

The less explored impacts of Sexual Selection: Male mate-choice on  
female fitness traits and functional senescence of the sexes in  
*Drosophila*

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## Abstract

Sexual selection is largely responsible for widespread sexual dimorphism. This includes extreme condition-dependent phenotypes that often characterise males, enhancing their sexual fitness because females prefer males with exaggerated traits. Females tend to be drabber and less frequently display their reproductive quality. While there are a number of classical explanations for this general pattern, it has only recently been suggested that sexual conflict could also be important, with females avoiding bright pigmentation and markers of their quality to avoid the costs of male sexual harassment but this idea has not been subjected to much testing. In addition to effects on sexual behaviours and morphology, sexual selection can also affect life-history strategies and in particular, aging. Aging, declines in fertility and increases in mortality with age, is widespread and sex differences in fertility declines and mortality increases with age are common, largely resulting from sexual selection. Much less is known about possible sex differences in functional senescence (i.e. how much and how quickly different traits lose function over age) and the role of sexual selection in causing different patterns of functional senescence. This thesis used insect models to investigate why sexual selection may not favour female signals of quality (Chapter 1) and whether the sexes differed in performance declines with age (Chapter 2). I first tested if male harassment of high quality females reduces female fitness and found no male preference or increased harassment directed towards high-quality females in *Drosophila simulans*. I found that long-term harassment reduces lifespan but overall increases fecundity. However, short-term harassment decreases fecundity early in life. When exploring the role of sexual selection in driving diverse patterns of functional

senescence, I found that the sexes broadly age in similar patterns and for the most part follow similar patterns of functional decline as they age. Although the patterns in aging are similar, I find that traits lose function at different rates which is contrary to traditional aging theory of functional senescence. Jointly, this thesis highlights the different affects of sexual selection across taxa and how this is true even in closely related species like *D. simulans* and *D. melanogaster*. Results are discussed in relation to sexual selection and aging theory.

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*“If you got one foot in the grave and one on a  
banana peel...*

*...you might as well dance.”*

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## General introduction

Sexual selection occurs due to reproductive competition between individuals of the same sex and species (Darwin, 1871; Andersson, 1994; Hosken & House, 2011). The two main mechanisms underlying sexual selection are usually male-male competition, where males compete for access to female ova, and female choice, where females pick from among competing males. Ultimately, these sex differences in behaviour arise because males tend to produce abundant, tiny, motile sperm whereas females produce fewer, larger, non-motile eggs (anisogamy).

This asymmetry was highlighted by Bateman (1948) who suggested female fitness is typically limited by access to the resources needed to produce large costly gametes, whereas male fitness is typically limited by access to females. This leads to sex roles where females tend to be the sex which invests more heavily in offspring and are 'choosy', while males often compete against rivals for access to females and are less choosy. As a result, sexual selection acts more strongly on males to direct resources towards attracting females (Trivers, 1972). Females then choose amongst these 'showy' males who compete between each other (male-male competition) for access to fertilize the female (female choice). Female preference can be driven by direct benefits (e.g. access to food, protection, parental care; Trivers, 1972) or through indirect benefits (genes) via the quality of their offspring (e.g. Kotiaho & Puurtinen, 2007; Pomiankowski, 1988).

It has recently been suggested that the general lack of sexual signals in females could be due to sexual conflict, where the sexes have different evolutionary optima for a shared trait or an interaction (Hosken *et al.*, 2016). Specifically, females that invest in sexual display may suffer from high levels of male harassment which reduce their

fitness (Hosken *et al.*, 2011). Previous explanations for lack of female signals have involved females needing to be more camouflaged compared to males (Wallace, 1889) or costs of ornamentation reducing fecundity, and these would not be replenished through male-choice which in turn would overall reduce female fitness (Gwynne, 2001). Furthermore, and as described in the traditional models of sexual selection above, males have been thought to maximise their fitness by mating with all available mates. However, we do observe males choosing mates in nature even if only basic species level choice (Bonduriansky, 2001). Moreover, males do make reproductive decisions based on female traits like body size (Gage, 1998; Martin & Hosken, 2002). Recently, evidence from *Drosophila melanogaster* suggests that males exhibit preference for high-quality females when presented with a broad range of females of variable quality (Byrne & Rice, 2006; Edward & Chapman, 2012; Nandy *et al.*, 2012). Additionally, there is evidence to suggest that due to this male choice, females of high-quality suffer reduced fecundity (Long *et al.*, 2009) which can be mediated by the environment (Yun *et al.*, 2017) and this may explain why females do not signal their quality. This is yet to be tested more widely in other species.

Evidently sexual selection and sexual conflict can affect life-history traits. As highlighted above, the costs of male harassment can reduce female fecundity or lifespan. However, the effects of sexual selection on life-history evolution go beyond the direct costs of mating, and sexual selection does not just affect sexual interactions but can also impact aging by affecting optimal strategies of investment in reproduction over age (Archer & Hunt, 2015). Aging is characterized by rising mortality, declining fertility and declines in physiological function with age over a lifespan of an organism (Baudisch & Vaupel, 2012). The evolutionary explanation for this phenomenon is that natural selection grows weaker over an organism's lifetime (Haldane, 1942; Hamilton,

1966). As natural selection weakens, it allows for an accumulation of alleles with costly late-acting effects on fitness. These alleles may be mutations that have costly effects on fitness late in life but no effects on early life fitness (Medawar, 1952). These alleles may have costly pleiotropic effects on fitness late in life but positive effects on fitness early in life (Williams, 1957). Or they might be alleles that favour high investment in fitness traits early in life, but lower investment late in life (Kirkwood, 1977). The accumulation of late-acting alleles with costly fitness effects will promote the evolution of senescence (an increase in mortality risk and fertility decline) seen in many multicellular species once they reach sexual maturity (Hamilton, 1966). For a long time, it was believed that senescence was inevitable (Hamilton, 1966) but as more species are studied it is becoming clear that some species escape senescence and have constant mortality and fertility over age, or even show negative senescence, with increases in fertility and declines in mortality risk over age. Allocation theory, the trade-offs that must occur when allocating limited resources to growth, maintenance, reproduction, and survival at different time points in an organism's life, provides a compelling and interesting explanation for this variation: fitness is maximised in different ways depending on the organism, resulting in different trajectories (Stearns, 1989, Zera & Harshman, 2001). Despite our understanding of how natural selection is clearly at the centre of the process of aging, we are still trying to understand how sexual selection and sexual conflict can affect aging in terms of male and female life history traits.

Aging research has typically focused on age-associated rises in mortality (actuarial senescence) rather than declining physical performance (functional senescence) (Nussey *et al.*, 2013). This means that changes in behavior and function over age are poorly understood (Petrosyan *et al.*, 2014; Hassall *et al.*, 2015; Anotaux *et al.*, 2016).

Because functional senescence has been largely overlooked in aging research we do not understand why particular traits deteriorate at different rates both within and between individuals. If any single trait loses function before any other, then natural selection should counter this early decline (Williams, 1957). Again, this is not the case and traits have been observed to lose function at different rates in individuals (Herndon *et al.*, 2002; Lailvaux *et al.*, 2011; Nussey *et al.*, 2009; Rivera-Gutierrez *et al.*, 2012). Although it is unclear what factors shape these diverse patterns of decline one process that might be involved is sexual selection (Lailvaux *et al.*, 2014). Very few studies have asked how variance in age-dependent strategies of reproductive success affects functional senescence across the sexes. However, traits often show sex-specific rates of functional decline over time (Bonduriansky *et al.*, 2008).

Here, I addressed both the lack of sexual signals in females and sexual selection effects on aging using insect models. In chapter 1 of the thesis I tested for the presence of male mate-choice in *Drosophila simulans* when males are presented with females that varied in quality. I also tested whether high-quality females were subject to greater harassment from males and whether this harassment resulted in reduced fitness - fecundity or lifespan. In chapter 2 of the thesis I tested for sex differences in the functional senescence of key physiological traits in *Drosophila melanogaster*. A General Discussion then follows to bring key findings together.

# Chapter 1

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*Male mate-choice and potential female fitness costs of male harassment in*

*Drosophila simulans*

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## 1.1 Introduction

Extravagant male ornamentation and colouration is one of the most common patterns in nature, with exaggerated sexual traits tending to be much rarer in females (Darwin, 1871; Anderson, 1994; Hosken, Alonzo & Wedell, 2016). This is because females tend to be the choosy sex and select males that compete to mate with them. Females are choosier because they make very few, large gametes (eggs) and this high per gamete investment means that females should ensure high quality males fertilize their ova. In contrast, males produce many tiny sperm and so male fitness is limited by how many ova then can fertilise. Accordingly males compete for access to females, using sexual signals to attract potential mates. Sexual signals tend to be costly, and hence high-quality males tend to develop larger or more exaggerated signals that attract more females (Hosken & House, 2011). However, in some species sex roles are reversed (i.e. males are the choosey sex and females compete and sexually signal), but even in taxa with standard sex roles, males may exert some mate choice. Put simply, even though females are generally the choosier sex this does not mean males mate indiscriminately, with evidence from a range of species indicating that males tend to prefer high-quality females as mates (Svensson & Petersson, 1988; Cote & Hunte, 1989; Olsson, 1993; Kraak & Bakker, 1998; Amundsen & Forsgren, 2001; for review see Anderson, 1994). Nonetheless, females tend not to signal sexual quality in standard sex-role taxa.

There are several reasons females might not sexually signal as much as males (reviewed in Hosken *et al.*, 2016). Crucially, male sexual harassment (repeated unsuccessful attempts to mate by males) of high-quality females may explain why it does not pay females to signal their quality (Hosken *et al.*, 2016). In effect, if female quality was signalled, high-quality females could suffer increased harassment and reduced fitness, which may be selectively disadvantageous. For this to be true, males must make mate choices and females that are preferred by males must suffer reduced fitness. Evidence from *Drosophila* supports both of these assumptions (Long *et al.*, 2009).

Foremost, there is clear evidence for male mate-choice in *Drosophila*, with male preference for high-quality females reported in two separate studies of *D. melanogaster* (Byrne & Rice, 2006, Edward & Chapman, 2012). However, only Byrne & Rice (2006) reported that male choosiness depended on male condition – with resource depleted males being choosier (also see Edward & Chapman, 2013). Other studies have reported that males were equally or more discriminating than females during pre-copulation courtship, as in both *D. melanogaster* and *D. pseudoobscura* where some females actively approaching males more than males approached females (Gowaty *et al.*, 2002; Gowaty *et al.*, 2003). Furthermore, it has also been reported that when males are sperm limited they often mate preferentially with younger and higher-quality females (Nandy *et al.*, 2012).

As well as male *Drosophila* being choosy, at least sometimes, it appears that being a preferred female can be costly. For example, in *D. melanogaster*, larger females are more fecund. Larger females are subject to greater male sexual-attention and pay a cost for this increased male harassment in terms of reduced lifetime reproductive

success (LRS) (the number of offspring raised throughout an individual's lifespan) (Long *et al.*, 2009). Smaller, less attractive females can therefore achieve similar or greater lifetime fitness than their larger counterparts when continuously exposed to males because they have lower costs of harassment.

Male mate-choice can therefore have costly consequences for female fitness. There are several possible mechanisms for this. For example, multiple experimental evolution studies have shown that male ejaculate components are toxic to females and these toxins can reduce female lifespan (Rice 1996; Wigby & Chapman, 2004). Evidence of male harm and male harassment targeting high-quality females extends beyond *Drosophila*. Coevolution of male and female weaponry (clasping and anti-clasping morphologies) has been extensively observed in water striders where female resistance to aggressive male mating attempts increases the female's chance of male harm and predation but larger females often experience less male harassment (Arnqvist & Rowe, 2002, Perry & Rowe, 2011). Clearly males can choose mates and this choice can be costly for females. However, it is currently unclear just how common this is.

Here, I tested whether male *D. simulans* preferentially target their courtship toward high-quality females and if there are fitness consequences for females as a result of any targeted male courtship. I aimed to determine whether (i) *Drosophila simulans* males' court/harass larger, more fecund females (ii) whether harassment of preferred females might reduce female fitness (fecundity and lifespan). *Drosophila simulans* and *Drosophila melanogaster* share a recent common ancestor (estimated divergence of 2 million years ago) and the comparison of the two is important for understanding of the evolutionary changes that may have occurred since their divergence (Capy, Pla &

David, 1993, 1994; Markow, 1996; Powell, 1997). *Drosophila simulans* has a polygamous mating system where both males and females mate multiple times (females directly increase their fitness by multiple mating. Taylor *et al.*, 2008b), and where no parental care occurs (Powell, 1997). Sexual selection in *D. simulans* has been well studied, particularly for understanding female mate-choice. In this taxon females base their mating decisions on male attractiveness, which is partly determined by cuticular hydrocarbon (CHC) bouquets (Sharma *et al.*, 2010; Sharma *et al.*, 2011; Ingleby *et al.*, 2013b). Female choice is heritable and influenced by the environment, particularly temperature (Sharma *et al.*, 2010; Ingleby *et al.*, 2014), and is genetically correlated to male CHC attractiveness (Ingleby, Hunt & Hosken, 2013a). However, male mate-choice has received very little attention in *D. simulans* and while the mechanisms underpinning male mate-choice have been explored (Shahandeh *et al.*, 2018), the presence and adaptive consequences are yet to be thoroughly investigated. As a result, it is unclear if male mate-choice occurs and if so, what its consequences are for female fitness.

## **1.2 Materials and methods**

### *1.2.1 Fly stocks*

The *wild-type* populations of *D. simulans* used in all male-female interaction assays were derived from iso-female lines collected in Australia in 2004. These have been maintained in large population cages (800 – 1000 flies per cage) with overlapping generations and harbour significant genetic and phenotypic variation for multiple behavioural and phenotypic traits (Taylor *et al.*, 2007, 2008a; Wright *et al.*, 2008). *Ebony* populations used in lifespan and fecundity assays were obtained from the Tucson stock centre and maintained as above for over 100 generations in large



population cages. *Ebony* is a recessive phenotypic body colour mutant with reduced fitness (Sondergaard, 1985). Flies were maintained on a Jazz Mix Medium (Fisher Scientific). All flies were maintained on 12/12 light/dark cycle in an incubator at 25°C.

### 1.2.2 Manipulating female quality

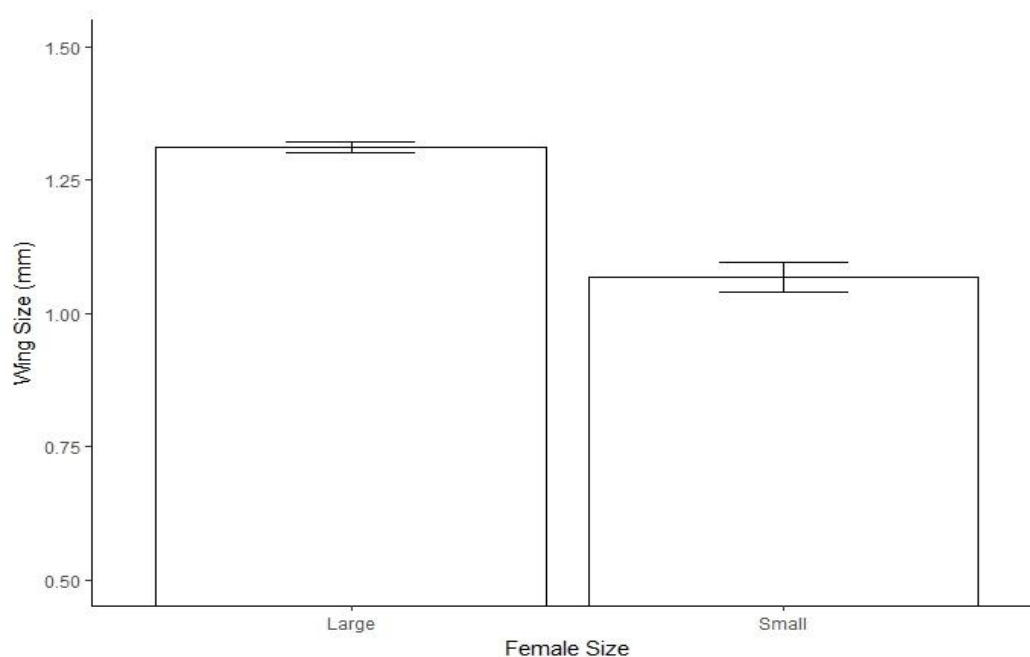
Female fecundity was our measure of quality, and high quality females hereafter refers to females that are more fecund (e.g. Parker *et al.*, 1999, Gage, 1998). To generate high and low-quality females I used diet-larval-density manipulations to affect female size, which is a key predictor of female fecundity with larger females being more fecund (see Long *et al.*, 2009). As this study is essentially a comparison of previous *Drosophila melanogaster* studies concerning male mate choice, I decided to follow female quality manipulations as closely as possible. Long *et al* 2009 found that both diet and larval manipulations heeded the best female size results which is supported by earlier studies of male mate choice in *Drosophila melanogaster* (Byrne & Rice, 2006). An apple juice-agar mix (50:50 distilled water to apple juice, 1 gram agar: 100ml total liquid) was poured into petri dishes and was sprinkled with dried yeast. The dishes were then placed into stock population cages overnight. Eggs were then individually picked from these plates with an egg pick and cultured in vials from which experimental flies were collected. Food inside these vials was arranged so the volume was constant (12 ml), but nutrition varied, and fly density was also adjusted to generate low and high-quality environments that generate small low-quality females and larger high-quality females. To produce large, high-quality females, 25 eggs were transferred to 40ml vials containing 5ml of water agar (which is nutritionally empty) and 7 ml of commercially available food (Jazz mix). To produce small, low-quality females, 180 eggs were added to vials containing 10 ml of water agar and 2 ml of Jazz Mix. All

males used were reared under standard conditions as described above. To confirm that I successfully manipulated body size, I used wing length as a measure of overall body size (Gilchrist & Partridge, 1999). This was measured with a Leica dissecting microscope connected to a PC imaging screen with SPOT basic software for image analysis. This manipulation consistently affected female body size ( $t$ -test:  $t = -16.726$ ,  $df = 81.154$ ,  $p < 0.0001$ ), with females held at low density of high nutrient availability being larger than the females held at high density of low nutrient availability (Figure 1.1 to observe size readings for large and small females).

### 1.2.3 Male mate preferences

I measured male preference for differential female quality when presented with a large and a small female and recorded all behavioural interactions. All flies used in male harassment video assays were derived from the *wild-type* population described above. 'Mating arenas' (10 x 8 x 11cm) used to house the flies during behavioural observations were designed such that flies had enough space for general movement and to display mating behaviours. These mating arenas were laser cut to a rectangular size and made from clear plastic so I could easily observe mating behaviours without disturbing the flies. The arena itself was made of 3 layers of individual plastic with the top layer cut so it could slide open and closed. A tiny hole was laser cut on the top surface where I could aspirate flies into the mating chambers. Mating assays were recorded using Chameleon3 1.3 MP Mono USB3 Vision (ON Semi PYTHON 1300) and Flea3 1.3 MP Mono USB3 Vision (VITA 1300) both fitted with Computar M3Z1228C-MP, 12 – 36mm Varifocal, Manual Iris Megapixel Lens and captured with StreamPix 6.5.0.0 (x64). These are speciality cameras which were small enough to be

hung above the mating arenas to record mating behaviours and not provide a 'fish-eyed' view on the recorded video. The Computer lenses aided the ability to zoom in on an object with perfect clarity. One large and one small female were aspirated into a mating chamber and a stock male (size was unmanipulated) was aspirated into the arena after females were given 2 minutes to settle. The assay began immediately after the male had been aspirated into the mating chamber. Each replicate was recorded for 10 minutes and the following behaviours recorded: first female approached by male (measured when male orientated himself to towards female for the first time) and male chasing female (measured when male is actively pursuing female), occurred). Mating assays were conducted at 17:00pm to 19:00pm to observe a greater level of female resistance to male attention as females are most receptive to mating in the morning, shortly after incubator lights come on.



**Figure 1.1 Manipulating female size.** Mean ( $\pm$ SE) female size (wing size) of large and small individuals when our manipulating female size technique was implemented.

#### 1.2.4 *The direct costs of male harassment on female lifespan and fecundity*

To measure high and low-quality female lifespan and fecundity and how this was affected by male harassment I first conducted assays across a female's lifespan. I used focal female flies derived from the *wild-type* and *ebony* cage populations described above. In each vial, one female was *ebony* and the other *wildtype*, but all experimental males were *ebony* mutants. This meant that I could determine maternity during subsequent offspring counts to assay female fitness. This is because *ebony* is a recessive mutation and so any *ebony* offspring must have an *ebony* mother and *ebony* father, while mating between *ebony* males and wild-type females will always produce wild-type offspring.

In continuous harassment treatments, 3 *ebony* males were housed continuously in a single vial containing one high-quality (large) female and one low-quality (small) female. In a minimal harassment treatment female pairs (high and low-quality) were exposed to three *ebony* males every 5 days for 3 hours, this ensured that females did not become sperm depleted and suffered a fecundity cost, but equally should experience minimal levels of male harassment (see Taylor *et al.*, 2008b). I had 120 replicates, that were separated into two blocks where the *wildtype* female was of higher phenotypic quality (larger) and 120 replicates where *ebony* females were of higher phenotypic quality equally split across the two harassment levels. Fecundity was measured using offspring counts where females laid eggs for between 2 – 5 days (depending on treatment and stage in lifespan) and then moved onto fresh food medium to avoid overlapping generations. Offspring vials were monitored for eclosion and could develop for 10 days after first eclosion, where they were then counted. Once females died, offspring from their current vial were counted. The sum of female

offspring counts are our measure of lifetime reproductive success. Survival was monitored daily and lifespan was measured from the first day of eclosion to the day the female died.

#### 1.2.5 *Short term fitness effects of male harassment*

The treatment above measures the direct effect of male harassment on female performance i.e. I assayed female fitness when they were in the presence of males. This means that any costs to female fitness could represent the immediate, direct interference caused by males and longer term, accumulated physiological damage that persists even in the absence of males. To better understand the costs of male harassment, I next tested whether exposure to males had long-lasting fitness costs even when males were absent, while also controlling for potential phenotypic bias during our lifespan assay where both *wild-type* and *ebony* flies were used in assays. To do this, I set up an assay identical to that described above but, *wild-type* flies were used exclusively (3 wildtype males housed together with one large and one small female). Once an initial 10 days of harassment was completed, I housed the females individually in separate vials, and measured their fecundity for 15 days thereafter.

### **1.3 Statistical analyses**

All analyses were conducted in R version 1.1.442 (R core development team 2018). For all models I assessed the significance of explanatory variables using backwards model simplification and terms were excluded if above threshold of  $p > 0.05$ . This is a standard statistical technique which can look for effects which otherwise could be obscured when power is reduced by the inclusion of non-significant terms. All

significant statistics are provided without non-signals terms inside models. Degrees of freedom are reported for the full and reduced models.

### *1.3.1 Male Preference*

To determine if males preferred large or small females, or if males harassed larger females more than small females, I used a generalized linear mixed-effects model (package: lme4) with a binomial error structure, with female first approached as the response variable, female size (large or small) as the explanatory variable and block as a random effect.

### *1.3.2 Male harassment effects on fecundity*

I implemented a linear mixed model (package: lme4) with a Poisson error structure, with fecundity as the response variable and female size, genotype (ebony or wildtype) and harassment as the explanatory variables, and block (n=2) and vial ID (i.e. the vial that female pairs were housed in) as random effects.

### *1.3.3 Male harassment effects on female lifespan*

I used a mixed effect cox model (package: coxme) with female age and censored as the survival object, female size, genotype and harassment as the explanatory variables, and block and vial as random effects.

### *1.3.4 Initial female exposure to male harassment*

I used a linear mixed model (package: lme4) with fecundity as the response variable, and female size, genotype and harassment as the explanatory variables, and block and vial as random effects.

## 1.4 Results

### 1.4.1 Do males prefer larger females and do larger females get more harassed?

When first approaching females, males did not differentiate between large and small females ( $\chi^2_{2,3} = 0.49$ ;  $P = 0.48$ ). To determine whether larger females were harassed more than smaller females I measured the amount of time females were chased by males. I found large and small females were not differentially chased by males ( $\chi^2_{2,3} = 1.84$ ;  $P = 0.18$ ; Figure 2).

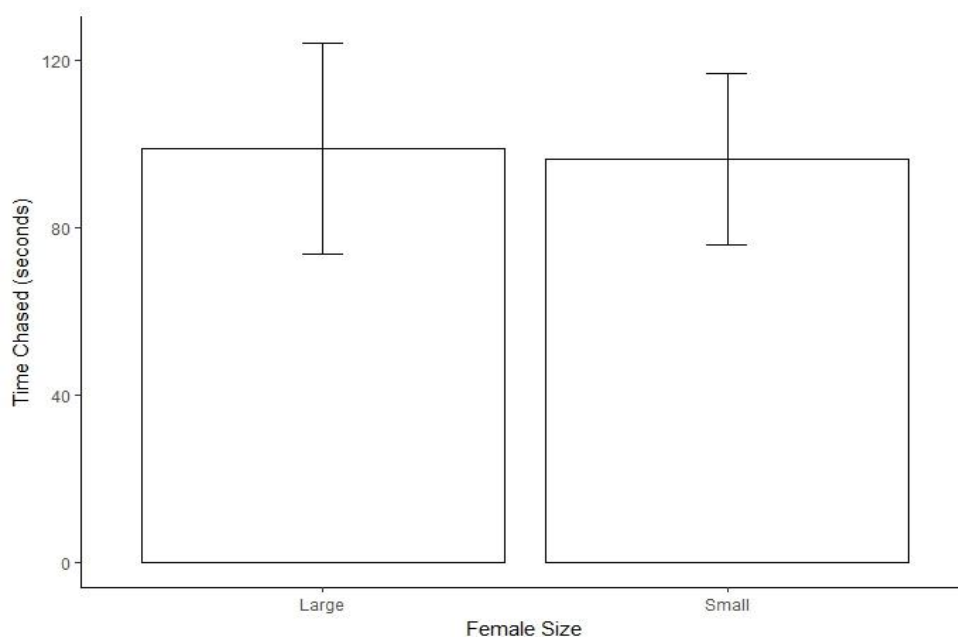
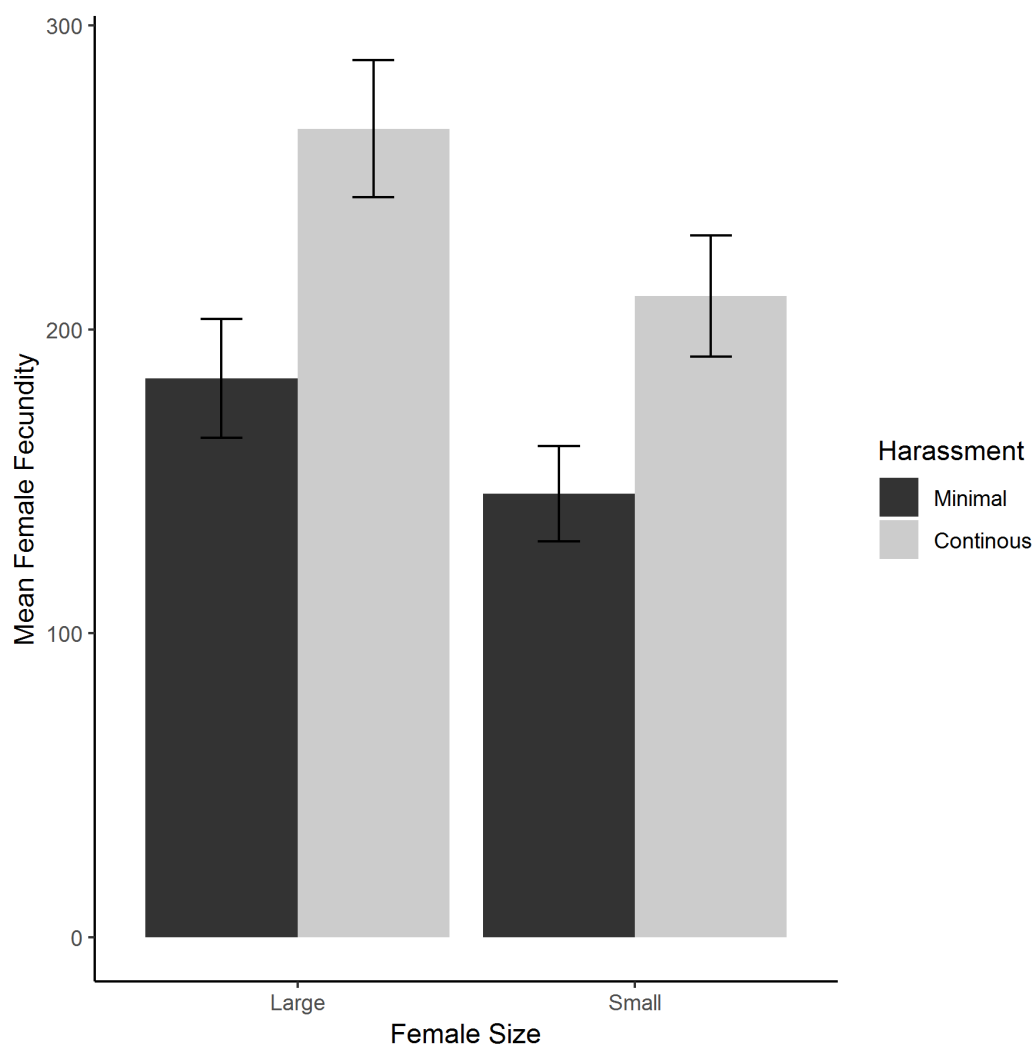


Figure 1.2. Time (seconds) male spent chasing large and small female.

### 1.4.2 Does continual male harassment affect fecundity over lifespan in females of differential attractiveness/quality?

Male exposure had a significant effect on female fecundity ( $\chi^2_{6,7} = 50.43$ ;  $P < 0.001$ ; Figure 3), with harassed females producing more offspring than non-harassed females. More so, I found the female size had a significant effect on our model ( $\chi^2_{6,7} = 22.21$ ;  $P < 0.001$ ), with larger females producing more offspring than smaller females irrespective of harassment treatment. The effect of size was consistent across treatments as there were no treatment by female size interactions ( $\chi^2_{9,10} = 0.566$ ;  $P = 0.45$ ).

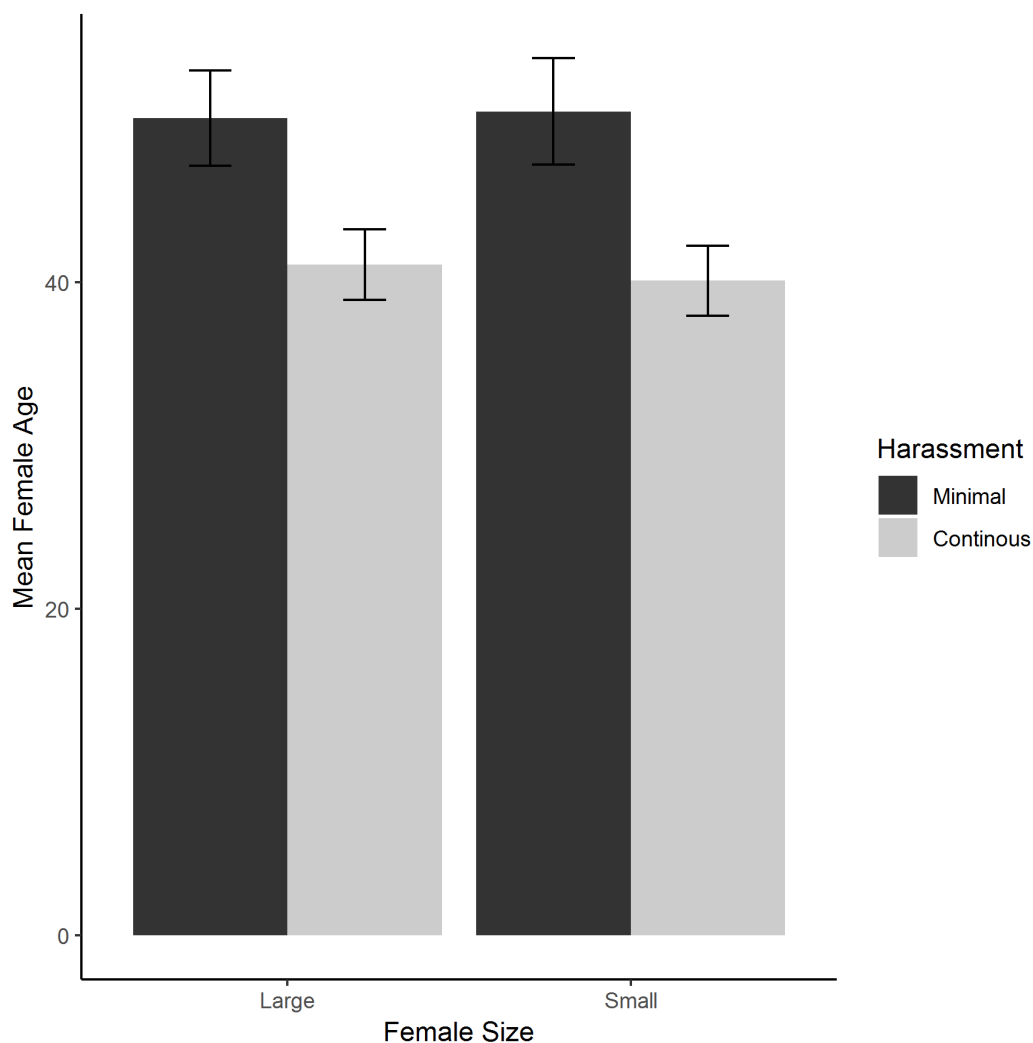


**Figure 1.3 Effect of male harassment on female lifetime fecundity.** Mean ( $\pm$ SE) lifetime fecundity (number of offspring produced) when large (high-fitness) and small (low-fitness) females are exposed to continuous harassment (blue line) and minimal non-harassment (red dotted line) of males. Harassed females produced significantly more offspring and larger females produced more offspring than smaller females irrespective of treatment.



### 1.4.3 Does continuous male harassment affect lifespan in females of differential attractiveness/quality?

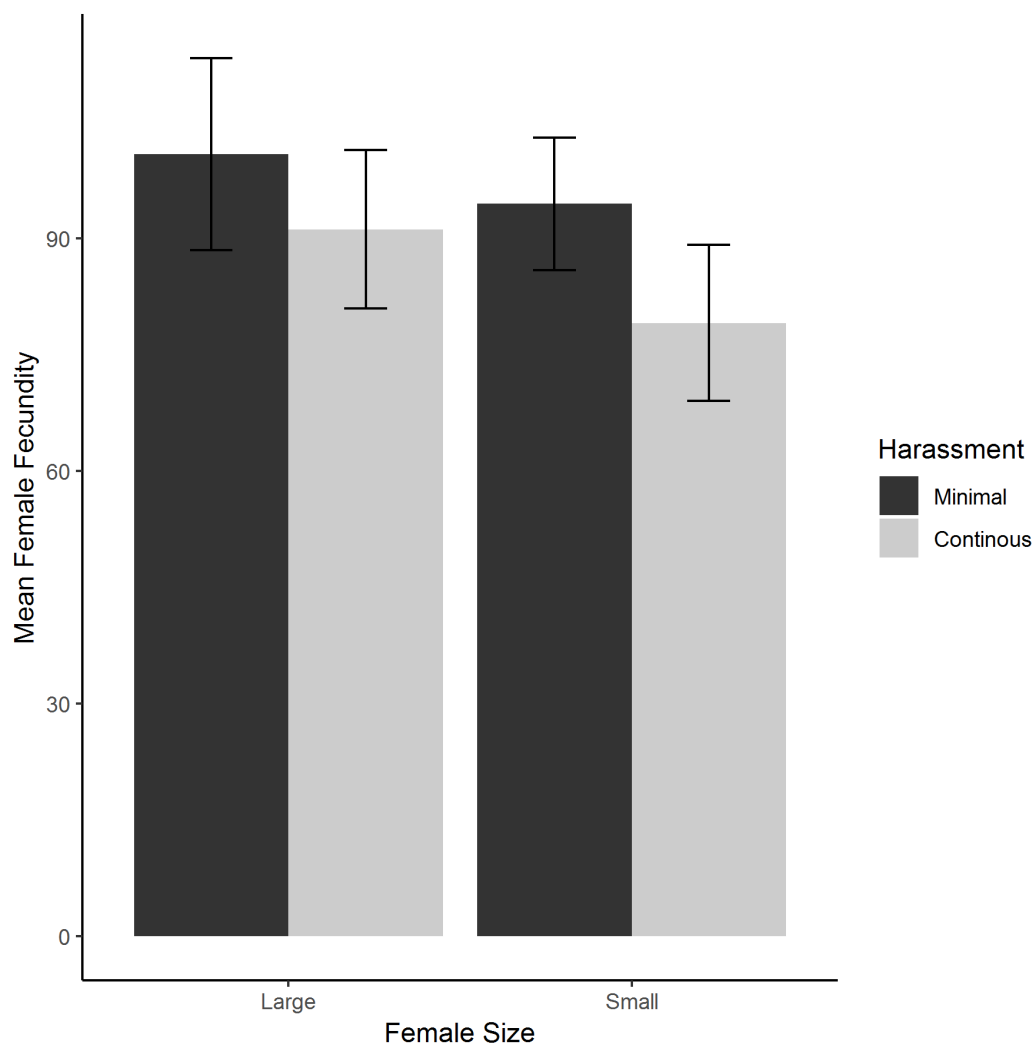
I found that harassment had a significant effect on lifespan ( $\chi^2_9 = 100.78$ ;  $P < 0.001$ ; figure 4) with harassed females living shorter lives than non-harassed females. I found no significant effect of size on lifespan, so manipulating female quality did not affect how long they lived ( $\chi^2_9 = 100.78$ ;  $P = 0.33$ ). Harassment had consistent effects on females, irrespective of their size. The effect of harassment is pronounced: females that are not harassed live on average 10 days longer than females that are harassed.



**Figure 1.4 Effect of male harassment on female lifespan.** Mean ( $\pm$ SE) lifetime fecundity (number of offspring produced) when large (high-fitness) and small (low-fitness) females are exposed to continuous harassment (black shade) and minimal non-harassment (grey shade) of males. Harassed females live significantly shorter lives and size was found to have no effect.

#### 1.4.4 Does initial harassment affect the fecundity in females of differential attractiveness/quality?

I found that harassment had a significant effect on female fecundity ( $\chi^2_{5,6} = 5.74$ ;  $P = 0.017$ ; Figure 5), with harassed females producing fewer offspring than non-harassed females. More so, these effects did not depend on female size ( $\chi^2_{5,6} = 3.32$ ;  $P = 0.07$ ) although larger females tended to produce more offspring than small females.



**Figure 1.5 The initial effect of male harassment on fecundity.** Mean ( $\pm$ SE) lifetime fecundity (number of offspring produced) when large (high-fitness) and small (low-fitness) females are exposed to continuous harassment (black shade) and minimal non-harassment (grey shade) of males. Females had a 10 day exposure to males and then separated.

## 1.5 Discussion

Generally, females are the choosier sex. However, males still exert some mate choice and, in some circumstances, can be choosier than females (Ah-King & Gowaty, 2016). When males exert choice, preferred females might be harassed so intensely that it reduces their fitness (Long *et al.*, 2009; Hosken *et al.*, 2016). Here, I tested if males exhibit preferences for high-quality female *D. simulans* and if male harassment of these females reduced their fitness (survival or fecundity). I found that males did not show any preferences for females on the basis of female quality, as measured by fecundity (size), and both high- and low- quality females were harassed equally. While harassment did reduce female lifespan and female fecundity early in life, long-term, harassment improved female LRS. This suggests that male *D. simulans* do not exhibit choice for high-quality females and the effects of male harassment are not entirely negative.

### 1.5.1 Males did not prefer high-quality females

Males are able to improve their fitness through multiple mating (Bateman, 1948) so males are expected to mate relatively indiscriminately. However, if high variation in female quality is present, males might increase their fitness by rejecting low-quality females and instead, trying to mate with high-quality females (Bonduriansky, 2001, Edward & Chapman, 2011). Here, there was pronounced variation in fitness between high and low-quality females: larger females were much more fecund than small females. Despite this, I did not find any signs of males preferring more fecund females. These results agree with classical evolutionary theory that suggests males should on average mate with any females that will accept them (Trivers, 1972). However, this result contrasts with multiple previous male mate-choice studies in *Drosophila*

*melanogaster* where authors found clear signs of male mate-choice in both virgin and mated males (Byrne & Rice, 2006; Edward & Chapman, 2012; Long *et al.*, 2009; Nandy *et al.*, 2012) and seems to be another example of the reproductive differences between these two closely related taxa (Taylor *et al.*, 2009).

The lack of choosiness I found may indicate that mating is not costly for the virgin males in this experiment, and that these males can readily re-mate. If males can remate readily, they do not need to choose between available females. Perhaps, had I tested sperm-limited or previously mated males I might have seen signs of male mate-choice as shown in previous studies (Byrne & Rice, 2006) as males will be resource depleted and mate choice might be more likely. Additionally, video behavioural analyses were conducted in the late afternoon/early evening when it is thought females are less receptive to mating. Males met with rejection by one female could immediately transfer their courtship activities to the other, that is, even if males had a preference for one female over the other, they may readily mate with a non-preferred female if they are rapidly rejected by their preferred mate and accordingly, I may not have detected any preference

### 1.5.2 *Male harassment reduces female lifespan*

As males did not prefer high-quality females, it may be anticipated that both low and high-quality females experience similar effects of harassment (continuous exposure to males) on their fitness. Alternatively, high-quality females may be better able to tolerate harassment costs. I found that harassment was associated with reduced lifespan in both high and low-quality females. On average, harassment reduced female lifespan by 10 days. This result is in keeping with a previous *D. simulans* study (Taylor *et al.*, 2008b), which found a decrease in female lifespan from continuous housing with

two males. More generally, prolonged male exposure and / or multiple mating often reduces female lifespan (e.g. Gay *et al.*, 2009). Bateman *et al.*, (2006) reported reduced female longevity in gryllid crickets (*Gryllus bimaculatus*) when housed with males. Crudgington & Siva-Jothy (2000) reported that multiple mating reduced female longevity via genital damage in the bean weevil (*Callosobruchus maculatus*). Reduced lifespan may reflect the direct damage caused by male mating (e.g. genital damage - Crudgington & Siva-Jothy 2000; toxins in the ejaculate – Eady *et al.*, 2007) or harassment (injury during courtship), or that females invest heavily in energy trying to reject male courtship attempts. Irrespective of the mechanism, these results add to a body of data showing that males pursuing, assessing, rejecting and copulating can carry heavy fitness costs for females.

### *1.5.3 Male harassment can improve female fecundity*

I measured the effects of harassment on females in two ways. Fecundity was either assayed in females exposed to continuous or minimal levels of male harassment throughout their lives, or in the absence of males after 10 days of continuous or minimal harassment early in life. In both assays, larger females were more fecund than small females and the effects of harassment on fecundity were similar for both large and small females. When females were exposed to harassment and then separated from males, I found that females from the continuous harassment treatment had lower fecundity than females from the minimal harassment treatment. This suggests that the costs of harassment are not just restricted to lifespan and extend to fertility. However, when females were exposed to harassment across their entire lives, females from the continuous harassment treatment lived shorter lives than females

from the minimal harassment treatment. Clearly, the effects of male harassment on female fecundity are more complicated than envisaged.

Indeed, data on how continuous male exposure affects female fecundity are mixed. Taylor *et al.*, (2008b) found that female fecundity in *D. simulans* was similar in females housed continuously with males and females given intermittent male exposure – these treatments are comparable to those I employed. In other species (e.g. seed beetles, bruchid beetles: Ronn *et al.*, 2006; Gay *et al.*, 2009) females housed continuously with males have lower lifetime egg production compared to females that were mated once and then isolated. Nonetheless, females often benefit from mating more than once. This might be because singly mated females become sperm limited or multiple mating allows females to exert cryptic female choice, picking the sperm of high-quality males. In support of this, Taylor *et al.*, (2008b) showed that in *D. simulans* continuous male exposure improves female fecundity relative to females mated just once. Similar results have been found in *D. melanogaster* (Chapman *et al.*, 1995: also see e.g. Savalli & Fox 1999). While these data suggest that not encountering enough males can reduce female fitness (i.e. sperm limitation), very high levels of exposure to males can also have a deleterious impact on female fitness. More so, recent research in *D. melanogaster* has shown that the environment can alter rates of sexual encounters which in turn impacts male harassment of high-fitness females and overall male harm can be reduced if the environment reduces male-female interactions (Yun *et al.*, 2017).

Two feasible explanations are feasible for why females that experienced continuous levels of harassment and were then separated had reduced fecundity, but females that experienced continuous levels of harassment across their entire lives did not experience reduced fecundity. Firstly, there may be a trade-off between lifespan and fecundity. This is because continuously harassed females lived significantly shorter

lives than minimally harassed females, but produced more offspring. The trade-off observed in our results is supported by a meta-analysis of 122 insects showing that females who were exposed to polyandry (increased male mating) had increased lifetime offspring production (Arnqvist & Nilsson, 2000). This was at the cost of reduced lifespan but direct benefits of multiple mating (increased offspring production) was as high as 30-70% - outweighing any negative effects on longevity. An alternative explanation is that females may pay an initial short-term cost to continuous levels of harassment as eager males persistently harassing them for mating's. However, over an entire lifespan the female has greater offspring production due to constant opportunity to mate.

In conclusion, I found no evidence to suggest male mate-choice is present in *D simulans*, or at least no choice for larger females. I also found no evidence for larger females paying a greater fitness cost due to male harassment than their smaller, lower-quality counterparts. These findings are interesting because in the closely related *D. melanogaster* the opposite is true. However, I found that females pay an initial short-term reduction in fecundity when housed continuously with males. Furthermore, females live significantly shorter lives when housed continuously with males, but this is offset by their increased fecundity over their lifespans. Further research should investigate whether male resource dependence influences male mate-choice in *D. simulans*, and the rate of sperm depletion in male remating.

## Chapter 2

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*Sexual selection and functional senescence across the sexes in Drosophila melanogaster*

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### 2.1 Introduction

Aging is characterized by increased mortality risk and declining fertility over the life of an organism (Baudisch & Vaupel, 2012). However, these demographic changes are often accompanied by deteriorating physiological performance. For example, in humans aging is associated with declines in cognitive capacity (Bishop, Lu & Yankner, 2010), muscle mass (Frontera, Zayas & Rodriguez, 2012) and immune function (Boraschi *et al.*, 2013). Species like orb web spiders species; (Anotaux *et al.*, 2016), red flour beetles (*Tribolium castaneum*) species; (Wexler *et al.*, 2016) and fruit flies species; (Martin & Grotewiel, 2006) become less mobile as they approach the end of their lives.

These functional declines can carry enormous fitness costs. In animal populations, reduced performance late in life can diminish foraging efficiency (Anotaux *et al.*, 2014) and increase vulnerability to predation (Wright *et al.*, 2006), and so affect survival and reproductive success in the wild. In humans, functional performance late in life influences the happiness of the elderly (Angner *et al.*, 2009). Despite the social and ecological importance of understanding declining performance over age, aging research has typically focused on age-associated rises in mortality (actuarial senescence) rather than declining physical performance (functional senescence) (Nussey *et al.*, 2013). This means that changes in behaviour and function over age



are poorly understood (Petrosyan *et al.*, 2014; Hassall *et al.*, 2015; Anotaux *et al.*, 2016).

Because functional senescence has been largely overlooked in aging research we do not understand why or even if particular traits deteriorate at different rates both within and between individuals. Traditional evolutionary theory predicts that aging evolved as a result of natural selection growing weaker throughout life (Hamilton, 1966) and so if any single trait loses function before any other, then natural selection should counter this early decline (Williams, 1957). This should mean that aging should entail a synchronized loss of function. This is not the case however, and traits lose function at different rates in individuals in the laboratory (Herndon *et al.*, 2002; Lailvaux *et al.*, 2011) and in the field (Nussey *et al.*, 2009; Rivera-Gutierrez, Pinxten & Eens, 2012). Although it is unclear what factors shape these diverse patterns of decline, one process that might be involved is sexual selection (Lailvaux, Wilson & Kasumovic, 2014).

Sexual selection is the reproductive competition that occurs between members of the same sex and species, and tends to act asymmetrically, usually being stronger on males (Anderson, 1994). Sexual selection might therefore affect how the sexes invest in survival versus reproduction throughout life (Bonduriansky *et al.*, 2008). For example, because reproduction frequently requires more time and energy investment from females, they are more likely to adopt a slow and steady strategy of reproductive effort (Bonduriansky *et al.*, 2008). In this case, patterns of actuarial, reproductive and functional senescence are likely to follow a pattern dictated by the progressive weakening of natural selection i.e. rising mortality risk, declining fertility and functional performance. However, sexual selection can promote altogether different strategies of age-dependent reproductive investment in males. If males maximize their fitness

by investing intensely in reproductive effort early in life to gain mates, even if this reduces their survival (Kokko, 1997), then males may be under selection for high early reproductive effort, followed by fast or severe actuarial and functional senescence. Male Australian field crickets (*Teleogryllus commodus*) adopt such a “live-fast-die-young” life-history strategy where high quality males call intensely early in life to attract females but, as a result, die earlier than both low quality males and females (Hunt *et al.*, 2004; also see Okada *et al.*, 2011).

Alternatively, males might invest more heavily in reproductive effort as they grow older, this may happen if reproductive success relies on a trait that takes time to develop or learn (e.g. large song repertoire, body mass). If this translates into an age-dependent rise in reproductive success, selection may favour a longer life in males than females. Similarly, we might expect that traits that promote reproductive success late in life are under stronger selection in males than females. This strategy is seen in male decorated field crickets (*Gryllodes sigillatus*), where males invest more intensely in reproductive effort as they age while females show age-associated declines in fecundity and in turn, die earlier than males (Archer *et al.*, 2012).

Very few studies have asked how variance in age-dependent strategies of reproductive success affects functional senescence across the sexes. However, traits often show sex-specific rates of functional decline over time. For example, in grey mouse lemurs, (*Microcebus murinus*) wild females are initially stronger than males but experience more rapid declines in strength such that the sexes are equally strong late in life (Hämäläinen *et al.*, 2015). In red flour beetles, declines in movement behaviours are steeper for males than females (Wexler *et al.*, 2016). Preliminary data collected in *Drosophila simulans* evolved under elevated or relaxed sexual selection suggest that sexual selection affects how quickly and how much different traits lose function in

either sex. Unfortunately, our understanding of how sexual selection affects age-dependent changes in function is incomplete due to a scarcity of studies quantifying sex-specific patterns of functional decline.

To better understand how sexual selection might shape sex differences in functional senescence, I characterise age-dependent performance in male and female *Drosophila melanogaster* from the 15 isofemale lines originating from the *Drosophila* Genetic Reference Panel. Because these are isogenetic lines, I test whether 1) there is genetic variation for the functional traits, 2) the sexes trade-off investment in particular traits differently, and 3) there are genetic correlations for these traits and, if so, characterise the direction of any correlations.

## **2.2 Materials and methods**

### *2.2.1 Fly stocks*

The *Drosophila* Genetic Reference Panel (DGRP) (Mackay *et al.*, 2012), consists of 205 *D. melanogaster* lines initiated from gravid wild-type females caught in Raleigh, North Carolina and maintained by full-sib mating for over 20 generations. I used 15 of these lines (ID = 28, 101, 136, 317, 360, 373, 382, 437, 443, 595, 703, 737, 765, 783, 796), Flies were maintained at the University of Exeter, under a 12:12 Light/Dark cycle at 25°C, with non-overlapping generations. During line maintenance flies were anaesthetized with CO<sub>2</sub>.

Experimental flies were collected as virgins and aspirated into individual 40 ml vials on their day of hatching with 8 ml of Jazz mix (Fisher Scientific, Loughborough, UK). Flies were checked every 48 hours to make sure that their food was free from mold or

bacterial growth; if vials were not sufficiently clean; flies were moved onto fresh food immediately. All flies were aspirated into fresh tubes every six days irrespective of the condition of the vial.

Flies used as mating partners to assess reproductive performance originate from the Dahomey stock caught in Raleigh, North Carolina. Tester flies that were used as mates were collected as virgins and kept in groups in single sex vials with excess food. All mates were aged between three and six days old when they were paired with experimental animals.

### 2.2.2 *Sampling regime*

A challenge with assaying functional senescence is that if a trait is assayed too late in life (i.e. in the very oldest old members of a population) it may overestimate physical performance by assaying particularly high-quality individuals. Conversely, if assay is performed too early then senescent declines may not be detected. Ivanov *et al.*, (2015) recorded lifespan in 25 virgin females for 197 DRGP lines, and for the lines used in the current study, median lifespan was 60 days (range: 33-78 days). I therefore sampled at four-time steps: days 5, 15, 25 and 35 post hatching. The final assay date exceeds (or is very close to) median lifespan in some experimental lines and so should reflect late life performance. Additionally, three weeks is sufficient to detect the beginnings of functional decline in *Drosophila* (Gargano *et al.*, 2005). However, 35 days old is less than median lifespan for most lines, which should minimise problems associated with selective disappearance, and be further reduced by using isolines.

A second challenge with measuring age-associated changes in function is that to really compare age-associated changes in multiple traits longitudinal data should be collected for a single cohort. However, this is inappropriate for some of the traits I assayed. For example, negative geotaxis performance improves with repeated testing (Piazza *et al.*, 2009) and repeated exposure to carbon dioxide can negatively affect mating behaviour (Barron, 2000). I therefore assayed each trait in different sets of 5 flies and use different flies in each time step (e.g. 5 males / females \* trait \* age class) to ensure innate age-associated changes in performance are not confounded by the effects of training or damage.

### 2.2.3 *Reproductive productivity*

Experimental flies were mated with virgins from our wildtype stock animals. Each experimental male was housed with three virgin tester females, aged between three and six days old, that had been left overnight in 40ml mating vials containing surplus food. Flies were then left for 48h, after which, females were transferred to a vial for oviposition (1B) for a further 48h, while males were removed and frozen. Females were then moved to one more vial for a further 48h, such that their egg laying over 6 days was recorded. Oviposition vials were then incubated at the temperature from which their sire originated, and offspring were counted 8 days after the first day of offspring eclosion (see Taylor *et al.*, 2008b). The same method was applied to females, but females were paired for 48h with two males.

### 2.2.4 *Recovery from CO<sub>2</sub> anaesthesia*

The ability to recover from hypoxia is indicative of mitochondrial function (Coquin *et al.*, 2008). I used recovery from CO<sub>2</sub> anaesthesia to assay age-associated changes

in mitochondrial function. I assayed 5 different flies of each sex in separate vials at each age class, from every inbred line (i.e. 5 flies x 2 sexes x 4 ages / line). To assay CO<sub>2</sub> recovery time, flies were transferred into separate vials where they were exposed to CO<sub>2</sub> (1ltr / min) for thirty seconds. The time until each fly stood upright was recorded. All assays were recorded within two hours of lights going on in the incubator of the flies (i.e. 9 - 11 am) and the order that flies were assayed was determined using a random number generator function in Excel.

#### 2.2.5 *Negative geotaxis*

Negative geotaxis (vertical climbing in response to shock) is a measure of motor ability that shows an age-dependent decline in *Drosophila* due to both reduced climbing speed and longer climb latency (Rhodenizer *et al.*, 2008). Once more, I assayed 5 different flies in separate vials of each sex at each age class, from every inbred line (i.e. 5 flies x 2 sexes x 3 ages / line). Flies were aspirated into 15ml vials and then placed inside a wider vial attached via epoxy glue to a wooden board 2cm apart. The wooden board was then raised 10cm and dropped, immediately in front of a cutting board marked every 5mm. A camera (Sony HDR-CX405) was used to video every trial. This allowed us to record (1) the number of striations an individual climbed past (geotaxis distance) and (2) the time taken to reach the top most striation (geotaxis time). Observations were stopped after two minutes if a fly did not begin climbing. Ten minutes later this assay was repeated and individual geotaxis distance and time taken for flies to pass all four striations were calculated from these two measurements.

## 2.3 Statistical analysis

To analyse the effects of age on functional performance I used general linear models (package: glm) in R version 1.1.442 (R core development team 2018). The response variable was the trait of interest and explanatory variables were sex (male or female), isoline (factor) and age (factor), and all interactions between them. Significance was assessed via backwards model simplification and non-significant terms removed when they had a non-significant effect on model fit ( $P < 0.05$ ). This is a standard technique which can look for effects which otherwise could be obscured when power is reduced by the inclusion of non-significant terms. All significant statistics are provided without non-signals terms inside models. Degrees of freedom are reported for the full and reduced models.

### 2.3.1 *Reproductive productivity*

To determine how productivity differs across the sexes, lines and age-classes, I used a general linear model, with a quasi-poisson error structure, with offspring numbers as the response variable, isoline, sex and age as explanatory variables. Because I found a significant interaction between isoline and sex on productivity, I analysed the sexes separately to better understand this interaction.

### 2.3.2 *Recovery from CO<sub>2</sub> anaesthesia*

To determine if an individual's ability to recovery from CO<sub>2</sub> differs between the sexes across age, I used a general linear model, with a quasi-poisson error structure, time

taken to recover from CO<sub>2</sub> as the response variable, isoline, sex and age as explanatory variables.

### 2.3.3 *Negative geotaxis*

To enable analyses given the non-normal distribution of the negative geotaxis data (what was the distribution), I conducted derived variable analyses, where I created a single average value for each isoline in each age, sex category. I then used a Kruskal-Wallis rank sum test to compare climbing ability in groups of different ages and sexes and then looked further into differences between groups by using a Dunn's test.

## 2.4 Results

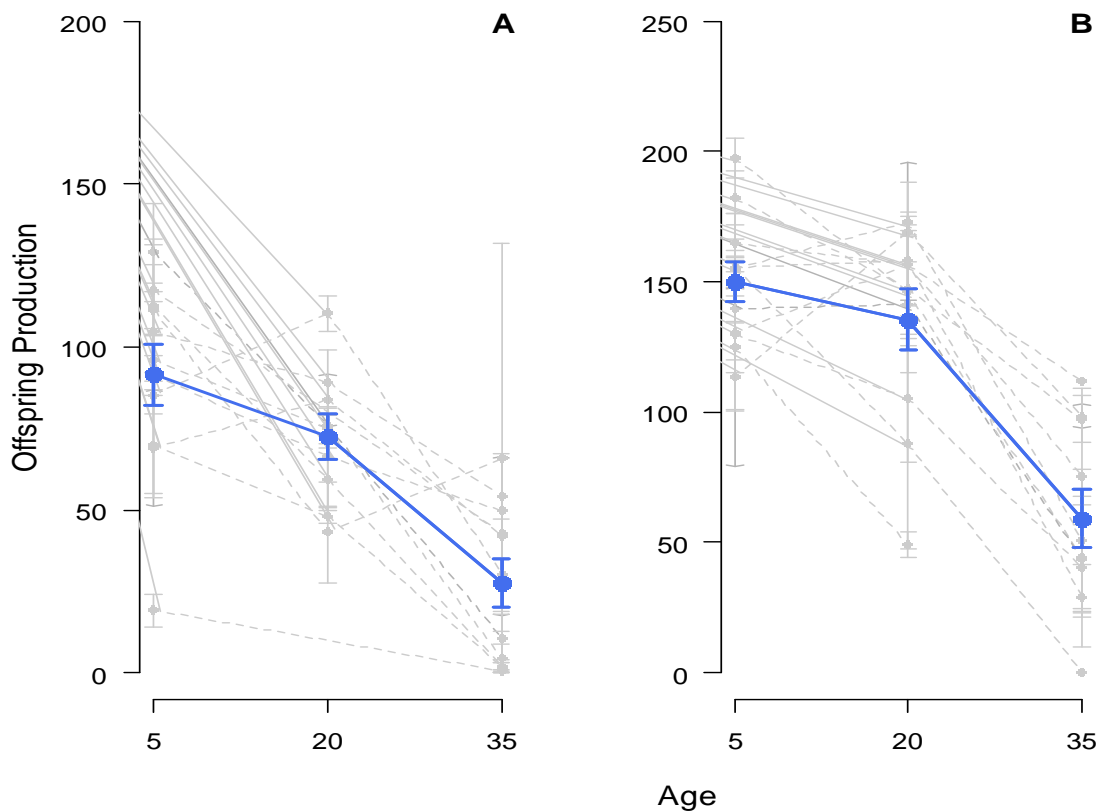
### 2.4.1 *Reproductive productivity*

I found no significant three-way interaction between isoline, sex or age on reproductive effort ( $\chi^2_{245,263} = 1.44$ ,  $P = 0.113$ ). There was a marginal non-significant interaction between age and isoline ( $\chi^2_{287,263} = 1.51$ ,  $P = 0.063$ ), which suggests all genotypes show broadly similar age-dependent declines (Figure 1). There was no interaction between age and sex ( $\chi^2_{287,289} = 2.49$ ,  $P = 0.084$ ) but there was a significant interaction between isoline and sex, suggesting genetic variation in how the sexes differ in productivity ( $\chi^2_{302,289} = 3.10$ ,  $P = < 0.001$ ).

To better understand this interaction, analyses were run separately for males and females using an identical model structure. In females, there was a significant interaction between age and line, meaning that there were genetic differences in how female productivity declines over age ( $\chi^2_{132,111} = 1.92$ ,  $P = 0.016$ ). In males, there was no such interaction between line and age ( $\chi^2_{155,134} = 1.29$ ,  $P = 0.192$ ) and lines did



not differ significantly in male productivity ( $\chi^2_{168,155} = 1.56, P = 0.103$ ) but, there was a strong effect of age ( $\chi^2_{157,155} = 47.71, P = <0.001$ ). The model coefficient shows that this difference was driven by the large declines in male productivity at age 35 relative to earlier ages ( $t = -8.694, P = <0.001$ ).

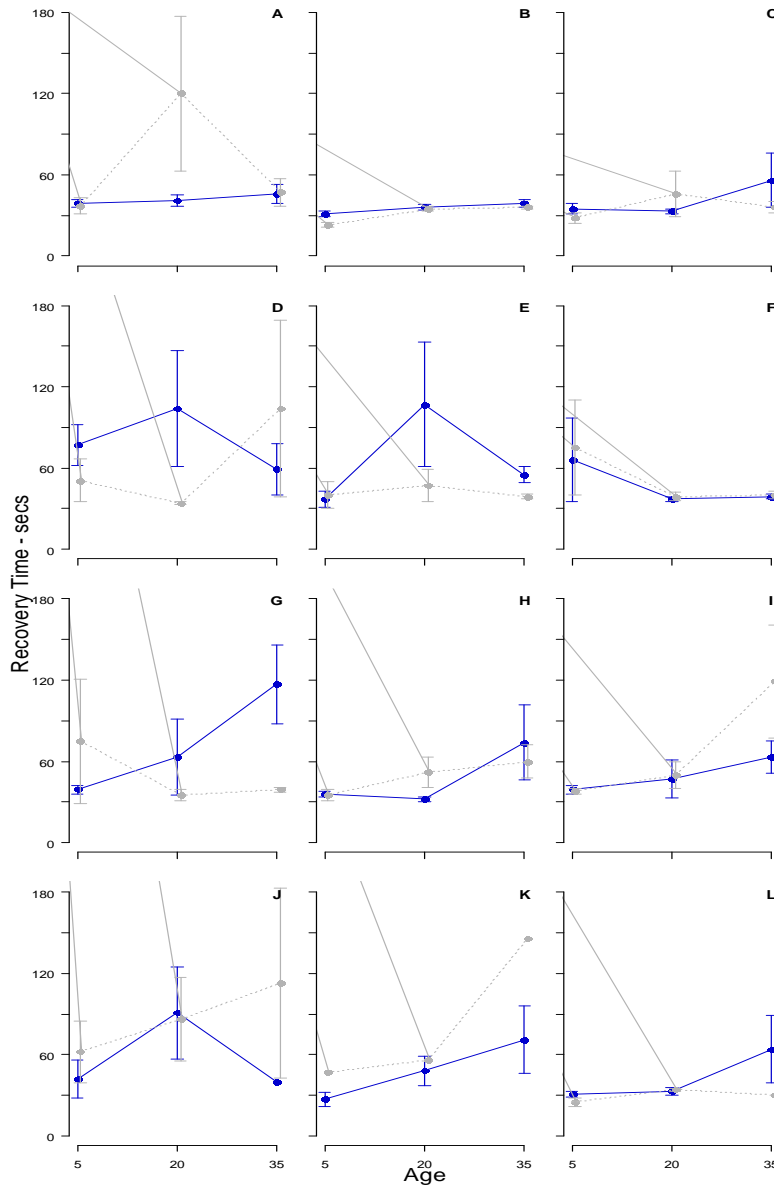


**Figure 1. The effect of age and sex on offspring production in females (Plot A) and males (Plot B).** Blue line represents the mean ( $\pm$ SE) trend of offspring production across isolines. Grey lines represent each isoline trend of offspring production over age.

#### 2.4.2 Recovery from CO<sub>2</sub>

The three-way interaction of line, sex and age did not significantly affect recovery time from CO<sub>2</sub> ( $F = 1.31, df = 353, 376, P = 0.15$ ). No significant interaction between age and sex, suggesting the sexes show a similar pattern of aging ( $F = 0.21, df = 378, 376, P = 0.81$ ). There was a marginal non-significant interaction between line and sex on recovery time with sex differences similar across lines ( $F = 1.58, df = 391, 378, P =$

0.09). Isolines showed different patterns of age dependent changes in trait expression as illustrated by a significant interaction between line and age ( $F = 1298.2$ ,  $df = 391$ ,  $415$ ,  $P = < 0.001$ ; Figure 2). Overall age did not significantly affect recovery from CO<sub>2</sub>.



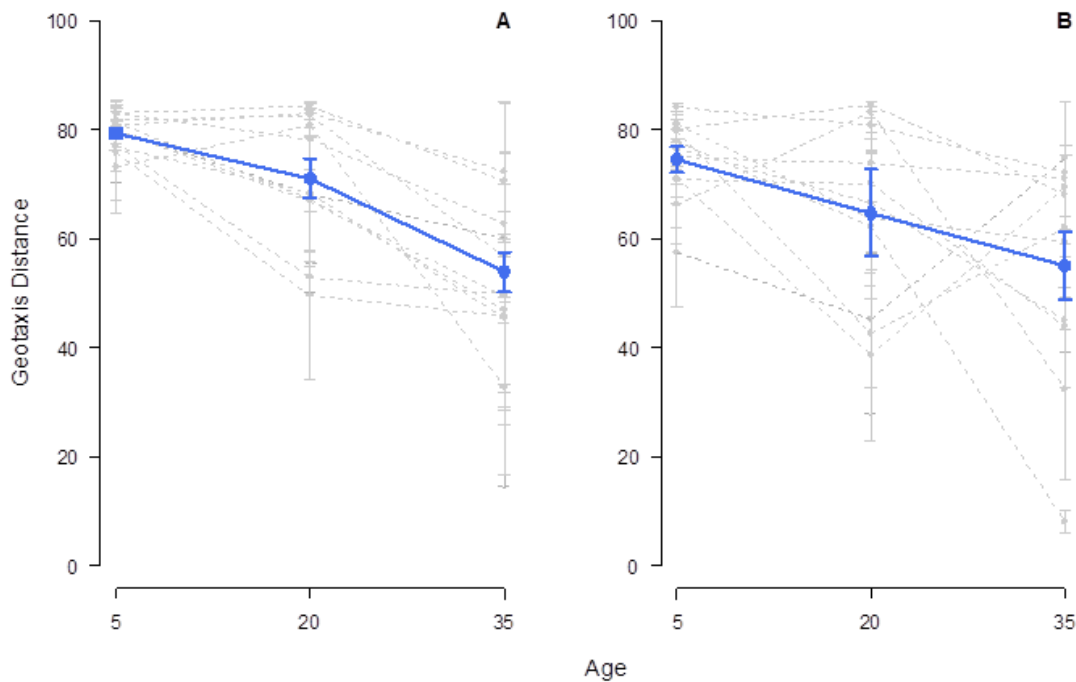
**Figure 2. Recovery time (seconds) from CO<sub>2</sub> over age across isolines.** There was a significant interaction between isoline (genotype) and age on recovery time indicating there was genetic variation in how aging affected CO<sub>2</sub> recovery. Each graph panel (A-L) represents a specific isoline and grey lines represent each specific isoline trend of recovery over age.

### 2.4.3 Negative geotaxis

There is a strong difference between groups who differed in age and sex in ability to vertically climb following being knocked to the base of a vial ( $\chi^2 = 32.6$ ,  $df = 5$ ,  $P = < 0.05$ ; Figure 3). We performed a Dunn's test to see which groups differed in geotaxis distance. The sexes show very similar patterns of decline over age in that, there are no differences in performance in age matched males and females (i.e. young males and young females show similar performance). The only sign of sex-differences in performance over age is that there is a significant difference between young and mid and old age females, but not between young and mid aged males. This probably reflects that there is greater variance in male performance in mid age (Table 1).

	Mid Female	Mid Male	Old Female	Old Male	Young Female
<b>Mid Male</b>	1.016586 0.1785				
<b>Old Female</b>	2.305674 <b>0.0226*</b>	1.328969 0.1254			
<b>Old Male</b>	1.84532 0.0542	0.868615 0.2063	-0.443607 0.3287		
<b>Young Female</b>	-2.504868 <b>0.0153*</b>	-3.521455 <b>0.0011*</b>	-4.712274 <b>0.0000*</b>	-4.25192 <b>0.0001*</b>	
<b>Young Male</b>	-1.053183 0.1827	-2.069769 0.0361	-3.31754 <b>0.0017*</b>	-2.857186 <b>0.0064*</b>	1.451685 0.1099

**Table1. Effect of age and sex on negative geotaxis between groups.** Significant P values are highlighted in bold and have \* symbol next to them. Values were only significant < 0.025. Young females differed from all groups.



**Figure 3. The effect age and sex on ability to vertically climb (mm) in females (Plot A) and males (Plot B).** Blue line represents the mean ( $\pm$ SE) trend of offspring production across isolines. Grey lines represent each specific isoline trend of Geotaxis Distance over age.

## 2.5 Discussion

Aging is the progressive decline in overall physiological performance that eventually leads to death (Arking, 1998). Aging is ultimately because natural selection grows weaker over an organism's lifetime. However, sexual selection can produce different strategies of age-dependent reproductive investment and this in turn, can affect the strength of selection on male and female survival and performance over age (Bonduriansky *et al.*, 2008). While sex differences in actuarial aging (i.e. how mortality risk changes over age) have been well studied (Baudisch & Vaupel, 2012; Nussey *et al.*, 2013), our understanding of sex differences in functional senescence is far from

complete. As a result, less is known about possible sex differences in functional declines over age.

Here, I attempted to partially fill this gap by testing for sex differences in age-dependent senescence in three measures of physiological performance (i.e. in functional traits). I measured recovery from reproductive productivity, CO<sub>2</sub> anaesthesia and negative-geotaxis. When analysing the sexes together to look at the reproduction production over age, I found that females and males show broadly similar patterns of productivity declines over age and there is no genetic variation in patterns of senescence (i.e. isolines show similar patterns of change over age). However, both these results were only marginally non-significant and when I analysed the sexes separately to better understand the significant interaction between line and sex, it became clear that the effects of genotype on aging trajectories were not the same in males and females. In females, there was genetic variation in patterns of productivity decline but in males, all genotypes declined in a similar way over age. I also found no difference between males and females over age concerning an individual's ability to recover from CO<sub>2</sub> anaesthesia and isolines differed tremendously in their patterns of age-dependent recovery time. Finally, I also found clear signs of aging in geotaxis (vertical climbing response after shock) and while there was some modest variation in the severity of these declines over age across the sexes, this variation was weak. I now discuss each of these results in turn.

### *2.5.1 Age-dependent reproductive productivity decline*

Reproductive productivity is central to an individual's fitness (Betzig *et al.*, 2012) and age-dependent declines in fertility are a hall-mark of aging (Kirkwood, 1977). However, sexual selection can promote sex differences in patterns of age-dependent

productivity that have knock on effects on functional senescence (Bonduriansky *et al.*, 2008). For example, typically, as individuals age reproductive productivity is predicted to decline, with few offspring produced by either sex near the end the lifespan (Snook & Promislow, 2003). However, sexual selection might cause patterns of sex-specific fertility to differ across the sexes. For example, if male reproductive success relies on a trait that takes time to develop (e.g. large body size), reproductive success may increase in males (but not females) with age (Archer *et al.*, 2012). Such sex-differences in patterns of fertility, might lead to sex-differences in lifespan. For example, if males only gain fitness if they reach advanced ages, but females have reproductive success across their lives, stronger selection for a long life may be expected in males. In other words, although classical theories of aging predict declining patterns of reproductive effort over age (Hamilton, 1966), sexual selection might lead to sex differences in the tempo or trajectory of age-dependent fertility declines and these can have a pronounced effect on aging overall. This means that to understand sex-differences in aging, it is vital that we characterise sex-specific patterns of reproductive success.

Here, I found that both sexes show signs of reproductive aging (i.e. productivity declines over age) but that these effects were broadly similar across the sexes. When analysing the sexes separately however, I found that the effects of genotype on age-dependent productivity differed between males and females as there was genetic variation in patterns of aging in females but not males.

Although, the interaction between age and sex were non-significant – they were marginally so, and a clear trend can be observed when visualizing the data and this shows a gradual decline in productivity with the lowest value being near the end of an individual's lifespan. However, although we might have expected males to decline

faster than females – they live fast and die young (Hamilton, 1966) – the data do not support this. Additional testing with more statistical power is probably warranted and hence sex-specific effects cannot be ruled out.

### 2.5.2 Sex and age effects on Negative geotaxis

Aging is associated with a range of age-associated functional declines (Arking, 1998) and *Drosophila* has proved a valuable model for researching the genetic basis of locomotor impairments (Grotewiel *et al.*, 2005). In humans, age related locomotor impairments are particularly important in the elderly due to mortality and injury risk (Boyd *et al.*, 2005; von Bonsdorff *et al.*, 2006). Negative geotaxis has been shown in a multitude of studies to decline with age in *Drosophila* (Arking & Wells, 1990; Orr & Sohal, 1994; Benguria *et al.*, 1996; Le Bourg & Minois, 1999; Minois *et al.*, 2001; Cook-Wiens & Grotewiel, 2002; Kang *et al.*, 2002; Goddeeris *et al.*, 2003; Simon, Liang & Krantz, 2006). In fact, the age-related decline in geotaxis is so well recorded that research now tests whether this is due to decreased speed of vertical movement or latency increases, with some research suggesting the former resulting from a decrease in jumping speed (Rhodenizer *et al.*, 2008). I found a very strong effect of age on geotaxis and some signs of sex-differences in patterns of decline over age. Sex-specific effects are consistent with previous research where differences in locomotor function between the sexes were also found (Fernandez *et al.*, 1999). I found that young females differed from all other groups apart from young males (which would suggest strong sex differences in like for like comparison), with young females able to climb vertically quicker than other groups. This may be due to females tending to be larger than males, covering greater distance in fewer steps and therefore able to outperform males. However, in males, there was no significant difference between

young and middle aged males although young males did perform better than old males and old females. While this hints at sex-differences in male and female patterns of decline between young and middle age, this probably reflects that there was more variation in male performance in middle age than in female performance, obscuring differences between these age classes. Clearly, this trait shows pronounced patterns of age-dependent declines that are broadly similar across the sexes. I hypothesize that, one reason why I found little sex differences in decline in motor function over age is because locomotor function is a particularly important trait linked to survival and increases injury risk over age leading to potential mortality and so we would expect there to be strong selection on this trait to be maintained over age in both sexes.

### *2.5.3 Age - sex interaction does not affect recovery time from CO<sub>2</sub>*

Carbon dioxide (CO<sub>2</sub>) anaesthesia is a common method for insect sorting in laboratories. There is much research showing effects of CO<sub>2</sub> including altered physiological and motor function behaviours. For instance, Bartholomew *et al.*, (2015) found that over five minutes of CO<sub>2</sub> exposure led to *D. melanogaster* displaying climbing and flight behaviour deficits that lasted for days. Furthermore, *D. melanogaster* longevity is directly reduced by exposure to CO<sub>2</sub> (Perron *et al.*, 1972). Little is known in terms of sex differences over age in ability to recover from CO<sub>2</sub> anaesthesia. I found the interaction between age and sex did not affect recovery from CO<sub>2</sub>, meaning the sexes recover at similar rates as they age. However, a strong interaction between age and isoline was found showing genetic variation in aging responses. In fact, the variation in how recovery times changed over age was pronounced, with some lines even improving with age. This finding contrasts with traditional aging theory which predicts increases in physiological decline over age as



natural selection becomes weaker with age (Baudisch & Vaupel, 2012). This may reflect selective disappearance, where we see improvements in trait expression over age because only the highest quality members of a cohort survive to reach old age. It may be that flies that performed poorly in this assay died earlier than flies that reached old age. Although the use of isolines should minimise the effects of selective disappearance, different traits often lose function at different rates even within clonal lines of individuals (e.g. Herndon *et al.*, 2002). Therefore, it is not possible to rule out selective disappearance driving these results. Whatever the mechanism, there is clearly genetic variation in aging affect.

In conclusion, I found that the sexes for the most part follow similar patterns of functional decline as they age. This may be due weak sexual selection effects and therefore patterns of age-dependent functional performance are relatively similar because natural selection is similar for males and females. Although the patterns in aging are similar, I find that traits lose function at different rates. In general, I find that individuals of both sexes decline in reproduction productivity and vertical climbing ability as they age but some individuals actually improve over age in their ability to recover from CO<sub>2</sub> anaesthesia. There was also considerable genetic variation in CO<sub>2</sub> recovery and reproductive productive patterns of aging but not in geotaxis. Furthermore, I found a pronounced decline in reproduction productivity at age 35 in males but not in females. This is interesting as traditional aging theory predicts all functional traits to decline at a similar time and tempo over age. There is very little research on sex-specific functional senescence trait loss and even fewer on the genetic variation for these traits. Here, I show there is genetic variation in function senescence. Thus, the results add to a growing body of data showing variable functional decline within individuals over age.

### 3.1 General discussion

The effects of sexual selection are many and broad, but the general awareness of its importance is only now being fully realised (Andersson, 1994; Hosken & House, 2011). This thesis is an investigation of some of the less explored impacts of sexual selection and explores a novel explanation for a widespread pattern in nature – why are elaborate sexual signals rarely found in females – and does sexual selection result in sex differences in functional senescence.

The lack of sexual signals in females has traditionally been thought to be due to females being the choosier sex that invests more heavily in reproduction and males tend to compete for access to these females (Trivers, 1972; Hosken *et al.*, 2016). Thus, female choice is often focused on as this method of sexual selection leads to a considerable amount of elaborate secondary sexual traits observed in males throughout the animal kingdom. However, males do make some mate choice decisions even if this choice is localised to the choice of the correct species or based on female body size as a function of increased fecundity (Bonduriansky, 2001). Moreover, even though females tend to be choosier than males this does not necessarily mean males are not choosy at all. Recent evidence in *D. melanogaster* suggests that the lack of female secondary signals is due to the increased sexual harassment these signals may bring and therefore reduce of female fitness (Long *et al.*, 2009; Hosken *et al.*, 2016). There are numerous recent reports of male mate-choice in *D. melanogaster* (Byrne & Rice, 2006; Edward & Chapman, 2012; Nandy *et al.*, 2012; Arbuthnott *et al.*, 2017) suggesting that the sexual selection implications of mate choice in males and the impacts on females are more nuanced than traditionally thought.

In the first data chapter of this thesis, I aimed to test how widespread these recent findings of male mate-choice were and if females pay of a cost of increased attractiveness due to male harassment in closely related *D. simulans*. My main findings suggest there is no male mate-choice in *D. simulans* nor do high-quality females incur more harassment than low-quality females. It may simply be that there is usually not enough variation in female quality for male mate-choice to evolve, or that finding any mate is so difficult in nature that indiscriminate mating by males has highest fitness returns. In any case, the findings of this chapter support a classical sex-role in *D. simulans*, where males are characterised as being far less choosy than females and mate indiscriminately

In Chapter Two, I investigated potential sex-specific aging using *D. melanogaster* as a model. Traditional aging theory predicts natural selection declines in strength over an organism's lifetime (Hamilton, 1966). This causes selection to be weak on removing late-acting deleterious genes from a population and therefore an accumulation of these late-acting deleterious genes occur in the genome (Medawar, 1952). Research over the last two decades has suggested that sexual selection may be important for aging (Promislow, 2003; Graves, 2007; Bonduriansky *et al.*, 2008; Archer & Hunt, 2015). Sexual selection can drive differences in sex specific aging by favouring reproductive effort between females and males. For example, if reproductive effort increases with age, sexual selection may promote the evolution of longer lifespan (Botero *et al.*, 2009). Sex specific aging across functional traits however, is far less understood and most aging research has focused on actuarial senescence (age-associated rises in mortality) and there is much less known about functional senescence (declining physical performance) despite its vast ecological and social importance.

I attempted to bridge this knowledge gap by testing whether there are sex differences in age-dependent functional traits and whether there is genetic variation for aging in functional traits. I found that there is little evidence for sex differences in functional decline but traits tend to lose function at different rates. Furthermore, there was genetic variation in some of these declines. Thus, my findings contradict some classical theory but equally do not always support predictions of sexual selection and aging. Clearly much more work needs to be done on this topic as the statistical power of some of my tests were fairly low.

There are various outstanding questions after the completion of this thesis. How widespread is male harassment of high-quality females? Work to date (this thesis and Long *et al.*, 2009) suggest this is not even common in the *D. melanogaster* clade. But male harassment of high-quality females happens frequently, how great is the effect relative to classical explanations for lack of female ornamentation and how big an effect would be needed to be biologically important? How much does sexual selection affect functional senescence and does sexual conflict over trait values magnify any impacts? And there are sure to be more questions than just these. Future studies would be wise to investigate if males vary their mate choice in *Drosophila simulans* when low on resources (sperm-limited, nutrient depleted etc.) as males are predicted to alter their mate preferences when mating becomes costly and high female variance in quality – we only investigated one of these variables.

Jointly, the chapters highlight the different affects of sexual selection across taxa and this is true even in closely related species like *D. simulans* and *D. melanogaster*. This is akin to what models of sexual selection predict: outcomes can be extremely contingent on starting conditions (Lande, 1981). Clearly additional work needs to be undertaken to assess how sexual selection affects aging, a point raised by many

others, and perhaps *Drosophila* are not the right taxon in which to conduct this work despite being an extremely popular aging model. After all they are largely sexually monomorphic, or at least do not display the stark sex differences seen in animals like guppies or stalk-eyed flies. Nonetheless, the work presented here is not without merit and can be seen as part of the initial steps towards a fully integrated understanding of sexual selection and its effects on aging and male and female traits.

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