ABSTRACT

VARIATION IN MATING PREFERENCES AND BEHAVIORS IN *DROSOPHILA MELANOGASTER*

By

Kimberly E. Dolphin

May 2015

I found that in inbred females *D. melanogaster,* physical condition plays a major role in the amount of polyandry. In some systems there is evidence that the ability to self assess allows inbred females to vary their reproductive behavior to increase promiscuity. I predicted that this may be true in *Drosophila melanogaster* females, but we found that inbred females behaved less promiscuously in three proxies than outbred females. Inbred females mated with fewer total males, fewer different males, and had longer copulation latency than their outbred conspecifics. However, male mate choice is not predicted in *Drosophila melanogaster* because males invest less than females, but recently the importance of male preference has been gaining support. How these males are making decisions is an important component to understanding the evolutionary impacts of the male's behaviors. I found that male mate choices are heavily influenced by previous experiences, and the lack of experience causes significant changes in courtship latency and overall preferences.

VARIATION IN MATING PREFERENCES AND BEHAVIORS IN *DROSOPHILA MELANOGASTER*

A THESIS

Presented to the Department of Biological Sciences

California State University, Long Beach

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biology

Committee Members:

Ashley J.R. Carter, Ph. D. (Chair)
Dessie Underwood, Ph. D.
Theodore Stankowich, Ph. D.

College Designee:

Dessie Underwood, Ph. D

By Kimberly E. Dolphin

B.S., 2012, University of California, Los Angeles

May 2015

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 - 1346

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Ashley J.R. Carter, for his patience and assistance at the various stages of my project. He taught me a lot about how to be a better scientist and the things I need to do, and not do, to succeed. I would also like to thank my committee members for their continued support over the last few years. They were always willing to be another pair of eyes for various issues that occurred in my project. One of the biggest components of making my project actually run were the army of helpers, who tirelessly helped me watch fly behaviors and maintain mutant lines. Without Brent DeShazo, Lilhac Medina, Annessa Du, Breanne Fletcher, Grace Lee, Natalie Lozano, Sam Degregori, Phuong Hyun, Marissa Andaloro, Amisha Thakkar, and Jose Cruz, I would never have been able to successfully complete these projects and obtained the sample sizes I needed. Finally, I have to extend a huge thank you to my ever-supportive father. He stubbornly supported me throughout the last few years despite the stress calls in the middle of the night and the occasional radio silence for a month when I was too busy to remember to call to check in.

This research was partially funded by LSAMP, grant number: HRD-0802628.

TABLE OF CONTENTS

Page

CHAPTER

LIST OF FIGURES

CHAPTER 1

OVERALL INTRODUCTION

General Background

The history of the study of sexual selection begins when Darwin (1871) initially described the process of modification of a species over time and included sexual selection as a possible mechanism driving this change he termed evolution. Darwin talked specifically about secondary sexual characteristics. Secondary sexual characteristics allow the opposite sex to identify greatest quality mates. The selection for this representation of increased quality leads to individuals possessing those qualities due to the increase in their reproductive success. Darwin also stated in his definition that males are the ones modified by this pressure, and that females are the choosers in the system.

This chooser versus chosen dynamic is explored in more depth by the parental investment theory, which explains that the parent that expends the most energy or risk towards reproduction will be the choosier of the sexes, this led to females consequently being considered the sole choosers (Trivers, 1972). This became the paradigm in discussions of sexual selection, but the paradigm had been previously established by Bateman's (1948) foundational paper. He found that in *Drosophila melanogaster*, male fitness was significantly more dependent on the number of mates rather than other factors. The female *D. melanogaster* were thus deemed the choosers, while males seemed to mate indiscriminately. Bateman's paper has been cited more than 2,000 times and is frequently used as a reference supporting a standard model in which females choose and males are indiscriminant.

Drosophila Mating Behavior Theory

Information used for learning can come from two sources: private and public. The type of learning that uses personal experiences and perception is defined as private information. Conversely, information that is provided to individuals from observing the actions and experiences of others is termed public information (Hebets and Sullivan-Beckers, 2010). Examples of the use of private information come from studies in which male *D. melanogaster* have been shown to change their preferences and learn from previous experiences based on whether they were previously accepted or rejected by females (Ejima et al., 2005; Dickson, 2008; Dukas and Dukas, 2012), and female *Drosophila melanogaster* have also been shown to vary their mating decisions based on previous personal experiences with varying quality of males (Dukas, 2005b; Mery et al., 2009). As an example of the use of public information, there is evidence that female *D. melanogaster* are able to vary their mate choice decisions based on which males they have observed successfully mating with conspecifics (Mery et al., 2009). The decision-making factors involved in determining whether to court a female or whether to accept a courting male are extremely diverse, and are easily biased based on information from both private and public information (Hebets and Sullivan-Beckers, 2010).

The incorporation of both private and public information into behaviors and the other various forms of learning found in *D. melanogaster* generates considerable plasticity within sexual selection behaviors, including courtship (Griffith and Ejima, 2009). Similar learning patterns have been found in other species of *Drosophila* as well (e.g., Dukas, 2008).

The use of these types of information can be used to create or modify the behavioral strategies of individuals and create specific tactics. Such a tactic has generally been called a "rule-of-thumb" in behavioral ecology, but has also been compared to a heuristic (Hutchinson and Gigerenzer, 2005). An aspect of the overall strategy of an individual is therefore the use of a specific heuristic, a decision making tool that allows an individual to choose between two or more options quickly based on simple rules or understandings (Tversky and Kahneman, 1974).

In *D. melanogaster* there is evidence of various rules-of-thumb being used as a strategy for quicker mating decisions. The Coolidge effect, for example, is the tendency of an individual, usually males, to select the novel female when choosing between options. Tan et al. (2013) found that male *D. melanogaster* demonstrated the Coolidge effect, and courted novel females significantly more than females with familiar phenotypes. There is also evidence that female *D. melanogaster* behave similarly and prefer to mate with novel males (Odeen and Moray, 2008). Additionally, there is evidence that male and female *D. melanogaster* are able to not only self-assess, but identify physical stresses in potential mates. Nutritional history for example, has been shown as an indicator of mating success; male *D. melanogaster* were found to significantly vary their choices based on both female age and the quality of their nutritional history (Nandy et al., 2012). The ability to use heuristics may not entirely be innate, but rather a behavior that is reinforced after continuous use. It was recently shown that male *D. melanogaster* under forced monogamous conditions for 100 generations eventually had a significant decline in their cognitive abilities (Hollis and Kawecki, 2014).

Additionally, certain *Drosophila* individuals may possess a trait that is rare in a population, for example eye color (Spiess and Kruckeberg, 2012), and these rare individuals are often found to have a higher mating success than those with the more common phenotype. Individuals having a rare trait may benefit from a version of the Coolidge effect due to their low frequency. Frequency dependent selection is an important factor in *D. melanogaster* sexual selection (Ayala and Campbell, 1974) and there is some evidence that this socially dependent sexual selection bias may be stronger in females (Billeter et al., 2012).

Frequently, experiments are designed to look at mate choice in terms of an acceptance threshold; a binary behavioral scale when a focal individual's trait value is above the threshold they are selected and when it is not the individual is rejected (Edward, 2014). While appealing conceptually and experimentally, we are now learning that this is not sufficient to tell the whole story of how mates are selected.

Current models do not include all the parameters needed to understand or predict mate choices. There has been work done that shows females can select males based on a relative scale rather than an absolute scale (Janetos, 1980) and this may have important ramifications for the interpretation of some studies. The sequential search theory, or acceptance threshold model, falls short in this area by failing to incorporate the varying strength of preference for certain traits arising from changes in preference occurring over time (Edward, 2014), namely the effects of the group quality on selection. Conversely, the fixed sample (best-of-n) misses important parameters by failing to incorporate the effects of previous experiences while selecting the best individual compared to others of a single group

One of the missing parameters not used in these models is mutual sexual selection. Mutual sexual selection is where both sexes have preferences and are displaying non-random sexual selection choices. There has been a significant amount of work looking at the comparative benefits of sequential search versus fixed sample (best-of-N) search. These two search models depend on a few important assumptions, including random male mating and unlimited time to search (Janetos, 1980; Real, 1990; Wiegmann et al., 2010; Wiegmann et al., 2013), and these theories rarely consider mutual mate choice (Fawcett and Bleay, 2008). These models will be explained in more depth in chapter 3. As previously mentioned there is increasing evidence of male mate choice in *Drosophila melanogaster,* so this introduces an important aspect of considering the effects of male mate choice on current search and mate choice theory.

Sexual Selection and Speciation

In addition to modifying the fitness of individuals, sexual selection has been suggested to be influential for speciation (Singh and Singh, 2014) and sexual conflict has been shown to have the potential to lead a population to extinction (Kokko and Brooks, 2003; Rankin et al., 2011). Additionally, it has been claimed that sexual antagonism may be what is driving evolution by sexual selection through females avoiding mating costs rather than selecting for better genes in mates (Hosken and Snook, 2005). This type of antagonistic co-evolution appears to be present with polyandrous *D. melanogaster* (Orteiza et al., 2005) and polyandry may increase rates of adaptation (Holman and Kokko, 2013). This antagonism creates an evolutionary arms race arising from sexual selection because of the extreme costs associated with mating, including death, which may encourage speciation (Gage, 2004).

There is presently a debate on the true importance of sexual selection for speciation. While there are a number of models showing that speciation by sexual selection is possible, there is doubt regarding the realism in these systems and the validity of predictions that sexual selection may be acting in conjunction with other processes rather than on its own (Ritchie, 2007). Sexual selection and non-sexual selection do appear to be misaligned in *Drosophila melanogaster* in some male fitness components. If sexual and non-sexual selection were aligned Arbuthott and Rundle (2014) predicted that males adapted to a certain environment would be more successful than males adapted to a different environment, but this was not the case in their analysis. This result and lack of alignment with non-sexual selection is an example of how sexual selection is an independent process. This independence allows for sexual selection to push species in a different evolutionary direction than would be predicted with non-sexual selection processes.

Conversely, recent work suggests that sexual selection may be acting counter intuitively even in basic Fisherian sexual selection models. Servedio and Bürger (2014) found that in their simple Fisherian population genetic models (i.e., basic sexual selection, where female preferences for a male trait are genetically encoded), once migration is introduced, stronger selection actually inhibits species isolation. More specifically, in their recent model they found that when gene flow is introduced between two allopatrically separated populations the population differences decline greatly with the strength of preference for those traits. The stronger the preference for a certain trait, the less likely there is for trait divergence to occur. This result is important because it highlights the fact that there are much more complex factors need to be added to sexual selection to verify its ability to aid in speciation.

Female Mate Choice

Female *Drosophila* use several types of information to select their mates. The aspects of males used in determining preference are both behavioral and physical, and are supposedly meant to represent the potential mate's ability to contribute to high fitness in the offspring.

During courtship, the male fly performs a very specialized courtship dance, which involves orienting towards the female, touching and licking the female, and also the vibrating the wings (Ewing, 1964; Ewing, 1983). This initial and clearly observable behavior is itself multimodal and includes both visual and auditory stimuli (Ewing, 1983; Boake et al., 1997; Ng and Kopp, 2008; Arthur et al., 2013).

Additionally, there are a number of different cuticular hydrocarbons (CHCs), which are pheromones released by the males and selected for by females (Rybak et al., 2002; Chenoweth and Blows, 2003) in various *Drosophila* species. There is also evidence that male size (Patridge et al., 1987; Markow, 1988; Bangham et al., 2002; Pavković-Lućić and Kekić, 2013), age (Price and Hansen, 1998), and the presence of sex combs (Ng and Kopp, 2008; Lee et al., 2011; Snook et al., 2013) are characteristics that female *Drosophila melanogaster* select for in males. In addition to behavioral and physiological characteristics presented by males, environmental factors have also been shown to sway mate choice, including the photoperiod in which the flies are raised (Oakeshotta, 1979).

Drosophila females tend to be polyandrous and mate with multiple males (Markow, 1988; Imhof et al., 1998). This behavior is counterintuitive because there is substantial evidence that shows multiple matings by females is deleterious (Brown et al., 2004), and have a negative effect on life span (Friberg and Arnqvist, 2003; Wigby and Chapman, 2005) and egg production, even causing an overall decrease in lifetime fitness (Chapman et al., 1993; Pitnick and Garcia-Gonzalez, 2002). The severity of these costs implies that there must be some benefits to justify the continued presence of their polyandrous mating strategy.

There are some fitness benefits that have been suggested to explain the evolution of polyandry in female *Drosophila.* For example, polyandry in *Drosophila pseudoobscura* appears to have a selective advantage in times of nutritional stress; females that mated multiply had a greater lifetime reproductive success than females that were only allowed to mate for shorter amounts of time (Turner and Anderson, 1983). However, more importantly, there is evidence that females that mate multiply provide benefits to their daughters. In *Drosophila melanogaster,* while the mother's lifetime reproductive success is greatly diminished by multiple matings, their daughters experience an increase in lifetime reproductive success (Priest et al., 2008; Barbosa et al., 2012). Finally, polyandry in females allows the opportunity for post-copulatory sexual selection to take place, where the males that have the most vigorous and healthy sperm preferentially fertilize the eggs (Ysui, 1997; Kvarnemo and Simmons, 2013). These types of benefits can explain why polyandry is so prolific in nature despite the apparently large negative costs.

Inbreeding in Female *D. melanogaster*

There has been a significant amount of work done looking at the effects of inbreeding on this species. Inbreeding has been shown to lower competitive ability and mating success (Sharp, 1984), decrease aversion learning (Nepoux et al., 2010), and increase sensitivity to stressful conditions (Robinson et al., 2009; Kristensen et al., 2011). These stresses may also act synergistically to drastically increase the risk of extinction in *D. melanogaster* (Bijlsma et al., 2000).

It is interesting to note that these negative effects of inbreeding may be unevenly distributed between the sexes, with inbreeding especially harmful towards males compared to females (Mallet and Chippindale, 2011). The lower severity of inbreeding depression in *D. melanogaster* females may have resulted in a beneficial intermediate level of inbreeding (Robinson et al., 2012b) and may be a reason why females of this species may select for males more closely related to themselves despite the inbreeding this entails (Loyau et al., 2012; Robinson et al., 2012b; Robinson et al., 2012a).

Another aspect of the harm to females that comes from mating arises from male-male competition. There is recent evidence that when male *Drosophila melanogaster* in a group are more closely related to each other, there is a reduction in male competition, and may be an example of kin selection within this species. This reduction in male-male competition leads to an increase in female lifetime fitness (Carazo et al., 2014; Pitnick and Pfennig, 2014). This reduction in costs to females from mating with related males may also explain some of the apparent inbreeding behavior and supports the concept of an optimal inbreeding level in this species (Robinson et al., 2012b).

Inbreeding does tends to result in less fit individuals though and if females alter their behavior based on their own genetic status, this implies that females also possess the ability to self-assess their own fitness and vary their behavior accordingly. There is evidence that females have this ability in several species, including humans and zebra finches (Hebets and Sullivan-Beckers, 2010).

Inbreeding effects arise from increased homozygosity so a selective pressure encouraging the presence of polyandry is its potential to increase the heterozygosity of the offspring of inbred parents (Brown, 1997). This benefit has been seen in birds; extra pair matings were shown to have a positive effect on the overall heterozygosity of offspring (Foerster et al., 2003). In the flour beetle (*Tribolium sp.*) there is evidence that inbred females are more promiscuous than outbred females, and the inbred females that mate multiply are able to increase their reproductive output in comparison to the inbred females that are monogamous (Michalczyk et al., 2011). Similar results have been found in field crickets (*Gryllus bimaculatus*) (Tregenza and Wedell, 2002). This could be an important factor for maintaining population health and numbers in populations that experience inbreeding depression frequently.

Studies of the relationship between inbreeding and behavioral promiscuity like those listed in the previous paragraph have not been done in *Drosophila melanogaster*.

In chapter 2, I describe the results from a study in which I looked at the relative promiscuity of inbred females compared to outbred females and the effects of this varied behavior on reproductive output.

Male Mate Choice

In contrast to female choice, it has generally been thought that sequential male mate choice is less likely to evolve and is probably negligible in many systems (Barry and Kokko, 2010). The argument against the presence of ubiquitous male mate choice largely arises from differences in the energetic contributions given to the offspring. As stated previously, females usually contribute more to offspring than males, and so should be choosier about their mates, to optimize the choice and offset of the costs, than males that make a much smaller contribution (Bateman, 1948; Trivers, 1972). However, the counter argument to this simplistic approach is that males also accrue multiple, non-negligible, costs while mating (Dewsbury, 1982; Cordts and Patridge, 1996). Both male and female mate choice therefore seem to play a possible evolutionary role in *Drosophila,* and neither should be over looked (Gowaty et al., 2003). With continued research, male mate choice has been found even in systems where it would not have normally been predicted, such as *Drosophila melanogaster* (Byrne and Rice, 2006; Edward and Chapman, 2012).

There is some evidence that the methodology used in the seminal study by Bateman may have been faulty and his conclusions therefore incorrect. A major component of his findings were based on using Mendelian laws of inheritance to assess the parentage of the offspring. Bateman made assumptions of paternity based on the phenotypes expressed by the *D. melanogaster* offspring, but when the trials were repeated the *D. melanogaster* lines did not follow these assumptions (Snyder and Gowaty, 2007; Gowaty et al., 2012; Gowaty et al., 2013). This error brings doubt to the significance of Bateman's findings, and motivates the consideration of male mate choice as a more important contributor in sexual selection than previously considered.

While male mate choice has not been entirely ignored, it is tested for much less often than female mate choice. With this gap now evident in the previous research literature, there is a revival of research on the effects of male mate choice on the evolution of species (Edward and Chapman, 2012). Several recent reviews on the presence of male mate choice have described evidence for male mate choice that counters the paradigm established by Darwin in 1871 (Bonduriansky, 2001; Edward and Chapman, 2011). Specifically, focus has shifted on the conclusion, based on the parental investment theory, which states that males will be less discriminant because they invest less than females (Trivers, 1972). In past evolutionary thinking, males were often assumed to have unlimited sperm resources, which would allow complete indiscriminate mating, but because males, including *D. melanogaster* (Fowler, 1973; Cook, 1975), ejaculate in batches with a limited supply, this resource is in fact finite (Dewsbury, 1982).

Male mate choice has been shown to increase some aspects of adult fitness of offspring in *D. melanogaster* (Promislow et al., 1998). There is evidence that males may be selecting for a variety of female traits, such as pheromones (Dickson, 2008), size (Bonduriansky, 2001; Dukas, 2005b), and aspects of physical condition such as nutritional history and age (Nandy et al., 2012).

As seen in females, there is evidence that males experience negative physiological costs associated with mating (Dewsbury, 1982; Cordts and Patridge, 1996; McKean and Nunney, 2001; Long et al., 2009), so completely indiscriminate mating would be detrimental for their lifetime reproductive output and lifespan. Males that are able to allocate time and resources toward greater quality females and more receptive females would have a selective advantage (Searcy, 1982; Dickson, 2008). This plasticity in behavior has been shown in some male courtships based on certain female traits, for example, males have been found to vary their courtship song and dance based on female movements, and those without olfaction were unable to plastically vary their behavior (Trott et al., 2012). Male mate choice, in a similar manner as seen for female mate choice, may have effects on speciation (Almeida and Abreu, 2003).

Male preference may provide an opportunity for the evolution of another form of antagonistic sexual selection due to females manipulating male mate choice (Edward and Chapman, 2011). Additionally, females selecting for certain male courtship behaviors may be encouraging the evolution of male mate choice, because males that are able to select more receptive females would be more successful than those male that are unable to determine the more receptive females (South et al., 2012).

Most previous studies of male mate choice have focused on recording successful mating events, but this confounds male preference and female choice. Male mate choice in *Drosophila* is different than female mate choice. Females either accept or reject male advances by allowing copulation by separating their wings (Ewing, 1983), but tend to not actively approach the preferred males. Males however, are in control of which females they decide to court (Dickson, 2008) and this courtship has been used as a proxy for mate choice or preference (Dukas, 2004; Dukas, 2005a). This difference is important when observing male sexual selection behaviors. Some experiments focused on observing successful copulations as a proxy of male mate choice (Okada et al., 2011; Edward and Chapman, 2012; Edward and Chapman, 2013). However, this success can be confounded with female receptivity because actual copulation tends to be directly associated with female choice (Dickson, 2008). Observing the number of courtship attempts and latency by males is therefore a better direct estimate of male choice than copulation (Eastwood and Burnet, 1977).

In chapter 3 I describe the results from a study in which I tested the effects of previous experiences on male mate choice using this courtship effort method.

Summary

Mate choice decisions are plastic and are reliant on the physical conditions of the chooser and the prospective mate, environmental conditions, and previous experiences. All of these factors play a role in mate search strategies and eventual mate choice. Understanding how different variables may alter eventual choices can provide important insight into the evolution of behaviors and strategies that may have been previously over looked in consideration of these systems. Self-assessment in females and variation in male choice both have a wide range of implications that I hoped to understand better with the experiments described in this thesis.

CHAPTER 2

INBREEDING DECREASES PROMISCUITY IN *DROSOPHILA MELANOGASTER* FEMALES

Introduction

In his classic paper Bateman (1948) concluded that males should be the indiscriminate sex while females should be choosy and restrict their mating to the best available male. However, there is evidence of many exceptions to this idea (Bonduriansky, 2001; Gowaty et al., 2003; Byrne and Rice, 2006; Edward and Chapman, 2011). Bateman's own study organism, *Drosophila melanogaster,* demonstrates polyandrous behavior (Marks et al., 1988), which presents a paradox because of the negative effects of mating events (Pitnick and Garcia-Gonzalez, 2002), including decreased life span (Friberg and Arnqvist, 2003) and fitness (Wigby and Chapman, 2005). Since one male can provide sufficient sperm to inseminate a female for several weeks (Lefevre and Jonsson, 1962), if females can identify the best male, then selection to avoid the negative effects of polyandry would be expected to have occurred and led to an absence of polyandry. Indeed, polyandry alone was not found to have direct fitness benefits in *Drosophila melanogaster* (Brown et al., 2004), which begs the question of the continued existence of this phenomenon. However, if the presence of polyandry in this species is able to rescue the fitness of some types of female *Drosophila*, specifically ones of lower quality, selection within these subsets of females may explain the presence this behavior.

One potential factor that may provide selection for polyandry is inbreeding. Inbreeding causes a wide range of impacts on individuals and populations; most predominately it causes inbreeding depression, which is defined as a lower mean fitness for a population due to decreased heterozygosity arising from inbreeding. This reduced heterozygosity causes an increased expression of recessive deleterious genes that would normally be hidden in heterozygotes (Charlesworth and Charlesworth, 1987). Inbreeding has been shown to cause lower egg-adult viability (Ehiobu et al., 1989; Robinson et al., 2009), lower fecundity (Ehiobu et al., 1989; Kristensen et al., 2011), and shortened life span (Valtonen et al., 2011) in *D. melanogaster*. Along with these life history traits, there is evidence that the ability of *Drosophila* to learn is also negatively affected by inbreeding (Nepoux et al., 2010). Learning appears to affect mate choice in female fruit flies (Dukas, 2005b) and creates greater reproductive success due to increased courtship of conspecifics over heterospecifics (Dukas, 2008; Delbarco-Trillo et al., 2010). This provides increased selection for those females that are able to compensate in states of high homozygosity.

With no evidence for pre or post-copulatory avoidance of inbreeding in *Drosophila* (Ala-Honkola et al., 2011; Tan et al., 2012), inbreeding may be a widespread issue in natural populations. Mechanisms that reduce the negative effects of inbreeding by increasing the genetic variation of the offspring may therefore be selected for. Michalczyk et al. (2011) found that inbred female flour beetles, *Tribolium castaneum,* were more promiscuous than their outbred conspecifics and that the more promiscuous inbred females were more fecund than the monogamous inbred females. Similarly, field crickets, *Gryllus bimaculatus*, appear to receive a fitness rescuing effect through polyandry in inbred females (Tregenza and Wedell, 2002).

I acknowledge that my experiment does vary from the previous experiments in degree of polyandry expressed in the model organism as well as design. These other experiments served as a motivation for a possible explanation to the presence of polyandry found in *Drosophila melanogaster* females that has been described many times (e.g., Marks et al., 1988; Brown et al., 2004; Byrne and Rice, 2005). However, many of the *Drosophila* experiments failed to find an explanation for the continued presence of this behavior. I therefore conducted this study to test the possible fitness rescuing effect of polyandry in inbred populations as an explanation for the presence of polyandrous behavior in these populations.

I therefore expected that a similar fitness rescuing strategy could be selected for in *D. melanogaster* and predicted that inbred females would be more promiscuous than their otherwise similar outbred counterparts. Additionally, I expected that those inbred females that were more promiscuous and indiscriminate would have a higher fecundity than the monogamous inbred females. To test these predictions I performed an experiment using two parallel lines of *D. melanogaster* that differed in their degree of inbreeding and recorded their mate choice behaviors and fecundities.

Methods

Fly Husbandry

Females used in the experiment were derived from a *D. melanogaster* wild type population obtained from Dr. Michael Rose; these lines are descended from 200 males and 200 females collected by P.T. Ives in South Amherst, Massachusetts in 1975 (Rose and Charlesworth, 1981a, 1981b). These genetically variable lines have been used in a number of other studies, but mainly for physiological evolution (e.g., Kimber and Chippindale, 2013; Mueller et al., 2013). Stock populations of *D. melanogaster ebony* and *yellow* mutants used to generate the males for the experiment were derived from two mutant lines supplied by the Bloomington Stock Center, Bloomington, Indiana (Lines numbers 1658 and 169 respectively). All flies were maintained in laboratory conditions under a 12:12h L:D cycle at 25℃ in shell vials (95 mm height, 30 mm diameter) with five females and five males per vial. Flies were fed with a standard corn meal based food mixture.

Fly Inbreeding

The inbred line was established by performing eight generations of full sibling-to-sibling matings. The resulting inbreeding coefficient, assuming no prior inbreeding effects, would therefore be F=0.7852. After the eight generations of inbreeding the lines were then maintained by performing random five by five matings in a similar manner as was done to the parallel outbred female lines as describes in the fly husbandry section described above.

Mate Choice

Mate choice was performed using virgin inbred wild type females, virgin outbred wild type females, and virgin *yellow, ebony,* and wild type males. The experimental vials contained standard food and were divided by a thin piece of plastic. One virgin female was placed on one side of the divider and one virgin male of each phenotype was placed on the other side for a total of three males. These vials were labeled with a number unique to the female and whether she was from the inbred or outbred line. These vials were all then placed in an incubator for 24 hours to allow the flies time to acclimate. After 24 hours the dividers were removed and the flies were watched continuously for 8 hours, and all copulations and phenotypes of the males copulated with were recorded along with the respective times in minutes. A 24-hour acclimation period prior to assessment of mate choice using these devices has been used previously (McKee et al., 2014).

Fecundity

After 8 hours of observations, the males were removed and the females remained in the vials. After 2 weeks the females were removed and the eclosing offspring were counted every morning for an additional 7 days.

Data Analysis

The data were analyzed using a combination of ANOVAs and Student's t-tests for the fecundity analysis and mate choices. A Mann-Whitney test was used to analyze the copulation latency data. These were performed using the Mini-tab program (v16.2.4).

Results

Mate Choice

Inbred females mated significantly fewer times than outbred females (t-test, t_{271}=3.00, p= 0.003) (Fig. 1A), and mated with a fewer number of different phenotypes (t-test, t_{262}=4.77, p= 2.77 x10^{-6}) (Fig. 1B). Inbred females also had significantly longer copulation latency than outbred females (Mann-Whitney, W=16175.5, N_1=147, N_2=148, p<0.001) (Fig. 1C).

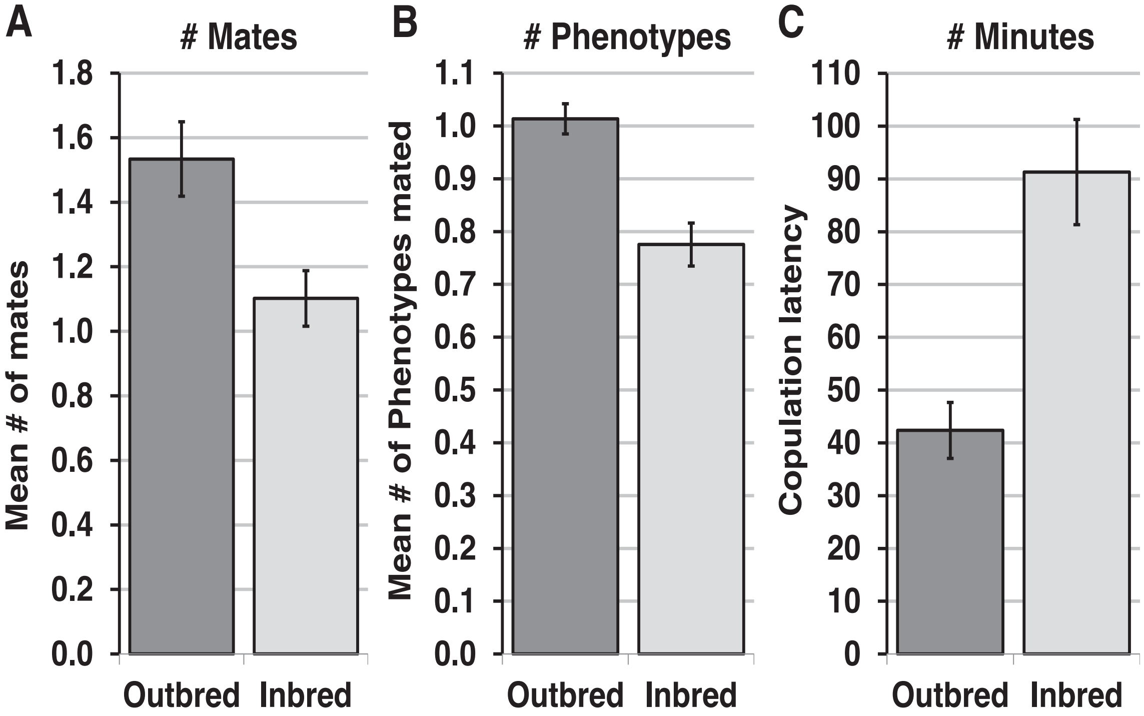

FIGURE 1. Results of inbred/outbred mate choice experimental trials. (A) Outbred females mated significantly more, and (B) with significantly more different males than inbred females. (C) Inbred females took significantly longer until copulation.

Fecundity

Inbred females produced significantly fewer offspring than outbred females (t-test, T_{242}=7.61, p = 1.42 x10^{-12}). Overall, with the combination of both groups of females, there was a significant drop in fecundity when individuals mated three or more times when both treatments were combined (One-way ANOVA, F_2=3.55 p=0.030), but this was not significant when analyzed separately for inbred and outbred females (One-way ANOVA, F_2=1.28, p=0.283 and F_2=2.22, p=0.112 respectively) (Fig. 2). Females that mated twice showed no significant increase in fecundity compared to inbred females that only mated once (unpaired heteroscedastic t tests; overall: t_{29}=0.956, p=0.056, inbred: t_{29}=0.769, p=0.297, outbred: t_{29}=0.915, p=0.107).

Discussion

Overall, the data did not confirm the initial predictions of increased promiscuity in inbred females and the converse was actually observed. My results therefore indicate that *D. melanogaster* do not exhibit the same behaviors reported for *Tribolium castaneum* (Michalczyk et al., 2011) and *Gryllus bimaculatus* (Tregenza and Wedell, 2002).

Relatively inbred females not only mated with fewer male phenotypes and had a fewer number of total matings, but they also had a significantly longer latency period before copulation (see Figure 1). Time to copulation and copulation successes are frequently used in mate choice experiments as proxies for mate preferences (e.g., Friberg and Arnqvist, 2003; Odeen and Moray, 2008; Tan et al., 2013), so my data seem to indicate that the inbred females were more selective instead of more indiscriminate relative to the outbred females.

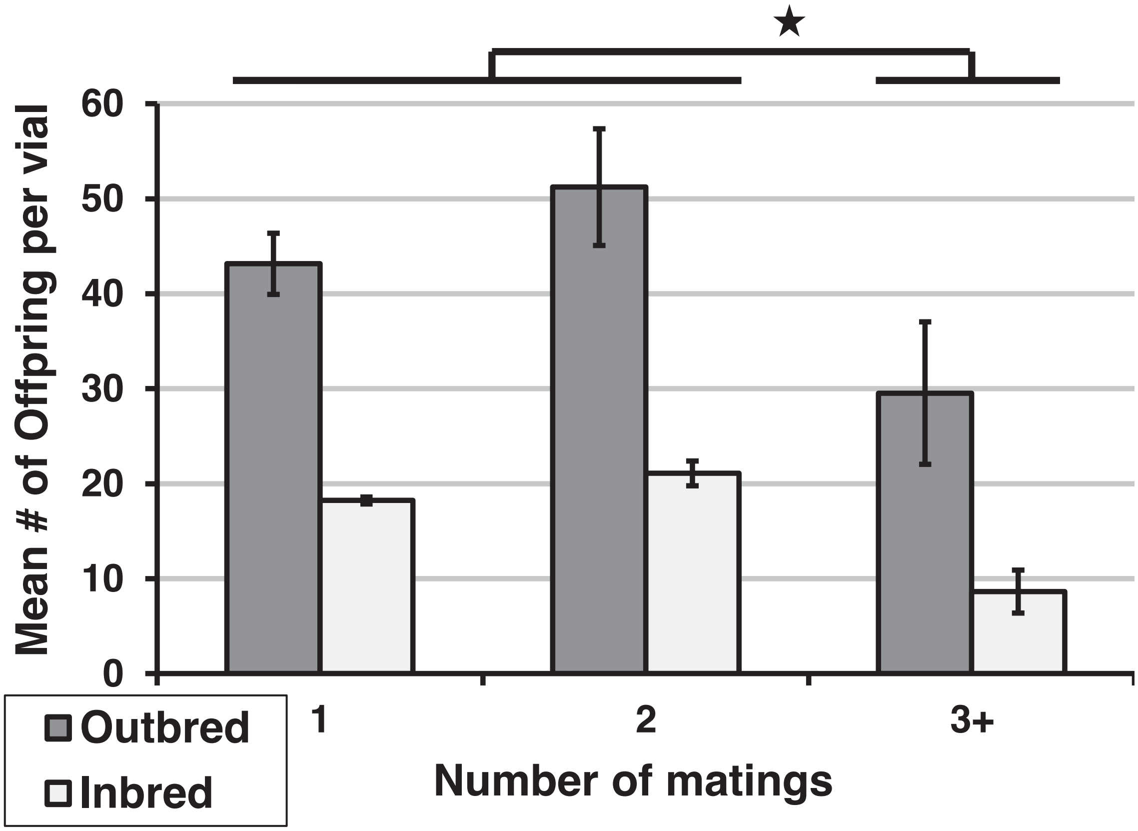

FIGURE 2. Results of the inbred/outbred fecundity measurements. There was a significant difference between the inbred and outbred fecundities in all mating groups, and there was a significant drop in fecundity in females that mated three or more times when the two treatment groups were combined.

My overall results therefore suggest that in *D. melanogaster*, inbreeding may actually increase female choosiness while decreasing promiscuity. Overall, inbred females had a significant decrease in fecundity compared to outbred females, as would be expected from multiple generations of sibling-sibling. I am confident that this reduction in fecundity is due to the inbreeding because our flies were raised in nearly identical conditions, which would eliminate the possibility of other factors causing variation in fecundity.

Being more selective may be advantageous for the inbred females because of the decrease in their fecundity when inbred females mated three or more times (see Figure 2). Females that mated once or twice, in both treatments, had significantly more offspring than females that mated three or more times. These results may support the previous findings for an optimal intermediate level of polyandry in females (Arnqvist and Nilsson, 2000). There was an unsurprising dramatic overall effect of being inbred on female fecundity; in the case of the inbred females, those that mated twice still did not have as many offspring as the least fecund group (three or more matings) of the outbred females. This drastic reduction in offspring output may accentuate selection within inbred females to mate fewer times in order to maximize their relative offspring output (e.g., inbred females that mated three or more times had on average 41% the number of offspring as those that mated twice, while outbred females that mated three or more times had 58% the number of offspring as those that mated twice).

There is evidence that polyandry may be beneficial for females in various species, such as increased offspring heterozygosity in blue tits (Foerster et al., 2003), evidence of the same in *Drosophila* (Imhof et al., 1998), increased offspring viability in *Drosophila*

(Gowaty et al., 2010), increase in offspring production in *Drosophila* (Taylor et al., 2008), and multiple paternities would allow more genetic variation within offspring clutches in *D. melanogaster* (Ochando et al., 1996). However, multiple matings have a wide range of costs (Friberg and Arnqvist, 2003; Wigby and Chapman, 2005) which inbred females may be less able to endure. Our results suggest that the balance between the benefits and costs of polyandry results in a higher degree of polyandry for outbred than for inbred females in *D. melanogaster*.

There is evidence of indirect benefits of polyandry in *D. melanogaster,* where mother suffers the costs, but benefits are passed onto the daughters (Priest et al., 2008). This effect would further support the explanation that the increase polyandry found in outbred females is beneficial, while the costs to fecundity is too great for the less fit inbred females.

If these differences in behavior are driven by such a balance, then changes in promiscuity and choosiness may rely on the existence of some mechanism by which females are able self-assess their own physiological state or quality. Females of poor quality, due to inbreeding, or some other factor, may have mechanisms to detect this fact and adjust their mating behaviors in an appropriate manner. This self-assessment ability has been reported for some species (Hebets and Sullivan-Beckers, 2010; Soper et al., 2014). In this case inbred females may be behaving in the manner that is most optimal for reproduction. Alternately, the loss of learning ability occasionally found due to inbreeding (Nepoux et al., 2010), may also account for the difference in choice behaviors I observed. If females rely heavily on their previous experience for mate choices (Billeter and Levine, 2012) or use some other complex cognitive selection procedure, then there may be problems in the mate choice abilities of the inbred females. These problems would explain the copulation latency I observed in the inbred females as arising not from increased choosiness, but rather from poorer decision-making abilities.

There is a need for more research to help detangle these two possible explanations for our observations and the contrasting observations seen in other taxa (Tregenza and Wedell, 2002; Michalczyk et al., 2011). At the least, our results show that the effects of inbreeding on behavior may be manifested in different ways in different systems, and reports from single species (e.g., Bateman, 1948) should not be taken as a universal rule or process.

CHAPTER 3

EVIDENCE THAT COURTSHIP LATENCY VARIES BY PREVIOUS EXPERIENCE IN MALE *DROSOPHILA MELANOGASTER*

Introduction

When originally described in 1871 by Darwin, sexual selection was presented as an evolutionary process guided by choosy females and competitive males. Bateman reinforced this view in his classic paper (Bateman, 1948) where he found that male *Drosophila melanogaster* fitness is significantly more variable and dependent on the number of mates than female fitness. This is explained more thoroughly by the parental investment theory, which states that the parent expected to be more selective (Trivers, 1972). This paradigm has been influential in guiding thought about sexual selection in nature, but now some papers have concluded that there is evidence of male mate choice in *D. melanogaster* and other systems in which it was not predicted (Bonduriansky, 2001; Gowaty et al., 2003; Byrne and Rice, 2006; Hebets and Sullivan-Beckers, 2010; Edward and Chapman, 2011; Gowaty et al., 2012; Tan et al., 2013). These papers tend to focus on various mechanistic and multimodal signaling involved with male mate choice, including some that elucidate the ability for mate choice learning in *D. melanogaster* (Ejima et al., 2005; Dickson, 2008; Griffith and Ejima, 2009), but there is less focus on the strategies used in decision-making by this species.

Mate choice theory predominately looks at two central strategies. The first is the sequential searching method, which is where an individual (generally considered the female) searches for a mate until a potential mate surpasses a fixed threshold of quality. The second is fixed sample strategy (best-of-n model) where an individual retains a memory of previously sampled prospects and uses a comparative model to pick the best of the group. The null for these two strategies is random choice (Wiegmann et al., 2010; Castellano et al., 2012; Wiegmann et al., 2013).

Additionally, private and public information have been shown as important factors for mate choice. Public information is utilized when an individual observes behaviors in others within the group and this biases their own behaviors (Hebets and Sullivan-Beckers, 2010). For example, a female observing a male successfully mating and then biasing her preference toward that male or similar males would be the utilization of public information (Mery et al., 2009). Conversely, private information is obtained when an individual's preferences are biased due to personal experiences (Hebets and Sullivan-Beckers, 2010). Private information would be utilized if a male is rejected and then biases his preferences away from that female or similar females in future choices (Ejima et al., 2005; Dickson, 2008; Dukas and Dukas, 2012).

There is considerable evidence that male *Drosophila* do not utilize a random choice strategy when selecting mates. For instance, there is some evidence that males select females based on fecundity (e.g., Byrne and Rice, 2006; Edward and Chapman, 2012; Edward and Chapman, 2013). It has been theorized that sequential search mate choice is unlikely to evolve in a system like *D. melanogaster* males because of the requirement to pass and sample all potential mates (Barry and Kokko, 2010). Based on the mating strategies of wild *Drosophila*, it seems the competition would be too great to allow the evolution of sequential mating strategies.

I therefore predicted that males may be utilizing a mate choice strategy that integrates previous private information into future decisions and that a variation of the fixed sample strategy may be present in males. In the standard fixed sample strategy definitions, individuals sample all of the prospective mates and then select the best of the sample. I propose that males may be using prior information in combination with a fixed sample strategy to allow males to modify their definition of "best" in the group based on previous experiences and allow better and quicker decision-making. This ability would allow males to utilize previous experiences in terms of private information to develop decision-making rules (rule-of-thumb) for choices.

In terms of *D. melanogaster*, quicker mate choices would be extremely beneficial. Congregations on fruit for mating and breeding may create a competitive environment (Marks et al. 1988), which would mean the males that are able to begin courtship quicker might have an advantage of first courtship with preferred females.

I tested for the presence of private information utilization in males by pre-exposing them to different female phenotypes and then observing their courtship behaviors in a second round of encounters with multiple females differing in phenotype. In addition to the observed male preferences, I measured the time to first courtship to examine how the degree to which the options were familiar or novel influenced decision-making speed (often used as a proxy for preference itself (e.g., Ewing, 1964; Dukas, 2005b; Okada et al., 2011)). I predict that if male *Drosophila* use an experience-biased decision-making rule in their decision making process, males that have had previous experience with one of the female phenotypes encountered in the second round will begin courting faster than those without such an experience.

Methods

Fly Maintenance

The *D. melanogaster* wild type population was obtained from Dr. Michael Rose, whose lines are descended from 200 males and 200 females collected by P.T. Ives in South Amherst, Massachusetts in 1975 (Rose and Charlesworth, 1981a; Rose and Charlesworth, 1981b). These lines have been used in a number of other studies, but mainly for physiological evolution (e.g., Kimber and Chippindale, 2013; Mueller et al., 2013). Stock populations of *D. melanogaster* mutants were derived from three mutant lines supplied by the Bloomington Stock Center, Bloomington, Indiana. The mutant traits chosen were *ebony* body, *Ocellarless*, and *sepia* eyes (Bloomington stock lines 1658, 77, and 1668, respectively). All flies were maintained in laboratory conditions under a 12:12 L:D cycle at 25℃ in shell vials (95 mm height, 30 mm diameter). Flies were fed with a standard corn meal based food mixture.

Preliminary Mate Choice

I used females with the *Ocellarless*, *sepia,* and *ebony* mutations in our experiment because they are easily distinguishable by eye and because preliminary trials indicated similar mating success when mated with virgin males. Time to courtship by males was not statistically different between the female phenotypes: *Ocellarless* v. *sepia* (p= 0.35), *sepia* v. *ebony* (p= 0.06), and *Ocellarless* v. *ebony* (p= 0.39) (Mann-Whitney U tests).

Previous Exposure Trial

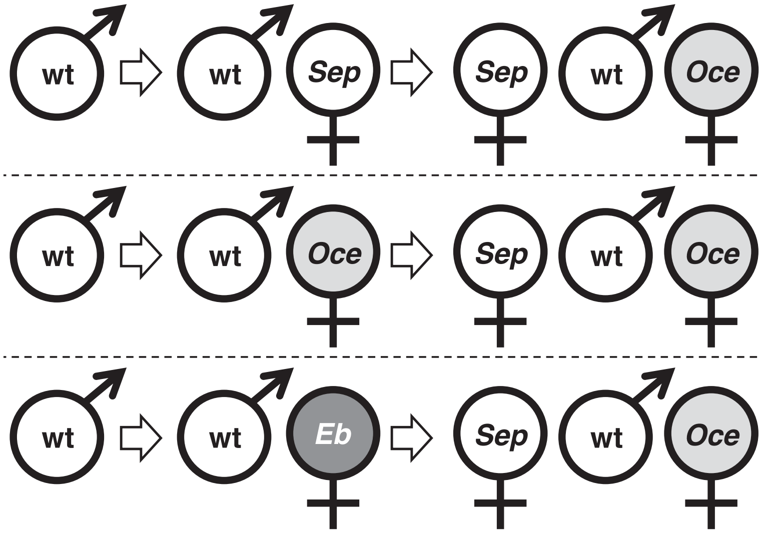

FIGURE 3. Schematic of exposure and mate choice set up by phenotypes.

A test for the influence of prior exposure on male mate preference for either *sepia* or *Ocellarless* was performed. The trials used virgin males and females aged seven to ten days old, which were housed in separate vials until put in the experimental vials. Three groups of 200 wild type males were used. Groups consisted of males paired with *ebony*, *sepia,* or *Ocellarless* females (see Figure 3). In the exposure period, individual virgin males and females were housed together and allowed to interact and mate for 24-hours. Each vial contained one male and one female, for a total of 600 vials. After this exposure period, the males were removed and placed in a divided vial with standard food with two novel virgin individuals, one *sepia* female and one *Ocellarless* female. Specialized caps on each of these vials held plastic dividers in place to keep the male separated from the females until preference was assessed after a 24-hour acclimation period. A 24-hour acclimation period prior to assessment of mate choice using these devices has been used previously (McKee et al., 2014). The caps allowed for easy removal of the divider for the initiation of each trial. After 24-hours the divider was removed and each mating vial was continuously monitored until the male courted a female. The courtship behavior was scored when the male oriented towards the female and began to wing flick, which is an easily visible and specific characteristic of the courtship behavior of *Drosophila* (Ewing, 1983). Time until courtship and which female phenotype was courted was recorded for all trials.

Virgin Choice Trial

I also performed a follow up experiment to test the relationship between fecundity and virgin male preferences. I predicted that if fecundity of females is the primary trait being selected by males, the similarity of the fecundity of *Ocellarless* females and *Sepia* females might have explained the lack of significant preference observed in the males previously exposed to either of these. I predicted that males should significantly prefer the more fecund females based on previous experiments.

A test for the presence or absence of male mate preference using wild type and *yellow* female virgins was performed. I used wild-type and females with the yellow mutation because they are easily distinguishable by eye and because preliminary trials indicated a large fecundity difference between the two genotypes. This significant difference would allow me to more clearly see whether any preference observed was correlated with the fecundity differences. Choice behaviors were assessed using the divided vials with the specialized caps previously described. The mating vials were set up with a virgin wild type male on one side of the divider, and a virgin wild type female and a virgin *yellow* female on the other side. All trials were conducted on the same day and each vial was continuously monitored after divider removal until the male courted a female. Time until courtship and which female phenotype was courted was recorded for all trials. Sample size differs from 200 because of overnight mortality, some flies not initiating courtship within the time frame of the trial, and flies crossing over the divider before the 24-hours.

Fecundity

To test the fecundity of the female mutant phenotypes, wild-type males were mated with a female for three days in vials containing a single male and female. After three days the adults were removed and the vials were retained and allowed to incubate for a week. After one week, all the newly eclosed adults were removed and counted every day at the same time for another week.

Statistical Analyses

To test for significant differences in choice frequencies I performed a binomial test using a null expected frequency of 0.5 for all three groups combined and individually for each of the exposure groups. I performed a Fisher's exact test to compare the frequencies of choices in each of the three groups to test for differences in preference. I analyzed the differences in mean time to courtship and mean fecundity values with Mann-Whitney and Kruskal-Wallis tests due to the unequal variances and highly skewed data values. All of these tests were done using R (v2.15.1) and Minitab (v16.2.4).

Results

Previous Exposure

Combining all groups there was a significant overall male preference for *Ocellarless* females over *sepia* females (binomial, N_{Oce}=305, N_{sep}=246, p=0.013) (Figure 4A). In the individual previous exposure treatments, the *ebony* exposed male flies significantly preferred to court *Ocellarless* females first (binomial test, N_{Oce}=107, N_{sep}=76, p=0.026), both the *Sepia* and *Ocellarless* exposed male flies also trended toward courting the *Ocellarless* females first, but these differences were not significant (binomial tests, N_{Oce}=100, N_{sep}=90, p=0.20 and N_{Oce}=98, N_{sep}=80, p=0.51 respectively). Among the treatments there were no significant differences in the degree of preference for the *Ocellarless* females (Fisher's exact test, X^2=1.2952, p= 0.52).

The overall difference among the courtship times in the three treatments was significant (Kruskal-Wallis test, H_2=11.24, N=549, p=0.004) (Figure 4B). Males previously exposed to ebony females took significantly longer to begin courting a female than the males that had been previously exposed to either *sepia* (Mann-Whitney U test, *W*= 36706.5, N_1=183, N_2=190, p=0.0148) or *Ocellarless* (Mann-Whitney U test, *W*=36005.0, N_1=183, N_2=176, p=0.0014) females. Males exposed to *sepia* females showed a nonsignificant trend for shorter courtship latency when they courted the novel *Ocellarless* compared to those that courted *sepia* females (Mann-Whitney U test, *W*= 8246.5, N_1=90, N_2=100, p=0.34). Males exposed to *Ocellarless* females showed a nonsignificant trend for shorter courtship latency when they courted the novel *sepia* females compared to those that courted *Ocellarless* females (Mann-Whitney U test, *W*=6776.0, N_1=80, N_2=96, p=0.35). Males exposed to *ebony* females show no significant difference in courtship latency when courting either of the novel female phenotypes (Mann-Whitney U test, *W*=7032.0, N_1=76, N_2=107, p=0.91).

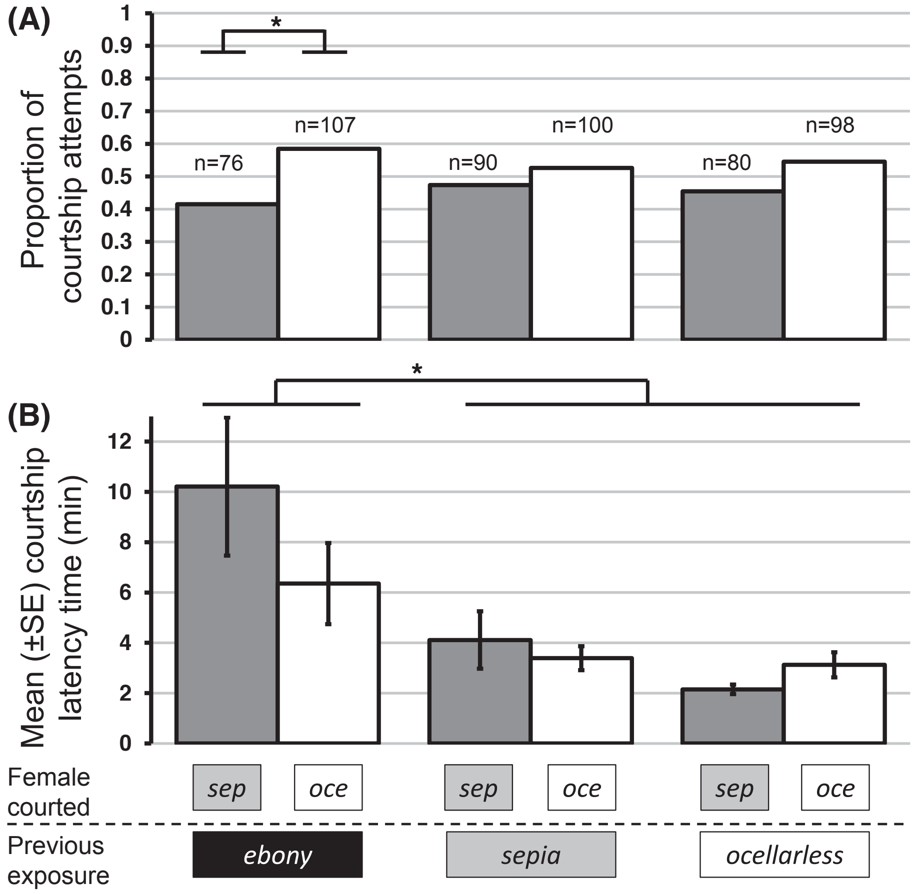

FIGURE 4. Results of previous exposure experiment. (A) Frequency of choices of the two focal phenotypes. (B) Time until first courtship. Significant differences denoted with asterisk (*).

Although there was evidence of an overall preference and some courtship latency differences, there was no strong evidence of a difference in fecundity; *Ocellarless* and *sepia* females did not differ significantly in the number of the offspring they produced when mated to wildtype males (unpaired heteroscedastic t test, t_{124}= 0.16, p=0.876).

Virgin Choice Trial

Virgin wild-type males presented with *yellow* and wild-type females showed no significant difference in courtship preference (binomial test, N_y=100, N_{wt}=92, p=0.61) (Fig. 5 A) or courtship latency values (Mann-Whitney U test, *W= 9266.5,* N_1=92, N_2=100, *p*=0.31) (Fig. 5 B). Although there was no evidence of preference or courtship differences, there was strong evidence of a difference in fecundity; wild-type females produced significantly more offspring than *yellow* females (unpaired heteroscedastic t test, $t_{87}=6.25$, $p<10^{-7}$) (Fig. 5 C).

Discussion

There was a minor, but significant, preference (55.3%) for the *Ocellarless* females over the *sepia* females, but no significant difference in the fecundity of the females. Our data indicate that slight male preference does exist, but it seems unrelated to any fitness advantage, like increased fecundity, that mating with those females may provide. This conclusion contrasts with previous studies that argue for preferences based on female fecundity (e.g., Byrne and Rice, 2006; Edward and Chapman, 2012; Edward and Chapman, 2013).

There was a major (160%) and significant (p=0.004) increase in the time taken to initiate courtship by males that were exposed to two novel phenotypes compared to males exposed to one novel and one familiar phenotype. I find this significantly increased latency time for *ebony*-exposed male flies particularly interesting.

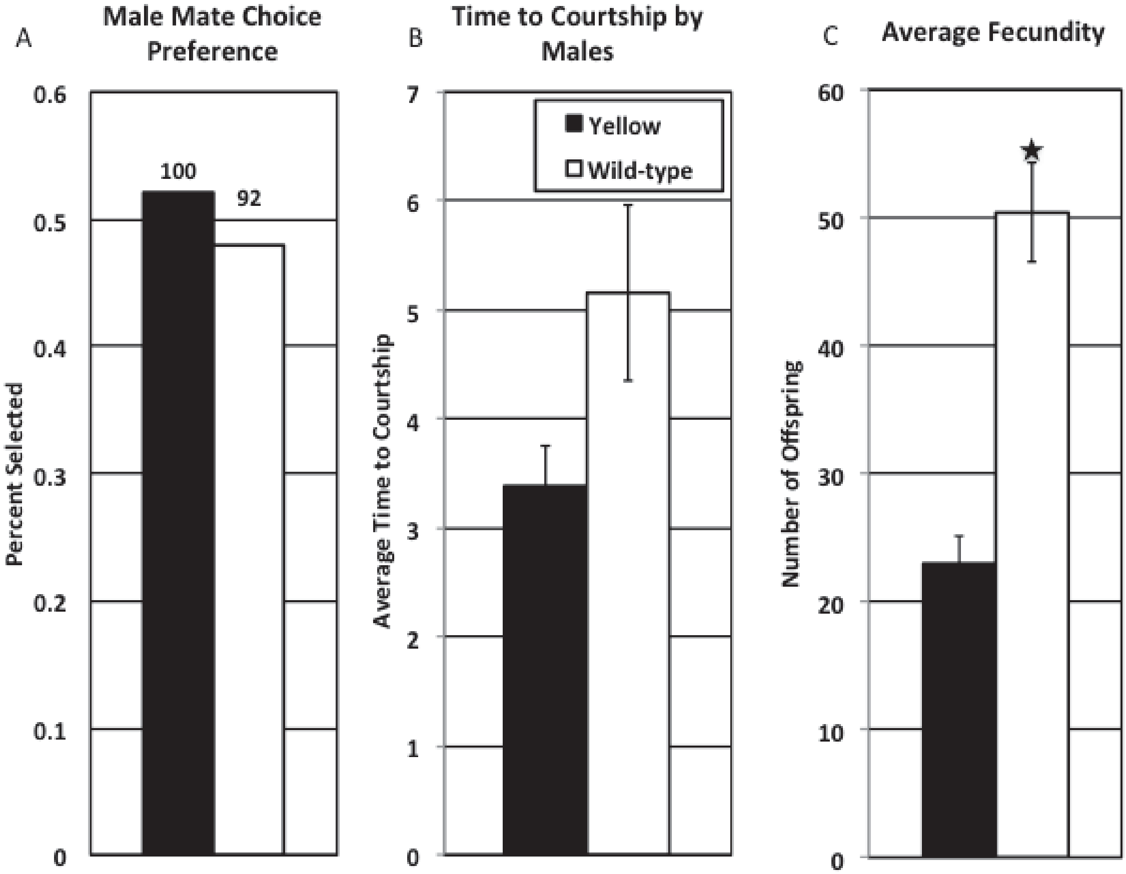

FIGURE 5. Results of virgin choice experiment. (A) Frequency of choices of the two focal phenotypes. (B) Time until first courtship. No significant difference in courtship latency toward either female phenotype was observed (C) Fecundity of females of each focal phenotype. A significant difference in the mean number of offspring produced between *yellow* and wild-type females was observed (unpaired heteroscedastic t test, $t_{87}=6.25$, $p<10^{-7}$).

This increased decision time is consistent with the use of a rule-of-thumb in which quicker decisions are made by subconsciously biasing choices based on previous experiences without measuring the choices independently for their true value (Tversky and Kahneman, 1974; Furnham and Boo, 2011). The lack of previous information about either of the two phenotypes may have left these males without an "anchor" reference and resulted in the longer overall latency times. It seems that this may be evidence for the usage of a fixed sample (best-of-n) strategy that the male *Drosophila* are using. When they have a previous exposure to females, their definition for the “best” of the group is changed toward the novel female. While insignificant in our study, this Coolidge effect has been found in other studies with *D. melanogaster* (e.g., Tan et al., 2013).

I suspect the presence of the usage of a modifiable best-of-n strategy and not the sequential search strategy because males are selecting the best out of the present group and not sampling each in search of a certain threshold. This is important to identify because our results are similar to those predicted in a model by Fawcett and Bleay (2008), who describe this as evidence for sequential search rules. Our interpretation is a deviation from the general definition of the two major search rules, and serves as almost a blend of the two theories. I propose that there could be instances where the usage of previous experiences affects a best-of-n search strategy as well as sequential search strategy.

The standard interpretation of best-of-n versus sequential search theories is based on assumptions that include unlimited mates and time, proposed by Janetos (1980) and Real (1990). The standard model tends to predict the superiority of the sequential search method. However, in a special model by Luttbeg (2002), he found that the best-of-n strategy out performs the sequential search strategy in systems where time and mates were limited and when assessment of quality distribution is uncertain and error prone. This model fits more closely with *D. melanogaster* male mate choice, where faster courtship and utilization of rule-of-thumb decision tools are apparently important for success. This model is consistent with our interpretation of the behaviors.

Our results are novel because they show that without the previous experience with the phenotypes being encountered mate choice times are significantly longer, implying the effect of a previous exposure experience allowing for a quicker decision. Evolutionarily this is significant because the males that select faster and begin courting sooner may stand a better chance of copulation than similarly matched males. *D. melanogaster* mate on the fruit where they are feeding. This tends to cause a significant amount of competition where certain males can be excluded from the breeding sites (Markow, 1988). This competition would cause an evolutionary advantage to those individuals that are able to select preferred females more quickly.

There may be alternative explanations for our data. The two phenotypes of females I used in the first experiment had insignificant differences between their fecundity, and the virgin males did not have a preference difference between the two female phenotypes that did have significant fecundity differences in the second. There is a possibility that in order for males to select optimal females, or the most fecund as described in other studies, they require a level of previous experience for comparison. However, in order to really distinguish this result from our previous explanation, I would need to redo this experiment with a combination of different quality females, to establish the effect on mate choice preferences by previous experience with females of varying degrees of fecundities.

Although there is some evidence for the use of personal, or private, information such as this in mate choice in female *Drosophila* and the males of other species (Hebets and Sullivan-Beckers, 2010), the majority of research reported is based on public information such as males being selected by females that have seen their previous successes (Mery et al., 2009). Some use of private information by males was reported to bias their preferences towards female phenotypes with which they were previously successful (Dickson, 2008), but the use of phenotypic familiarity and novelty for biasing mate choice is relatively new for studies of mate choice decision making in *Drosophila* (Tan et al., 2013). While I cannot conclusively demonstrate that this is the only explanation for our data, I can say that previous experiences do seem to have a significant effect on male courtship latency. This is an important addition to current understanding and work with male mate choice studies.

There seems to be considerable effort to understanding the neurological circuitry in mate choice behaviors, but less attention paid to why specific decisions are made; perhaps due to the organism specific nature of certain cues and the innate complexity of multiple strategies (Griffith and Ejima, 2009). Behavioral studies like this one, in addition to being informative in their own right, also provide additional motivation and context for those neurological studies (Byrne and Rice, 2006). Additionally, this study is important because many mate searching theories are made under the assumption that males are mating indiscriminately (Wiegmann et al., 2010; Wiegmann et al., 2013), which I can show here is clearly not the case. Also I have provided evidence that current simplistic search and mate choice theories may not apply correctly to male mate choice and should be revisited.

LITERATURE CITED

LITERATURE CITED

Ala-Honkola O, Manier MK, Lupold S, Pitnick S. 2011. No evidence for postcopulatory inbreeding avoidance in *Drosophila melanogaster*. Evolution 65:2699-2705.

Almeida CR, Abreu FV. 2003. Dynamical instabilities lead to sympatric speciation. Evolutionary Ecology Research 5:739-757.

Arbuthnott D, Rundle HD. 2014. Misalignment of natural and sexual selection among divergently adapted *Drosophila melanogaster* populations. Animal Behaviour 87:45-51.

Arthur BJ, Sunayama-Morita T, Coen P, Murthy M, Stern DL. 2013. Multi-channel acoustic recording and automated analysis of *Drosophila* courtship songs. BioMed Central Biology 11:11.

Ayala FJ, Campbell CA. 1974. Frequency- dependent selection. Annual Review of Ecology and Systematics 5:115-138.

Bangham J, Chapman T, Patridge L. 2002. Effects of body size, accessory gland and testis size on pre- and postcopulatory success in *Drosophila melanogaster*. Animal Behaviour 64:915-921.

Barbosa M, Connolly SR, Hisano M, Dornelas M, Magurran AE. 2012. Fitness consequences of female multiple mating: A direct test of indirect benefits. BMC Evolutionary Biology 12:185-185.

Barry KL, Kokko H. 2010. Male mate choice: Why sequential choice can make its evolution difficult. Animal Behaviour 80:163-169.

Bateman AJ. 1948. Intra-sexual selection in *Drosophila*. Heredity 2:349-368.

Bijlsma R, Bundgaard J, Boerema AC. 2000. Does inbreeding affect the extinction risk of small populations?: predictions from *Drosophila*. Journal of Evolutionary Biology 13:502-514.

Billeter J-C, Jagadeesh S, Stepek N, Azanchi R, Levine JD. 2012. *Drosophila melanogaster* females change mating behaviour and offspring production based on social context. Proceedings. Biological sciences / The Royal Society 279:2417-2425.

Boake CR, DeAngelis MP, Andreadis DK. 1997. Is sexual selection and species recognition a continuum? Mating behavior of the stalk-eyed fly *Drosophila heteroneura*. Proceedings of the National Academy of Sciences of the United States of America 94:12442-12445.

Bonduriansky R. 2001. The evolution of male mate choice in insects: a synthesis of ideas and evidence. Biological Reviews 76:305-339.

Brown JL. 1997. A theory of mate choice based on heterozygosity. Behavioral Ecology 8:60-65.

Brown WD, Bjork A, Schneider K, Pitnick S. 2004. No evidence that polyandry benefits females in *Drosophila melanogaster*. Evolution 58:1242-1250.

Byrne PG, Rice WR. 2005. Remating in *Drosophila melanogaster*: An examination of the trading-up and intrinsic male-quality hypotheses. Journal of Evolutionary Biology 18:1324-1331.

Byrne PG, Rice WR. 2006. Evidence for adaptive male mate choice in the fruit fly *Drosophila melanogaster*. Proceedings of the Royal Society B-Biological Sciences 273:917-922.

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

Castellano S, Cadeddu G, Cermelli P. 2012. Computational mate choice: Theory and empirical evidence. Behavioural Processes 90:261-277.

Chapman T, Hutchings J, Partridge L. 1993. No reduction in the cost of mating for *Drosophila melanogaster* females mating with spermless males. Proceedings. Biological sciences / The Royal Society 253:211-217.

Charlesworth D, Charlesworth B. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics 18:237-268.

Chenoweth SF, Blows MW. 2003. Signal trait sexual dimorphism and mutual sexual selection in *Drosophila serrata*. Evolution 57:2326-2334.

Cook R. 1975. The attractiveness to males of female *Drosophila melanogaster:* effect of mating, age,and diet. Animal Behaviour 23:521-526.

Cordts R, Patridge L. 1996. Courtship reduces longevity of male *Drosophila melanogaster*. Animal Behaviour 52:269-278.

Darwin C. 1871. The Decent of Man. London: Murray. 864 p.

Delbarco-Trillo J, McPhee ME, Johnston RE. 2010. Adult female hamsters avoid interspecific mating after exposure to heterospecific males. Behavioral Ecology and Sociobiology 64:1247-1253.

Dewsbury DA. 1982. Ejaculate cost and male choice. The American Naturalist 119:601-610.

Dickson BJ. 2008. Wired for sex: The neurobiology of *Drosophila* mating decisions. Science 322:904-909.

Dukas R. 2004. Male fruit flies learn to avoid interspecific courtship. Behavioral Ecology 15:695-698.

Dukas R. 2005a. Experience improves courtship in male fruit flies. Animal Behaviour 69:1203-1209.

Dukas R. 2005b. Learning affects mate choice in female fruit flies. Behavioral Ecology 16:800-804.

Dukas R. 2008. Learning decreases heterospecific courtship and mating in fruit flies. Biology Letters 4:645-647.

Dukas R, Dukas L. 2012. Learning about prospective mates in male fruit flies: Effects of acceptance and rejection. Animal Behaviour 84:1427-1434.

Eastwood L, Burnet B. 1977. Courtship latency in male *Drosophila melanogaster*. Behavior Genetics 7:359-372.

Edward DA. 2014. The description of mate choice. Behavioral Ecology 00:1-10.

Edward DA, Chapman T. 2011. The evolution and significance of male mate choice. Trends in Ecology and Evolution 26:647-654.

Edward DA, Chapman T. 2012. Measuring the fitness benefits of male mate choice in *Drosophila melanogaster*. Evolution 66:2646-2653.

Edward DA, Chapman T. 2013. Variation in male mate choice in *Drosophila melanogaster*. PLoS One 8:e56299.

Ehiobu NG, Goddard ME, Taylor JF. 1989. Effect of rate of inbreeding on inbreeding depression in *Drosophila melanogaster*. Theoretical and Applied Genetics 77:123-127.

Ejima A, Smith BPC, Lucas C, Levine JD, Griffith LC. 2005. Sequential learning of pheromonal cues modulates memory consolidation in trainer-specific associative courtship conditioning. Current Biology 15:194-206.

Ewing AW. 1964. The influence of wing area on the courtship behaviour of *Drosophila melanogaster*. Animal Behaviour 12:316-320.

Ewing AW. 1983. Functional aspects of *Drosophila* courtship. Biological Reviews 58:275-292.

Fawcett TW, Bleay C. 2008. Previous experiences shape adaptive mate preferences. Behavioral Ecology 20:68-78.

Foerster K, Delhey K, Johnsen A, Lifjeld JT, Kempenaers1 B. 2003. Females increase offspring heterozygosity and fitness through extra-pair matings. Nature 425:714-717.

Fowler GL. 1973. Some aspects of the reproductive biology of *Drosophila:* Sperm transfer, sperm storage, and sperm utilization. Advances in Genetics 17:293-360.

Friberg U, Arnqvist G. 2003. Fitness effects of female mate choice: Preferred males are detrimental for *Drosophila melanogaster* females. Journal of Evolutionary Biology 16:797-811.

Furnham A, Boo HC. 2011. A literature review of the anchoring effect. The Journal of Socio-Economics 40:35-42.

Gage M. 2004. Evolution: Sexual arms races. Curr Biol 14:R378-380.

Gowaty PA, Kim Y-K, Anderson WW. 2012. No evidence of sexual selection in a repetition of Bateman's classic study of *Drosophila melanogaster*. PNAS 109:11740-11745.

Gowaty PA, Kim Y-K, Anderson WW. 2013. Mendel's law reveals fatal flaws in Bateman's 1948 study of mating and fitness. Fly 7:28-38.

Gowaty PA, Steinichen R, Anderson WW. 2003. Indiscriminate females and choosy males: Within- and between- species variation in *Drosophila*. Evolution 57:2037-2045.

Griffith LC, Ejima A. 2009. Courtship learning in *Drosophila melanogaster*: Diverse plasticity of a reproductive behavior. Learning and Memory 16:743-750.

Hebets EA, Sullivan-Beckers L. 2010. Mate choice and learning. Encyclopedia of Animal Behavior 2:389-393.

Hollis B, Kawecki TJ. 2014. Male cognitive performance declines in the absence of sexual selection. Proc Biol Sci 281:20132873.

Holman L, Kokko H. 2013. The consequences of polyandry for population viability, extinction risk and conservation. Philos Trans R Soc Lond B Biol Sci 368:20120053.

Hosken D, Snook R. 2005. How important is sexual conflict? The American Naturalist 165:S1-S4.

Hutchinson JM, Gigerenzer G. 2005. Simple heuristics and rules of thumb: Where psychologists and behavioural biologists might meet. Behavioural Processes 69:97-124.

Imhof M, Harr B, Brem G, Schlotterer C. 1998. Multiple mating in wild *Drosophila melanogaster* revisited by microsatellite analysis. Molecular Ecology 7:915-917.

Janetos AC. 1980. Strategies of Female Mate Choice: A Theoretical Analysis. Behavioral Ecology and Sociobiology 7:107-112.

Kimber CM, Chippindale AK. 2013. Mutation, condition, and the maintenance of extended lifespan in *Drosophila*. Current Biology 23:2283-2287.

Kokko H, Brooks R. 2003. Sexy to die for? Sexual selection and the risk of extinction. Ann. Zool. Fennici 40:207-219.

Kristensen TN, Knudsen MR, Loeschcke V. 2011. Slow inbred lines of *Drosophila melanogaster* express as much inbreeding depression as fast inbred lines under semi-natural conditions. Genetica 139:441-451.

Kvarnemo C, Simmons LW. 2013. Polyandry as a mediator of sexual selection before and after mating. Philos Trans R Soc Lond B Biol Sci 368:20120042.

Lee J, Malagon JN, Larsen E. 2011. The case of fly sex combs: Using a model organism to infer mechanisms of morphological evolution. Journal of Undergraduate Life Sciences 5:29-30.

Lefevre G, Jonsson UB. 1962. Sperm transfer, storage, displacement, and utilization in *Drosophila melanogaster*. Genetics 47:1719-1736.

Long TAF, Pischedda A, Stewart AD, Rice WR. 2009. A cost of sexual attractiveness to high-fitness females. PLoS Biology 7:e1000254.

Loyau A, Cornuau JH, Clobert J, Danchin E. 2012. Incestuous sisters: Mate preference for brothers over unrelated males in *Drosophila melanogaster*. PLoS One 7:e51293.

Luttbeg B. 2002. Assessing the robustness and optimality of alternative decision rules with varying assumptions. Animal Behaviour 63:805-814.

Mallet MA, Chippindale AK. 2011. Inbreeding reveals stronger net selection on *Drosophila melanogaster* males: Implications for mutation load and the fitness of sexual females. Heredity 106:994-1002.

Markow TA. 1988. Reproductive behavior of *Drosophila melanogaster* and *D. nigrospiracula* in the field and in the laboratory. Journal of Comparative Psychology 102:169-173.

Marks RW, Seager RD, Barr LG. 1988. Local ecology and multiple mating in a natural population of *Drosophila melanogaster*. The American Naturalist 131:918-923.

McKean KA, Nunney L. 2001. Increased sexual activity reduces male immune function in *Drosophila melanogaster*. Proceedings of the National Academy of Sciences of the United States of America 98:7904-7909.

McKee AA, Newton SM, Carter AJR. 2014. Influence of Inbreeding on Female Mate Choice in Two Species of *Drosophila*. Insect Behavior 27:613-625.

Mery F, Varela SaM, Danchin E, Blanchet S, Parejo D, Coolen I, Wagner RH. 2009. Public versus personal information for mate copying in an invertebrate. Current Biology 19:730-734.

Michalczyk Ł, Millard AL, Martin OY, Lumley AJ, Emerson BC, Chapman T, Gage MJG. 2011. Inbreeding promotes female promiscuity. Science 333:1739-1741.

Mueller LD, Joshi A, Santos M, Rose M. 2013. Effective population size and evolutionary dynamics in outbred laboratory populations of *Drosophila*. Journal of Genetics 92:349-361.

Nandy B, Joshi A, Ali ZS, Sen S, Prasad NG. 2012. Degree of adaptive male mate choice is positively correlated with female quality variance. Sci Rep 2:447.

Nepoux V, Haag CR, Kawecki TJ. 2010. Effects of inbreeding on aversive learning in *Drosophila*. Journal of Evolutionary Biology 23:2333-2345.

Ng CS, Kopp A. 2008. Sex combs are important for male mating success in *Drosophila melanogaster*. Behavior Genetics 38:195-201.

Oakeshotta JG. 1979. Effects of Genotype and Light on Mating Preference in *Drosophila melanogaster*. Aust. J. Biol. Sci. 32:607-614.

Ochando MD, Reyes A, Ayala FJ. 1996. Multiple paternity in two natural populations (orchard and vineyard) of *Drosophila*. Proceedings of the National Academy of Sciences of the United States of America 93:11769-11773.

Odeen A, Moray CM. 2008. *Drosophila melanogaster* virgins are more likely to mate with strangers than familiar flies. Die Naturwissenschaften 95:253-256.

Okada K, Blount JD, Sharma MD, Snook RR, Hosken DJ. 2011. Male attractiveness, fertility and susceptibility to oxidative stress are influenced by inbreeding in *Drosophila simulans*. Journal of Evolutionary Biology 24:363-371.

Orteiza N, Linder JE, Rice WR. 2005. Sexy sons from re-mating do not recoup the direct costs of harmful male interactions in the *Drosophila melanogaster* laboratory model system. Journal of evolutionary biology 18:1315-1323.

Patridge L, Hoffmann A, Jones JS. 1987. Male size and mating success in *Drosophila melanogaster* and *D. pseudoobscura* under field conditions. Animal Behaviour 35:468-476.

Pavković-Lućić S, Kekić V. 2013. Developmental temperature , body size and male mating success in fruit flies, *Drosophila melanogaster* (Diptera : Drosophilidae). European Journal of Entomology 110:31-37.

Pitnick S, Garcia-Gonzalez F. 2002. Harm to females increases with male body size in *Drosophila melanogaster*. Proc. R. Soc. Lond. B 269:1821-1828.

Pitnick S, Pfennig DW. 2014. Brotherly love benefits females. Nature 505:626-627.

Price DK, Hansen TF. 1998. How does offspring quality change with age in male *Drosophila melanogaster*? Behavior genetics 28:395-402.

Priest NK, Galloway LF, Roach DA. 2008. Mating frequency and inclusive fitness in *Drosophila melanogaster*. American Naturalist 171:10-21.

Promislow DE, Smith Ea, Pearse L. 1998. Adult fitness consequences of sexual selection in *Drosophila melanogaster*. Proceedings of the National Academy of Sciences of the United States of America 95:10687-10692.

Rankin DJ, Dieckmann U, Kokko H. 2011. Sexual conflict and the tragedy of the commons. Am Nat 177:780-791.

Real L. 1990. Search theory and mate choice. I. Models of single-sex discrimination. The American Naturalist 136:376-405.

Ritchie MG. 2007. Sexual selection and speciation. Annu. Rev. Ecol. Evol. Syst. 38:79-102.

Robinson SP, Kennington WJ, Simmons LW. 2009. No evidence for optimal fitness at intermediate levels of inbreeding in *Drosophila melanogaster*. Biological Journal of the Linnean Society 98:501-510.

Robinson SP, Kennington WJ, Simmons LW. 2012a. Assortative mating for relatedness in a large naturally occurring population of *Drosophila melanogaster*. J Evol Biol 25:716-725.

Robinson SP, Kennington WJ, Simmons LW. 2012b. Preference for related mates in the fruit fly, *Drosophila melanogaster*. Animal Behaviour 84:1169-1176.

Rose M, Charlesworth B. 1981a. Genetics of life history in *Drosophila melanogaster.* I. Sib analysis of adult females. Genetics 97:173-186.

Rose M, Charlesworth B. 1981b. Genetics of life history in *Drosophila melanogaster.* II. Exploratory selection experiments. Genetics 97:187-196.

Rybak F, Sureau G, Aubin T. 2002. Functional coupling of acoustic and chemical signals in the courtship behaviour of the male *Drosophila melanogaster*. Proceedings of the Royal Society: Biological Sciences 269:695-701.

Searcy WA. 1982. The evolutionary effects of mate selection. Annual Review of Ecology and Systematics 13:55-85.

Servedio MR, Burger R. 2014. The counterintuitive role of sexual selection in species maintenance and speciation. Proc Natl Acad Sci U S A 111:8113-8118.

Sharp PM. 1984. The effect of inbreeding on competitive male-mating ability in *Drosophila melanogaster*. Genetics 106:601-612.

Singh A, Singh BN. 2014. Role of sexual selection in speciation in *Drosophila*. Genetica 142:23-41.

Snook RR, Gidaszewski NA, Chapman T, Simmons LW. 2013. Sexual selection and the evolution of secondary sexual traits: Sex comb evolution in *Drosophila*. J Evol Biol 26:912-918.

Snyder BF, Gowaty PA. 2007. A reappraisal of Bateman's classic study of intrasexual selection. Evolution 61:2457-2468.

South SH, Arnqvist G, Servedio MR. 2012. Female preference for male courtship effort can drive the evolution of male mate choice. Evolution 66:3722-3735.

Spiess EB, Kruckeberg JF. 2012. Minority Advantage of Certain Eye Color Mutants of *Drosophila melanogaster*. II. A behavioral basis. The American Naturalist 115:307-327.

Tan CKW, Løvlie H, Greenway E, Goodwin SF, Pizzari T, Wigby S. 2013. Sex-specific responses to sexual familiarity, and the role of olfaction in *Drosophila*. Proceedings of the Royal Society B-Biological Sciences 280:20131691.

Tan CKW, Løvlie H, Pizzari T, Wigby S. 2012. No evidence for precopulatory inbreeding avoidance in *Drosophila melanogaster*. Animal Behaviour 83:1433-1441.

Tregenza T, Wedell N. 2002. Polyandrous females avoid costs of inbreeding. Nature 415:71-73.

Trivers RL. 1972. Parental investment and sexual selection. In:Chicago, IL: Aldine Publishing Company. pp 136-179.

Trott AR, Donelson NC, Griffith LC, Ejima A. 2012. Song is modulated by female movement in *Drosophila* males. PLoS One 7:e46025.

Turner ME, Anderson WW. 1983. Multiple Mating and Female Fitness in *Drosophila pseudoobscura*. Evolution 37:714-723.

Tversky A, Kahneman D. 1974. Judgment under Uncertainty: Heuristics and Biases. Science 185:1124-1131.

Valtonen TM, Roff Da, Rantala MJ. 2011. Analysis of the effects of early nutritional environment on inbreeding depression in *Drosophila melanogaster*. Journal of Evolutionary Biology 24:196-205.

Wiegmann DD, Angeloni LM, Seubert SM, Wade JG. 2013. Mate choice decisions by searchers. Current Zoology 59:184-199.

Wiegmann DD, Seubert SM, Wade GA. 2010. Mate choice and optimal search behavior: Fitness returns under the fixed sample and sequential search strategies. Journal of Theoretical Biology 262:596-600.

Wigby S, Chapman T. 2005. Sex peptide causes mating costs in female *Drosophila melanogaster*. Current Biology 15:316-321.

Ysui Y. 1997. A "good-sperm" model can explain the evolution of costly multiple mating by females. The American Naturalist 149:573-584.