2016

Causes and consequences of variation in female mate choice and its relation to sexual conflict in Drosophila melanogaster

David CS Filice
Wilfrid Laurier University, fili2950@mylaurier.ca

Causes and consequences of variation in female mate choice and its relation to sexual conflict in *Drosophila melanogaster*

By

David Carmine Steven Filice

Honours BSc Biology & Psychology, University of Toronto, 2014

THESIS

Submitted to the Department of Biology

Faculty of Science

in partial fulfillment of the requirements for

Master of Science in Integrative Biology

Wilfrid Laurier University, 2016

ABSTRACT

Female mate choice is a significant driving force of evolutionary change and can explain the evolution of exaggerated male traits and/or displays, and dimorphism between the sexes. Females are thought to choose mates based on the greatest provision of direct or indirect benefits. Despite this, we often still see substantial individual variation in female mate choice behaviours both within and across populations. Recent studies suggest that female mate choice is a complex decision-making process that involves many context-dependent factors. However, the precise sources of this variation, such as previous mating experience, are not completely understood. In *Drosophila melanogaster*, mating can be harmful and have costly effects on a female's lifetime fitness. As such, sexual conflict theory predicts that females may make trade-offs in their mate choice decisions to balance the direct costs and indirect benefits associated with mating. In this thesis, I set out to understand if the harmfulness of a previous mating experience influences a female's subsequent mate choice behaviours. In chapter two of this thesis, I assessed the effect of male exposure on female fitness by measuring the change in their fecundity (a meaningful metric of fitness) across a brief and prolonged period of exposure. In this experiment, we found that the degree that different males harm their mates across this time period largely depended on the male's genetic background. Using these results, I was able to quantify the harmfulness of 26 male hemiclone lines that each possess a unique genetic background. In chapter three of this thesis, I used these quantified males to examine if the direct costs of a previous mating experience has an effect on subsequent female mate choice behaviours and to quantify the degree of additive genetic variation associated with this effect. The results of my studies suggest that females alter their mate

choice behaviours based on previous mating experiences, and that the degree to which these behaviours change has a genetic basis. I discuss how these results are significant for our understanding of the evolution of female mate choice, and the maintenance of variation in harmful male traits.

ACKNOWLEDGEMENTS

There are many people I have to thank for their help and support during my time at Laurier that made this work possible.

Firstly, I would like to thank my supervisor Dr. Tristan Long for taking me as a student and for all his help and involvement throughout my project. I think it's safe to say my journey would have been much more difficult without Tristan's unmatched dedication to his students' success. I would also like to thank my committee members, Dr. Scott Ramsay and Dr. David White for their assistance and guidance.

I am grateful towards all members of the Long Lab, past and present, who assisted me with many hours of research in the lab. I am also thankful to have had a fun and awesome cohort of grad students to share this adventure with - I'm not sure if I would have made it without all the late afternoon rounds of golf and beers.

Lastly, I would like to thank my family and friends back home in Toronto (spams & zboys), particularly my parents who have supported me in so many ways. As much as I act like I can handle everything on my own, I know I couldn't have achieved my goals without them.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

Context-dependent mate choice and its relation to sexual conflict

Introduction

Sexual selection, the differential success of individuals in a population associated with acquiring and securing mates, often leads to the evolution of exaggerated traits and/or displays in males and dimorphism between males and females of the same species (Darwin, 1891; Andersson, 1994). This differential success can be the outcome of intra-sexual selection (competition for access to mates, typically male-male) and/or inter-sexual selection (choice of mates, typically by females). In many species, females exhibit mate choice as an evolutionary means of selecting a mate whom yields the greatest benefits towards her lifetime reproductive success(Kokko *et al.*, 2003). These benefits may directly improve a female's fitness such as through the provision of nuptial gifts, parental care and protection (Heywood, 1989), or may indirectly do so by transmitting "good genes", resulting in offspring with increased attractiveness and/or survivability (Kirkpatrick, 1996).

Since Darwin's time, questions as to how and why mate choice evolves have been vigorously debated (Kirkpatrick & Ryan, 1991). In the past 30 years, a large amount of empirical and theoretical research has improved our understanding of the underlying mechanisms of sexual selection and how they influence the evolution of species (Andersson, 1994; Jennions & Petrie, 1997; Kokko *et al.*, 2003). Recently, the concept of sexual conflict has particularly sparked much interest. Sexual conflict may arise when males and females of the same species have different and incompatible strategies for

maximizing their individual reproductive success (Parker, 1979), which typically results in antagonistic coevolution between the sexes (Chapman *et al*, 2003a; Arnqvist & Rowe, 2005). Frequently, males will evolve harmful traits to exploit females, and in response, females will coevolve traits to resist this harm (Arnqvist & Rowe, 2005). Although Darwin (1891) alluded to this concept in chapter 8 of *The descent of man, and selection in relation to sex,* it wasn't until the explosion of sexual conflict research in the 1990s that these concepts were empirically demonstrated (Rice, 1992; Rowe *et al*, 1994; Arnqvist, 1997). Ultimately, the integration of sexual conflict into sexual selection theory has broadened our once narrow view of mate choice. Instead of viewing mate choice as a process where females choose "ideal" mates to maximize benefits, biologists may alternatively consider if sexually antagonistic coevolution results in females evolving biases against harmful mates to resist the direct costs associated with mating. Holland & Rice (1998) first described this idea in their chase-away sexual selection model, wherein females evolve resistance (decreased attraction) to male displays or traits that are directly harmful to their lifetime fitness. As a result, males are expected to evolve exaggerated display traits in attempt to continuously exploit female mating thresholds (Holland & Rice, 1998).

In addition to sexual conflict, recent studies that demonstrate individual variation in female mate choice behaviours have broadened our understanding of the operation of sexual selection and its evolutionary implications (Jennions & Petrie, 1997; Widemo & Sæther, 1999). Traditionally, biologists have viewed female mate preferences as static and uniform, but empirical evidence has shown that environmental factors and individual genotypes can influence a female's mate choice phenotype (Moore & Moore, 2001; Hunt

et al., 2005). Collectively, these studies imply that plasticity in female mate choice behaviours is common (Jennions & Petrie, 1997), and may be an adaptation to mitigate the costliness of expending time and resources while searching for a mate (Qvarnström, 2001). Although our understanding of the causes and consequences of individual variation in female mate choice has improved to date, no one has ever tested how the costliness of a previous mating experience (i.e. via sexual conflict) affects the expression of mate choice behaviours.

In this thesis, I performed a series of experiments in order to examine if female mate choice decisions are influenced by the degree of direct costs incurred from previous mating experiences and the social context of that encounter (amount of time exposed to males and genotype of mates). In this chapter, I review the relevant background literature on sexual conflict and individual variation in mate choice, and how these topics may be integrated to better understand how sexual selection operates.

Mate choice: Preference and choosiness

Mate choice is important in understanding the evolution of traits in many sexually reproducing species as it influences the reproductive success of individuals (Andersson, 1994). Selective behaviours toward prospective mates can impact the evolution of traits considered to be "attractive" (Pomiankowski & Iwasa, 1998), and also accelerate the evolution of traits favoured by natural selection (Servedio *et al.*, 2011). Therefore, quantifying a species' mate choice behaviours can offer insight into understanding how and why mate choice behaviours and the traits they influence can evolve.

Modern evolutionary biologists typically quantify mate choice by measuring two components: preference and choosiness (Jennions & Petrie, 1997). Preference is defined

as how "attractive" an individual finds a potential mate, where "attractiveness" is characterized by an individual's ranking of potential mates based on their phenotypic traits and/or displays (Jennions & Petrie, 1997). Preference can be evaluated by placing a female in a scenario where she can actively choose who to mate or associate with (*e.g.* Sharon *et al*., 2010). On the other hand, choosiness has to do with the amount of effort or energy an individual is willing to put in to assess or discriminate between potential mates (Jennions & Petrie, 1997), which can be quantified by measuring latency before mating with males that vary in the intensity of a trait and/or display (*e.g.* Hunt *et al.,* 2005). Despite the potential for fitness benefits, mate choice can be costly, particularly for individuals that are too choosy (Kokko *et al.*, 2003). The act of searching for a suitable mate requires the expenditure of time and resources (Parker, 1983; Gibson & Bachman, 1992; Etienne *et al.*, 2014), and may put an individual at greater risk of predation and/or injury (Forsgren, 1992; Hedrick & Dill, 1993; Godin & Briggs, 1996; Booksmythe *et al.*, 2008). Thus, females who are too choosy may risk not mating at all in their lifetime, or have decreased net reproductive success compared to individuals whom are less choosy (Kokko *et al.*, 2003).

Although the phenomenon and evolution of mate choice has been extensively described in a number of ways (i.e. Fisher's run-away hypothesis (Fisher, 1930), the "good genes" hypothesis (Williams, 1966; Ryan, 1996), the direct benefits hypothesis (Price *et al.*, 1993) and the sensory bias hypothesis (Endler & Basolo, 1998)), there are aspects to mate choice that are relatively new to the literature. Particularly, the causes and consequences of mating biases against certain phenotypes (via sexual conflict) and individual variation in mate choice behaviours are not well understood. In the following

two sections, I discuss the relevance of these topics in regards to our understanding as to how and why mate choice may evolve, and suggest what specific areas require more research.

Sexual conflict and male-induced harm

One way in which sexual selection may operate is via sexual conflict, a process arising when males and females of the same species have different (and incompatible) strategies for maximizing their individual reproductive success (Chapman *et al.,* 2003a). Typically, this conflict is rooted in anisogamy, where parental investment into offspring production between the sexes is asymmetrical (Trivers, 1972). Sexual conflict may be manifested when the fitness optimum for a trait controlled at a specific locus is different between males and females (intra-locus sexual conflict), or when males and females express traits or behaviours that are beneficial to their own reproductive success, but not of their mates' (inter-locus sexual conflict) (Arnqvist & Rowe, 2005). In the latter case, males and females frequently engage in a sexually antagonistic arms race, a continuous co-evolutionary cycle of adaptation and counter-adaptation. One way in which sexually antagonistic co-evolution may proceed is males evolve selfish traits that harm females, and in response, females evolve traits to resist the consequences of such exploitation (Chapman, 2006). The harm induced by males can occur before, during, and after mating (Arnqvist & Rowe, 2005). For example, male water striders in the genus *Gerris* frequently harass females to mate, which is costly to females. Males have evolved grasping structures that increase the efficiency of copulation at a cost to females, while females have evolved antigrasping structures to resist this harm (Arnqvist & Rowe, 2002). Male bedbugs, *Cimex hermipterus*, traumatically inseminate their mates by

physically puncturing their abdomens and injecting sperm directly into the bloodstream. These encounters leave visible melanized scars (Walpole, 1988) and reduce the lifespan of females (Newberry, 1989). This harm can be a great selective pressure for females, and therefore females are expected to evolve traits and/or strategies to resist this harm (Holland & Rice, 1999; Wigby *et al.*, 2004).

Theoretically, one way females may resist the harm associated with sexual conflict is by avoiding harmful mates via mate choice (Holland & Rice, 1998; Gavrilets *et al.*, 2001). The chase-away sexual selection hypothesis states that females may evolve resistance (decreased attraction) to reduce the direct costs of harmful male traits, and in response, males may evolve exaggerated traits to overcome the new female mating threshold (Holland & Rice, 1998; Figure 1). However, this hypothesis is highly controversial (Brooks & Jennions, 1999; Rice & Holland, 1999) and has only been theoretically demonstrated by Gavrilets *et al.* (2001) using quantitative genetic models. As such, there is a clear need to empirically investigate the possibility of male-induced harm influencing the evolution of female mate choice behaviours. In order for this type of mate choice (resistance) to evolve, there needs to be heritable individual variation in the amount of harm that males inflict via mating. A challenge in testing this model is the need to be able to accurately quantify the amount of harm individual males inflict, especially in natural populations. However, one model species has been extensively used to perform empirical tests of questions regarding mate choice and sexual conflict. The fruit fly, *Drosophila melanogaster*, is frequently used to understand the causes and consequences of individual variation in male-induced harm (Civetta & Clark, 2000; Sawby & Hughes, 2001; Lew & Rice, 2005).

In *D. melanogaster*, males harm their mates via the toxic-side effects of accessory gland proteins (Acps) transferred in their ejaculate (Chapman *et al.*, 1995; Rice, 1996) and through courtship and copulation behaviours (Partridge & Fowler, 1990; Kamimura, 2007). Although male-induced harm is well documented in *Drosophila,* the factors that influence the extent of harm a male inflicts during mating (i.e. the degree of reduction to his mate's fitness) are not well understood. A handful of studies demonstrate that this harm may have an additive genetic basis (Friberg, 2005; Lew & Rice, 2005; Fiumera *et al.*, 2006), but all of these studies are limited in their quantification of female fitness. Firstly, they quantified harm by measuring the fitness of females that carried multiple deleterious mutations and were derived from different source populations than the males. The presence of multiple deleterious mutations represents a clear limitation as it could result in spontaneous decreased fitness from epistatic interactions (Simmons & Crow, 1977; Otto & Feldman, 1997). Additionally, using males and females derived from different source populations may yield results of questionable evolutionary relevance. As sexual conflict theory predicts that males and females co-evolve harmful and resistance traits respectively, using two isolated populations may not be representative of the male-induced harm that exists in natural populations (Chapman *et al.*, 2003; Long *et al.*, 2006). Secondly, they quantified female fitness by measuring the longevity of females, a fitness metric of limited value in lab-reared organisms cultured in short, non-overlapping generations who do not have the opportunity to reproduce the entire duration of their natural lifespan. As an alternative, I argue that measuring egg production during key oviposition windows yields a more evolutionary relevant measure of fitness (Long *et al.*, 2006).

In chapter two of this thesis, I describe how I quantified the degree of additive genetic variation in the magnitude of harm that males impose by measuring the fecundity of females exposed to males of different genetic backgrounds over a brief and prolonged period of time. Using these quantified male backgrounds, it is possible to answer questions regarding the influence of male-induced harm on individual variation in female mate choice behaviours.

Variation in mate choice

As with many traits and behaviours, females often exhibit a wide degree of individual variation in their mate choice behaviours both within and between populations (Jennions & Petrie, 1997; Widemo & Sæther, 1999). Understanding the causes and consequences of this variation is fundamental to our understanding the operation of sexual selection because variation among female preferences and choosiness can influence both the rate and direction of the evolutionary change (Jennions & Petrie, 1997). Variation in mate preferences can be characterized by two functions: peak preference and preference selectivity. A change in peak preference is when there is a shift in the trait value with the highest attractiveness (Rodríguez *et al.*, 2013; Figure 2a), while a change in preference selectivity indicates that the peak attractiveness stays the same, but the range of traits that a female may find attractive changes (Rodríguez *et al.*, 2013; Figure 2b). Characterizing the type of changes in female preference functions (and the sources of these changes) can help infer the evolutionary consequences of individual variation in mate preferences, as they can help predict what male traits and/or displays are the most attractive in specific contexts (Bailey, 2008; Rodríguez *et al.*, 2013).

One potential source of variation in mate preferences is an individual's genotype

(Brooks & Endler, 2001; Ritchie *et al.*, 2005; Ratterman *et al.*, 2014; Tennant *et al.*, 2014). Research by Ritchie *et al.* (2005) suggests that this variation is present both within and between populations. Quantifying this variation is important, because for mate choice behaviours to (co-)evolve, there must be heritable additive variation within the population (Jennions & Petrie, 1997). Additionally, the degree of individual variation in female choice within populations can mediate the maintenance of genetic variation in male display traits (Ratterman, 2014, *but see* Tennant *et al.*, 2014). Quantifying the degree of genetic variance associated with individual variation in female mate choice can therefore improve our understanding of the potential for evolutionary change and the factors that contribute to the maintenance of this variation (Chenoweth & Blows, 2006).

In addition to genetic influences, individual variation in mate choice behaviours may arise from a variety of environmental factors (Jennions & Petrie, 1997; Widemo & Sæther, 1999). Qvarnström (2001) argued that a large amount of this variation may originate from context-dependent plasticity. In some contexts, the costs associated with mate choice (i.e. resource expenditure and risk of predation) may be greater in comparison to other contexts. Therefore, a female that is able to alter her mate choice behaviours across environments may be able to maximize her potential reproductive success over multiple contexts (Qvarnström, 2001). This hypothesis has been empirically tested across numerous factors such as population size (Berglund, 1994), nutritional state (Hunt *et al.*, 2005), age (Moore & Moore, 2001), and social experience (Bailey & Zuk, 2009). For example, Hunt *et al.* (2005) showed that in the black field cricket, *Teleogryllus commodus*, females reared on high-nutrition diets exhibited a stronger preference for males whose mating calls were of a higher frequency and more dominant

compared to those females reared on low-nutrition diet. They argued that the costs associated with mate choice might be higher for under-nourished individuals, so less choosy under-nourished individuals may have a reproductive advantage over individuals who are choosier. To fully understand the causes and consequences of individual variation in mate choice behaviours, it is therefore absolutely necessary that we explore all the potential sources that contribute to this variation.

Despite the considerable amount of scientific investigation that has gone into understanding the factors that influence the evolution and maintenance of individual variation in mate choice behaviours, very few studies have considered the potential role of mating experience. Amongst the studies that have, none have considered the potential role of male-induced harm in shaping female mate choice behaviours. In the next and penultimate section of this chapter, I discuss my proposed synthesis between sexual conflict theory and experience-dependent mate choice that I hope will pave the way for many exciting questions regarding the dynamics between the evolution and maintenance of variation in mate choice behaviours and antagonistic coevolution between the sexes.

A new synthesis: Sexual conflict and experience-dependent mate choice

In many species, an individual's experience can shape their subsequent behaviours in a variety of ways, including mate preferences (Verzijden *et al.*, 2012; Rodríguez *et al.*, 2013). Although previous studies have looked at the effect of courtship experience (Dukas, 2005; Rebar *et al.*, 2009) and mating experience (Rebar *et al.*, 2011) on female mate choice behaviours, no one has ever considered the role of direct costs associated with mating (i.e. via sexual conflict) as a factor in these changes. For example Rebar *et al.* (2011) analyzed the effect of mating experience in Pacific field crickets, *Teleogryllus*

oceanicus and found that females that had mated with an "attractive" male 24h prior to a new mating encounter mounted more slowly compared to females who mated with an "unattractive" male 24h earlier. Although this study demonstrates that mating experience can influence female choosiness, the authors did not look at its potential impact on the differential expression of female preferences. Plasticity in preferences is important to consider, as it may influence the direction of sexual selection depending on the stability of different contexts that may trigger this plasticity. Additionally, the authors did not discuss their results in the context of sexual conflict. As sexual conflict has not been extensively studied in this species, a better model for studying the relationship between sexual conflict and behavioural plasticity in mate choice would be *D. melanogaster*.

While much is known about sexual selection in *D. melanogaster*, the effect of mating experience on subsequent female mate choice behaviours has only been investigated in a single study. Dukas (2005) looked at the effect of previous courting experience on mate choice by experimentally pairing virgin *D. melanogaster* females with males of varying body sizes and analyzing their behaviours the following day. He found that females previously courted by smaller males were more likely to mate with both small and large males compared to females previously courted by larger males. This result demonstrates that females do exhibit plasticity in their mate choice behaviours as a result of male exposure, however it does not take into account the potential effects of mating (copulation and ejaculate transfer). In *D. melanogaster,* copulation with a male can cause damage to females' genitalia (Kamimura, 2007) and the transfer of Acps in their mate's ejaculate can have toxic-side effects (Chapman *et al.*, 1995; Rice, 1996). More specifically, mating can elicit changes in a female's physiological and behavioural

phenotypes by increasing egg production (Soller *et al.*, 1999), decreasing mating receptivity (Chen *et al.*, 1988; Chapman *et al.*, 2003b), decreasing lifespan (Chapman *et al.*, 1995), and increasing feeding behaviour (Carvalho *et al.*, 2006). Given that courtship experience can affect female mate choice behaviours and mating can influence a female's physiology and behaviour, it is plausible to hypothesize that mating experience may also influence an individual's mate choice behaviours.

In chapter 3 of my thesis, I explore the role of mating experience on subsequent female mate choice behaviours in the context of the direct costs incurred via mating (i.e. male-induced harm). I argue that similar to how females may exhibit behavioural plasticity to reduce the costs associated with mate choice, or increase their indirect benefits by finding a more attractive mate (Rodríguez *et al.*, 2013) females may also alter their mate choice behaviours in order to reduce the direct costs incurred from previous mating experiences.

Significance, objectives and hypotheses

A review of recent literature reveals a pressing need to investigate the genetic basis and maintenance of variation in male-induced harm, and its potential role as a source of individual variation in female mate choice behaviours. The major focus of this thesis was to investigate the effect of mating experience and male-induced harm on female mate choice behaviours. To do this, I had to first quantify the degree to which individual males vary in their harmfulness, leading me to ask:

1.) Is there phenotypic variation in the effect of length of male exposure (courtship & copulation) on female fitness, and does this variation have a genetic basis?

To answer this question, I quantified the effect of male-exposure on female fitness across 26 genetic backgrounds using an evolutionary relevant metric of fitness (fecundity) and a protocol that mimicked the developmental conditions of our laboratory population. Next, I was able to use these lines of quantified male-induced "harm" to ask the question:

2.) Do females differ in their individual mate choice behaviours depending on the harmfulness of a previous mating experience, and does this variation itself have a genetic basis?

To answer these questions, I investigated a (poorly understood) source of individual variation in female mate choice behaviours ,mating experience,and examined the causes and consequences of this variation from a novel perspective (relationship to sexual conflict).

In these studies I predicted that:

1.) Males would phenotypically vary in their impact on female fitness between a brief and prolonged period of exposure, and there would be underlying additive genetic variation associated with this phenotypic variation.

2.) Female mate choice behaviours will differ depending on the harmfulness of their previous mate(s). Furthermore, I predict that there will be differences in the expression of individual female behavioural phenotypes associated with their genetic background.

In the following two chapters, I describe my two major experiments, designed to test these questions and expand our understanding of harmful male traits, individual variation in female mate choice (and the potential interactions between the two).

References

Andersson, M. B. (1994). *Sexual selection*. Princeton University Press, Princeton, NI.

Arnqvist, G. (1997). Evolution of water strider mating systems: causes and consequences of sexual conflicts. *Evolution of mating systems in insects and arachnids*.

Arnqvist, G., & Rowe, L. (2002). Antagonistic coevolution between the sexes in a group of insects. *Nature*, *415*(6873), 787-789

Arnqvist, G., & Rowe, L. (2005). *Sexual conflict*. Princeton University Press, Princeton, NI.

Bailey, N. W. (2008). Love will tear you apart: different components of female choice exert contrasting selection pressures on male field crickets. *Behavioral Ecology*, *19*(5), 960-966.

Bailey, N. W., & Zuk, M. (2009). Field crickets change mating preferences using remembered social information. *Biology letters*, *5*(4), 449-451.

Berglund, A. (1994). The operational sex ratio influences choosiness in a pipefish. *Behavioral Ecology*, *5*(3), 254-258.

Booksmythe, I., Detto, T., & Backwell, P. R. (2008). Female fiddler crabs settle for less: the travel costs of mate choice. *Animal Behaviour*, *76*(6), 1775-1781.

Brooks, R., & Jennions, M. D. (1999). The dark side of sexual selection. *Trends in ecology & evolution*, *14*(9), 336-337.

Brooks, R., & Endler, J. A. (2001). Female guppies agree to differ: Phenotypic and genetic variation in mate choice behavior and the consequences for sexual selection. *Evolution*, *55*(8), 1644-1655.

Carvalho, G. B., Kapahi, P., Anderson, D. J., & Benzer, S. (2006). Allocrine modulation of feeding behavior by the sex peptide of *Drosophila*. *Current Biology*, *16*(7), 692-696.

Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F., & Partridge, L. (1995). Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature*, *373*(6511), 241-244.

Chapman, T., Arnqvist, G., Bangham, J., & Rowe, L. (2003a). Sexual conflict. *Trends in Ecology & Evolution*, *18*(1), 41-47.

Chapman, T., Bangham, J., Vinti, G., Seifried, B., Lung, O., Wolfner, M. F., ... & Partridge, L. (2003b). The sex peptide of *Drosophila melanogaster*: female post-mating

responses analyzed by using RNA interference. *Proceedings of the National Academy of Sciences*, *100*(17), 9923-9928.

Chen, P. S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M., & Böhlen, P. (1988). A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. *Cell*, *54*(3), 291-298.

Chenoweth, S. F., & Blows, M. W. (2006). Dissecting the complex genetic basis of mate choice. *Nature Reviews Genetics*, *7*(9), 681-692.

Civetta, A., & Clark, A. G. (2000). Correlated effects of sperm competition and postmating female mortality. *Proceedings of the National Academy of Sciences*, *97*(24), 13162-13165.

Darwin, C. (1859). *On the origin of species by means of natural selection, or the preservation of favoured race in the struggle for life.* John Murray, London, UK.

Darwin, C. (1891). *The descent of man, and selection in relation to sex.* John Murray, London, UK.

Endler, J. A., & Basolo, A. L. (1998). Sensory ecology, receiver biases and sexual selection. *Trends in ecology & evolution*, *13*(10), 415-420.

Etienne, L., Rousset, F., Godelle, B., & Courtiol, A. (2014). How choosy should I be? The relative searching time predicts evolution of choosiness under direct sexual selection. In *Proc. R. Soc. B* (Vol. 281, No. 1785, p. 20140190). The Royal Society.

Fisher, R. A. (1930). *The genetical theory of natural selection: a complete variorum edition*. Oxford University Press.

Forsgren, E. (1992). Predation risk affects mate choice in a gobiid fish. *The American Naturalist*, *140*(6), 1041-1049.

Friberg U. (2005). Genetic variation in male and female reproductive characters associated with sexual conflict in *Drosophila melanogaster*. *Behavioural Genetics* 35, 455-462.

Fiumera AC, Dumont BL, Clark AG. (2006). Natural variation in male-induced 'cost-of-mating' and allele-specific association with male reproductive genes in *Drosophila melanogaster*. *Proceedings of the Royal Society of London B: Biological Sciences*. 361, 355-361.

Gavrilets, S., Arnqvist, G., & Friberg, U. (2001). The evolution of female mate choice by sexual conflict. *Proceedings of the Royal Society of London B: Biological Sciences*, *268*(1466), 531-539.

Gibson, R. M., & Bachman, G. C. (1992). The costs of female choice in a lekking bird. *Behavioral Ecology*, *3*(4), 300-309.

Godin, J. G. J., & Briggs, S. E. (1996). Female mate choice under predation risk in the guppy. *Animal Behaviour*, *51*(1), 117-130.

Hedrick, A. V., & Dill, L. M. (1993). Mate choice by female crickets is influenced by predation risk. *Animal Behaviour*, *46*(1), 193-196.

Heywood, J. S. (1989). Sexual selection by the handicap mechanism. *Evolution*, 1387-1397.

Holland, B., & Rice, W. R. (1998). Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution*, 1-7.

Holland, B., & Rice, W. R. (1999). Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proceedings of the National Academy of Sciences*, *96*(9), 5083-5088.

Hunt, J., Brooks, R., & Jennions, M. D. (2005). Female mate choice as a condition dependent life history trait. *The American Naturalist*, *166*(1), 79-92.

Jennions, M. D., & Petrie, M. (1997). Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews*, *72*(2), 283-327.

Kamimura, Y. (2007). Twin intromittent organs of *Drosophila* for traumatic insemination. *Biology Letters*, *3*(4), 401-404.

Kirkpatrick, M., & Ryan, M. J. (1991). The evolution of mating preferences and the paradox of the lek. *Nature*, *350*(6313), 33-38.

Kirkpatrick, M. (1996). Good genes and direct selection in the evolution of mating preferences. *Evolution*, 2125-2140.

Kokko, H., Brooks, R., Jennions, M. D., & Morley, J. (2003). The evolution of mate choice and mating biases. *Proceedings of the Royal Society of London B: Biological Sciences*, *270*(1515), 653-664.

Lew, T. A., & Rice, W. R. (2005). Natural selection favours harmful male *Drosophila melanogaster* that reduce the survival of females. *Evolutionary Ecology Research*, *7*(4), 633-641.

Long, T. A., Montgomerie, R., & Chippindale, A. K. (2006). Quantifying the gender load: can population crosses reveal interlocus sexual conflict?. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *361*(1466), 363-374.

Moore, P. J., & Moore, A. J. (2001). Reproductive aging and mating: the ticking of the biological clock in female cockroaches. *Proceedings of the National Academy of Sciences*, *98*(16), 9171-9176.

Newberry, K. (1989). The effects on domestic infestations of *Cimex lectularius* bedbugs of interspecific mating with *C. hemipterus*. *Medical and veterinary entomology*, *3*(4), 407-414.

Otto, S. P., & Feldman, M. W. (1997). Deleterious mutations, variable epistatic interactions, and the evolution of recombination. *Theoretical population biology*, *51*(2), 134-147.

Parker, G. A. (1979). Sexual selection and sexual conflict. *Sexual selection and reproductive competition in insects*, 123-166. Academic Press Inc, New York, NY.

Parker, G. A. (1983). Mate quality and mating decisions. *Mate choice*, *141*, 164.

Partridge, L., & Fowler, K. (1990). Non-mating costs of exposure to males in female *Drosophila melanogaster*. *Journal of Insect Physiology*, *36*(6), 419-425.

Qvarnström, A. (2001). Context-dependent genetic benefits from mate choice. *Trends in Ecology & Evolution*, *16*(1), 5-7.

Pomiankowski, A., & Iwasa, Y. (1998). Runaway ornament diversity caused by Fisherian sexual selection. *Proceedings of the National Academy of Sciences*, *95*(9), 5106-5111.

Price, T., Schluter, D., & Heckman, N. E. (1993). Sexual selection when the female directly benefits. *Biological Journal of the Linnean Society*, *48*(3), 187-211.

Ratterman, N. L., Rosenthal, G. G., Carney, G. E., & Jones, A. G. (2014). Genetic variation and covariation in male attractiveness and female mating preferences in *Drosophila melanogaster*. *G3: Genes| Genomes| Genetics*, *4*(1), 79-88.

Rebar, D., Bailey, N. W., & Zuk, M. (2009). Courtship song's role during female mate choice in the field cricket Teleogryllus oceanicus. *Behavioral Ecology*, arp143.

Rebar, D., Zuk, M., & Bailey, N. W. (2011). Mating experience in field crickets modifies pre-and postcopulatory female choice in parallel. *Behavioral Ecology*, *22*(2), 303-309.

Rice, W. R. (1992). Sexually antagonistic genes: experimental evidence. *Science*, *256*(5062), 1436-1439.

Rice, W. R. (1996). Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature*, *381*(6579), 232-234.

Rice, W. R., & Holland, B. (1999). Reply to comments on the chase-away model of sexual selection. *Evolution*, *53*(1), 302-306.

Ritchie, M. G., Saarikettu, M., & Hoikkala, A. (2005). Variation, but no covariance, in female preference functions and male song in a natural population of *Drosophila montana*. *Animal Behaviour*, *70*(4), 849-854.

Rodríguez, R. L., Rebar, D., & Fowler-Finn, K. D. (2013). The evolution and evolutionary consequences of social plasticity in mate preferences. *Animal Behaviour*, *85*(5), 1041-1047.

Rowe, L., Arnqvist, G., Sih, A., & Krupa, J. J. (1994). Sexual conflict and the evolutionary ecology of mating patterns: water striders as a model system. *Trends in Ecology & Evolution*, *9*(8), 289-293.

Sawby, R., & Hughes, K. A. (2001). Male genotype affects female longevity in *Drosophila melanogaster*. *Evolution*, *55*(4), 834-839.

Servedio, M. R., Van Doorn, G. S., Kopp, M., Frame, A. M., & Nosil, P. (2011). Magic traits in speciation:'magic'but not rare?. *Trends in Ecology & Evolution*, *26*(8), 389-397.

Sharon, G., Segal, D., Ringo, J. M., Hefetz, A., Zilber-Rosenberg, I., & Rosenberg, E. (2010). Commensal bacteria play a role in mating preference of Drosophila melanogaster. *Proceedings of the National Academy of Sciences*, *107*(46), 20051-20056.

Simmons, M. J., & Crow, J. F. (1977). Mutations affecting fitness in *Drosophila* populations. *Annual review of genetics*, *11*(1), 49-78.

Soller, M., Bownes, M., & Kubli, E. (1999). Control of oocyte maturation in sexually mature *Drosophila* females. *Developmental biology*, *208*(2), 337-351.

Tennant, H. M., Sonser, E. E., & Long, T. A. (2014). Hemiclonal analysis of interacting phenotypes in male and female *Drosophila melanogaster*. *BMC evolutionary biology*, *14*(1), 95.

Trivers, R. (1972). Parental investment and sexual selection. *Sexual Selection & the Descent of Man, Aldine de Gruyter, New York*, 136-179.

Verzijden, M. N., Ten Cate, C., Servedio, M. R., Kozak, G. M., Boughman, J. W., & Svensson, E. I. (2012). The impact of learning on sexual selection and speciation. *Trends in Ecology & Evolution*, *27*(9), 511-519.

Walpole, D. E., & Newberry, K. (1988). A field study of mating between two species of bedbug in northern KwaZulu, South Africa. *Medical and veterinary entomology*, *2*(3), 293-296.

Widemo, F., & Sæther, S. A. (1999). Beauty is in the eye of the beholder: causes and consequences of variation in mating preferences. *Trends in Ecology & Evolution*, *14*(1), 26-31.

Wigby, S., & Chapman, T. (2004). Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution*, *58*(5), 1028-1037.

Williams, G. C. (1966). *Adaptation and natrual selection: a critique of some current evolutionary thought* (No. 574.52 W5).

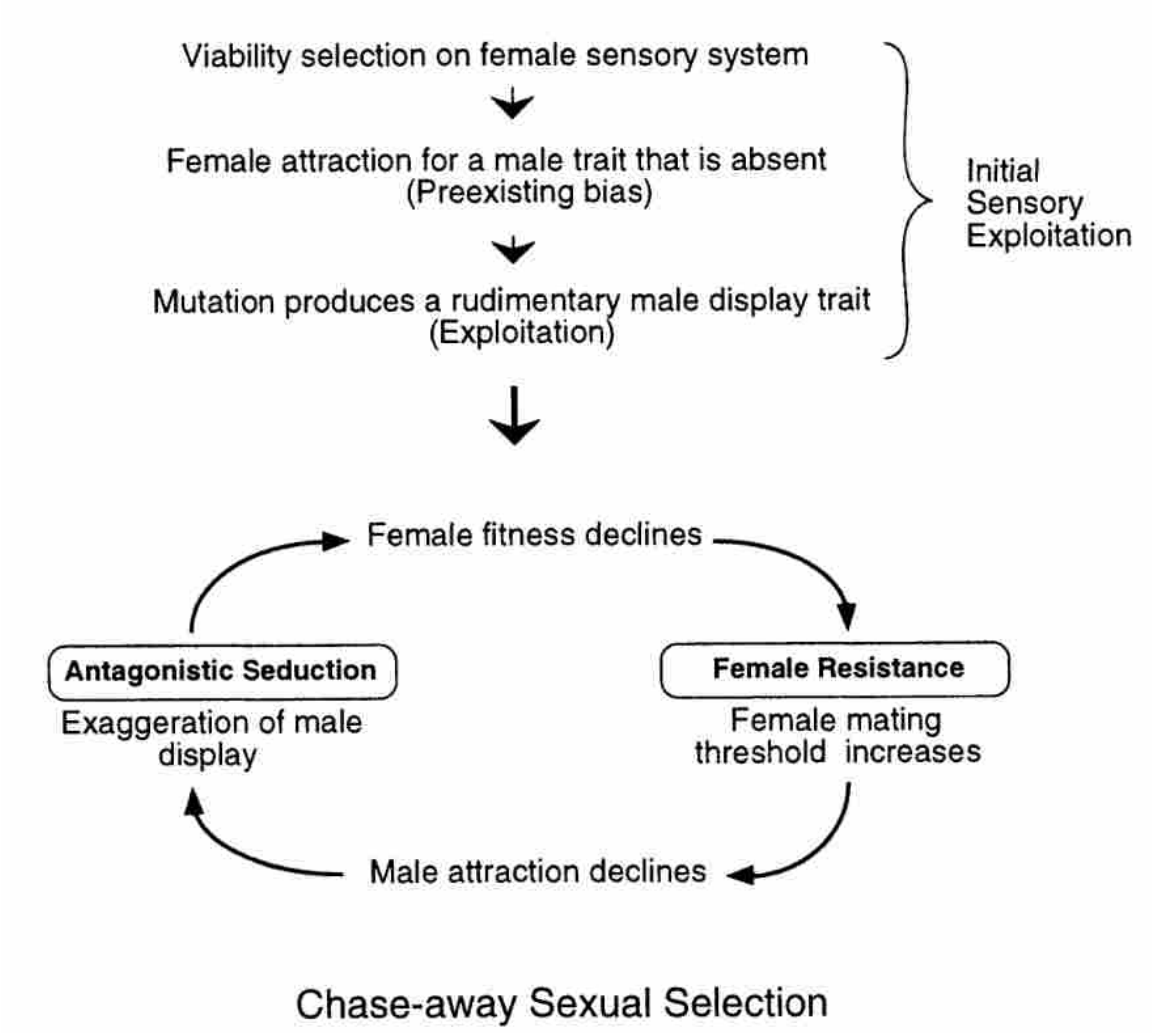

Figure 1.1: The chase-away sexual selection model for the evolution of elaborate male display traits. A male display trait is defined as any phenotype trait that attracts females, and thereby increases the probability of mating (Adapted from Holland & Rice, 1998).

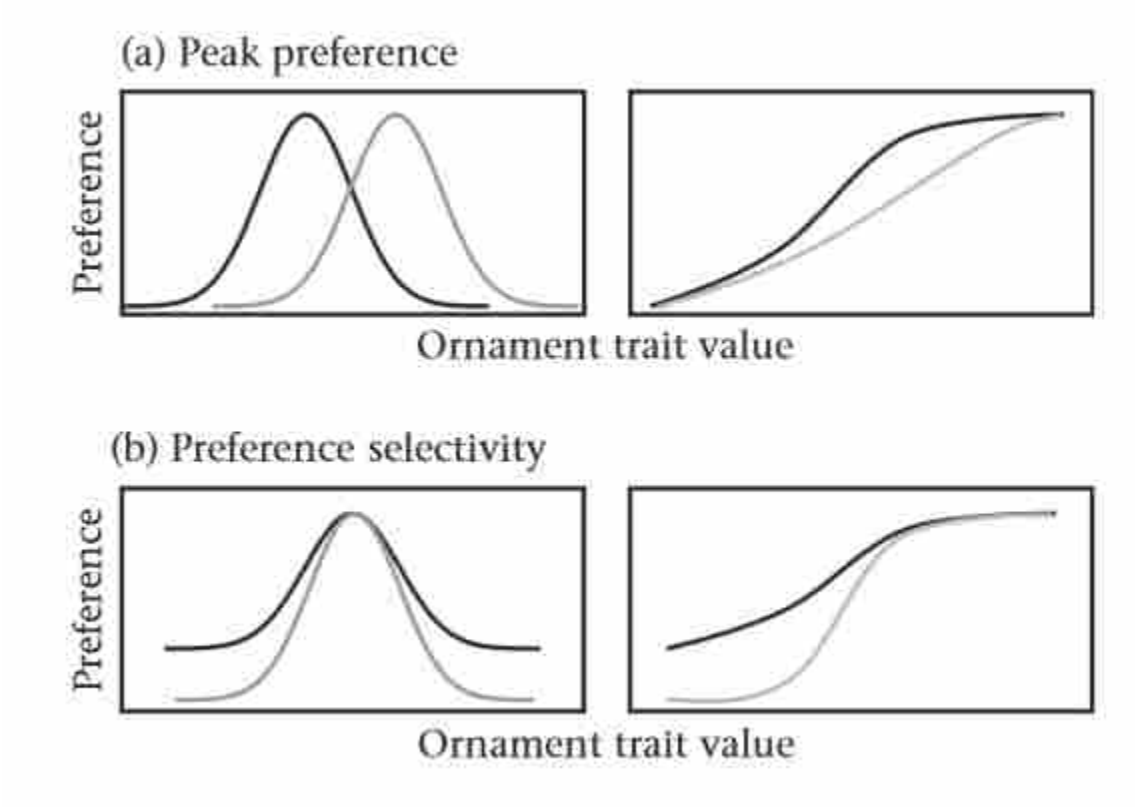

Figure 1.2: Describing variation in mate preference functions. Mate preferences may differ in shape. They may be 'closed' (unimodal), as shown in the left hand panels in (a) and (b), or 'open-ended', as shown in the right-hand panels in (a) and (b). Mate preferences of either shape can be further characterized with two main traits: (a) 'peak preference' is the ornament trait value with highest attractiveness; each panel in the top row shows two mate preferences that differ in peak preference; (b) 'preference selectivity' summarizes variation in aspects of preference shape other than peak; each panel in the bottom row shows two mate preferences that differ in selectivity (Adapted from Rodríguez *et al*., 2013).

Preamble

The following chapter was written in the style of *Biology Letters*, where it was published on April 27, 2016. Due to word count and figure limitations enforced by the journal, the following chapter was slightly modified from the published version to include details from the supplementary materials section. Figure and table numbers have been slightly altered for consistency with the rest of this thesis.

CHAPTER 2

Genetic variation in male-induced harm in *Drosophila melanogaster*

David C.S. Filice[1,2], Tristan A.F Long[1]

[1.] Department of Biology, Wilfrid Laurier University

[2.] Corresponding author: Department of Biology, Wilfrid Laurier University, 75 University Avenue West, Waterloo, ON N2L 3C5, Canada.

Phone: +1 519-884-0710 x2888 Email: fili2950@mylaurier.ca

Keywords: *Drosophila melanogaster*, male-induced harm, mate choice, sexual conflict, fitness, population genetics

Abstract

In *Drosophila melanogaster*, prolonged exposure to males reduces the longevity and fecundity of females. This harm arises from the effects of male courtship behaviours and the toxic-side effects of the accessory gland proteins (Acps) in their seminal fluids. Here, we examine the relationship between male exposure and its harmful effect on the lifetime fitness of his mates, and quantify the genetic basis for this variation. We found significant additive genetic variation in the magnitude of harm that males impose on females by exposing females to males from a variety of hemiclonal backgrounds for either a brief or prolonged period of time and measuring their fecundity, a meaningful fitness index. Furthermore, we discovered a strong *negative* correlation between the magnitude of harm and the short-term effects of male exposure on female fitness. We discuss the evolutionary significance of these results with regards to potential life-history trade-offs in females, and its relationship to male body size.

Introduction

Selection acting on males to increase their individual reproductive success can sometimes favour the evolution of traits that are ultimately deleterious to the fitness of their mates [1]. This inter-locus sexually antagonistic selection may be manifested through physical harm that males inflict on their mates during courtship and copulation [2,3]. The male-specific benefits associated with harm can be substantial, and are likely to be under strong directional selection [4]. Despite this, we see considerable phenotypic variation in the magnitude of individual male-induced harm within populations (possibly due to segregating genetic variation) [5,6]. As such, we set out to study the ecological and genetic underpinnings of male-induced harm in order to gain insight in the forces that shape its evolution.

Our study used fruit flies, *Drosophila melanogaster*, a model species in which the mechanisms of male-induced harm has been extensively investigated. Male flies transfer numerous Acps in their ejaculate to their mates, some of which have toxic side-effects [7,8]. Additionally, males harm females during courtship and copulation [9,10]. This harm is manifested as decreased female fecundity and/or longevity, and thus constitutes an important selective pressure on females in this species [11]. The extent to which this trait is heritable will therefore have important consequences for the rate and/or trajectory of inter-sexual co-evolution. Despite its importance, only a handful of attempts have been made to determine if there is additive genetic variance for male-induced harm [12,13,14]. Additionally, these studies have been limited in several ways. Firstly, they only examined variation present on a single chromosome and/or quantified harm using females carrying multiple deleterious mutations that were obtained from populations with a different

evolutionary origin from which the males' genetic material was derived. Secondly, harm was quantified by examining female mortality rates, a fitness metric of limited value in laboratory-reared organisms cultured with short, non-overlapping generations. Instead, measuring egg production during key oviposition-windows yields more meaningful estimates of fitness [15], and avoids genotype-by-environment interactions for performance under novel test conditions [16].

Here, we set out to determine if phenotypic variation in the magnitude of harm that males inflict on their mates reflects the presence of additive genetic variation. Our assays were conducted using a large, outbred population, and used a protocol that mimicked (as closely as possible) the environment to which fruit flies were adapted [16]. We used hemiclonal analysis techniques [17 *& Supplemental Methods*] to quantify the degree of additive genetic variation responsible for male-induced harm to female fecundity, where harm is quantified for each hemiclone line as the difference in fecundity of females that were briefly exposed to males, and those continuously housed with males [18]. Furthermore, we also examined the potential relationship between harm and male body size, as these two traits are often assumed to be positively correlated with each other [19,20].

Materials and Methods

In this experiment, we used fruit flies derived from the large (~3500 adults per generation), wild-type population *Ives* (hereafter "IV") which has been cultured under standardized conditions for hundreds of generations [15 *& Supplemental Methods*]. This population harbors considerable genetic variation for many traits, and has been used in a wide range of behavioral ecology and population genetics studies [5,16,21]. The

population is cultured on a non-overlapping two-week schedule, in which they are combined *en masse* every 14 days and distributed onto fresh media. After 2-3 hours, adult flies are discarded and the density of eggs in each vial is standardized to ~100 apiece. The number of eggs laid by females during this brief period of time is thus of great relevance to their fitness, as it is their only opportunity to directly pass on their genes to the next generation. From this population, we established 26 whole haploid-genome clone lines using cytogenetic cloning techniques [17,22], which we subsequently expressed in a hemiclonal state using established protocols [17,22 & *Supplemental Methods*].

To quantify the genetic basis for variation in the magnitude of male-induced harm, 200 hemiclone males were collected from each line, and housed in single-sex vials. Simultaneously, 1300 females were collected as virgins (within 8h of eclosion) from standard IV culture vials. The larvae pupated on acetate inserts [15], which were transferred to holding vials prior to adult eclosion, allowing us to save the "natal" vials (containing spent media) for use as mating chambers. On the morning corresponding to the 11th day of the flies' culture cycle, in each of two replicate blocks, we created two mating chambers for each hemiclonal line, consisting of 25 females and 50 males placed into a "natal" vial. Males and females were left in one of these vials for 180 minutes (the "short-exposure" treatment), which is typically sufficient to ensure all females will have mated once (TAFL, *pers. obs.*). Following this, males were removed from these vials, and females were retained for an additional 45h. In the other vial (the "long-exposure" treatment), females and males we housed together for the full 48h period. At the end of this period, females in both treatments were removed from their vials, and individually

placed into test tubes (containing 3mL of fresh media) for 18h before being discarded and their eggs immediately counted. This protocol attempts to mimic the phenology & developmental conditions historically experienced by this population as closely as possible (see *Supplemental Methods*). All males were collected, frozen, and later dried overnight at 70°C to be weighed on a Sartorius M5 microbalance to the nearest 0.001mg.

Results

For both the long- and short-exposure treatments, male line had a significant effect on the observed phenotypic expression of their mates' fecundity (Table 2.1). Furthermore, prolonged exposure to male flies resulted in a substantial decrease in the fitness of female flies (Treatment Effect: $\chi_1^2 = 233.871$, $p < 2.2 \times 10^{-16}$). However, the male effects on female fecundity that were associated with each of the hemiclonal lines were not homogenous across exposure treatments (Treatment x Clone Line: $p < 0.0001$), indicating the presence of significant additive genetic variation in male-induced harm (Table 2.1 & Figure 2.1).

We found no significant correlation between the number of eggs laid by females mated to hemiclones in the short-exposure treatment, and those laid by females mated to the same hemiclones in the long-exposure treatment. (Spearman's rho= 0.156, S= 2470, p= 0.446). Similarly, there was no correlation between body mass and the magnitude of harm (the difference in the number of eggs laid between short- and long-exposure treatments [18]) associated with each hemiclone line (Spearman's rho= 132, S= 2538, p= 0.518). However, a strong negative correlation was detected between the number of eggs laid in the short-exposure treatment and the magnitude of harm (Spearman's rho= -0.785, S= 5222, $p = 4.42 \times 10^{-6}$, Figure 2.2).

Discussion

The outcome of intra- and inter-sexual selection processes in *D. melanogaster* has important consequences for the variation in individual reproductive success in both sexes. For females, male identity has been associated with a sizeable percentage of the phenotypic variation in female fecundity [23]. Here, we demonstrated that this variation is due (in part) to the presence of segregating additive genetic variation, which can account for ~10% of female fecundity variation. Consistent with previous research [13], we found prolonged exposure to males was associated with decreased female fecundity (i.e. harm), presumably as a side-effect of traits that have evolved to benefit male fitness [1,2]. Interestingly, the effect of male hemiclone line on female fitness was not consistent between exposure times (Table 1), indicating that variation in the magnitude of harm has a genetic basis. Furthermore, our assay revealed that the impact of a male hemiclone line on a female's fecundity following a brief interaction was negatively correlated with the magnitude of harm associated with their prolonged interaction. This observation may help explain the maintenance of additive genetic variation for harmfulness in this species, if females vary in their preference for males depending on the temporal stability of their social environment and/or their extrinsic mortality risk. When associations are ephemeral, females may prefer mating with males that provide the greatest short-term benefits, but in more stable groups they may prefer to associate and mate with males that are inflict less harm over the long-term. More broadly, these results may prove enlightening to the study of life-history trade-offs between longevity and fecundity [23,24]. In environments where survivorship of females is relatively high, it may be advantageous to mate with males whose harmful effects are less deleterious to later-life fecundity, compared to situations

where there are high rates of adult mortality. We look forward to conducting future empirical and theoretical tests of these hypotheses.

A second surprising observation was the lack of any correlation between male body size and variation in short-exposure fitness, long-exposure fitness or magnitude of harm. Interestingly, these findings are at odds with previous studies that have found male body size and harm to be associated with each other [19,20]. However, in these studies body size variation was achieved by manipulating larval densities/nutrition [19,20], while our males were reared under the standardized conditions that the IV population has evolved under for decades. It is possible that phenotypic correlations may have arisen due to trade-offs resulting from this methodology. While the evolution of Acps is often viewed in the framework of increased post-copulatory success [7,8], there appears to be no genetic correlation between sperm competitive ability and male body size (at least for genes on the 2^{nd} and 3^{rd} chromosomes) [25]. Therefore, the biochemical and morphological traits associated with male-induced harm may actually not scale with male body size. Ultimately, the continued exploration of the relationships between male body size, harm, female preferences, and fitness variation under controlled genetic and environmental conditions are promising avenues for future research.

Ethics. The research conducted in this study did not require approval from an ethics committee.

Data accessibility. The data and codes have been deposited to Dyrad: http://dx.doi.org/10.5061/dryad.6008n

Authors' contributions. DCSF carried out the experiments. Both authors designed the experiments, contributed to the data analysis, writing, and editing process of the

manuscript. Both authors approve of the final manuscript submission and hold accountability for the accuracy and integrity of its contents.

Competing interests. The authors declare no competing interests.

Funding. TAFL was funded by a Discovery Grant from the Natural Sciences and Engineering Council of Canada (NSERC).

Acknowledgements. We thank members of the Long Lab for their "fly pushing."

References

1. Parker G. 1979 Sexual selection and sexual conflict. *Sexual selection and reproductive competition in insects.*, 123-166.

2. Morrow EH, Arnqvist G, Pitnick S. 2003 Adaptation versus pleiotropy: why do males harm their mates?. *Behav. Ecol.* **14**, 802-806.

3. Rice WR 2000. Dangerous liaisons. *Proc. Natl. Acad. Sci. USA.* **97**, 12953-12955. (DOI 10.1073/pnas.97.24.12953 [doi]).

4. Arnqvist G, Rowe L 2005. Sexual conflict. *Princeton University Press, Princeton, NI.*

5. Sawby R, Hughes, KA. 2001. Male genotype affects female longevity in *Drosophila melanogaster. Evolution.* **55**, 834-839.

6. Civetta A, Clark AG. 2000 Correlated effects of sperm competition and postmating female mortality. *Proc. Natl. Acad. Sci. U. S. A.* **97**, 13162-13165. (DOI 10.1073/pnas.230305397 [doi]).

7. Chapman T, Liddle, LF, Kalb JM, Wolfner, MF, Partridge L. 1995 Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature.* **373**, 241-244.

8. Rice WR. 1996 Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature.* **381**, 232-234. (DOI 10.1038/381232a0 [doi]).

9. Partridge L, Fowler K. 1990 Non-mating costs of exposure to males in female *Drosophila melanogaster. J. Insect Physiol.* **36**, 419-425.

10. Kamimura Y. 2007 Twin intromittent organs of *Drosophila* for traumatic insemination. *Biol. Lett.* **3**, 401-404. (DOI X527L59421J0P125 [pii]).

11. Stewart AD, Morrow EH, Rice WR. 2005 Assessing putative interlocus sexual conflict in *Drosophila melanogaster* using experimental evolution. *Proc. Biol. Sci.* **272**, 2029-2035. (DOI W1800378707321N5 [pii]).

12. Friberg U. 2005 Genetic variation in male and female reproductive characters associated with sexual conflict in *Drosophila melanogaster. Behav. Genet.* **35**, 455-462.

13. Lew TA, Rice WR 2005 Natural selection favours harmful male *Drosophila melanogaster* that reduce the survival of females. *Evol. Ecol. Res.* **7**, 633-641.

14. Fiumera AC, Dumont BL, Clark AG. 2006 Natural variation in male-induced 'cost-of-mating' and allele-specific association with male reproductive genes in *Drosophila*

melanogaster. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **361**, 355-361. (DOI 10.1098/rstb.2005.1791 [doi]).

15. Long TAF, Montgomerie R, Chippindale AK. 2006 Quantifying the gender load: can population crosses reveal interlocus sexual conflict?. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **361**, 363-374. (DOI 10.1098/rstb.2005.1786 [doi]).

16. Rose MR, Passananti HB, Matos M. 2004 Methuselah flies: a case study in the evolution of aging. *World Scientific*.

17. Abbott JK, Morrow EH. 2011 Obtaining snapshots of genetic variation using hemiclonal analysis. *Trends Ecol. Evolut.* **26**, 359-368.

18. Linder J, Rice WR. 2005 Natural selection and genetic variation for female resistance to harm from males. *J. Evol. Biol.* **18**, 568-575.

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

20. Pitnick S, Garcia-Gonzalez F. 2002 Harm to females increases with male body size in *Drosophila melanogaster*. *Proc. Biol. Sci.* **269**, 1821-1828. (DOI 10.1098/rspb.2002.2090 [doi]).

21. Martin ES, Long TAF. 2015 Are flies kind to kin? The role of intra- and inter-sexual relatedness in mediating reproductive conflict. *Proc. Biol. Sci.* **282**, 10.1098/rspb.2015.1991. (DOI 10.1098/rspb.2015.1991 [doi]).

22. Chippindale AK, Gibson JR, Rice WR. 2001 Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proc. Natl. Acad. Sci. U. S. A.* **98**, 1671-1675. (DOI 10.1073/pnas.041378098 [doi]).

23. Tennant HM, Sonser EE, Long TA. 2014 Variation in male effects on female fecundity in Drosophila melanogaster. *J. Evol, Biol*. **27**, 449-54.

24. Travers LM, Garcia-Gonzalez F, Simmons LW. 2015 Live fast die young life history in females: evolutionary trade-off between early life mating and lifespan in female Drosophila melanogaster. *Sci Rep*. **5**.

25. Nandy B, Gupta V, Sen S, Udaykumar N, Samant MA, Ali SZ, Prasad NG. 2013 Evolution of mate-harm, longevity and behaviour in male fruit flies subjected to different levels of interlocus conflict. *BMC Evol Biol*. **13**, 212.

26. Zhang R, Amah L, Fiumera AC. 2008 Autosomal variation for male body size and sperm competition phenotypes is uncorrelated in *Drosophila melanogaster*. *Biol. Lett.* **4**, 500-503. (DOI 10.1098/rsbl.2008.0283 [doi])

27. Bates D, Mächler M, Bolker B, Walker S. 2014 Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:1406.5823.*

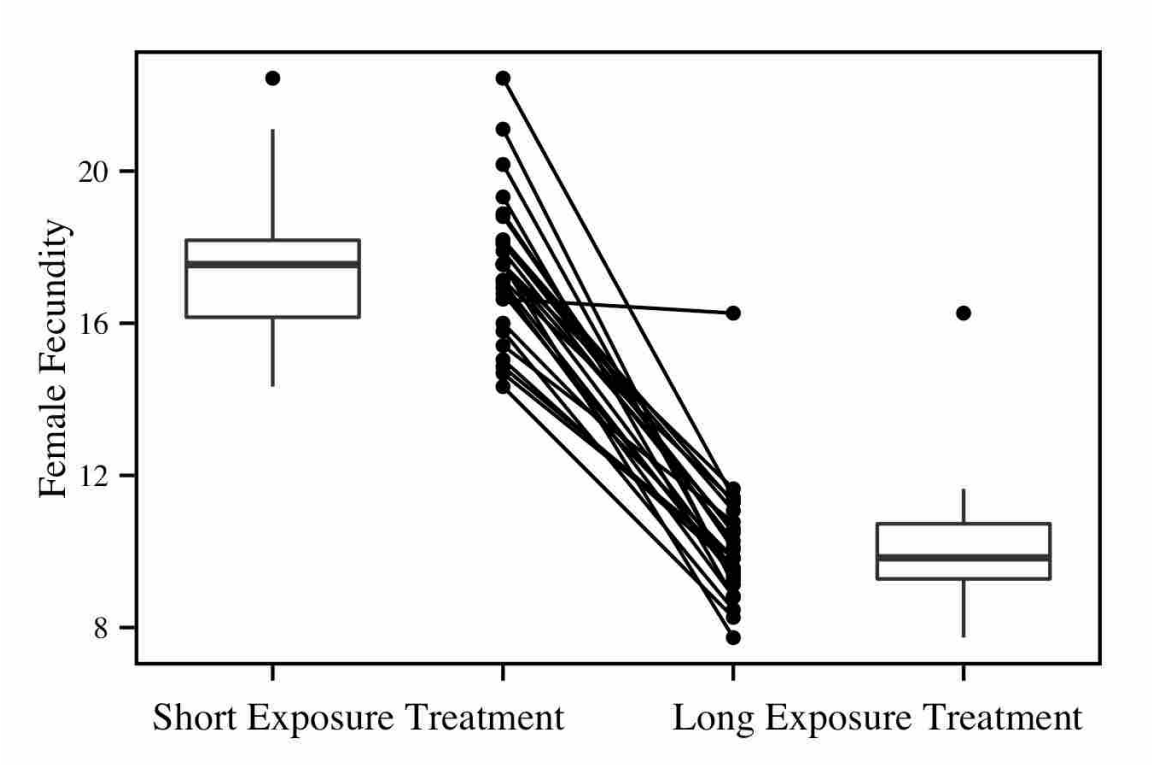

Figure 2.1: Effect of male genotype and exposure treatment on fecundity (number of eggs laid on Day 14 of the culture cycle) of female *Drosophila melanogaster*. The reaction norm plot at the centre depicts female fecundity for each of the 26 hemiclone lines across the two male-exposure duration treatments, while boxplots illustrate the overall differences between the treatments. The boxes enclose the middle 50% of each distribution (inter-quartile range, IQR), with the horizontal bars indicating the location of the medians. Values >±1.5x the IQR are outliers, and are indicated by closed circles, and whiskers extend from the margins of the box to the minimum and maximum values that are not outliers.

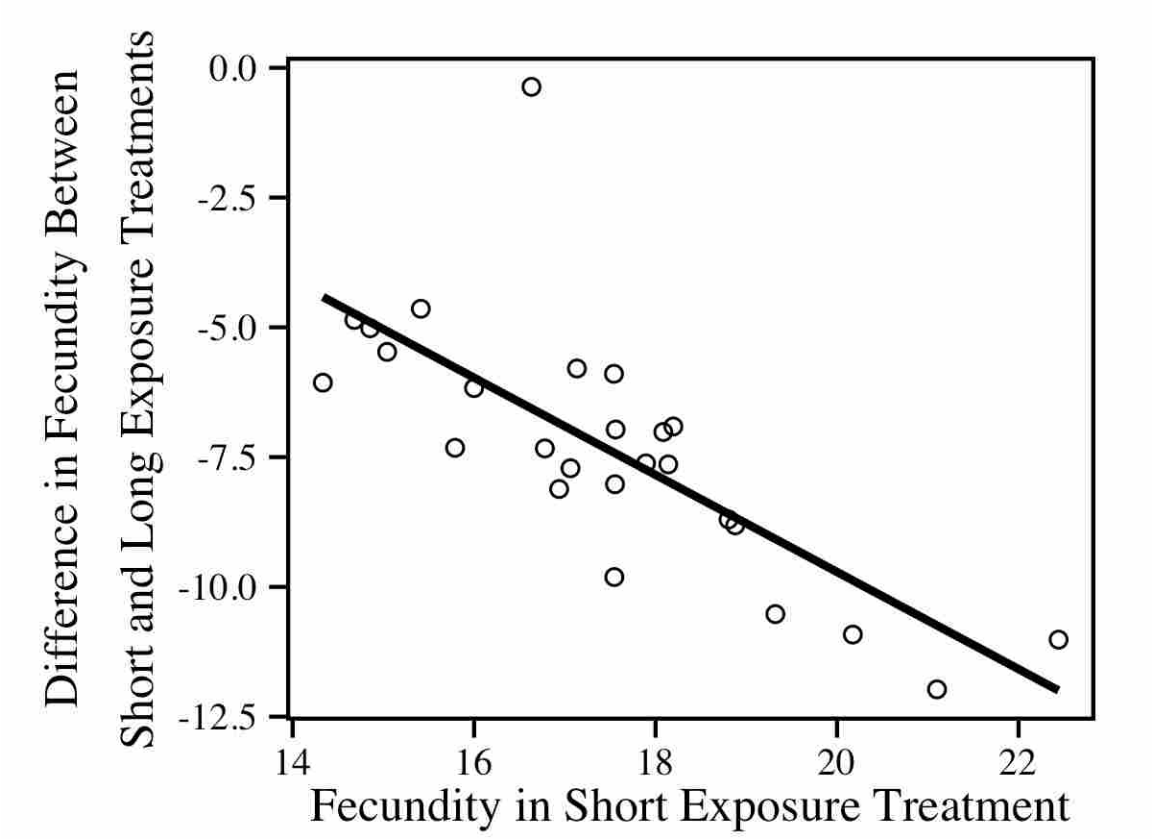

Figure 2.2: Scatterplot and regression line illustrating the negative relationship between fecundity of females in the short-term treatment, and the net-cost of prolonged male exposure in female *Drosophila melanogaster* that had been exposed to 26 different male hemiclone lines. Fecundity is defined as the number of eggs laid by females on Day 14 of their culture cycle.

Table 2.1: Variance components estimated using generalized linear mixed models fit by maximum likelihood for phenotypic variation in fecundity (egg production on Day 14 of their culture cycle) of female *Drosophila melanogaster* exposed to male hemiclones for either a short or a long period of time. We created models that included exposure treatment as a fixed effect, and clone line and interaction between exposure treatment and clone line as treated as random effects, as well as models for each treatment separately. Variance components and bootstrapped CI values were calculated using *lme4* package [26]. The statistical significance of each variance component was determined using a permutation test approach with 10,000 samples (*see Supplemental Methods).* A significant interaction between treatment and hemiclone line indicated that the fitness consequence of associating (and mating) with males from a given hemiclone background vary depending on their length of exposure to males.

Fitness	Source of Variance	Variance (SD)	Bootstrapped Upper & Lower 95% CI	% of Variance explained	P-value
Short-term exposure	Clone Line	3.194 (1.787)	5.524, 1.400	10.19	<0.0001
	Residual	28.143 (5.305)			
Long-term exposure	Clone Line	2.297 (1.516)	3.975, 1.047	12.84	<0.0001
	Residual	15.589 (3.948)			
Both Short- and Long Exposures	Treatment x Clone Line	2.517 (1.586)	3.851, 1.104	10.23	<0.0001
	Clone Line	0.232 (0.481)	1.155, 0.000	0.94	0.0257
	Residual	21.853 (4.674)			

Supplemental Methods

Source population history & culture protocols

The ultimate source of the genetic variation in our assays was the *Ives* (hereafter IV) population of *Drosophila melanogaster*. This outbred, wild-type, population was founded in 1975 from a sample of 200 females and 200 males collected in South Amherst, MA, USA. Since 1980, this population has been at large census size (>1000 adults/generation), on non-overlapping generations on a standardized culture protocol (Rose *et al.* 1984, Long *et al.* 2006, Martin & Long 2015). Flies are cultured in vials, each of which contains ~10ml of media consisting of banana, agar, corn syrup, barley malt & killed yeast. IV flies are maintained in an 25°C, 50% relative humidity environment on a 12L:12D diurnal light cycle. Our lab's population of IV is maintained at ~3500 adult flies generation^{-1} and was obtained from Adam Chippindale (Queen's University, Kingston) in 2011, who, in turn, obtained them from Michael R. Rose (UC Irvine, Irvine) in 2002 (Long et. al 2006).

At the start of each culture generation (Day "0") all eclosed adult flies are removed from their "natal" vials (the vials in which they has developed) using light CO_2 anesthesia, and mixed *en masse*, before being divided into equal groups and transferred to 35 new "oviposition" vials (vials containing fresh media). Flies are left in these vials for ~2-3 hours before being removed, and the eggs that are laid during this time are culled (by hand) to a density of 100 eggs vial^{-1}. These vials become the "natal" vials for the next generation of flies. Females start eclosing from their pupae as adults ~Day 8, and males start eclosing as adults ~Day 9. All flies are kept in these vials until the end of the generation (Day 14).

In our assays we also used flies obtained from the IV-*bw* population, which was created by repeatedly backcrossing a recessive brown-eyed mutant (bw1) into the IV genetic background. The IV-*bw* population is maintained under the same culture protocol as the IV population, and is regularly backcrossed to the IV population to ensure that the two populations have not diverged.

The selective environment under which the IV population has been cultured under for >900 generations has favoured the evolution of semelparous life-history traits in female flies. The brief window in which female flies find themselves in the oviposition vials is effectively the only opportunity that they have to make a contribution to the next generation. While adult females in the IV population will mate (repeatedly) in their natal vials, they largely delay ovipositing until they are transferred to the "oviposition" vial (Long *et al.* 2006). Thus measuring egg production on Day 14 of a female's life cycle (using a protocol that mimics as closely as possible the conditions to which flies have adapted) provides a meaningful metric of her individual lifetime fitness (*see* Rice et al. 2006). In our experiments we replicated the developmental environment of flies' early-adulthood by placing recently-eclosed flies into vials containing "spent" media (in which larvae had developed) (following protocols described in Long *et al.* 2006), which approximates the pre-"Day 14" culture conditions experienced by these flies. Since we wanted to measure individual female egg-production, we placed females into individual test-tubes, and measured egg laying over a 18h period (*as per* Tennant *et al*. 2013, Tennant *et al*. 2014), which is longer than typically afforded to females, but reduces the impact of stochastic variation associated with the anesthetization and transfer of individual females from vials to test-tubes.

Cytogenetic cloning & hemiclonal analyses

From the IV population we established 26 clone lines, using cytogenetic cloning techniques, which were subsequently expressed in a hemi-clonal male genetic background. Each clone line was created following established protocols (Chippindale *et al.* 2001, Tennant *et al.* 2014), and consists of a nearly-complete haploid genomes, maintained and propagated in a unrecombined state (*see* Chippindale et al. 2001, Rice *et al*. 2006, Abbott & Morrow 2011).

Clone lines are created and maintained by mating males randomly sampled from the IV population with females from a "clone-generator" population, who possess a random Y chromosome, a conjoined "double -X" chromosome [C(1)DX, *y, f*], and are homozygous for translocated autosomes [T(2;3) *rdgC st in ri* p^{P} bw^{D}]. Establishment, propagation, and maintenance of clone lines is possible due to the lack of recombination in male *D. melanogaster*, and the phenotypic expression of the artificial cytogenetic constructs in offspring (Chippindale et al. 2001, Rice et al. 2006, Tennant *et al*. 2014, Abbott & Morrow 2011). Together these allow us to track the haploid genome as it is passed on from father to sons, generation after generation.

These haploid genomes can be then expressed in a male "hemi-clonal" state (paired with a random genetic background) by crossing clone males with flies from the "DX-IV" population, that contained the "double-X" chromosome, but otherwise possess a random sample of autosomes originating from the IV population. In these crosses, fathers contribute the X-chromosome to their sons, which receive their Y-chromosome from their mother. Due to the high (75%) inviability of the offspring resulting from these crosses (due to chromosomal imbalances), eggs produced from these crosses are placed

into vials in sets of 200, along with 50 similarly-aged IV-bw eggs, in order to maintain a developmental environment that is similar to that which has been historically experienced by the IV population (i.e. 100 viable eggs $vial^{-1}$).

Statistical analyses associated with generalized linear mixed models

Data on female fecundity (egg production on Day 14 of their culture cycle) in female *D. melanogaster* was analyzed using generalized linear mixed models created in R (v 3.1.2; R Core Team) using the *lme4* (Bates *et al.*, 2014) package. In our initial model, we included exposure treatment (short or a long period of time), replicate vial, and their interaction as fixed effects, and clone line and the interaction between exposure treatment and clone line as treated as random effects. The significance of fixed effects was first tested using Wald Chi-square tests implemented in the *Anova* function of the *car* package (Fox & Weisberg, 2013). As neither the effect of vial, nor the interaction between treatment and vial were significant (χ_1^2= 0.082, p=0.774; & χ_1^2= 0.632, p=0.437, respectively), they were removed from subsequent models, while the significant (χ_1^2 = 233.871, $p<2.2x10^{-16}$) effect of treatment was retained.

We next created models that included treatment as a fixed effect, and clone line and the interaction between exposure treatment and clone line as treated as random effects. Additional models were created for each treatment separately, in which clone line was included as a random effect. For each of these models, we used the *bootMer* function (in the *lme4* package) to obtain 95% confidence intervals for our variance estimates. We then determined, using a permutation approach, the probability of obtaining (by random chance) our variance component estimates. Following the recommendations of Manly (2007), we randomized the fecundity data (without replacement) across all females, re-

ran the model and extracted the relevant variance component(s) from the model. This procedure was repeated 10,000 times. We then determined the fraction of repetitions in which the calculated variance estimates exceeded the magnitude of the values obtained from the model analyzing the original data. This constitutes our p-value in Table 1.

Supplemental methods references

Abbott JK, Morrow EH. 2011 Obtaining snapshots of genetic variation using hemiclonal analysis. *Trends Ecol. Evolut.* **26**, 359-368.

Rose MR, Charlesworth B. 1981 Genetics of life history in Drosophila melanogaster. II. Exploratory selection experiments. *Genetics*. 1;97(1):187-96.

Chippindale AK, Gibson JR, Rice WR. 2001 Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila. Proc. Natl. Acad. Sci. U. S. A.* **98**, 1671-1675.

Long TAF, Montgomerie R, Chippindale AK. 2006 Quantifying the gender load: can population crosses reveal interlocus sexual conflict?. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **361**, 363-374.

Rice WR, Stewart AD, Morrow EH, Linder JE, Orteiza N, Byrne PG. 2006 Assessing sexual conflict in the Drosophila melanogaster laboratory model system. *Philos. Trans. R. Soc. Lond. B. Biol. Sci*. **361**, 287-99.

Tennant HM, Sonser EE, Long TA. 2014 Hemiclonal analysis of interacting phenotypes in male and female Drosophila melanogaster. *BMC evolutionary biology*. **3;14(1)**:95.

Tennant HM, Sonser EE, Long TA. 2014 Variation in male effects on female fecundity in Drosophila melanogaster. *J. Evol, Biol*. **27**, 449-54.

Martin ES, Long TAF. 2015 Are flies kind to kin? The role of intra- and inter-sexual relatedness in mediating reproductive conflict. *Proc. Biol. Sci.* **282**, 10.

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

Bates D, Mächler M, Bolker B, Walker S. 2014 Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:1406.5823.*

Fox J, Weisberg S. 2011 An {R} Companion to Applied Regression, Second Edition. *Thousand Oaks*.

Manly, BFJ. 2007 Randomization, Bootstrap, and Monte Carlo Methods in Biology (3rd ed.). London: Chapman & Hall.

Preamble

The following chapter was written as manuscript in the style of *Animal Behaviour*, where we plan to submit it for review. Figure numbers have been slightly altered for consistency with the rest of the thesis.

CHAPTER 3

Individual variation in female mate choice in *Drosophila melanogaster* is influenced by her mating experience and genotype

David C.S. Filice[1,2], Tristan A.F Long[1]

[1.] Department of Biology, Wilfrid Laurier University

[2.] Corresponding author: Department of Biology, Wilfrid Laurier University, 75 University Avenue West, Waterloo, ON N2L 3C5, Canada.

Phone: +1 519-884-0710 x2888 Email: fili2950@mylaurier.ca

Females typically choose mates based on the greatest provision of direct or indirect benefits. Despite this, we still see significant variation in female mate choice

behaviours both within and across populations. Recent studies suggest that female mate choice is a complex decision-making process that involves many context-dependent factors. In *Drosophila melanogaster*, mating can have costly effects on a female's lifetime fitness. As such, sexual conflict theory predicts that females may make trade-offs in their mate choice decisions to balance direct costs and indirect benefits associated with mating. Here, we examined if the direct costs of a previous mating experience has an effect on subsequent female mate choice behaviours (assay 1) and quantified the degree of additive genetic variation associated with this effect (assay 2). In assay 1, randomly sampled females either remained virgin, or were mated to a male from a high, medium, or low harm genetic background. These treatments were replicated for either a short (3hrs) or long (48hrs) exposure to males before mate choice was scored. We found significant differences in the resulting female mate choice behaviours. In assay 2, a hemiclonal analysis was performed to quantify the additive genetic variation associated with experience-dependent mate choice behaviours. We discuss the significance of these results with regards to the evolution of plasticity in female mate choice behaviours and the maintenance of variation in male traits.

Introduction

Inter-sexual selection is traditionally envisioned as individuals of one sex (typically males) competing for the reproductive interest of individuals of the opposite sex (typically females) (Andersson, 1994) where individuals of the "choosy" sex bias their mating decisions based on the anticipated direct and/or indirect benefits associated with those that are selected for mating (Wiley & Poston, 1996). Models of inter-sexual selection often assume that while males may vary in their phenotypic display traits, female preference for these traits are consistent within populations (Jennions & Petrie, 1997), treating female choice as a fixed behaviour and ignoring the possibility of individual variation between females. However, recent empirical studies have found that individual variation in female mate choice behaviours is common (Jennions & Petrie, 1997; Widemo & Sæther, 1999). Understanding the causes and consequences of this variation is crucial for our understanding of sexual selection, as the underlying factors associated with individual variation in mate choice can influence the strength and direction of evolution via sexual selection (Jennions & Petrie, 1997). Here, we explore some of the factors that may explain the presence of individual variation in female mate choice.

It is possible that some of the observed individual variation in female mate choice behaviours may be attributed to an organism's specific environmental and genetic history (as well as the interaction between these factors). With regards to environmental factors, individual female mate choice may vary depending on context-dependant factors including physiological state (Hunt *et al.*, 2005; Hebets *et al.*, 2008), age (Moore & Moore, 2001; Anjos-Duarte & Costa, 2011; Abraham *et al.*, 2016), and social experience (Dukas, 2005; Bailey & Zuk, 2009; Rebar *et al.*, 2011). As mate choice is often a costly

behaviour (Kokko *et al.*, 2003), it is possible that the expression of choice may be traded-off as other life-history traits under different contexts. Qvarnström (2001) argued that in some circumstances, plasticity in mate choice behaviours may be favoured by selection over static behaviours. In some conditions, the expenditure of resources and risk of injury/predation associated with choosing between mates may be at a greater cost compared to other conditions. If a female can change her mate choice behaviours, she could potentially maximize her potential reproductive success over multiple contexts. For instance, in the black field cricket, *Teleogryllus commodus*, females that had been fed high-nutrition diets were more sexually responsive and demonstrated a stronger preference for males that exhibited mating calls at faster rates and at a more dominant frequency compared to females reared on low-nutrition diets (Hunt *et al.*, 2005). The authors concluded that female mate choice operated as a condition-dependent life-history trait. Theoretically, the costs associated with mate choice may be higher for an individual whom is under-nourished, and therefore, individuals with reduced choosiness may have a reproductive advantage. Similarly, Moore & Moore (2001) observed that in cockroaches, *Nauphoeta cinerea*, older females were significantly less choosy for mates than younger females. They argued that older females were less choosy in order to reduce the costs associated with mate choice and to avoid their risk of failing to find a mate. The context of social experiences may also influence the expression of female mate choice behaviours. Female Pacific field crickets, *Teleogryllus oceanicus*, modify the expression of both their pre- and post-copulatory choice mechanisms depending on the specific context of a previous mating experience (Rebar *et al.*, 2011). Females that had mated to an "attractive" male 24h earlier mounted new males more slowly compared to females

that had previously mated with an "unattractive" male, suggesting that females may use the attractiveness of their previous mates as a proxy to evaluate the attractiveness of subsequent mates (Rebar *et al.,* 2011). For example, a female that previously mated to an unattractive male may be less choosy because on average, her prospective mates will be more attractive than her previous encounter. This type of plasticity may help a female ensure that she is choosing the most attractive mate available in her social environment (Rebar *et al.,* 2011). Collectively, these studies indicate that individual plasticity in female mate choice behaviours can be influenced by a variety of context-dependent environmental factors.

In addition to environmental influences, individual variation in female mate choice may have a genetic basis (Brooks & Endler, 2001; Ritchie *et al.,* 2005; Ratterman *et al.,* 2014; Tennant *et al.,* 2014). Quantifying this variation is important because in order for elaborate mate choice behaviours to co-evolve with male display traits, there must be heritable additive genetic variation present in these behaviours. In fruit flies, *Drosophila montana,* females differ in their expressed preferences for male courtship frequencies (both within and across families) suggesting the presence and maintenance of genetic variation in preferences within the gene pool (Ritchie *et al.,* 2005). Furthermore, the phenotypic expression of mate choice behaviours may be shaped by the interactive effects between an individual's genotype and their environment (GxE). Although an array of empirical results suggests that female genotype and environmental factors can have an interactive effect on sexual trait expression (i.e. courtship behaviour (Etges *et al.,* 2007) and sperm length (Morrow *et al.,* 2008)), few studies have demonstrated that female choice can similarly be affected (Ingleby *et al.,* 2010). In the only study to date to

investigate this specific type of interaction (in the lesser waxmoth, *Achroia grisella*,) different families of females showed variation in their preference for male courtship signals (pulse rate) across two rearing temperatures (22ºC and 25ºC) (Rodríguez & Greenfield, 2003). Clearly, this topic is an important but under-investigated area of research.

When considering the sources of variation in female mate choice behaviours, researchers typically frame plasticity as either a mechanism of increasing the acquisition of benefits provided by their mates, or reducing the costs associated with mate choice such as resource expenditure. However, the role of the direct costs associated with physical mating experience (i.e. arising via sexual conflict) on variation in mate choice behaviours has not received any specific attention. Sexual conflict is predicted to arise when males and females of the same species have different and incompatible strategies for maximizing their individual reproductive fitness (Parker, 1972; Chapman *et al.*, 2003; Arnqvist & Rowe, 2005). In many species, males may physically harm their mates as the pleiotropic side effect of traits that have evolved to increase individual male success (Morrow *et al.*, 2003). This harm can be costly to a female's lifetime fitness as it can cause physical genitalia damage (Kamimura, 2007) and may reduce both her longevity and/or fecundity (Lew & Rice, 2005; Filice & Long, 2016).

In the fruit fly, *Drosophila melanogaster*, a model species for the study of sexual selection and conflict, males harm their mates via toxic side-effects of accessory proteins (Acps) in their ejaculate (Chapman *et al.*, 1995; Rice, 1996), and through physical harassment during courtship and copulation (Partridge & Fowler, 1997; Kamimura, 2007). Furthermore, substantial evidence suggests individual variation in the degree of

male-induced harm has an additive genetic basis (Friberg, 2005; Lew & Rice, 2005; Fiumera *et al.,* 2006; Filice & Long, 2016). In addition to their harmful side-effects, Acps in male ejaculates can also influence the physiological state and/or behaviour of females (Chapman & Davies, 2004; Wong & Wolfner, 2006; Bonduriansky & Day, 2013).

As such, we can synthesize these features with our understanding of individual variation in mate choice behaviours to formulate some interesting predictions. Firstly, if male phenotypes have an impact on the physiological state and/or behaviours of their mates, then we should predict that virgin flies will exhibit different mate choice behaviours than flies that have mated. In this study, we empirically tested this hypothesis by comparing the mate choice behaviours of virgin and mated flies. Secondly, if males vary in the magnitude of their male-induced harm exerted on females, then we predict that female mate choice behaviours will differ depending on the degree of harm associated with their mate's phenotype. To test these predictions we examined if female mate choice behaviours were influenced by a) the duration of male exposure and b) the magnitude of harm associated with the genotype of a previous mate. Furthermore, *Drosophila melanogaster* females may exhibit genetic variation in how they rank male "attractiveness" and in how much they discriminate against certain males (Ratterman *et al.*, 2014; *but see* Tennant *et al.*, 2014). As such, we also examined if there was genetic variation associated with female behavioural response to mating experience. If there is a genetic basis for individual variation in female mate choice behaviours, then we may also expect to see genetic variation associated with changes in behaviour due to experience (i.e. a GxE interaction). By studying the potential role that male-induced harm

contributes to individual variation in female male choice, this study helps advance our understanding of the complex nature of evolutionary change via sexual selection.

Methods

Fly stock and hemiclone generation

The flies used in our experiments were derived from the large outbred wild-type *Ives* (hereafter "IV") population. This population originated from a sample of 200 females and 200 males collected in South Amherst, MA, USA, 1975. Since 1980, this population has been cultured at a large census size (>1000 adults/generation) on non-overlapping generations and standardized protocols (Rose *et al.* 1984, Long *et al.* 2006, Martin & Long 2015; Filice & Long, 2016). Flies are cultured in vials containing 10mL of a banana-agar-killed yeast medium, and stored in an incubator maintaining a consistent temperature of 25°C, humidity of 60%, and light:dark cycle of 12h:12h. At the start of each generation, flies are collected from their "natal" vials as adults, mixed *en masse*, and transferred into equal groups into "oviposition" vials containing fresh media. Flies are left in this vials for ~2-3 hours to allow oviposition. After this period, the adult flies are removed and the eggs that were laid are trimmed (by hand) to a density of 100 eggs/vial. These oviposition vials become the natal vials for the next generation of flies.

From the IV population we established 26 male clone lines using cytogenetic cloning techniques (Chippindale *et al.* 2001, Tennant *et al.* 2014), which were subsequently expressed in a male or female "hemiclonal" background. Clone lines were created and maintained by mating males chosen from the IV population to females from a "clone-generator" (CG) population, who possess a random Y chromosome, a conjoined

"double -X" chromosome [C(1)DX, *y, f*], and are homozygous for translocated autosomes [T(2;3) *rdgC st in ri* p^{P} bw^{D}]. The resulting clone males all possess one full haplotype originating from the base IV population maintained in an unrecombined state and with the translocated autosomes inherited from their CG mother. To express the haploid genome in a male hemiclonal state, clone males are crossed with virgin females from the "DX-IV" population (which possess the "double-X" chromosome and autosomes originating from the IV population). To express the haploid genome in a female hemiclonal state, clone males are crossed to virgin females from the base IV population. Ultimately, all target hemiclones resulting from one of these crosses posses one haplotype identical to all other individuals in the line, and one randomly inherited haplotype.

Prior to this study, we quantified the magnitude of male-induced harm in 26 hemiclone lines by measuring the fecundity (a meaningful metric of fitness (Rice *et al.,* 2006)) of IV females that were exposed to males of different genetic backgrounds for either a short (3hrs) or long (48hrs) period. By measuring the difference in fecundity between the two treatments for each of the male lines, we were able to estimate their harmfulness (*see* Filice & Long, 2016). From these 26 lines, we identified the 2 lines with the greatest mean effect on female fecundity (high harm males), the two lines that had the lowest mean effect (low harm males) and one line that had the median effect (medium harm males).

Assay 1: Does mating experience influence female mate choice behaviours?

Experience phase

In this assay, we were interested in examining if mating experience has an effect on female mate choice behaviours. The experiment began by collecting 400 female flies as virgins (upon 8 hours from ecolosion) from the base IV population, reared at standard culture densities. These flies were all placed into individual vials and assigned to four different treatments. In the control treatment, 100 females remained virgins throughout the experiment. The other 300 females were randomly assigned to one of three "mating" treatments: "low-harm", "medium-harm", and "high-harm". In the low-harm treatment, each female was housed with a single male derived from the least harmful hemiclone line. In the medium-harm treatment, each female was housed with a single male derived from the hemiclone line that had the median impact on female fecundity. Finally, in the high-harm treatment, each female was housed with a single male derived form the most harmful hemiclone line. Next, males were removed from half of the vials in each treatment after 3h of exposure (short-term treatment). In the short-term treatment, all vials were observed to assure at least one mating occurred. The other half of the females remained with males for an additional 45h (total of 48h) until the mate choice assay (long-term treatment), allowing for continuous courtship and multiple matings to occur.

Mate choice assay

Following the "experience phase" of our experimental protocol, individual females were placed into mate choice chambers (using light CO_2 anesthesia) in order to observe their subsequent mate choice behaviours (Figure 3.1). These chambers consisted of a 41 x 41 x 8mm main area (Fisher brand weighing boat 08-732-112) covered with a

sheet of clear styrene (Evergreen Scale Models, Inc.), held in place with a bulldog-clip, thereby creating an arena where females could freely move. Inside the arena we installed four sub-chambers attached to the base of the main chamber (Micrewtube brand, Simport Scientific Inc.). One of these sub-chambers was filled with 60μL of media for the female, and the other three caps were filled with 20μL of media for males, who were physically blocked from the main chamber by a ½" (OD) 149-micron polypropylene mesh disk (AmazonSupply.com). The mesh restricts the males from physically interacting with the female (and each other), but does not disrupt olfactory and auditory signals between males and females. Thus, this design allows for females to sample (some) male display traits but eliminates the potential for male-male competition and harassment to confound the expression of a female's mate choice.

We placed males into their sub-chambers 18 hours before the start of the mate choice assay. In each mate choice chamber, we placed a single high-harm male and a single low-harm male (both from different hemiclonal backgrounds from the males used in the experience phase) into individual sub-chambers, and left the third sub-chamber empty (Figure 3.1). All mate choice chambers were placed randomly over a light board. The light emitted from below the chambers ensured the females would appear in strong contrast to the background in our videos. We filmed chambers from above using JVC Everio GZ-HM440U video cameras on a time-lapse setting (1 frame s^{-1}) for ~4 hours.

Assay 2: Does variation in experience-dependent female choice have a genetic basis?

In this assay, we set out to quantify the amount of additive genetic variation underlying individual variation in female mate choice behaviours following different mating

experiences. This experiment's protocol was similar to that described above for assay 1, except that in this assay we experimentally controlled female genotype instead of using randomly-sampled IV females. In this assay we used the 21 clone-lines that were not used to generate the low-, medium-, and high-harm males, used in experiment 1. From each of these lines we obtained 24 females hemiclones by crossing clone males to virgin IV females. In this assay, we restricted the number of mating treatments during the experience phase to just low- and high-harm males. In this assay 6 females derived from each hemiclone line were either mated to males from the low- or high-harm hemiclonal backgrounds used in assay 1. Females were housed at a 2:1 male:female ratio (12 males & 6 females/vial). Half of these females remained together for 3h and were then separated from the males (short-term exposure) while the other half remained together for the 48hrs until they were filmed (long-term exposure).

Mate choice assay

Following the four combinations of treatments during the experience phase (low harm or high harm x short-term or long-term) across the 21 female hemiclone lines, we transferred all flies into mate choice chambers and filmed their interactions in the same manner as described above for assay 1.

Video Analysis

Videos files were analyzed using the program DTrack (Courtesy of Dr. Scott Pletcher, University of Michigan) in order to track the physical position of the individual females in each frame of the video. Using this software, we counted the number of frames that each female spent on the surface of each sub-chamber containing either a "high-harm male", "low-harm male", or were elsewhere in the chamber (Figure 3.1).

Statistical Analysis

To understand female mate choice behaviours, we were interested in analyzing females' interest in males and male preference. Interest in males was defined by the total amount of time (i.e. the number of frames) a female spent associating with males compared to the entire duration of the assay. This was calculated by dividing the sum of frames a female spent on the surface of a chamber containing the high- and the low-harm males by total number of frames recorded in the assay.

Preference was defined by the amount of time (number of frames) a female spent with high harm male compared to the amount of time spent with either male. This was calculated by dividing the number of frames a female spent on the surface of the chamber containing the high-harm male by the number of frames spent on the surface of the chamber containing the low-harm male added to the number of frames spent on the surface of the chamber containing the high-harm male. Our decision to use high-harm males as the numerator was arbitrary. Although our results would be inversed if we used the low-harm males as the numerator, the conclusions and implications of these results would remain the same.

All data analyses were conducted using R v3.1.2 (R Core Team, 2014). For assay 1, we constructed generalized linear models (GLMs) with quasibinomial errors and used the *Anova* function in the *car* package (Fox and Weisberg, 2011) to determine whether virgin and mated females differed in their interest in males and preferences. Next, we used GLMs with quasibinomal errors and the *Anova* to determine the effect of mating treatment (high and low), length treatment (short and long), and their interaction had an effect on female interest in males and preference. To determine differences within

treatments, we used a Tukey's post-hoc from the *multcomp* package (Horthorn *et al.* 2008).

For assay 2, data collected from hemiclonal females were analyzed using Generalized Linear Mixed Models (GLMMs), created using the *lme4* package (Bates *et al.,* 2014). As the response variables in both models (examining the overall female attractio to males, and the female's preference for the harmful male) are binomial, the models were fit with a logit link function. Initially the models included mating treatment (high or low-harm 1st mate), mating exposure treatment (brief or prolonged) and their interaction as fixed effects, with hemiclone line (and all of it possible interactions with the previous effects) entered as random effects. First, the significance of fixed effects was first determined using the *Anova* function, and models were simplified by the removal of non-significant effects (and their corresponding random-effects). Next, using the *bootMer* function 95%CI were calculated for the random effect variables based on 1,000 bootstrap samples. The statistical significance of each variance component was determined using a permutation test approach (Manley, 2007) whereby the magnitude of our model's variance component was compared to the distribution of 10,000 variance components each derived a randomize set of the experimental data.

In order to better understand the nature of the interaction between clone line and mating treatments, we calculated the mean fraction of time females from each clone line, in each of the two treatments (mated to high-harm male or mated to low-harm males) spent associating with the high-harm male in the male-choice chamber. We then calculated the correlation between these two variables, and obtained the Standardized Major Axis (SMA) method (Sokal & Rolf, 2012) using the *lmodel2* package (Legendre,

2014). A SMA regression was calculated, as both the x-and y-axes were subject to natural variation and measurement error. For each of these statistics we calculated 95% CI by bootstrapping the data 10,000 times each.

Results

Assay 1: Does mating experience influence female mate choice behaviours?

Our first objective was to compare the mate choice behaviours of mated and virgin females. Our analysis revealed that females that remained virgin throughout the experiment spent approximately twice the amount of time in association with males in the mate choice phase compared to females who had mated ($\bar{x}_{virgin} = 0.0826$, $\bar{x}_{mated} = 0.0463$ $\chi^2=15.43$, Df=1, $p=8.55x10^{-5}$)(Figure 3.2), but did not differ in their preference of males ($\chi^2=2.32$, Df=1, p=0.128).

Our next analysis looking at the effect of mating experience on mate choice behaviours (i.e. harmfulness of first mate and length of exposure) revealed a significant effect of length of male exposure (p=0.0056), no effect of mating treatment (p=0.1416) and a significant interaction between length and mating treatments (p=0.0001)(Figure 3.3; Table 3.1). A Tukey's post-hoc test revealed that females that had previously mated with a low-harm or medium-harm male spent the same amount of time with males between the short- and long-term treatments. However, females previously mated to high-harm males spent significantly more time with males in the short-term treatment compared to the long-term treatment (Figure 3.3; Table 3.1). For male preference, there was a significant effect of mating treatment (p=0.0047), but no significant effect of length treatment (p=0.2258) or the interaction between the mating and length treatments

(p=0.0575)(Figure 4, Table 1). A Tukey's post-hoc test revealed that females that had previously mated with a medium-harm male spent significantly more time associating with the high-harm male compared to those females that had previously mated with a high-harm male, but the same amount of time as females that had previously mated with a low-harm male. (Figure 3.4, Table 3.1).

Assay 2: Does variation in experience-dependent female choice have a genetic basis?

When analyzing the degree of genetic variation associated with experience-dependent mate choice behaviours, we found a significant effect of mating treatment on interest in males (χ^2=20.57, Df=1, p=5.76x10^{-6}), but no effect of exposure length (χ^2=0.3797, Df=1, p=0.5378) nor for the interaction between length and mating treatment (v^2=0.0059, Df=1, p=0.9388). When including random effects, we found that although clone line alone was not a significant factor and explains almost none of the variation, the interaction between mating treatment and clone line is significant and explains 4.2% of the observed variation in the amount of time females spent associating with males (Figure 3.5, Table 3.2). To better understand how each hemiclone line was affected by the two mating treatments, we looked at the correlation in overall interested in males of the clone lines between females previously mated to a high-harm male and females previously mated to low-harm males and detected a strong negative correlation

When looking at female preference, no fixed effects had a significant effect (mating treatment (χ^2=1.1147, Df=1, p=0.2911), length treatment (χ^2=2.1131, Df=1, p=0.1469), and their interaction (χ^2=0.0013, Df=1, p=0.9711)). Upon analysis of the random effects, clone line was not a significant factor and explains none of the observed variation, but the interaction between clone line and mating treatment was significant and

explained ~10.4% of the observed phenotypic variation in female preferences (Figure 3.6, Table 3.3). When we examined the behaviour of the hemiclone females that had been mated to either high-harm or low-harm males, we found a significant, negative correlation, of weak-to-moderate effect size (correlation [bootstrapped 95%CI]: -0.298 [-0.025 -0.557]). Similarly, the SMA regression also has a significant negative slope (slope [bootstrapped 95%CI]: [-0.424,-1.539])(Figure 3.7).

Discussion

Individual variation in female mate choice can have important consequences for the direction and/or strength of the evolution of sexually selected traits (Jennions & Petrie, 1997). In many animals, individual experiences can shape and modify behaviours in a variety of ways, including mate choice behaviours (Verzijden *et al.*, 2012; Rodríguez *et al.*, 2013). Here, we demonstrate that some of the individual phenotypic variation in female mate choice behaviours is shaped by previous mating experience. While previous studies have identified plasticity in mate choice behaviours (Dukas, 2005; Rebar *et al.*, 2009; Rebar *et al.,* 2011), we are the first to identify a genetic component underlying this variation and to identify a link to sexual conflict. Our results indicate that i.) the magnitude of direct costs a female incurs from a her previous mating experience(s) may shape her subsequent mate choice behaviours, ii.) some of his variation is rooted in the presence of additive genetic variation in the population and iii.) the expression of this genetic variation is highly plastic (*i.e.* exhibits GxE effects). Our study's results advance our understanding of the causes and consequences of individual variation in female mate choice behaviours, and the potentially important role of sexual conflict.

Interest in males, but not preferences, influenced by individual mating status

In our first assay, we found that virgin females spent more time on the surface of sub-chambers containing males compared to mated females. This suggests that virgin females may be more interested in general in associating with males than those females that had previously mated. This result is consistent with data that shows virgin flies are more receptive to courtship than mated flies (Manning, 1967), and that Acps transferred by previous mates decrease female receptivity to later males' courtship (Chen *et al.*, 1988; Aigaki *et al.*, 1991). There are two possible (but not mutually-exclusive) explanations for this observation that are related to sexually-antagonistic coevolution between the sexes. Firstly, mated females may avoid males in order to avoid the direct costs associated with courtship and/or mating. As prolonged exposure to males and multiple matings has been shown to reduce both the longevity (Lew & Rice, 2005) and fecundity (Filice & Long, 2016) of females, it may be in a female's best interest to focus on oviposition until her stored sperm is depleted (Chapman *et al.*, 1995). A second hypothesis, proposed by Johnstone & Keller (2000), is that the decreased female receptivity to males following remating is due to manipulation by the earlier mate, who is attempting to increase his share of paternity by reducing his partner's interest in other mates. Their model predicts that the size and potency of the manipulative substances transferred to females should increase in species with greater second-male advantage, a phenomena well documented in *Drosophila* (Price, 1997; Price *et al.*, 1999). To test these hypotheses, future studies could quantify how changed in female interest in males changes with time since mating, and its relationship to remating rates, female egg production and the outcome of sperm competition.

Somewhat surprisingly, there was no effect of mating status on the expression of female preferences. Both virgin and mated females spent, on average, the same amount of time associating with the sub-chamber containing the “high-harm” male as they spent over sub-chamber containing the “low-harm” male. This is possibly due to the phenomenon observed in our second assay, that the effect of mating treatment on female preference largely depended on female's genetic identity. Since females were all randomly selected IV females (of unknown genetic identities), any behavioural differences in female preferences due to mating status may have been obscured by the wide range of phenotypes expressed by females in each treatment group.

Interest in males influenced by identity of previous mate and interaction with genotype

In assay 1, we also observed that amongst mated females, the differences in individual mate choice behaviours were associated with differences in the phenotype of her mate and the length of her exposure to that mate. In the short-term exposure treatment, females that were previously mated to a high-harm male spent significantly more time on the surface of sub-chambers containing males compared to females that had been previously housed with high-harm males for a prolonged period (Figure 3.2). This result might be explained in part by the relationship between the length of exposure to males, and the effect of those males on a female’s fecundity. In Filice & Long (2016), we found that males who are the most harmful to female fitness over a prolonged period of time tended to be the most beneficial to female fitness after a single mating. Thus, it is possible that females in the "high-harm, long-term exposure" treatment were the least interested in males because they incurred the greatest amount of costs to their lifetime fitness and may be less interested in remating as a means to reduce the costs (decreased fecundity,

longevity) associated with continuous male harassment and mating. Alternatively, as several studies have also found that increased exposure to the toxic side-effects of Acps reduces female longevity (Chapman *et al.*, 1995; Rice, 1996) and receptivity to mating (Chen *et al.*, 1988; Aigaki *et al.*, 1991), it is possible that the more harmful a previous mating experience was (i.e. increased exposure to the toxic side-effects of Acps) the less willing a female would be to be receptive to the courtship of other (harmful) males.

Together, these results suggest that the observed plasticity in female mate choice behaviours may be an adaptation that allows females to reduce the direct costs associated with previous mating encounters. This hypothesis is consistent with models that predict experience-dependent plasticity in female mate choice may evolve as an adaptation (Fawcett & Bleay, 2009), as the benefits associated with mate choice can frequently be context dependent (Qvarnström, 2001). For example, a female that incurs low physiological costs from a previous mate may benefit from remating if they are able to acquire and select higher quality sperm via sperm competition and/or cryptic mate choice (Dickinson, 1997; Jennions & Petrie, 2000). However, a female that incurs relatively higher physiological costs may not receive a net-benefit from remating, even if it means potentially producing higher quality offspring.

The presence of mate-choice plasticity can potentially explain the maintenance of variation of deleterious alleles within a population's gene pool. It is possible that male-induced harm is acting as an indirect genetic effect (IGE) which arise when the phenotypic expression of an individual's genes influence the phenotype of another (Wolf, 2000). In Filice & Long (2016), we showed that there is significant genetic variation associated with phenotypic variation in male-induced harm. Our current study may

explain how this variation is maintained: if females that have mated with a harmful male are less likely to remate, then one would predict increased frequency of alleles associated with male-induced harm represented in the next generation.

In assay 2, we did not detect an identical pattern, but our results still support this hypothesis. The effect of treatment length was not significant, and in both the short- and long-exposures, females previously mated to low-harm males spent more time with males than those previously mated to high-harm males (Figure 3.5). We suspect that this could have occurred due to the fact that in this assay females were housed at a 2:1 male:female ratio (12 males and 6 females) rather than the 1:1 ratio (single male and single female) in assay 1. This may have resulted in increased male harassment and male aggression due to increased competition, even in the short-term exposure. Interestingly, we also observed a significant interaction between individual female genotype and mating treatment, which accounted for ~4.19% of the total observed variation in interest in males (Table 2). This means that not all female lines that had mating experience with "high-harm" males responded by spending less time with males in the choice trial compared to females from the same line but experience mating with "low-harm" males. However, this inconsistency was only observed in 3 of the 21 lines (Figure 3.5), and the effect of this interaction is quite weak ($p=0.048$, Table 3.2). As such, we suspect that these lines are outliers, and that the negative trend between mating treatments is relatively consistent across genotypes.

Female preferences influenced by identity of previous mate and interaction with genotype

In our analysis of preference in assay 1, we did not find any statistical differences between the behaviours of females previously mated to high- and low-harm males did not differ in their preferences, contrary to our initial predictions. However, the results in assay 2 shed some light on the reason no differences between treatments were detected. Here, we found a significant interaction between female genotype and experience that was characterized by a negative correlation between the female preference phenotype exhibited by hemiclonal females previously mated to a high harm male and females from the same hemiclonal line that had previously mated to a low harm male. This means that a female genotype that exhibited a strong preference for low-harm males after having a previously mated with a "high-harm" male tended to exhibit a strong preference for high-harm male if female of the same genotype had previously mated with a "low-harm" male. This surprising result yields many exciting implications for our understanding of the causes and consequences of individual variation in female mate preferences. Firstly, it suggests that the plasticity we observed in female choice is due an interaction between mating individual experience and genotype. Secondly, it may explain the maintenance of genetic variation in populations despite some females consistently choosing phenotypes in males over others to acquire the greatest indirect benefits. This phenomenon, known as the "lek paradox", asks how genetic variation is maintained in the face of directional sexual selection (Kokko & Heubel, 2008). Models have predicted that GxE interactions between experience and genotype may act as a mechanism to maintain genetic variation

(Kokko & Heubel, 2008; Ingleby *et al.*, 2010), and here we provide empirical evidence of such a mechanism.

Plasticity as resistance?

Our study's results have interesting implications regarding the evolution of plasticity in mate choice behaviours and female resistance to male harm. Rodríguez *et al.* (2013) proposed five hypotheses that may explain the evolution of behavioural plasticity in mate preferences. All five of these hypotheses are explained by two general functions: a) that females alter their preference to ensure mating and reduce the costs associated with mate choice (*i.e.* resource expenditure, time searching, predation risk) and b) that females alter their preference to ensure mating with an "attractive" mate, or to prevent mating with an "unattractive" mate. While it is possible that our results support the latter function, we have no evidence to suggest if males from the "high-harm" line are inherently more or less attractive than males from the "low-harm" line. Although it is often assumed that "harmful males" are "attractive" (*e.g.* Friberg & Arnqvist, 2003), virgin females from our first assay spent nearly the exact same time on average on the surface the "high-harm" chamber as they did over the "low-harm" chamber ($\bar{x}$= 0.519). We showed here that females could alter both their interest in prospective mates (receptivity) and preference based on the harmfulness of a previous mate. Therefore, we suggest that plasticity in mate choice behaviours may operate as a mechanism of female "resistance" to male-induced harm.

In previous research demonstrating genetic variation (Linder & Rice, 2005; Lew *et al.*, 2006) and an evolutionary basis (Holland & Rice, 1999; Wigby *et al.*, 2003) for female resistance, the actual mechanisms that mediate female resistance were not well

characterized. Holland & Rice (1998) suggested in their "chase-away" sexual selection hypothesis that females may resist the direct costs of mating by evolving biases against traits that stimulate them to mate. Since then, theoretical models have inferred that females can indeed evolve mate choice behaviours as a means of reducing the direct costs of mating (Gavrilets *et al.*, 2001), but to date no studies have empirically demonstrated this. Therefore, it is integral for future studies to continue investigating the dynamics between sexual conflict theory and mate choice. In order to better understand evolutionary significance of our results, studies should consider how individual components of harm (i.e. Acp concentrations, physical condition) influence mate choice, and how female fitness is affected by changes in mate choice behaviours.

Conclusions

In this experiment, we identified mating experience as a novel source of variation in female mating choice behaviours in the model species *D. melongaster*. By comparing the mate choice behaviours of virgins and mated females, we showed that virgin females were significantly more interested in spending time over male chambers, but did not differ in their preference of males. We found that the duration of exposure to males had a interactive effect with the type of male a female was exposed to on her subsequent interest in males, and that mating treatment had a significant effect on her mate preference. We also identified an interaction between mating experience and individual genotype (a GxE effect) on interest in males and female preferences, making this the second study to observe such an effect (*other than* Rodríguez & Greenfield, 2003). Our results offer new insight into the maintenance of variation of male traits and the evolution of plasticity in female mate choice behaviours.

References

Abbott, J. K., Morrow, E. H. (2011). Obtaining snapshots of genetic variation using hemiclonal analysis. *Trends in Ecology and Evolution*, 26, 359-368.

Abraham, S., Contreras-Navarro, Y., & Pérez-Staples, D. (2016). Female Age Determines Remating Behavior in Wild Mexican Fruit Flies. *Journal of Insect Behavior*, 1-15.

Aigaki, T., Fleischmann, I., Chen, P. S., & Kubli, E. (1991). Ectopic expression of sex peptide alters reproductive behavior of female *D. melanogaster*. *Neuron*, *7*(4), 557-563.

Andersson, M. B. (1994). *Sexual selection*. Princeton University Press.

Anjos-Duarte, C. S., Costa, A. M., & Joachim-Bravo, I. S. (2011). Influence of female age on variation of mate choice behavior in Mediterranean fruit fly (Diptera: *Tephritidae*). *Journal of insect behavior*, *24*(1), 11-21.

Arnqvist, G., & Rowe, L. (2013). *Sexual conflict*. Princeton University Press.

Bailey, N. W., & Zuk, M. (2009). Field crickets change mating preferences using remembered social information. *Biology letters*, *5*(4), 449-451.

Bates, D., Mächler, M., Bolker, B., Walker, S. (2014). Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:1406.5823.*

Brooks, R., & Endler, J. A. (2001). Female guppies agree to differ: Phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution*, *55*(8), 1644-1655.

Brooks, R. (2002). Variation in female mate choice within guppy populations: population divergence, multiple ornaments and the maintenance of polymorphism. *Genetica*, *116*(2-3), 343-358.

Bonduriansky, R., & Day, T. (2013). Nongenetic inheritance and the evolution of costly female preference. *Journal of evolutionary biology*, *26*(1), 76-87.

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

Chapman, T., Arnqvist, G., Bangham, J., & Rowe, L. (2003). Sexual conflict. *Trends in Ecology & Evolution*, *18*(1), 41-47.

Chapman, T., & Davies, S. J. (2004). Functions and analysis of the seminal fluid proteins of male *Drosophila melanogaster* fruit flies. *Peptides*, *25*(9), 1477-149

Chen, P. S., Stumm-Zollinger, E., Aigaki, T., Balmer, J., Bienz, M., & Böhlen, P. (1988). A male accessory gland peptide that regulates reproductive behavior of female *D. melanogaster*. *Cell*, *54*(3), 291-298.

Chippindale, A. K., Gibson, J. R., & Rice, W. R. (2001). Negative genetic correlation for adult fitness between sexes reveals ontogenetic conflict in *Drosophila*. *Proceedings of the National Academy of Sciences*, *98*(4), 1671-1675.

Dickinson, J. L. (1997). Multiple mating, sperm competition, and cryptic female choice in the leaf beetles (Coleoptera: *Chrysomelidae*). *The evolution of mating systems in insects and arachnids. Cambridge University Press, Cambridge*, 164-183.

Etges, W. J., De Oliveira, C. C., Gragg, E., Ortíz-Barrientos, D., Noor, M. A., & Ritchie, M. G. (2007). Genetics of incipient speciation in *Drosophila mojavensis*. I. Male courtship song, mating success, and genotype x environment interactions. *Evolution*, *61*(5), 1106-1119.

Filice, D. C., & Long, T. A. (2016). Genetic variation in male-induced harm in *Drosophila melanogaster*. *Biology Letters*, *12*(4), 20160105.

Friberg, U., & Arnqvist, G. (2003). Fitness effects of female mate choice: preferred males are detrimental for *Drosophila melanogaster* females. *Journal of evolutionary biology*, *16*(5), 797-811.

Friberg U. 2005 Genetic variation in male and female reproductive characters associated with sexual conflict in *Drosophila melanogaster*. *Behav. Genet.* **35**, 455-462.

Fiumera AC, Dumont BL, Clark AG. 2006 Natural variation in male-induced 'cost-of-mating' and allele-specific association with male reproductive genes in *Drosophila melanogaster*. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **361**, 355-361. (DOI 10.1098/rstb.2005.1791 [doi]).

Hebets, E. A., Wesson, J., & Shamble, P. S. (2008). Diet influences mate choice selectivity in adult female wolf spiders. *Animal Behaviour*, *76*(2), 355-363.

Holland, B., & Rice, W. R. (1998). Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution*, 1-7.

Holland, B., & Rice, W. R. (1999). Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proceedings of the National Academy of Sciences*, *96*(9), 5083-5088.

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical journal*, *50*(3), 346-363.

Hunt, J., Brooks, R., & Jennions, M. D. (2005). Female mate choice as a condition-dependent life-history trait. *The American Naturalist*, *166*(1), 79-92.

Ingleby, F. C., Hunt, J., & Hosken, D. J. (2010). The role of genotype-by-environment interactions in sexual selection. *Journal of evolutionary biology*, *23*(10), 2031-2045.

Jennions, M. D., & Petrie, M. (1997). Variation in mate choice and mating preferences: a review of causes and consequences. *Biological Reviews*, *72*(2), 283-327.

Jennions, M. D., & Petrie, M. (2000). Why do females mate multiply? A review of the genetic benefits. *Biological Reviews of the Cambridge Philosophical Society*, *75*(01), 21-64.

Johnstone, R. A., & Keller, L. (2000). How males can gain by harming their mates: sexual conflict, seminal toxins, and the cost of mating. *The American Naturalist*, *156*(4), 368-377.

Kamimura Y. 2007 Twin intromittent organs of *Drosophila* for traumatic insemination. *Biol. Lett.* **3**, 401-404. (DOI X527L59421J0P125 [pii]).

Kokko, H., & Heubel, K. (2008). Condition-dependence, genotype-by-environment interactions and the lek paradox. *Genetica*, *132*(2), 209-216.

Legendre, P. (2014). lmodel2: Model II Regression. R package version 1.7-2. http://CRAN.R-project.org/package=lmodel2

Lew TA, Rice WR 2005 Natural selection favours harmful male *Drosophila melanogaster* that reduce the survival of females. *Evol. Ecol. Res.* **7**, 633-641.

Long TAF, Montgomerie R, Chippindale AK. (2006). Quantifying the gender load: can population crosses reveal interlocus sexual conflict?. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **361**, 363-374.

Manly, BFJ. (2007). *Randomization, Bootstrap, and Monte Carlo Methods in Biology (3rd ed.). London: Chapman & Hall.*

Manning, A. (1967). The control of sexual receptivity in female *Drosophila*. *Animal Behaviour*, *15*(2), 239-250.

Martin ES, Long TAF. (2015). Are flies kind to kin? The role of intra- and inter-sexual relatedness in mediating reproductive conflict. *Proc. Biol. Sci.* **282**, 10.

Morrow, E. H., Arnqvist, G., & Pitnick, S. (2003). Adaptation versus pleiotropy: why do males harm their mates?. *Behavioral Ecology*, *14*(6), 802-806.

Morrow, E. H., Leijon, A., & Meerupati, A. (2008). Hemiclonal analysis reveals significant genetic, environmental and genotype× environment effects on sperm size in *Drosophila melanogaster*. *Journal of evolutionary biology*, *21*(6), 1692-1702.

Moore, P. J., & Moore, A. J. (2001). Reproductive aging and mating: the ticking of the biological clock in female cockroaches. *Proceedings of the National Academy of Sciences*, *98*(16), 9171-9176.

Nakagawa, S. & Schielzeth, H. (2010). Repeatability for Gaussian and non-Gaussian data: a practical guide for biologists. Biological Reviews of the Cambridge Philosophical Society, 85, 935–56.

Partridge, L., Fowler, K. (1990). Non-mating costs of exposure to males in female *Drosophila melanogaster*. *Journal of Insect Physiology.* 36, 419-425.

Price, C. S. (1997). Conspecific sperm precedence in *Drosophila*. *Nature*, *388*(6643), 663-666.

Qvarnström, A. (2001). Context-dependent genetic benefits from mate choice. *Trends in Ecology & Evolution*, *16*(1), 5-7.

Price, C. S., Dyer, K. A., & Coyne, J. A. (1999). Sperm competition between *Drosophila* males involves both displacement and incapacitation. *Nature*, *400*(6743), 449-452.

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

Ratterman, N. L., Rosenthal, G. G., Carney, G. E., & Jones, A. G. (2014). Genetic variation and covariation in male attractiveness and female mating preferences in *Drosophila melanogaster*. *G3: Genes| Genomes| Genetics*, *4*(1), 79-88.

Rebar, D., Bailey, N. W., & Zuk, M. (2009). Courtship song's role during female mate choice in the field cricket *Teleogryllus oceanicus*. *Behavioral Ecology*, arp143.

Rebar, D., Zuk, M., & Bailey, N. W. (2011). Mating experience in field crickets modifies pre-and postcopulatory female choice in parallel. *Behavioral Ecology*, *22*(2), 303-309.

Rebar, D., & Rodríguez, R. L. (2013). Genetic variation in social influence on mate preferences. *Proceedings of the Royal Society of London B: Biological Sciences*, *280*(1763), 20130803.

Rice WR. (1996), Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature.* **381**, 232-234. (DOI 10.1038/381232a0 [doi]).

Rice, W. R., Stewart, A. D., Morrow, E. H., Linder, J. E., Orteiza, N., & Byrne, P. G. (2006). Assessing sexual conflict in the *Drosophila melanogaster* laboratory model system. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *361*(1466), 287-299.

Ritchie, M. G., Saarikettu, M., & Hoikkala, A. (2005). Variation, but no covariance, in female preference functions and male song in a natural population of *Drosophila montana*. *Animal Behaviour*, *70*(4), 849-854.

Rodríguez, R. L., & Greenfield, M. D. (2003). Genetic variance and phenotypic plasticity in a component of female mate choice in an ultrasonic moth. *Evolution*, *57*(6), 1304-1313.

Rodríguez, R. L., Rebar, D., & Fowler-Finn, K. D. (2013). The evolution and evolutionary consequences of social plasticity in mate preferences. *Animal Behaviour*, *85*(5), 1041-1047.

Rose, M. R., & Charlesworth, B. (1981). Genetics of life history in *Drosophila melanogaster*. II. Exploratory selection experiments. *Genetics*, *97*(1), 187-196.

Stewart AD, Morrow EH, Rice WR. (2005). Assessing putative interlocus sexual conflict in *Drosophila melanogaster* using experimental evolution. *Proc. Biol. Sci.* **272**, 2029-2035.

Sokal, R.R. & Rolhlf F.J. (2012). Biometry: The principles and practices of statistics and biological research. 4th edition. W.H. Freeman and Company, New York, NY.

Tennant, H. M., Sonser, E. E., & Long, T. A. (2014). Hemiclonal analysis of interacting phenotypes in male and female *Drosophila melanogaster*. *BMC evolutionary biology*, *14*(1), 95.

Verzijden, M. N., Ten Cate, C., Servedio, M. R., Kozak, G. M., Boughman, J. W., & Svensson, E. I. (2012). The impact of learning on sexual selection and speciation. *Trends in Ecology & Evolution*, *27*(9), 511-519.

Widemo, F., & Sæther, S. A. (1999). Beauty is in the eye of the beholder: causes and consequences of variation in mating preferences. *Trends in Ecology & Evolution*, *14*(1), 26-31.

Wigby, S., & Chapman, T. (2004). Female resistance to male harm evolves in response to manipulation of sexual conflict. *Evolution*, *58*(5), 1028-1037.

Wiley, R. H., & Poston, J. (1996). Perspective: indirect mate choice, competition for mates, and coevolution of the sexes. *Evolution*, 1371-1381.

Wolf, J. B. (2000). Indirect genetic effects and gene interactions. *Epistasis and the evolutionary process*, 158-176.

Wong, A., & Wolfner, M. F. (2006). Sexual behavior: a seminal peptide stimulates appetites. *Current Biology*, *16*(7), R256-R257.

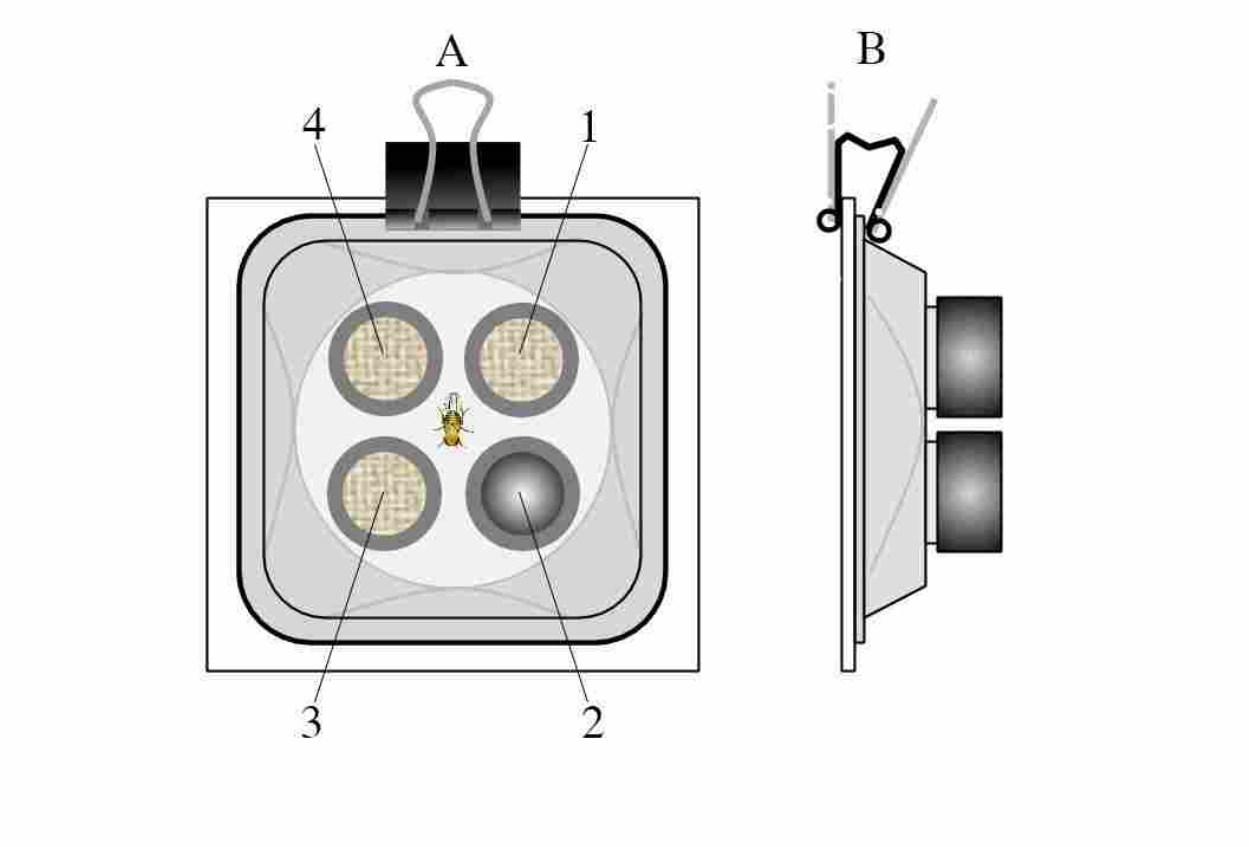

Figure 3.1: Illustration of experimental mate choice chambers and setup. Figure **A** depicts the chamber from a top (bird's eye) perspective, and figure **B** depicts the chamber from a side-view perspective. Sub-chamber **1** contained a male from a high-harm hemiclonal background, sub-chamber **2** contained media for the experimental female, sub-chamber **3** contained a male from a low-harm hemiclonal background, and sub-chamber **4** remained empty as a control.

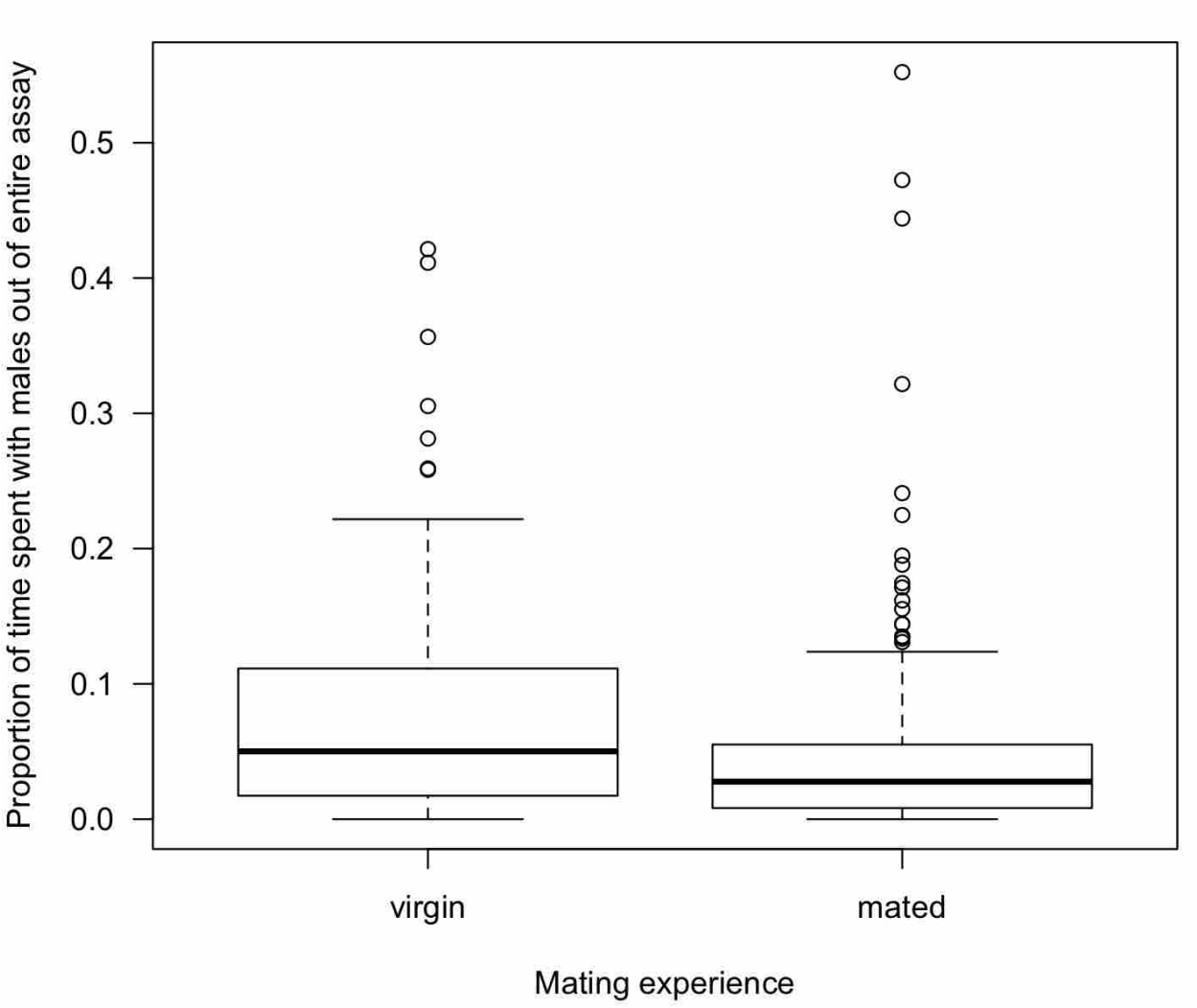

Figure 3.2: Effect of mating status on interest in associating with males. The boxplots illustrate the differences between virgin and mated females in the proportion of time females spent over a sub-chamber containing a male out of the entire duration of the assay. The boxes contain the middle 50% of data (inter-quartile range, IQR), and the horizontal lines represent the medians. Values > ± 1.5x IQR are outliers and are represented by open circles, and all other values that are not outliers are represented by the whiskers above and below each box.

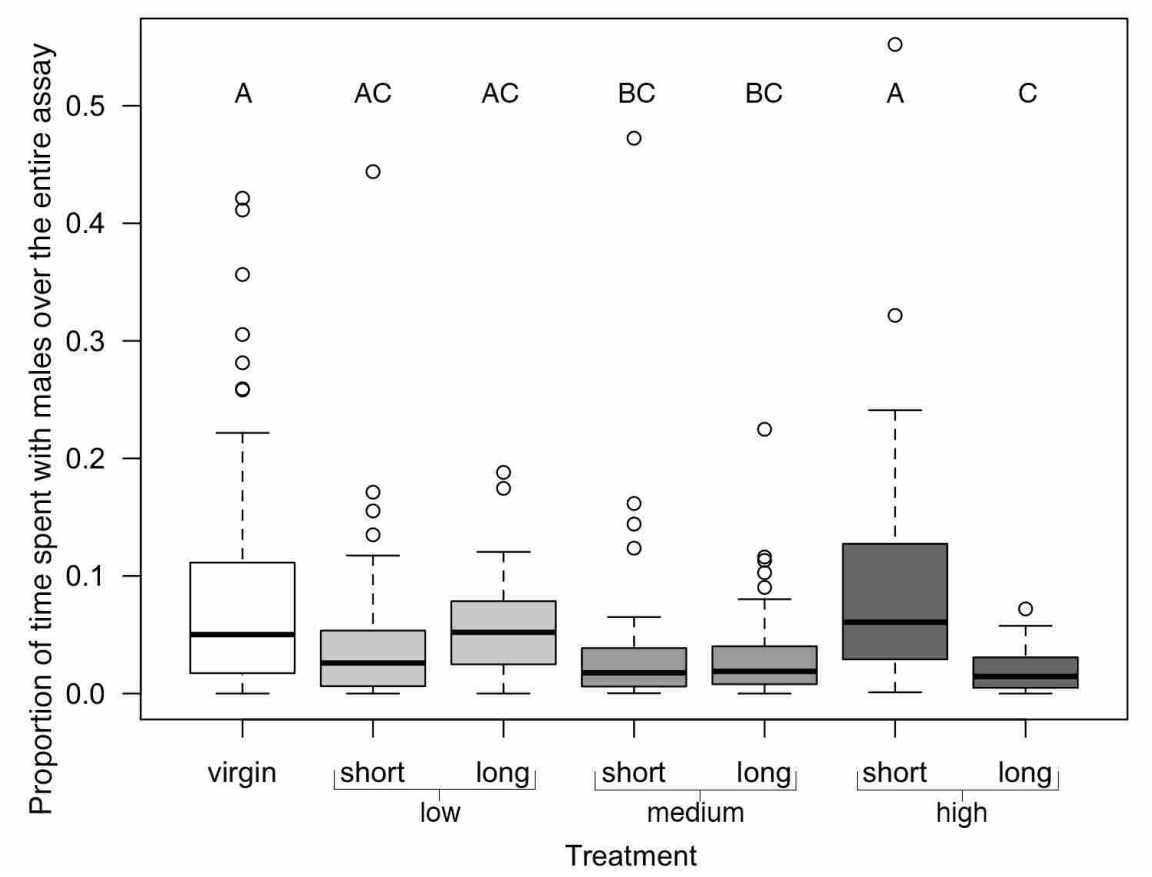

Figure 3.3: Effect of previous mating experience and length of exposure to that mate on the proportion of time females spent with males during the mate choice assay (interest in males). The box plots show variation in the data distributions of each treatment combination. The boxes contain the middle 50% of data (inter-quartile range, IQR), and the horizontal lines represent the medians. Values > ± 1.5x IQR are outliers and are represented by open circles, and all other values that are not outliers are represented by the whiskers above and below each box. The letters beside each line indicate the statistical similarity of the means using a Tukey's post-hoc test (Table 1).

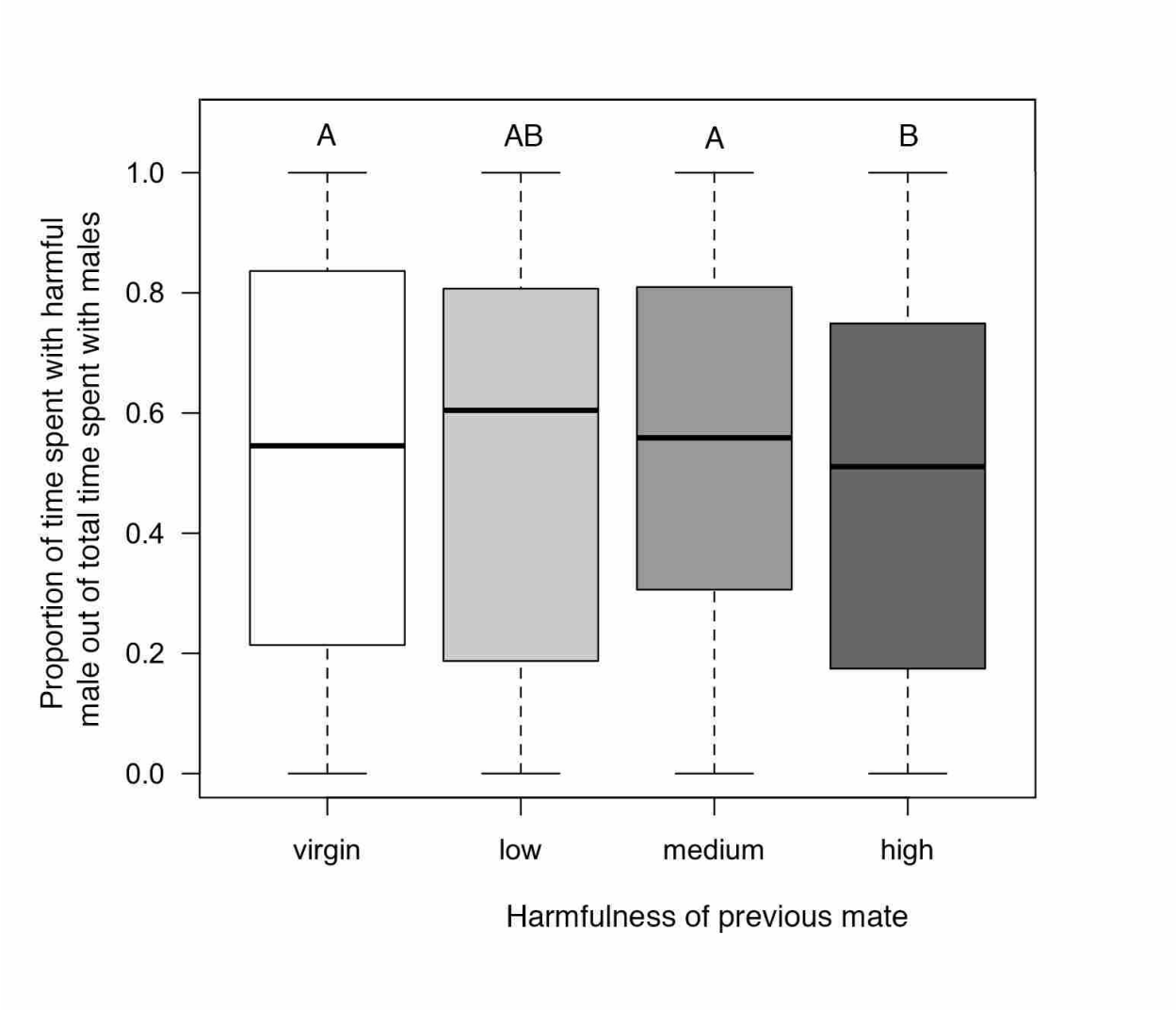

Figure 3.4: Effect of previous mating experience on the proportion of time females spent with the high harm male out of their total time spent with males during the mate choice assay (preference for high harm male). The box plots show variation in the data distributions of each mating treatment. The boxes contain the middle 50% of data (inter-quartile range, IQR), and the horizontal lines represent the medians. Values > ± 1.5x IQR are outliers and are represented by open circles, and all other values that are not outliers are represented by the whiskers above and below each box. The letters beside each line indicate the statistical similarity of the means using a Tukey's post-hoc test (Table 3).

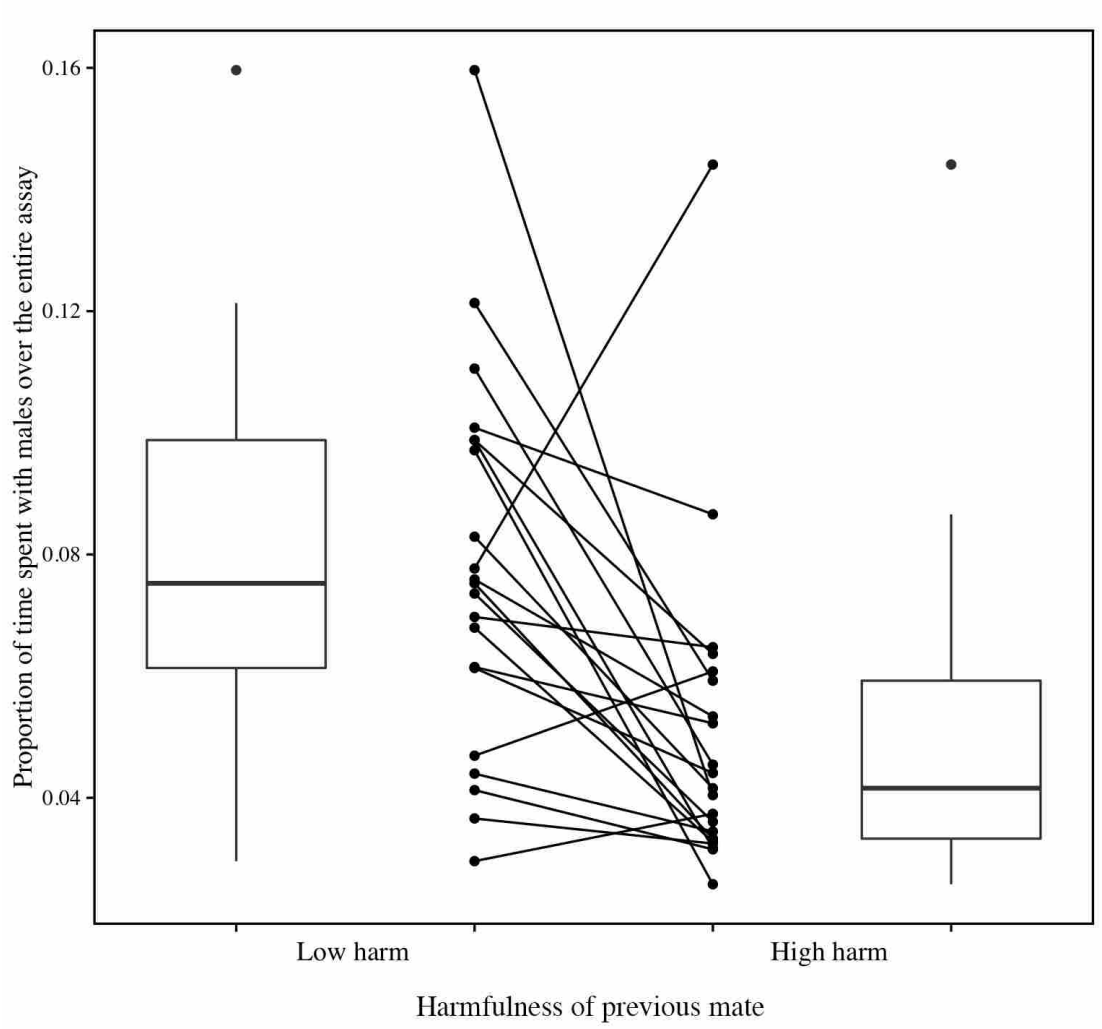

Figure 3.5: Effect of previous mating experience and female genotype on female interest in associating with males in *Drosophila melanogaster*. The reaction norm plot in the centre depicts the proportion of time each female hemiclone line spent over a sub-chamber containing a male over the entire duration of the assay across the two mating experience treatments, while the boxplots depict the distribution of data independent of hemiclonal background. The boxes contain the middle 50% of data (inter-quartile range, IQR), and the horizontal lines represent the medians. Values > ± 1.5x IQR are outliers and are represented by closed circles, and all other values that are not outliers are represented by the whiskers above and below each box.

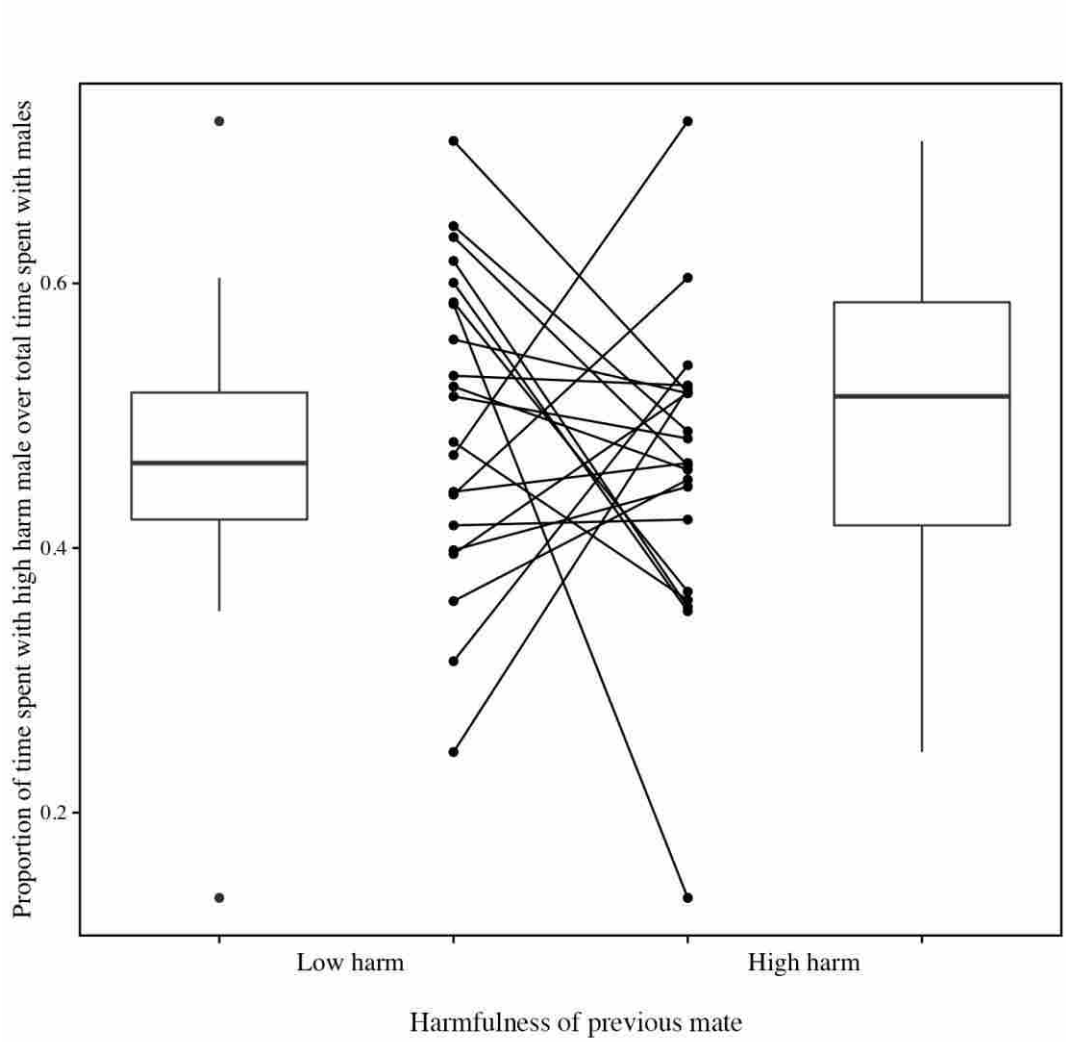

Figure 3.6: Effect of previous mating experience and female genotype on female preference in *Drosophila melanogaster*. The reaction norm plot in the centre depicts the proportion of time each female hemiclone line spent with the high harm male over the total time she spent with males across the two mating experience treatments (degree of preference for high-harm male), while the boxplots depict the distribution of data independent of hemiclonal background. The boxes contain the middle 50% of data (inter-quartile range, IQR), and the horizontal lines represent the medians. Values > ± 1.5x IQR are outliers and are represented by closed circles, and all other values that are not outliers are represented by the whiskers above and below each box.

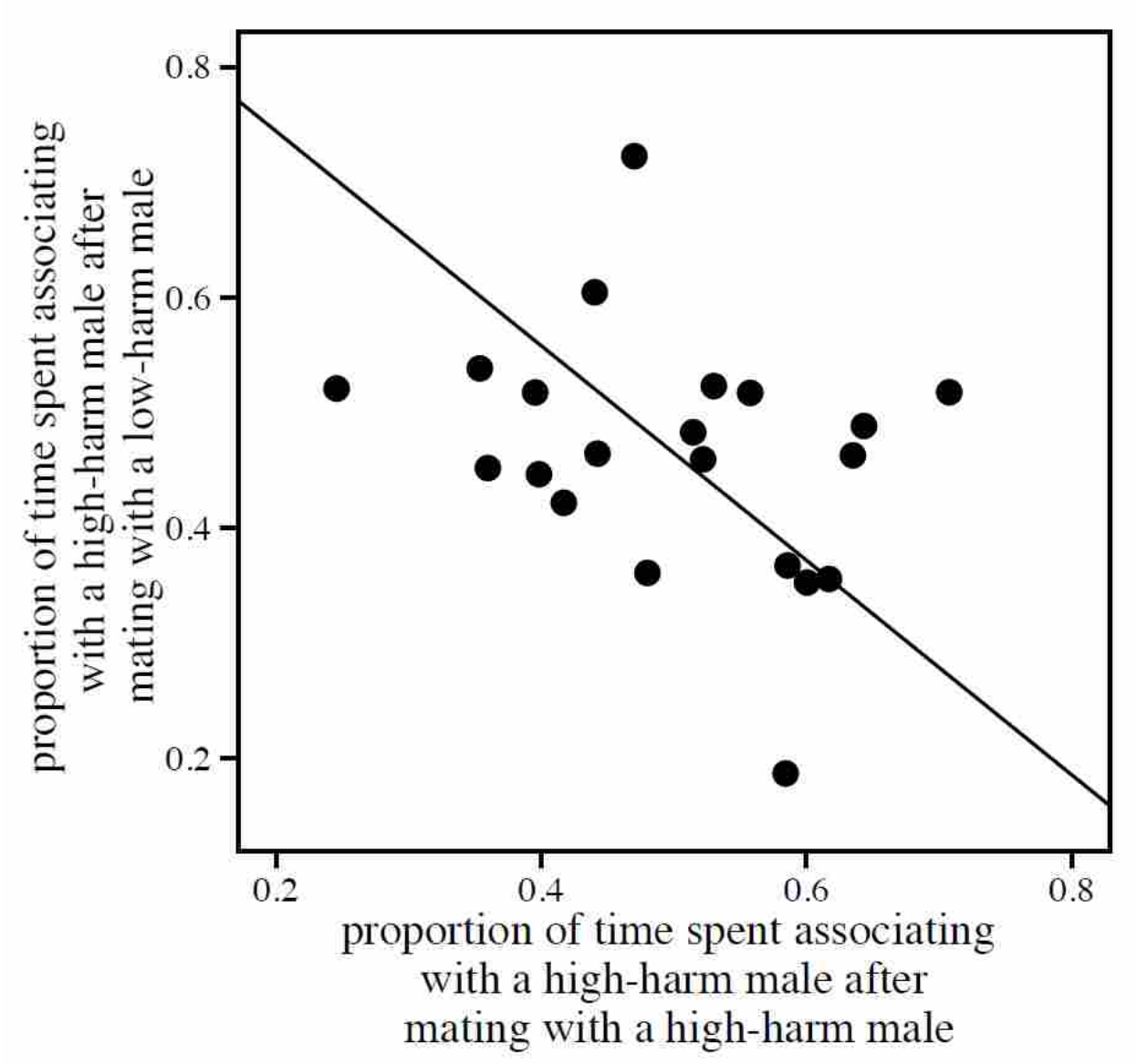

Figure 3.7: Scatterplot and regression line illustrating the negative relationship between the amount of time spent with the high-harm male compared to the low-harm male between females previously mated to a high-harm male and females previously mated to low-harm males (degree of preference for high harm male) of 21 different hemiclone lines (correlation [bootstrapped 95%CI]: -0.298 [-0.025 -0.557]).

Table 3.1: Anova results of our GLMs for interest in males and degree of preference of the female *Drosophila melanogaster* from assay 1. Females had been previously mated to a low-harm male, medium-harm male, or high-harm male (mating treatment) for either a short or long period of time (length treatment).

Response variable	Factor	χ^2	df	p-value
Interest in males	Length treatment	7.675	1	0.0056
	Mating treatment	3.909	2	0.1416
	Length treatment x Mating treatment	17.661	2	0.0001
Degree of preference for high-harm male	Length treatment	1.4669	1	0.2258
	Mating treatment	10.717	2	0.0047
	Length treatment x Mating treatment	5.710	2	0.0575

Table 3.2: Variance components estimated using a generalized linear mixed model (GLMM) fit by maximum likelihood (Laplace Approximation) for hemiclonal *Drosophila melanogaster* female interest in males. Females had previously been mated to either a high-harm or a low-harm male. The 95% CI values for the variance components were based on 1,000 bootstrapped samples of the data. The statistical significance of each variance component was determined using a permutation test approach (Manley 2007) whereby the magnitude of each model's variance component was compared to the distribution of 10,000 variance components obtained from models by randomizing the identity of the original data.

Source of Variance	Variance (SD)	Bootstrapped Upper & Lower 95% CI	% of Variance Explained	P-value
Clone	0.0397 (0.1992)	0.1214341 <0.001	1.142358	0.0615
Clone x Treatment	0.1457 (0.3817)	0.2232277 0.06597954	4.192482	0.0476
Residual*	3.289868			

*Residual variance value of $\pi^2/3$ as per Nakagawa & Schielzeth (2010) for a GLMM binomial model fit with logit link function.

Table 3.3: Variance components estimated using a generalized linear mixed model (GLMM) fit by maximum likelihood (Laplace Approximation) for hemiclonal *Drosophila melanogaster* female preference of harmful males. Females had previously been mated to either a high-harm or a low-harm male. The 95% CI values for the variance components were based on 1,000 bootstrapped samples of the data. The statistical significance of each variance component was determined using a permutation test approach (Manley 2007) whereby the magnitude of each model's variance component was compare to the distribution of 10,000 variance components obtained from models by randomizing the identity of the original data.

Source of Variance	Variance (SD)	Bootstrapped Upper & Lower 95% CI	% of Variance Explained	P-value
Clone	<0.001 (<0.001)	0.1681924 0.000	0.00	0.5043
Clone x Treatment	0.3834 (0.6192)	0.5094962 0.1755919	10.43757	0.0021
Residual*	3.289868			

*Residual variance value of $\pi^2/3$ as per Nakagawa & Schielzeth (2010) for a GLMM binomial model fit with logit link function.

CHAPTER 4

The role of experience-dependent mate choice in the maintenance of genetic variation and evolution of species

The primary purpose of this thesis was to determine if there was an effect of mating experience on subsequent mate choice behaviours in females. Specifically, I was interested in investigating if the degree of male-induced harm experienced by females caused changes in mate choice behaviours, and if these changes had a genetic basis. To do this, I first quantified the harmfulness of 26 male hemiclone lines. These lines were used to experimentally manipulate the amount of degree of harm experienced by females, and to measure her subsequent preferences for males. The data from my two experimental chapters have yielded interesting results that have shed light on the understudied relationship between male harm and female choice (Kokko *et al.,* 2003). In this chapter, I discuss these implications, and conclude with a discussion about the integrative techniques I used throughout my research.

Maintenance of male-induced harm and male competitive success

Although it is well understood that male-induced harm typically arises from the sexually-antagonistic arms-race between the sexes (Rice, 1996; Morrow *et al.*, 2003; Arnqvist & Rowe, 2005), the specific causes and consequences underlying individual variation in harmful male traits are not fully understood. In chapter 2, we showed that the magnitude of harm a female experiences via courtship and/or mating depended on her length of exposure to males and the genotype of her mates. This study is the first to date to show that phenotypic variation in female fecundity is affected by the genetic background of

their mates, and indicates the presence of an indirect genetic effect (Wolf, 2000). We also found that males who are more harmful tend to provide the greatest benefits to female fecundity if the exposure was brief (i.e. a single mating), which was a somewhat surprising result, as it indicates that the male that provides the greatest direct-benefits to the female is strongly context-dependent. In our second experiment, we found that in the long-exposure treatment, females that were previously housed with a harmful male are subsequently less interested in associating with males compared to females that were previously housed with a less harmful male, but in the short-exposure treatment, females showed no differences in their interest in males. From these results, we can infer that females may vary in their preferences depending on the temporal stability of their social environment. In situations where encounters to males may be low/brief, females may prefer to mate with a more harmful male, but in environments where encounters are high/prolonged, a female may prefer to mate with a more harmful male. This phenomenon may help explain the maintenance of additive genetic variation for harm in this species.

In order to better understand how harmful traits evolve and are maintained in populations, future research should compare the potential relationship between male-induced harm and male competitive success over time. This could be tested by combining "target males" that vary in their degree of harm with females and random "competitor males" and measuring the reproductive success of the "target males" over four different days. If increased harm is associated with increased competitive success (i.e. positively correlated), then this could serve as an alternate explanation for the evolution and maintenance of variation in harm. Rather than harm evolving only as a pleiotropic by-

product of improved sperm competition and copulation success (Morrow *et al.*, 2003), such a result would imply that harm could be the by-product of improved male-male competition. By examining this relationship over multiple days, we would also gain insight if harmfulness is a continuously optimal strategy. For example, if harmful males are very successful on day one, but consistently drop off in success over the next three days, then this would imply the strategies associated with harm are only beneficial in the short-term. Such a result could be another explaining factor for the maintenance of harmful male traits in populations. Together with our results, these hypothetical results would strongly suggest that multiple factors can lead to the evolution of male-induced harm, and variation in these harmful traits can be maintained due to a variety of context-dependent factors.

Evolution of plasticity in female mate choice and female fitness

The idea that plasticity in female mate choice behaviour evolves as an adaption is relatively new (Qvarnström, 2001; Hunt *et al.,* 2005), but has been actively investigated (Rodríguez *et al*., 2013). However, the role of mating experience on mate choice behaviours, particularly in the context of the direct costs of mating, has not previously received any study. In chapter 3, we showed that mating experience can influence a female's subsequent mate choice behaviours, and that some of this variation has a genetic basis. Specifically, we found that females that previously mated with a "high-harm" male were subsequently less interested in associating with males compared to females that previously mated with a "low-harm" male. We hypothesized that females may be

changing their mate choice behaviours in order to reduce the direct costs associated with a previous mating experience.

In order to test this hypothesis, future studies should attempt to quantify the fitness effects on females that alter their mate choice behaviours. This could be done by experimentally mating females to males that vary in harmfulness (identical to our protocol in chapter 3), giving them the option to remate, and comparing the fecundity and/or longevity between females that did and did not remate. If females that had been mated to a harmful male and didn't subsequently remate had greater fitness than those that remated, then it would suggest that the decision to delay remating is adaptive. Additionally, future studies should investigate the role of post-copulatory (cryptic) female choice, as this can influence the evolution of male traits and shed light on what traits may be beneficial (Eberhard, 1991). This can be easily tested by comparing the reproductive success (offspring produced) between the first and second males in the protocol described above. Answering these questions would allow us to better understand if mate choice behaviours can evolve as a means of female resistance, as predicated by Holland & Rice (1998).

An integrative perspective

One challenge I faced while designing and executing my thesis work was how to approach my research questions from a wide variety of biological perspectives. Throughout my work, I focused on techniques and literature from the disciplines of evolutionary biology, behavioural ecology, and population (quantitative) genetics. I encourage future students in the Long Lab (and elsewhere) to continue integrating new

perspectives into similar questions. Specifically, a biochemical analysis of the relationship between male-induced harm and female choice appears to be an exciting avenue. In *D. melanogaster*, cuticular hydrocarbons (CHCs) play an important role in communication and mate choice (Ferveur, 2005). By examining the relationship between the CHC profile of males and their harmfulness, we could potentially gain answers as to how females discriminate against males that vary in harmfulness. Additionally, as increased exposure to Acps has been shown to have deleterious effects on females (Chapman *et al.*,1995; Rice, 1996), investigating the relationship between harmfulness and Acp profiles may shed some light on the exact factors that make "harmful" males more harmful. Ultimately, it is important for biologists to take an integrative approach in order to place their specific questions into the bigger picture. Although I sometimes wish I could take a completely naturalistic approach to my research, my work throughout this thesis has taught me the importance of thinking about the same question from a variety of perspectives.

References

Arnqvist, G., & Rowe, L. (2005). Sexual conflict. *Princeton University Press, Princeton, NI*.

Eberhard, W. G. (1991). Copulatory courtship and cryptic female choice in insects. *Biological Reviews*, *66*(1), 1-31.

Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F., & Partridge, L. (1995). Cost of mating in *Drosophila melanogaster* females is mediated by male accessory gland products. *Nature*, *373*(6511), 241-244.

Ferveur, J. F. (2005). Cuticular hydrocarbons: their evolution and roles in Drosophila pheromonal communication. *Behavior genetics*, *35*(3), 279-295.

Holland, B., & Rice, W. R. (1998). Perspective: chase-away sexual selection: antagonistic seduction versus resistance. *Evolution*, 1-7.

Hunt, J., Brooks, R., & Jennions, M. D. (2005). Female mate choice as a condition-dependent life-history trait. *The American Naturalist*, *166*(1), 79-92.

Kokko, H., Brooks, R., Jennions, M. D., & Morley, J. (2003). The evolution of mate choice and mating biases. *Proceedings of the Royal Society of London B: Biological Sciences*, *270*(1515), 653-664.

Morrow, E. H., Arnqvist, G., & Pitnick, S. (2003). Adaptation versus pleiotropy: why do males harm their mates?. *Behavioral Ecology*, *14*(6), 802-806.

Rice, W. R. (1996). Sexually antagonistic male adaptation triggered by experimental arrest of female evolution. *Nature*, *381*(6579), 232-234.

Rodríguez, R. L., Rebar, D., & Fowler-Finn, K. D. (2013). The evolution and evolutionary consequences of social plasticity in mate preferences. *Animal Behaviour*, *85*(5), 1041-1047.

Qvarnström, A. (2001). Context-dependent genetic benefits from mate choice. *Trends in Ecology & Evolution*, *16*(1), 5-7.

Wolf, J. B. (2000). Indirect genetic effects and gene interactions. *Epistasis and the evolutionary process*, 158-176.