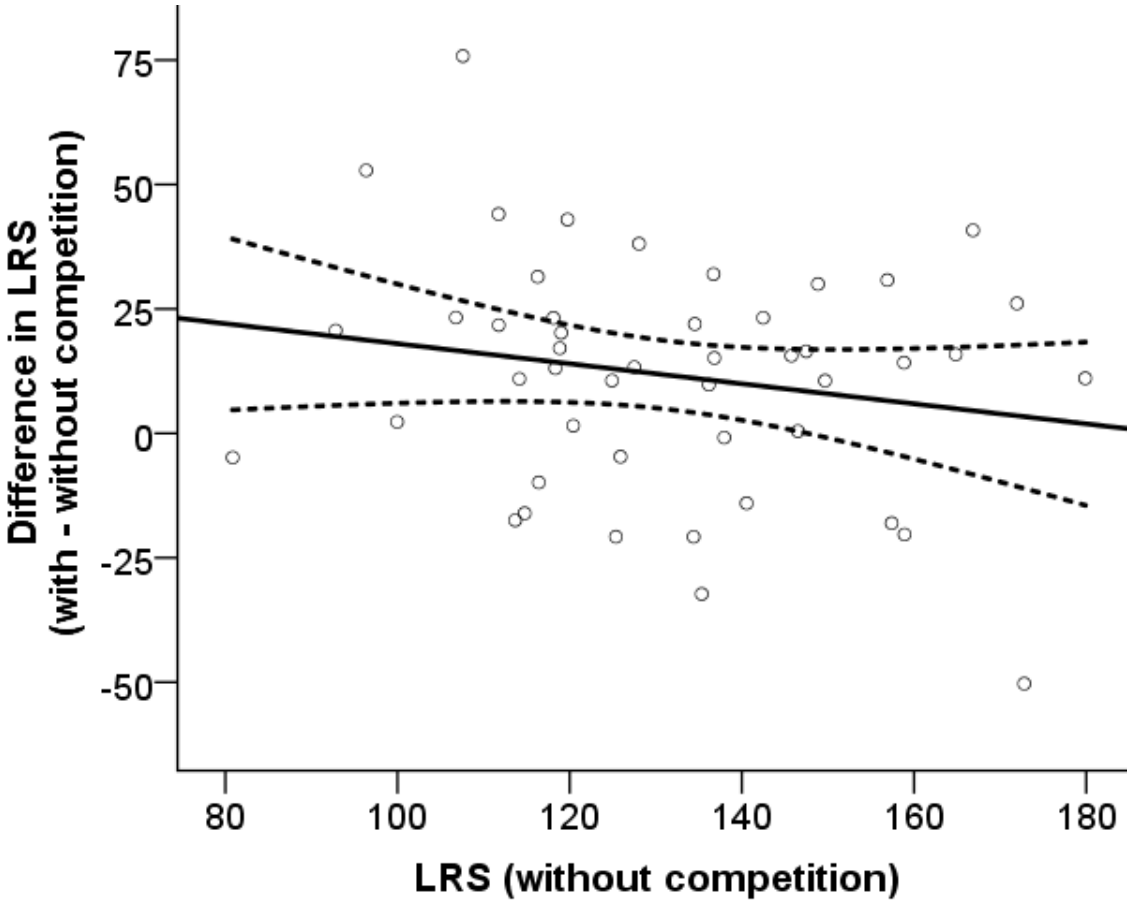


Figure B.3 Difference in lifetime reproductive success (LRS, a measure of productivity) between the experimental treatment (competition where the first male has only Acps) and the control (without competition) regressed on productivity of the control treatment (without competition). This detects whether the increase in productivity due to competition was greater for males who have low productivity or high productivity when not in competition. A Linear Model (LM) regression was used ($R^2 = 0.036$, d.f. = 45, $P = 0.196$). Dashed lines represent 95% CI.

Appendix C: Chapter 6 supplementary material

Table C.1 Identity of high and low quality male lines for each isofemale line for all five fitness measures. Shaded combination crosses were not performed. Isofemale line 4 was lost at the time of experiment. Therefore, any female line that had a corresponding male line 4 as a high or low quality male was replaced with the next highest or lowest male line (as shown in parenthesis).

Female Line	**Male Line**									
	Productivity		**Productivity of F_1 sons**		**Productivity of F_1 daughters**		**Mating success**		**Overall fitness measure**	
	High	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**	**High**	**Low**
1	10	1	8	7	9	1	1	10	8	4(7)
2	1	3	10	7	3	2	3	7	10	7
3	10	2	8	4(5)	8	3	1	6	8	4(2)
4										
5	9	1	5	1	10	5	8	7	9	1
6	8	1	6	1	1	6	2	9	8	9
7	4(9)	1	4(8)	9	8	7	2	5	8	7
8	3	1	7	1	10	8	1	7	3	1
9	8	2	8	2	8	2			8	2
10	8	4(9)	8	4(2)	8	9	3	9	8	9

Table C.2 Various models for mating analysis. Variables that were not significant from the log likelihood test were removed in the reduced model unless they were significant in a higher order interaction.

Model	**Respone variable**									
Percentage of high quality and low quality males that courted (See Figure 6.2)	**Male quality**		**Female line**		**Female line * Male quality**		**Male thorax size**		**Male quality * Male thorax size**	
	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***
A. Productivity	19.1070	<0.0001	26.3020	<0.0001	1.1145	0.2911	9.1646	0.0024	7e-04	0.9792
B. Productivity of sons	12.8720	0.0003	0.4481	0.5033	0.8979	0.3433	0.0808	0.7762	0	1
C. Productivity of daughters	1.5039	0.2201	0	1	3.0500	0.0807	0.5764	0.4477	0.1760	0.6748
D. Mating success	0	0.9977	0.4375	0.5084	6.6291	0.0100	0.4740	0.4912	0	1
E. Combined fitness traits	11.5140	0.0006	2.4148	0.1202	0	1	9.8984	0.0016	0	1

Model	**Respone variable**									
Time taken for high quality and low quality males to start courting (See Figure 6.3)	**Male quality**		**Female line**		**Female line * Male quality**		**Male thorax size**		**Male quality * Male thorax size**	
	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***
A. Productivity	4.2276	0.0397	0	1	47.3160	<0.0001	59.0760	<0.0001	0.2102	0.6466
B. Productivity of sons	9.5710	0.0019	2.5488	0.1104	5.0240	0.0250	13.6080	0.0002	0	1
C. Productivity of daughters	2.355	0.1249	0.8232	0.3642	7.8714	0.0050	55.9730	<0.0001	0	1
D. Mating success	0.2821	0.5953	0	1	23.7500	<0.0001	44.3170	<0.0001	0	1
E. Combined fitness traits	14.2680	0.0007	27.5530	<0.0001	0	1	0	1	4.4306	0.0353

Model	**Respone variable**									
Courtship duration (See Figure 6.4)	**Male quality**		**Female line**		**Female line * Male quality**		**Male thorax size**		**Male quality * Male thorax size**	
	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***
A. Productivity	0.4424	0.5060	0.8296	0.3624	0.5553	0.4562	62.1460	<0.0001	0	1
B. Productivity of sons	3.0886	0.0788	0.3331	0.5639	0.4560	0.4995	14.5440	0.0001	0	1
C. Productivity of daughters	2.0623	0.1510	4.8185	0.0281	0	1	63.7320	<0.0001	0	1
D. Mating success	0.2990	0.5845	2.0968	0.1476	0	1	47.8060	<0.0001	0	1
E. Combined fitness traits	2.3991	0.1214	5.0120	0.0251	2.0442	0.1528	42.1750	<0.0001	0.4049	0.5246

Model	**Respone variable**									
Percentage of high and low quality males that mated out of those that courted (See Figure 6.5)	**Male quality**		**Female line**		**Female line * Male quality**		**Male thorax size**		**Male quality * Male thorax size**	
	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***
A. Productivity	1e-04	0.9919	0	1	5.6797	0.01716	4.5922	0.0321	0	1
B. Productivity of sons	2.7492	0.0973	0.2089	0.6477	9.6254	0.0019	0.2004	0.6544	0	1
C. Productivity of daughters	0.3225	0.5701	0.0925	0.761	5.7772	0.0162	5.4680	0.0193	0	1
D. Mating success	0.3727	0.5415	5.1265	0.0235	0	1	1.1900	0.2753	0	0.9999
E. Combined fitness traits	0.1015	0.7501	0.5986	0.4391	6.8154	0.0090	0.7877	0.3748	0	1

Model	**Respone variable**									
Percentage of high and low quality males that mated out of total replicates (See Figure 6.6)	**Male quality**		**Female line**		**Female line * Male quality**		**Male thorax size**		**Male quality * Male thorax size**	
	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***
A. Productivity	1.2245	0.2685	0	1	10.9230	0.0009	5.0531	0.0245	0	1
B. Productivity of sons	4.4465	0.0349	0.1968	0.6573	11.1160	0.0008	0.4278	0.5131	0	1
C. Productivity of daughters	0	0.9994	0	0.9999	9.8994	0.0016	5.5963	0.0180	0	1
D. Mating success	0.3909	0.5319	1.4179	0.2338	1.8422	0.1747	1.5907	0.2072	0	1
E. Combined fitness traits	1.0323	0.3096	0.8762	0.3492	5.8594	0.0154	4.3479	0.0370	0	1

Model	**Respone variable**									
Copulation duration (See Figure 6.7)	**Male quality**		**Female line**		**Female line * Male quality**		**Male thorax size**		**Male quality * Male thorax size**	
	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***	$\chi^2_{(1)}$	***P***
A. Productivity	0.9025	0.3421	0	1	0.8735	0.3500	111.4700	<0.0001	0	1
B. Productivity of sons	7.9129	0.0049	0	1	0	1	13.7790	0.0002	0	1
C. Productivity of daughters	0.3478	0.5553	0	1	0	1	112.6300	<0.0001	0	1
D. Mating success	1.3497	0.2453	1.8122	0.1782	0	0.9999	85.0350	<0.0001	0	1
E. Combined fitness traits	7.2763	0.0069	0	1	3.7529	0.05272	92.0690	<0.0001	0	1

Table C.3 Various models for postcopulatory selection analysis. Variables that were not significant from the log likelihood test were removed in the reduced model unless they were significant in a higher order interaction.

Model	Response variable					
	Male quality		Female line		Female line * Male quality	
	$\chi^2_{(1)}$	*P*	$\chi^2_{(1)}$	*P*	$\chi^2_{(1)}$	*P*
Productivity in Acps competition (See Figure 9)						
A. Productivity	0.1396	0.7087	0.3694	0.5433	9.6090	0.0019
B. Productivity of sons	0.8922	0.3449	0.5398	0.4625	21.4820	<0.0001
C. Productivity of daughters	1.1608	0.2813	0.1893	0.6635	12.2020	0.0004
D. Mating success	3.1591	0.0755	0	1	16.863	<0.0001
E. Combined fitness traits	4.6018	0.0319	0.9664	0.3256	8.7383	0.0031
Fertilization success of the second mated male in sperm and Acps competition (See Figure 10)						
A. Productivity	2.2579	0.1329	0	1	0	1
B. Productivity of sons	2.5764	0.1085	13.2420	0.0002	0.6300	0.4274
C. Productivity of daughters	2.6979	0.1005	0	1	0.3518	0.5531

D. Mating success	3.1554	0.0756	0	1	4.4753	0.0343
E. Combined fitness traits	17.5640	<0.0001	1.3867	0.2390	1.7111	0.1908

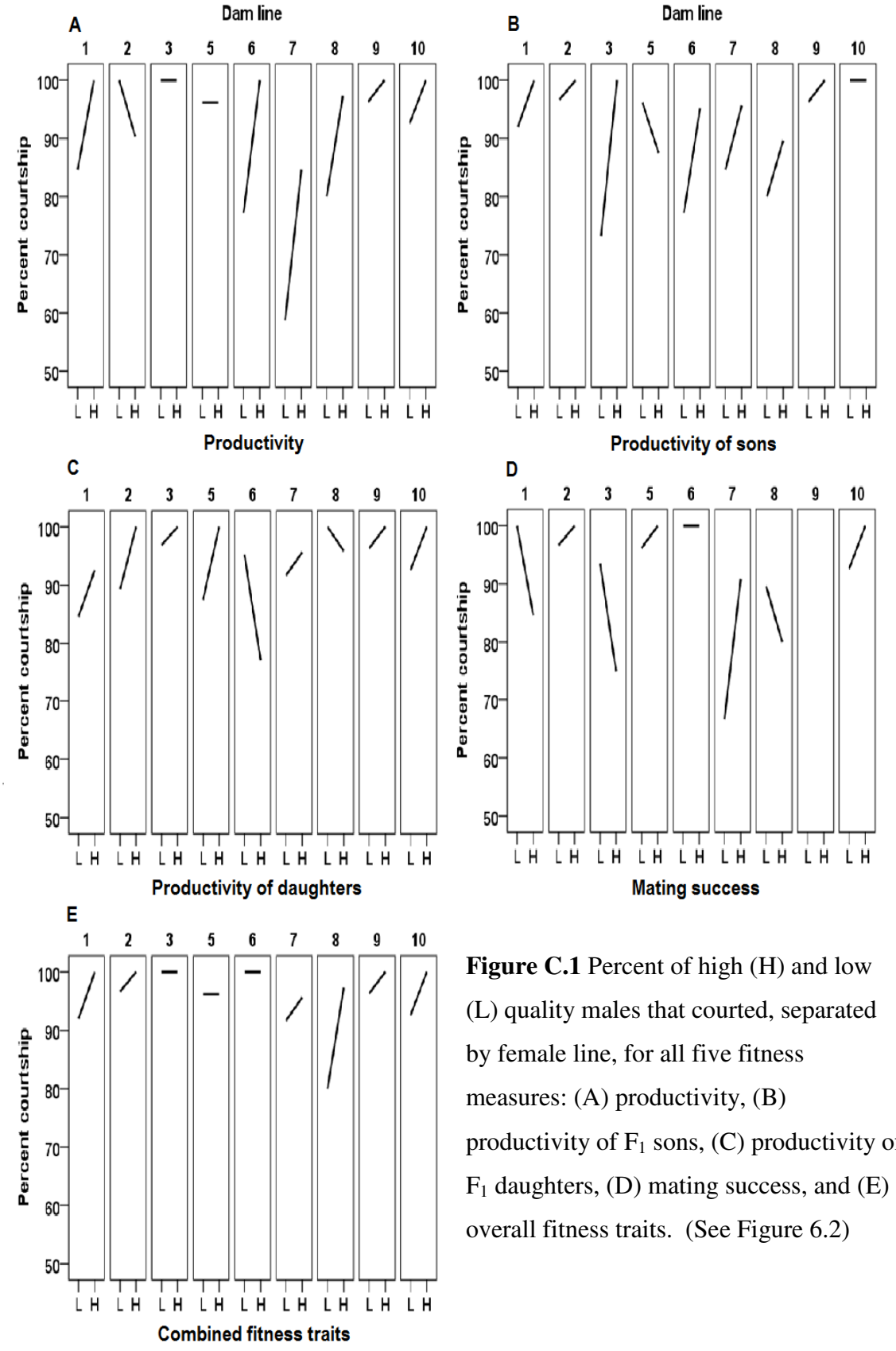


Figure C.1 Percent of high (H) and low (L) quality males that courted, separated by female line, for all five fitness measures: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. (See Figure 6.2)

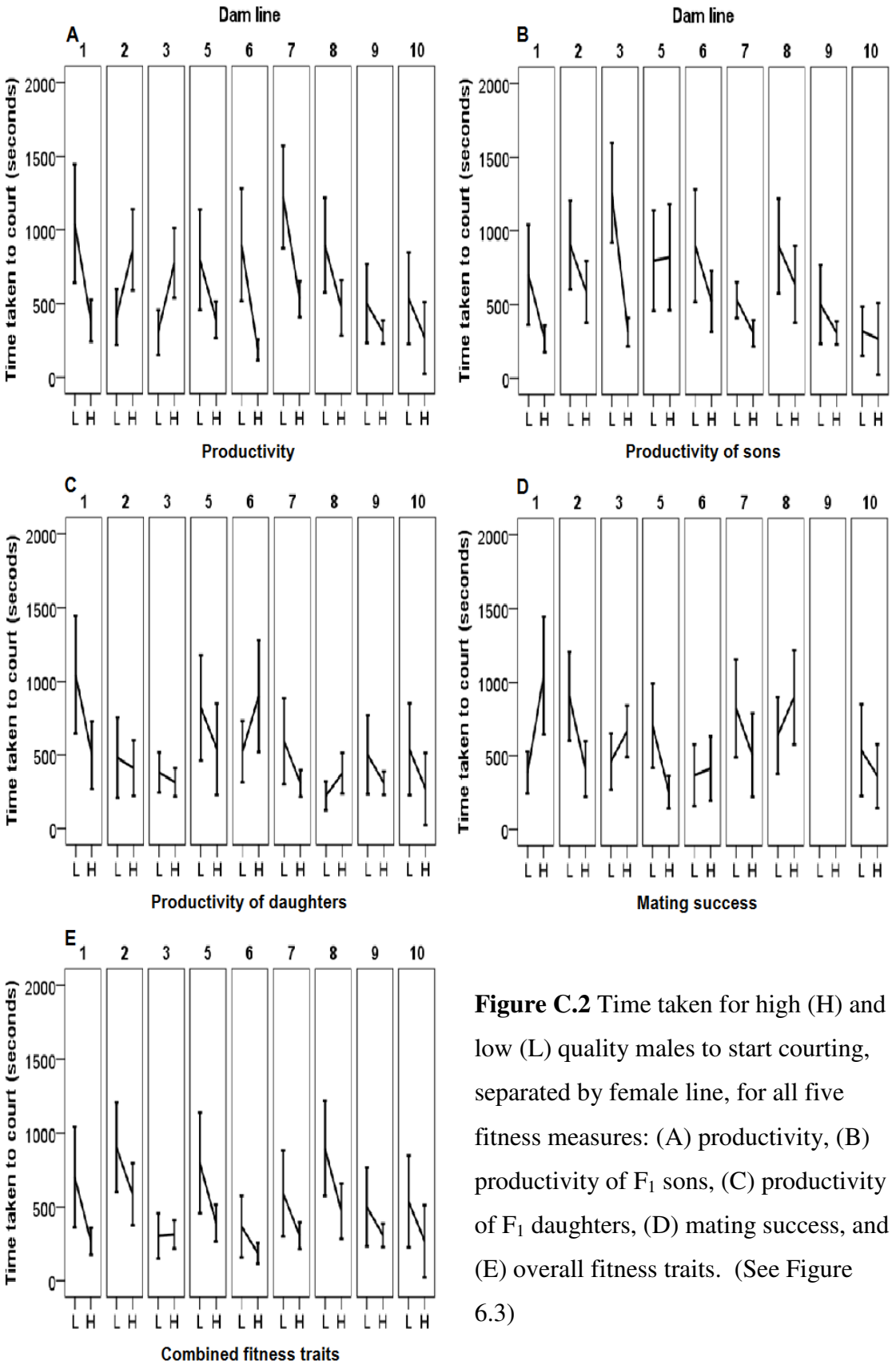


Figure C.2 Time taken for high (H) and low (L) quality males to start courting, separated by female line, for all five fitness measures: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. (See Figure 6.3)

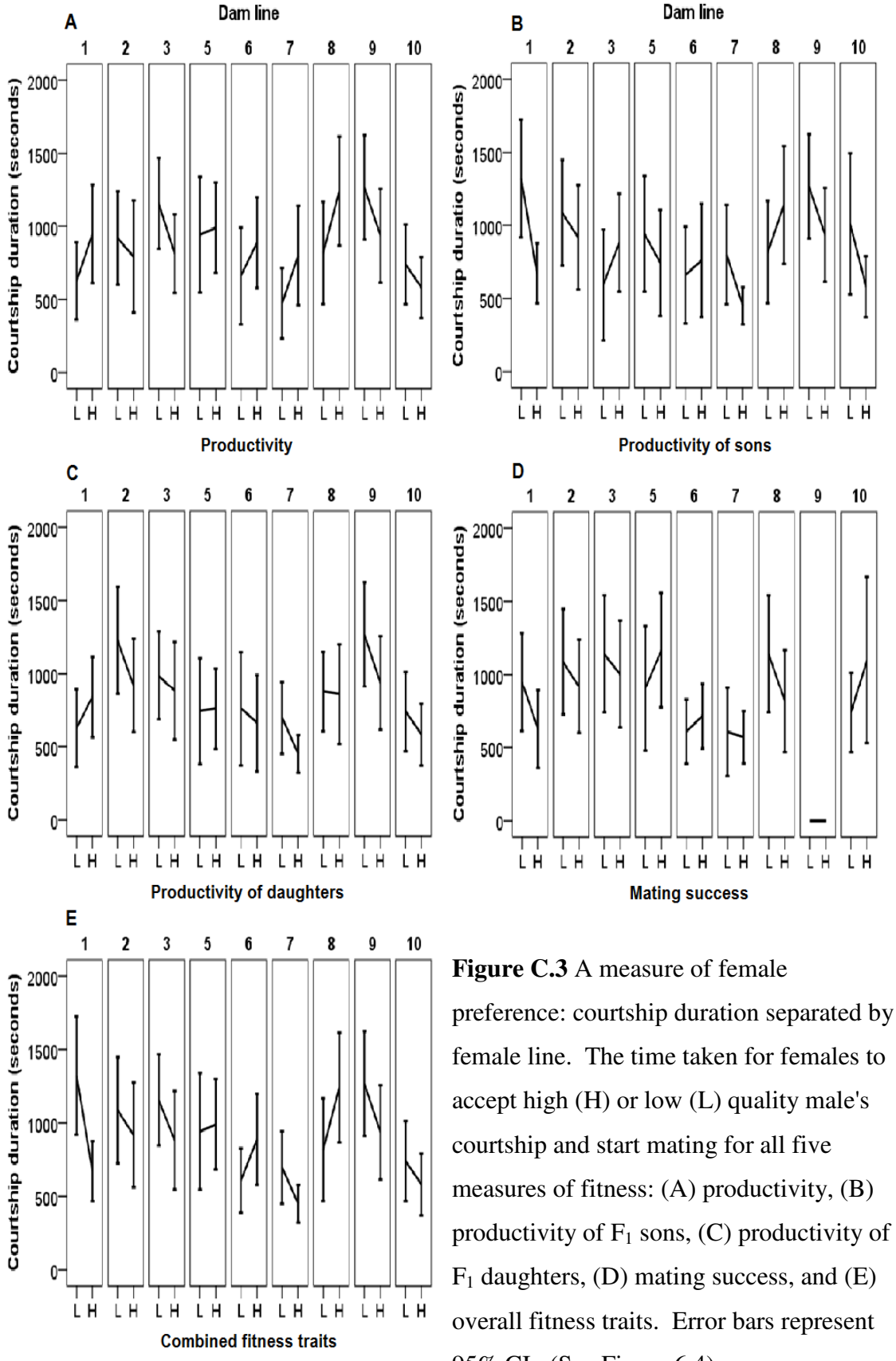


Figure C.3 A measure of female preference: courtship duration separated by female line. The time taken for females to accept high (H) or low (L) quality male's courtship and start mating for all five measures of fitness: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Error bars represent 95% CI. (See Figure 6.4)

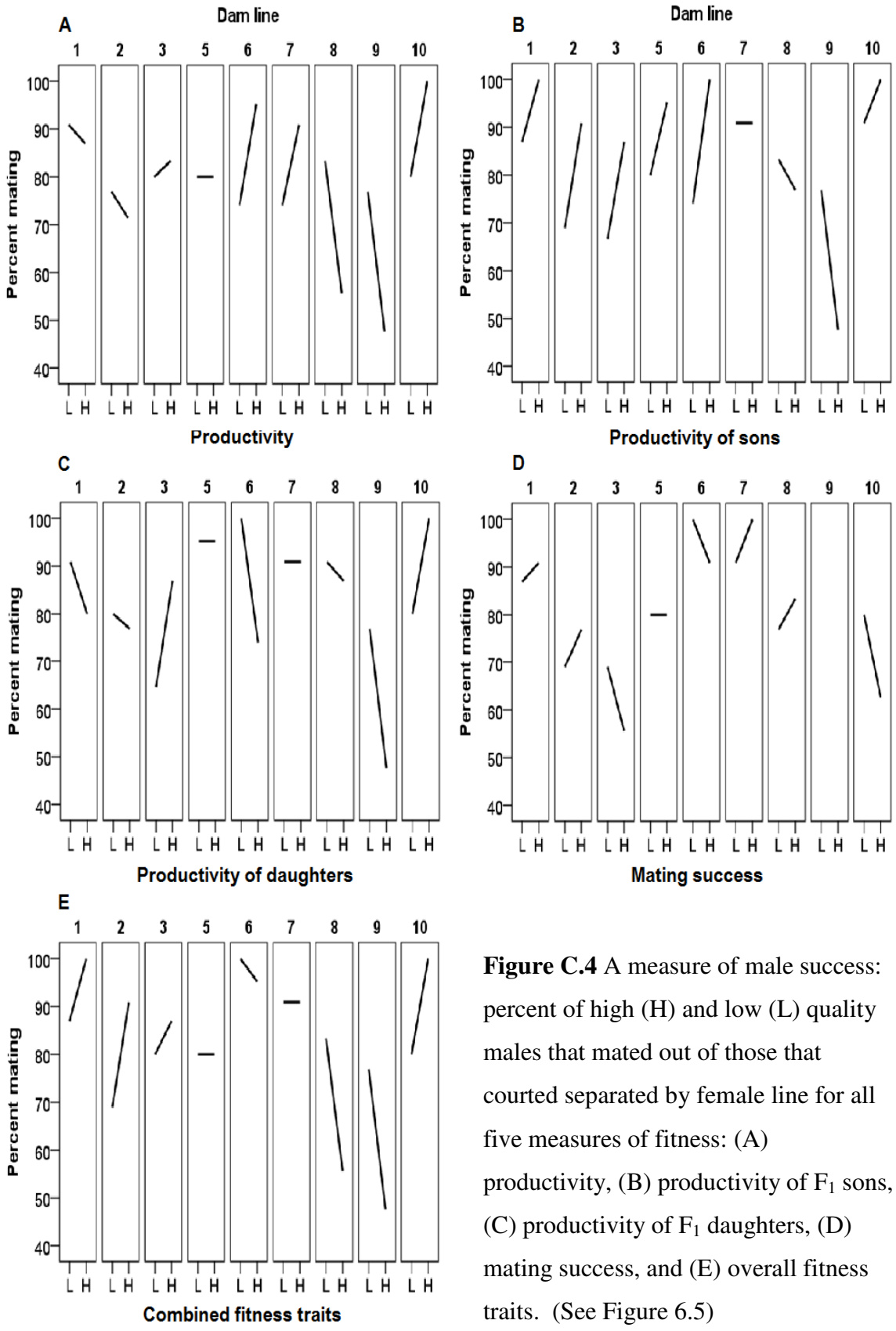


Figure C.4 A measure of male success: percent of high (H) and low (L) quality males that mated out of those that courted separated by female line for all five measures of fitness: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. (See Figure 6.5)

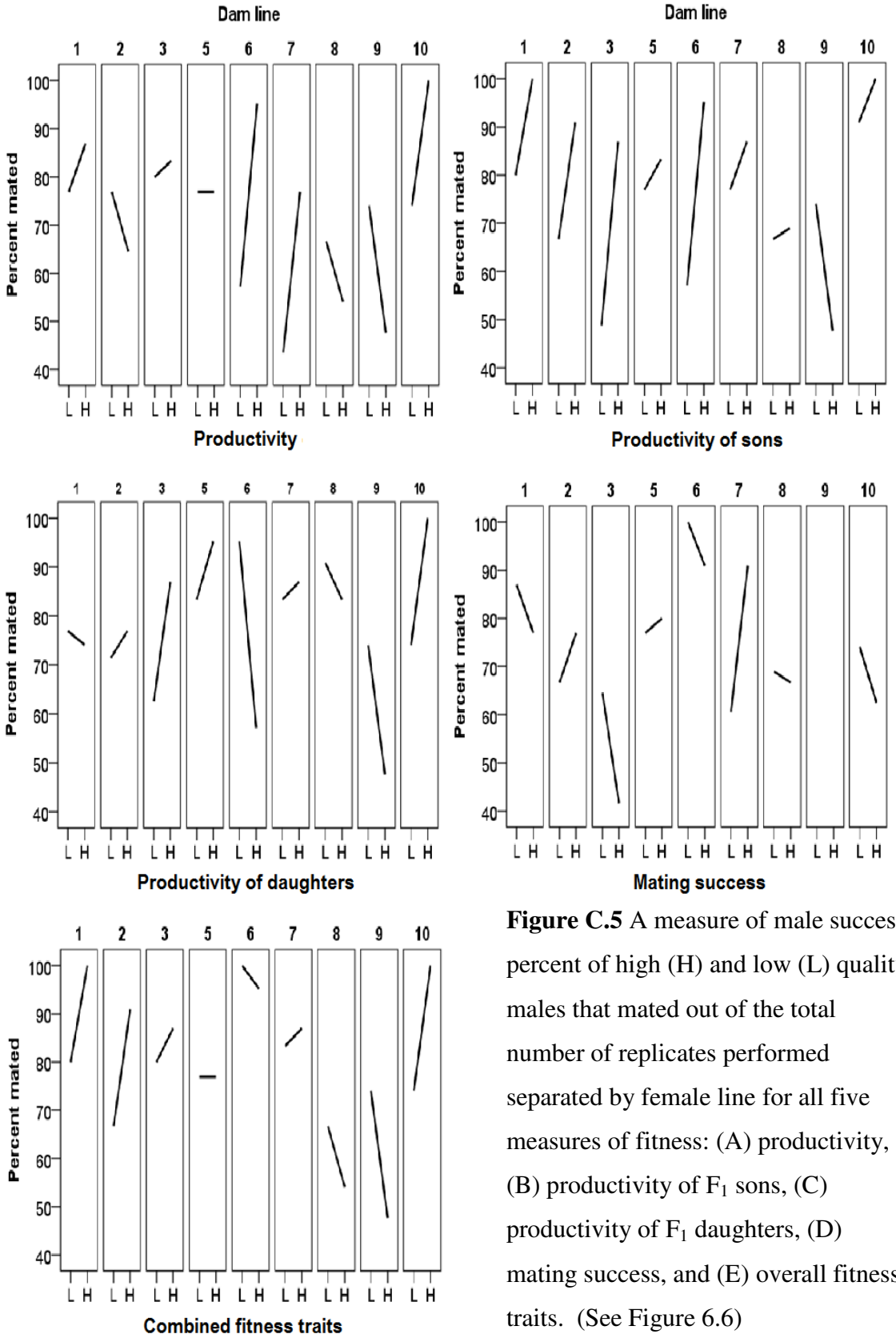


Figure C.5 A measure of male success: percent of high (H) and low (L) quality males that mated out of the total number of replicates performed separated by female line for all five measures of fitness: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. (See Figure 6.6)

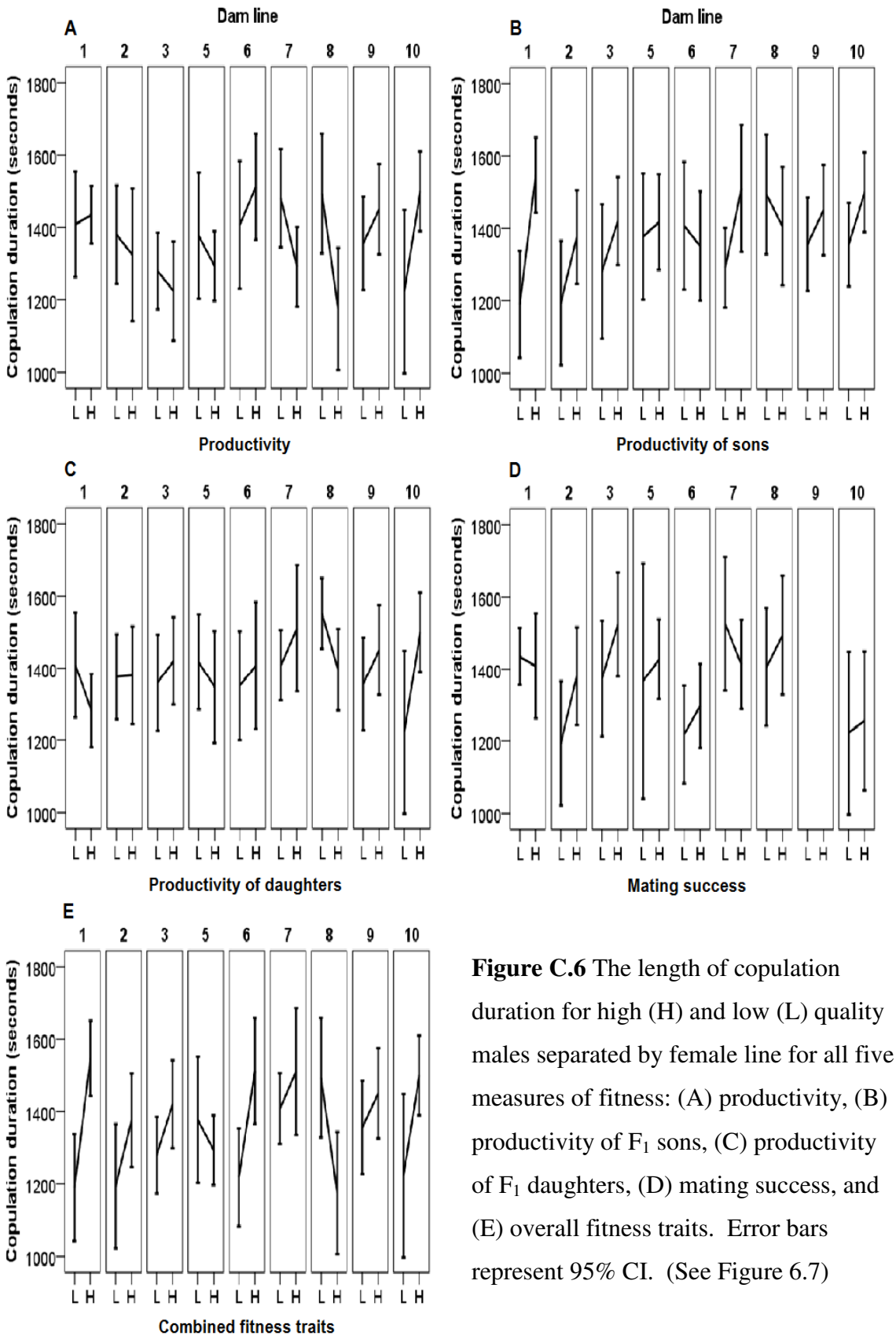


Figure C.6 The length of copulation duration for high (H) and low (L) quality males separated by female line for all five measures of fitness: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Error bars represent 95% CI. (See Figure 6.7)

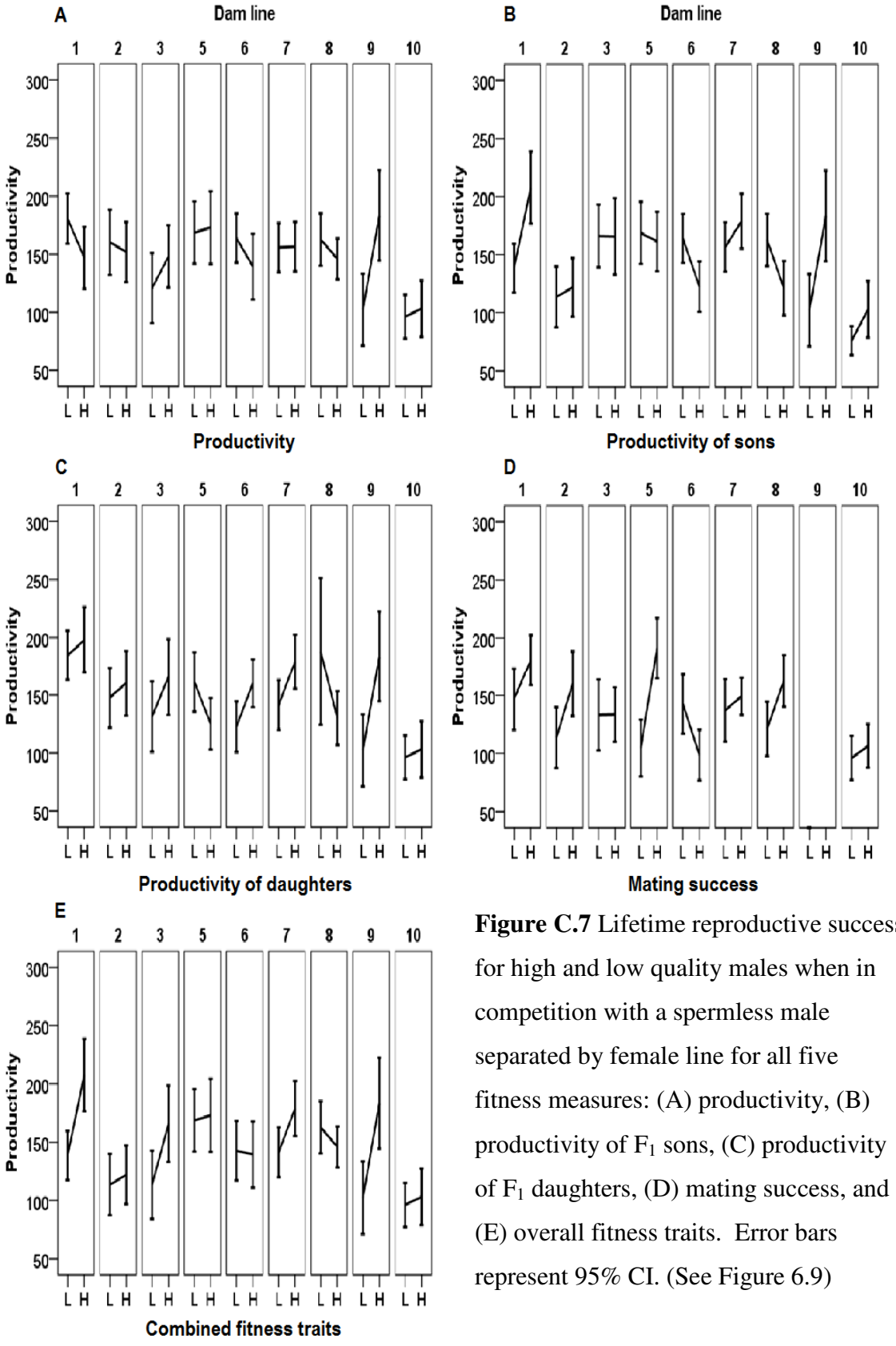


Figure C.7 Lifetime reproductive success for high and low quality males when in competition with a spermless male separated by female line for all five fitness measures: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Error bars represent 95% CI. (See Figure 6.9)

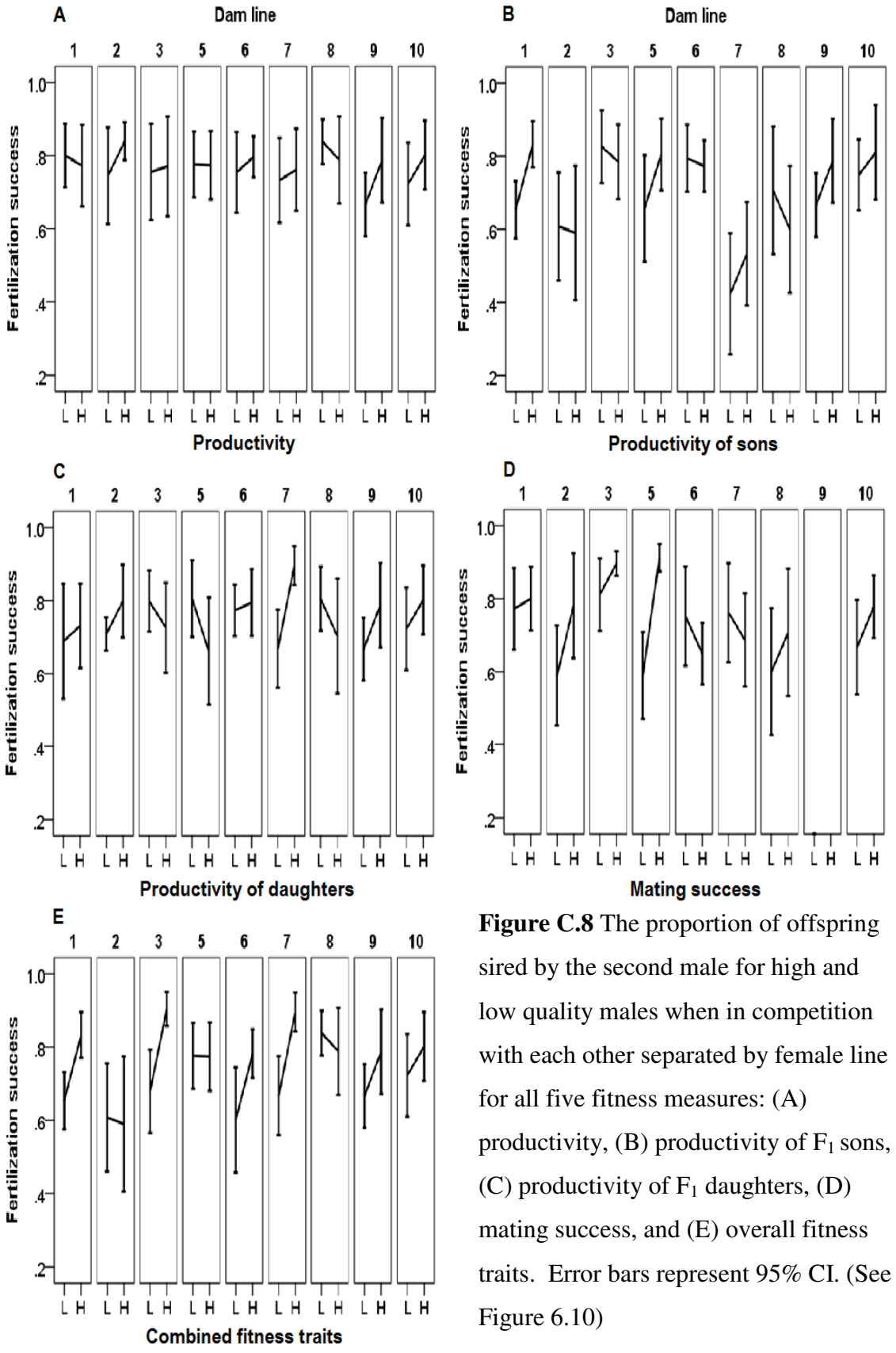


Figure C.8 The proportion of offspring sired by the second male for high and low quality males when in competition with each other separated by female line for all five fitness measures: (A) productivity, (B) productivity of F_1 sons, (C) productivity of F_1 daughters, (D) mating success, and (E) overall fitness traits. Error bars represent 95% CI. (See Figure 6.10)

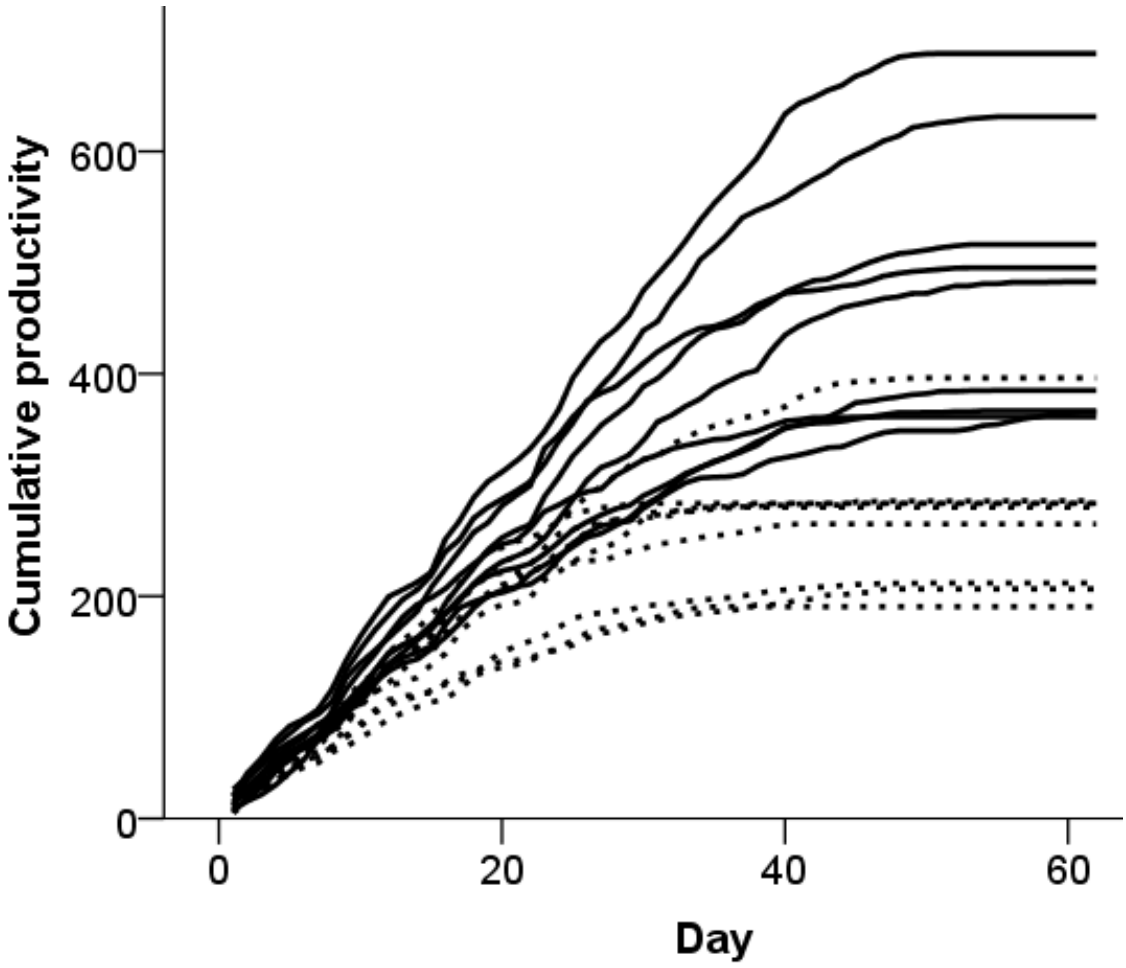


Figure C.9 Cumulative daily number of offspring for multiply mated female controls for each female line. Solid lines represent high quality males, dashed lines represent low quality males. Males are categorized as either high or low quality based on the combined fitness traits measure. Error bars are not shown as they would obscure the ability to visualize differences among the averages.

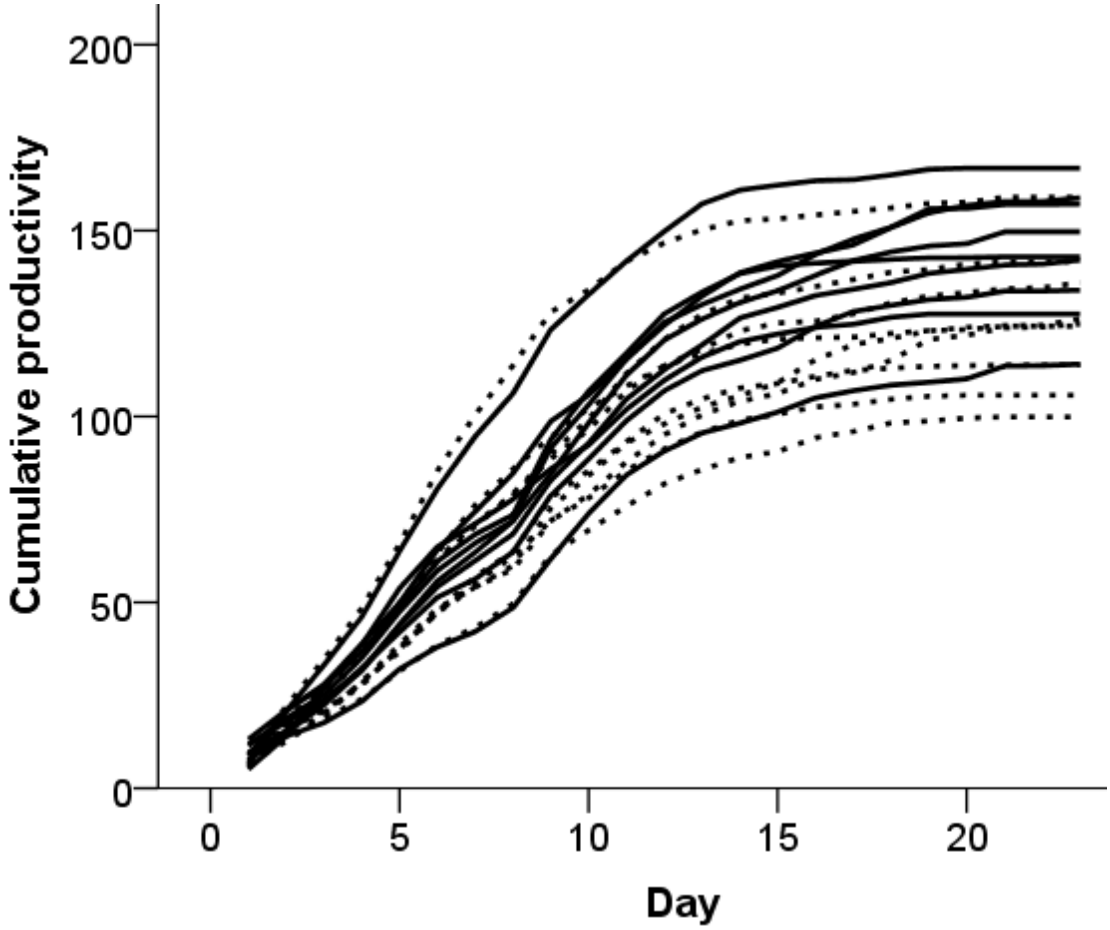


Figure C.10 Cumulative daily number of offspring for singly mated female controls for each female line. Solid lines represent high quality males, dashed lines represent low quality males. Males are categorized as either high or low quality based on the combined fitness traits measure. Error bars are not shown as they would obscure the ability to visualize differences among the averages.

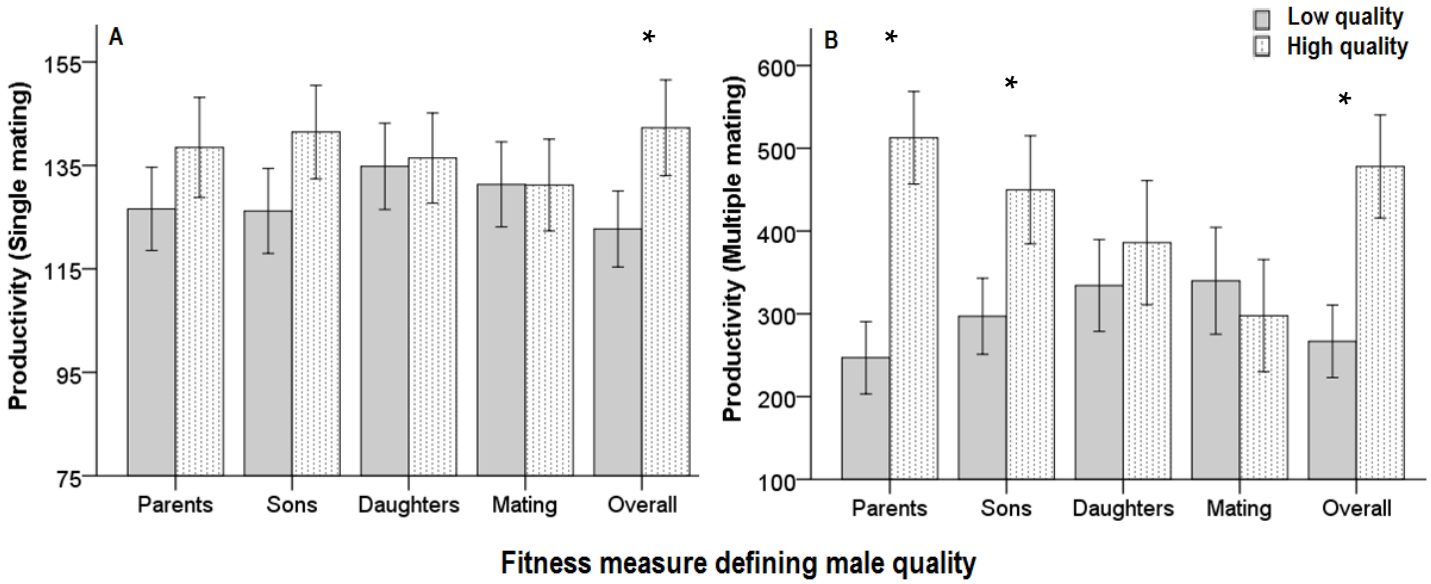
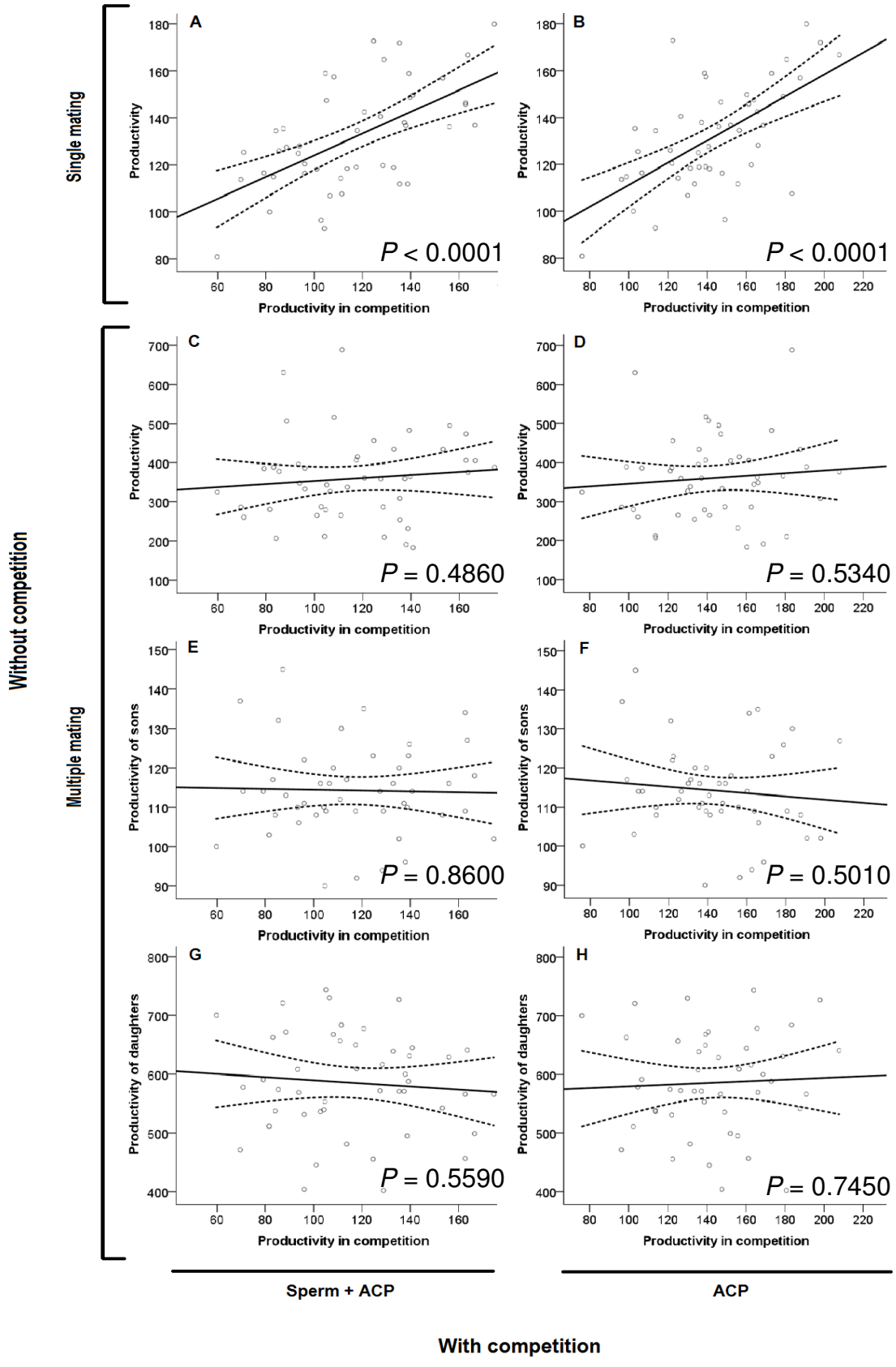


Figure C.11 Total lifetime reproductive success (productivity) in (A) singly mated controls and (B) multiply mated controls for high and low quality males defined by all five fitness measures. A t-test was performed to detect differences in productivity between low quality and high quality males. Asterisks represent significant differences ($P < 0.05$) between low quality and high quality males defined by the particular fitness measure. Error bars represent 95% CI. (Single mating control: Parents; t = -1.862, d.f. = 319.690, P = 0.064, Sons; t = -2.464, d.f. = 334.634, P = 0.014, Daughters; t = -0.262, d.f. = 333.953, P = 0.793, Mating; t = 0.019, d.f. = 298.415, P = 0.985, Overall; t = -3.270, d.f. = 314.395, P = 0.001. Multiple mating control: Parents; t = -7.611, d.f. = 66.208, $P < 0.0001$, Sons; t = -3.887, d.f. = 62.755, $P < 0.0001$, Daughters; t = -1.126, d.f. = 64.481, P = 0.265, Mating; t = 0.919, d.f. = 61.852, P = 0.362, Overall; t = -5.628, d.f. = 62.910, $P < 0.0001$).

Figure C.12 Summary of potential relationships of productivity with various treatments. All y-axis values were without competition with other males and all x-axis values were with competition. A LM was performed. For those that did not meet the criteria for a LM, a GLM was performed with a quasipoisson distribution. (A; $F_{(1, 45)} = 23.94$, $R^2 = 0.3327$, $P < 0.0001$, B; $F_{(1, 45)} = 27.10$, $R^2 = 0.3620$, $P < 0.0001$, C; pseudo $R^2 = 0.0112$, d.f. = 45, $P = 0.4860$, D; pseudo $R^2 = 0.0089$, d.f. = 45, $P = 0.5340$, E; pseudo $R^2 = 0.0006$, d.f. = 45, $P = 0.8600$, F; pseudo $R^2 = 0.0101$, d.f. = 45, $P = 0.5010$, G; $F_{(1, 45)} = 0.3464$, $R^2 = -0.0144$, $P = 0.5591$, H; pseudo $R^2 = 0.0023$, d.f. = 45, $P = 0.7450$).


Without competition
Single mating
Multiple mating
A
B
C
D
E
F
G
H
Productivity
Productivity of sons
Productivity of daughters
Productivity in competition
P < 0.0001
P < 0.0001
P = 0.4860
P = 0.5340
P = 0.8600
P = 0.5010
P = 0.5590
P = 0.7450
Sperm + ACP
ACP
With competition

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Service

Biology Graduate Research Forum, Western University (2014)

Biology Graduate Research Forum, Western University (2012)

North America Forensic Entomology Association, Windsor Canada (2010)

Publications:

Nguyen, T. T. X., A. J. Moehring. (submitted) Daughters affected most strongly by good genes and inbreeding depression. Evolution.

Nguyen, T. T. X., A. J. Moehring. (in press). Accurate alternate measurements for female lifetime reproductive success in *Drosophila melanogaster*. PLoS ONE.

Nguyen, T. T. X., J. K. Tomberlin, and S. Vanlaerhoven. (in press). Ability of black soldier fly (Diptera: Stratiomyidae) larvae to recycle food waste. Environ Ent.

Nguyen, T. T. X., J. K. Tomberlin, and S. Vanlaerhoven. 2013. Influence of resource on *Hermetia illucens* (Diptera: Stratiomyidae) larval development. J Med Ent. 50(4): 898-906

Tubman, J., J. Maimaiti, M. E. Masri, S. Fox-Wasylyshyn, D. Kane, T. T. X. Nguyen, E. Maticka-Tyndale, and L. A. Porter. 2011. pH Effects on the bio-permeability of polymers used in prophylactics. Am J Bio Med Sci. 3: 292-300.

Conferences

Evolution 2014 - June 2014
Raleigh, North Carolina
Oral presentation

1st Joint Conference CSEE CSZ SCL - May 2014
Montreal, Canada
Oral presentation

Ontario Ecology, Ethology and Evolution Colloquium - May 2014
Guelph, Canada
Oral presentation

North America Forensic Entomology Association - July 2010
Windsor, Canada
Oral presentation

Entomological Society of America - April 2010
Cancun, Mexico
Oral presentation

North American Forensic Entomology Association - July 2009
Florida, USA
Oral presentation

Ontario Biology Day - March 2008
Guelph, Cana
Oral presentation