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Causes and consequences of female mate choice in Drosophila melanogaster: A hemiclonal analysis

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Causes and consequences of female mate choice in *Drosophila melanogaster*: A hemiclonal analysis

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ABSTRACT

One of Darwin's greatest questions, the reason why females prefer elaborate sexually selected male traits and displays, was elucidated by the Fisherian coevolution of male traits and female preferences. While variation in male attractiveness and ornamentation has received much attention, there has been little attempt to evaluate the causes and consequences of intraspecific variation in components of female preference. Furthermore, demonstrating a genetic basis to female preference does not answer the question of how within-population genetic variation is maintained.

Understanding the sources of variation in potential mating interactions between males and females is important because this variation determines the strength and the direction that evolution via sexual selection will proceed. Using cytogenetic cloning techniques developed for *Drosophila melanogaster* – an important model species for sexual selection and sexual conflict research – I examined not only the contribution of genetic variation from in each sex to observed phenotypic variation in biologically important traits such as mating speed, copulation duration, and subsequent offspring production, but also quantified the magnitude of intersexual genetic correlations (Chapter 2). By decomposing the genetic components of interacting phenotypes in mating behaviours between the sexes, we identified possible mechanisms maintaining genetic variation (i.e. sexual conflict) due to the presence of a negative genetic correlation between male attractiveness and female choosiness. These results may provide a framework to improve theoretical models of sexual selection and to provide a more cohesive understanding of the coevolutionary dynamics between male attractiveness and female choosiness for future empirical studies.

Even traits that have a strong genetic basis can be profoundly influenced by environmental conditions, such that the same genotype may yield quantitatively or qualitatively different phenotypes in different environments. While Chapter 2 confirmed genetic variation for female responsiveness, whether or not components of female preference, mainly choosiness, varied with individual condition had yet to be determined. In Chapter 3 I experimentally manipulated female condition by varying the larval density for hemiclonal females (the same lines from Chapter 2) to determine if a genotype-by-environment (GxE) existed for female choosiness. The absence of a GxE interaction for female choosiness suggests that this component of female preference may not be condition dependent. Since GxE interactions may be potentially important to sexual selection, especially if both sexually selected male traits and female preferences are subject to GxEs (and genetic correlations between the two are central to many models of sexual selection), more empirical work on the condition-dependence of female choosiness is needed to strengthen predictions of GxEs for sexually selected traits.

These results demonstrate, to the best of our knowledge, findings regarding the causes and consequences of variation in female mate choice using hemiclonal analysis. Furthermore, the importance of quantifying genetic variation in female mate choice – including how it is maintained – is necessary for theoretical models of sexual selection.

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CHAPTER 1:

EVOLUTIONARY AND POPULATION GENETICS OF FEMALE MATE CHOICE

Introduction

Females often assess multiple traits when choosing potential mates, and many of these traits vary continuously among males (Chenoweth & Blows, 2006). Since Darwin (1817) first proposed that female preferences could be responsible for the evolution and maintenance of sexually selected male traits, the evolutionary consequences of mate preferences (the sensory and behavioural properties that influence the propensity of individuals to mate with certain phenotypes (Jennions & Petrie, 1997)) have received less attention (Andersson, 1994). Studying female mate choice allows us to determine the degree to which females are attracted to males of different phenotypes and enables us to define components of female behaviour, such as responsiveness and choosiness. Female responsiveness (the likelihood a female will respond to a potential mate) provides insight into female motivation to mate (Bailey, 2008; Ratterman *et al.*, 2014). Female choosiness (the degree to which females discriminate amongst potential mates) measures the time taken to assess potential mates (Narraway *et al.*, 2010); choosey females are more variable in their responses to males of varying attractiveness (Gray & Cade, 1999; Brooks & Endler, 2001; Bailey, 2008; Ratterman *et al.*, 2014). The empirical investigation of mate choice is problematic, with issues that stem from studying the genetic basis of mate choice. Males produce complex signals and mating displays that may consist of a combination of acoustic, visual, chemical, and behavioural phenotypes (Hall, 1994). Furthermore, female preferences for these male traits are particularly challenging to quantify. During mate choice, genes not only affect the phenotypes of the focal

individual, but can influence the expression of phenotypes in other individuals (Moore *et al.,* 1997; Wolf, 2000). Phenotypes can either be enhanced or inhibited depending on the nature of direct and indirect genetic effects or IGEs (*see below*). Quantification of the genetic basis of female choosiness and female responsiveness is important to test predictions of sexual selection theory since genetic correlations between ornaments contributing to male attractiveness and components of female mate choice play a central role in models of preference evolution (Lande, 1981; Mead & Arnold, 2004).

Genetic covariance between male attractiveness and female choosiness

The coevolutionary dynamics between sexually selected male traits and female mate preferences have been the subject of an ongoing debate from which few generalizations have emerged (Ratterman *et al.,* 2014). Quantitative genetic models of sexual selection have described genetic variance and covariance for the elaboration of male displays and female mate preferences which characterizes the Fisherian runaway process: female mate choice selects for male attractiveness and the resulting linkage disequilibrium between female preference and male attractiveness alleles generates indirect selection for female preference (Fisher, 1931; Lande, 1981; Mead & Arnold, 2004). Fisherian runaway hypothesizes that females choose "attractive" male(s) with the most exaggerated ornaments and/or displays based solely upon the males' possession of that ornament. This sexual selection process of females choosing males to whom they find "attractive" can undermine the direction of natural selection (a key component of Fisherian process) by selecting for an ornament that may otherwise be non-adaptive and selected against in natural selection. This results in male offspring more likely to possess the preferred trait and female offspring more likely to possess the preference for that trait.

Over subsequent generations this can lead to the runaway selection by means of a positive feedback mechanism for males who possess the most exaggerated ornaments. Fisherian runaway also predicts that female mate choice and ornamentation in males are both genetically variable and heritable (Fisher, 1931). Substantiated by genetic models of sexual selection (Lande, 1981, Kirkpatrick, 1982), the Fisherian process is theoretically sound but is sorely lacking in consistent empirical data. Some studies have found a transient positive genetic correlation that disappears after one generation of random mating (Bakker & Pomiankowski, 1999; Gray & Cade 1999; Blows, 1999), or no correlation at all (Hall *et al.,* 2004; Zhou *et al.,* 2011; Ingleby *et al.,* 2013). While a positive genetic correlation between male attractiveness and female choosiness is essential to the element of Fisherian runaway selection, it is not essential to all models of sexual selection (Fuller, 2005). Other models such as sensory bias (males that evolve traits to exploit the female sensory system become favoured by female mate choice; *see* Ryan, 1998) and good genes (*see below*) (Houle & Kondrashov, 2002), do not require a particular genetic correlation between male attractiveness and female choosiness. Furthermore, the sexual conflict model predicts a negative correlation between female choosiness and male attractiveness due to interlocus sexual conflict (Gavrilets 2000; Chippindale *et al.,* 2001). Sexual conflict theory predicts that fitness maximizing strategies of males and females are incompatible and traits that increase fitness in one sex decrease fitness in the other sex. This conflict can arise from sexually antagonistic alleles and indirectly, potentially resulting in a negative genetic correlation between female choosiness and male attractiveness.

Genetic variance in female mate choice

While the evolution and maintenance of genetic variation in male secondary sex traits has been the subject of considerable scientific investigation, similar studies regarding genetic variation in female preference for male traits are much more rare (Jennions & Petrie, 1997; Ratterman *et al.,* 2014). Understanding the causes and consequences of genetic variation in female mate choice is fundamental to the field of evolutionary biology because genetic variation among females may influence the rate, strength and direction of sexual selection acting on sexually selected male traits (Andersson, 1994; Jennions & Petrie, 1997) and thus influence a population's evolutionary trajectory and/or speciation. Changes in sexually selected male traits male traits can occur if genetic variation in female mate choice allowed evolutionary change in the average preferences of a population (Houde, 1988). To date, the genetic basis of female preference has been explored in numerous taxa and the majority of empirical studies have focused on determining additive genetic variation in components of female mate choice. Early work with fruit flies, *Drosophila melanogaster,* (Heisler, 1984), ladybirds, *Adalia bipunctata* (Majerus, 1986), and guppies, *Poecilia reticulata* (Houde, 1988) found differences among females in their preferences for male traits originating from different populations. Knowledge of genetic variation between populations may provide insight on the extent to which female mate choice is subject to sexual selection (Jennions & Petrie, 1997; *but see* Houde, 1993). For example, variation in sexually selected male traits affects female mating behaviour and differences in female mating behaviour lead to differential male mating success. If variation in female mate choice is heritable, differential mating success (i.e sexual selection) can result in evolutionary change. Results from Houde (1988) suggest that female mate choice differs genetically

within a species and that differences in female mate choice may have contributed to the variation in sexually selected male traits. Both the Fisherian model of sexual selection and the "good genes" model predict considerable genetic variation in female mate choice, both within and among populations (Lande, 1981). According to the good genes model, female choice provides offspring with increased viability, whereas Fisher's sexual selection model provides choosey females with attractive male offspring (*see* Andersson, 1994). Since females may benefit from being choosey if there are differences in the genetic quality of males, the evolution of female mate choice requires genetic variation in male fitness. Thus, any hypothesis for the evolution of female choice for indirect fitness benefits requires a mechanism for the maintenance of genetic variation in both male and female traits.

Several genetic models (genic capture (Rowe & Houle, 1996), sexual conflict (Chippindale *et al.*, 2001; Bonduriansky & Chenoweth, 2002)) reveal a life-history trade-off for male survival and male mating success, resulting in genetic variation in sexually selected male traits (Kirkpatrick & Ryan, 1991). The degree of elaboration of sexually selected male traits that produce this trade-off likely depends on female preference for the male traits. Each individual female exhibiting a different preference imposes unique selection on preferred male traits so that each male therefore is subject to a different set of selection pressures depending on which females he encounters (Ratterman *et al.*, 2014). Measuring heritable, individual-level variation in female preference is necessary to the understanding of intersexual selection acting on a population, and a growing body of empirical work (Chenoweth & Blows, 2006; Ratterman *et al.*, 2014) is now attempting

to establish which specific mechanism(s) contribute to the evolution and maintenance of genetic variation in female choice.

Indirect genetic effects maintaining genetic variation in female mate preference

The abiotic environment, the biotic environment, and the interaction between the two may shape the phenotypic expression of genetic variation in female choosiness and, consequently, influence the nature of sexual selection acting on the population. There are a variety of proposed mechanisms for the maintenance of genetic variation for female choosiness (Jennions & Petrie, 1997; Widemo & Sæther, 1999; Chenoweth & Blows, 2006). One way in which the additive genetic variation can be maintained is through the action of indirect genetics effects (IGEs). IGEs arise when the expression of genes in one individual affect the phenotype of conspecifics (Wolf, 2000) and are of interest to evolutionary biologists because they modify the relationship between genotype, phenotype, and the resulting genetic variance components (Wolf, 2000). Even when individuals interact at random, IGEs may generate positive or negative phenotypic covariance between interacting individuals, depending on the degree to which the expression of a trait in the focal individual is expressed in another (Moore *et al.,* 1997). Historically, investigation of IGEs has focused on the influence of parents on offspring (e.g. maternal effects (Wolf, 2000)) but, this viewpoint has expanded to include interactions between genetically unrelated individuals (Wolf *et al*., 1998; Wolf, 2000). Differences in social and/or environmental conditions have been seen to result in changes in the chemical composition of cuticular hydrocarbons (CHCs) in species of *Drosophila.* Male *D. melanogaster* alter their CHC expression in response to the genotype of males in their environment (Kent *et al*, 2008), and *D. serrata* males alter their CHC expression in

response to the genotype of interacting females (Petfield *et al*, 2005), suggesting that interactions within species of *Drosophila* may be subject to IGEs (Krupp *et al*, 2008). Furthermore, a genetic correlation was found between female body condition and the expression of male CHCs (Petfield *et al*, 2005), suggesting that the genes responsible for variation in female body condition may be linked to different genes in males which influence the expression of male pheromones. Female phenotypes, including choosiness, may be determined by manipulating female social interactions and measuring changes to the expression of genes present in interacting males. Evaluating female choosiness as the focal trait and using male phenotypes as known or fixed genetic background interacting traits allows empirical description of how female choosiness changes in response to interactions with male conspecifics (Bailey & Zuk, 2012). For example, both the strength and direction of interactions between male and female *Teleogryllus oceanicus* differed between populations for female choosiness and male calling song. The acoustic environment generated by male *T. oceanicus* calling songs not only influenced the expression of female choosiness, but also affected female size, suggesting that IGEs may affect both behavioural and morphological traits (Bailey & Zuk, 2012).

IGEs are predicted to influence selective outcomes whenever interactions between social partners (i.e. mating partners) affect the variance of interacting traits (Wolf *et al.*, 1998; Wolf, 2000). Theoretical models also suggest that IGEs can accelerate or decelerate the rate of evolution of interacting traits (Moore *et al.*, 1997). Although it is unclear if IGEs are widespread, the complex interaction between males and females during mating (Hall, 1994) suggests that sexually selected traits – in both sexes – are likely to be influenced by IGEs. Little is known about the fitness consequences of these

interactions and empirical work on the effects of IGEs on sexual selection is warranted (Chenoweth & Blows, 2006).

Interacting phenotypes and sexual conflict

Many traits can be considered 'interacting phenotypes' whose expression may be dependent on or influenced by interactions between conspecifics (Moore *et al.*, 1997). Interactions between unrelated individuals may have profound effects on expression of certain shared phenotypes. For example, the probability of mating between two individuals may depend on both male attractiveness and female preference (Bateson, 1983); the duration of copulation may depend on both female resistance and the male's ability to overcome female resistance (Friberg, 2005; Mazzi, 2009); and the number of sperm that a female stores may depend on female sperm storage phenotype and the amount and type of sperm ejaculated by the male (Miller & Pitnick, 2002; Miller &Pitnick, 2003). Interacting phenotypes are unique in that they may act as both the targets and agents of sexual selection. Interacting phenotypes can rapidly increase the strength, direction, and rate of evolution of the focal trait(s) differently than non-interacting trait(s) (Moore *et al.*, 1997, Simmons & Moore, 2009) by increasing the amount of phenotypic covariance between interacting individuals.

It is also important to consider that the reproductive interests of males and females are not always compatible (Arnqvist & Rowe, 2005) so that many shared traits may evolve under sexual conflict. Sexual conflict is manifested in two genetically different forms: interlocus sexual conflict which involves selection acting on different genes in each sex; and intralocus sexual conflict which involves selection in different directions on genes shared by the sexes. Allelic variation then results in opposite fitness

effects when expressed in different sexual environments (Pischedda & Chippindale, 2006). Two evolutionary consequences arise from this: the costs of sexual reproduction ("gender load," see Long *et al.*, 2006) and the maintenance of genetic variation for fitness resulting from strong sexual selection (Rice, 1984; Kirkpatrick & Ryan, 1991; Gibson *et al.*, 2002).

Sexual conflict may give rise to sexually antagonistic selection and may potentially influence the genetic architecture of interacting phenotypes. It is predicted that strong selection may deplete additive genetic variation (Kirkpatrick & Ryan, 1991), yet if a trait has developed under sexual conflict, the differential pattern of sexually antagonistic selection acting in the opposite sexes may maintain genetic variation via balancing selection (Foerster *et al.*, 2007; Hall *et al.*, 2010). Rice (1984) was one of the first to suggest that sexually antagonistic selection could maintain genetic variation for fitness-related traits. To understand the coevolution of such traits, it is necessary to estimate the additive genetic effects from both males and females in an interacting phenotype. For example, Edwards *et al*. (2014) studied the genetic contributions from both males and females to phenotypic variation in fecundity and copulation duration in *D. melanogaster.* These two traits were treated as interacting phenotypes (rather than considering the genetic contribution from each sex independently). As a result, the experiment provides a more complete picture of the genetic architecture underlying fecundity and copulation duration. Contrary to their predictions that egg production would be determined by variation in genetic contribution from both males and females, only female genetic background contributed to variation in fecundity, possibly indicating that sexual conflict was not present for this trait, or there was a lack of genetic variation

in male ability to stimulate egg production. In contrast, copulation duration exhibited the characteristics of an interacting phenotype since the genotypes of both males and females contributed to the phenotypic variation in this trait. This finding suggests there is enough genetic variation to 'fuel' sexually antagonistic coevolution in this species. The lack of quantitative genetic studies using interacting phenotypes in males and females means that it is tricky to make the generalizations about this phenomenon that are necessary to understand constraints and limitations to sexual selection (Snook *et al.,* 2010).

Empirical estimates of individual-level genetic variation

Recent attention has focused on the evolutionary significance of individual-level genetic variation in female mate choice (Ritchie *et al.,* 2005; Klappert *et al.,* 2007). Examining the genetic variability among individual females within a population can be a time-consuming and labour-intensive process because replication is necessary both at the level of the male stimulus and the level of the individual female (Wagner, 1998; Ratterman *et al.,* 2014). Facilitating this process is the use of genetically identical individuals (e.g. isogenic female lines), producing descriptions of mating patterns at the genotypic level. Since each genotype is considered a unique genetic individual, this reduces the efforts to obtain individual-level traits and reduces confounds associated with repeated testing of single individuals (Chenoweth & Blows, 2006). As an added bonus, any differences observed between isogenic lines can be attributed to heritable genetic variation and direct tests of models of the evolution of female preference can be made.

Hemiclonal analysis is a modern genetic technique used to provide estimates of additive genetic variation by measuring the total phenotypic variation in multiple groups of individuals that all share a (nearly) complete haploid genome. The techniques for laboratory hemiclonal systems were developed to mimic the natural hemiclone systems

found in nature where a single haploid genome is clonally transmitted without undergoing recombination (Rice, 1996). Instead of relying on balancer chromosomes, which only suppress recombination when on a single chromosome, this system makes use of the chromosomal constructs available in *D. melanogaster*. Recombination between homologous chromosomes is extremely rare in males, making hemiclonal analysis possible (Chippindale *et al.*, 2001). In the laboratory, hemiclonal analysis is performed using females with a "target" genetic make-up to generate "clone" males (see Fig 1.1). A single wild-type male is mated to groups of these "clone-generator" (GC) females (Step 1). These CG females possess 2 X-linked chromosomes, a free Y chromosome, and a translocation between the major 2 and 3 chromosomes (sex determination is determined by the X:autosome ratio in *Drosophila*). The combination of X and Y chromosomes in CG females allows for the paternal transmission of the X chromosomes from father to son (Abbott & Morrow, 2011) and the transmission of the Y from GC mother to son. From the resulting progeny, a single heterozygous male (brown eyed; Step 2) is retained and mated to many GC females, resulting in amplification of a singular haploid genome. In each subsequent generation during clone culture, the sons carrying the "target" haploid genome from these crosses are mated to many CG females to produce a clonal amplification line. The translocation of genetic material between chromosome 2 and 3 means that viable heterozygous clone males from this step inherit both chromosomes as a unit (represented as a long white bar; Step 3) and individuals that inherited only chromosome 2 or 3, but not both, are inviable. These haploid genomes can then be expressed in either sex, in combination with a random genetic background (Steps 4 & 5).

There are several advantages to using hemiclones over techniques that involve balancer chromosomes, isogenic lines, or target chromosomes in evolutionary genetics studies in *D. melanogaster*. First, an unlimited number of individuals with identical haplotypes can be produced from one hemiclonal system, which enables precise measurements of low levels of genetic variation by removing sample size limitations. Secondly, propagation of hemiclones is relatively simple, allowing easy preservation of generations of hemiclone lines for future experiments. Hemiclonal analysis gives researchers the tools to test the same known haplotype in a variety of environments or experimental conditions (see Rice *et al.*, 2005; Abbott & Morrow, 2011). Hemiclonal males have been reared in different environmental conditions to examine the maintenance of genetic variation in fitness-related traits due to condition dependence (Morrow *et al.*, 2008) but, to the best of our knowledge, no study has examined the effect of environmental condition *and* genetic identity on female mate choice using female hemiclones. Hemiclonal analysis can also take place in a relatively short period of time compared to the time-consuming inbreeding process. Undesirable chromosomal recombinations (which may occur when using balancer chromosomes) are eliminated in hemiclones due to the lack of recombination in *D. melanogaster* males and the removal of balancer chromosomes when creating the hemiclones. Finally, hemiclonal systems allows for selection variation covering all major chromosomes, contrasting introgression techniques, which focus on only a single specific chromosome (Abbott & Morrow, 2011). Hemiclonal analysis allows for the "capture" of the standing genetic variation for a given trait by sampling multiple hemiclone lines from the same source population.

Goals

Despite the empirical evidence regarding genetic variation within and between populations, questions remain about the maintenance and evolution of female preference and its coevolutionary dynamics with heritable male attractiveness. Here we take advantage of hemiclonal analysis developed for *D. melanogaster* to address three fundamental questions related to the genetic basis of female mate choice. First, I determine the extent of genetic variation for interacting phenotypes among female genotypes in a population and how this varies with respect to male genetic identity. Second, I quantify the genetic covariance between male attractiveness and female choosiness in order to test predictions of models of sexual selection. Finally, I examine the plasticity of female mate choice to determine if a genotype x environment interaction (GxE) is present for female choosiness. Together, these studies provide a multi-faceted perspective on the maintenance of genetic variation in female preference and how it relates to variation in male phenotype, how the two traits coevolve, and how this impacts evolution and influences sexual selection

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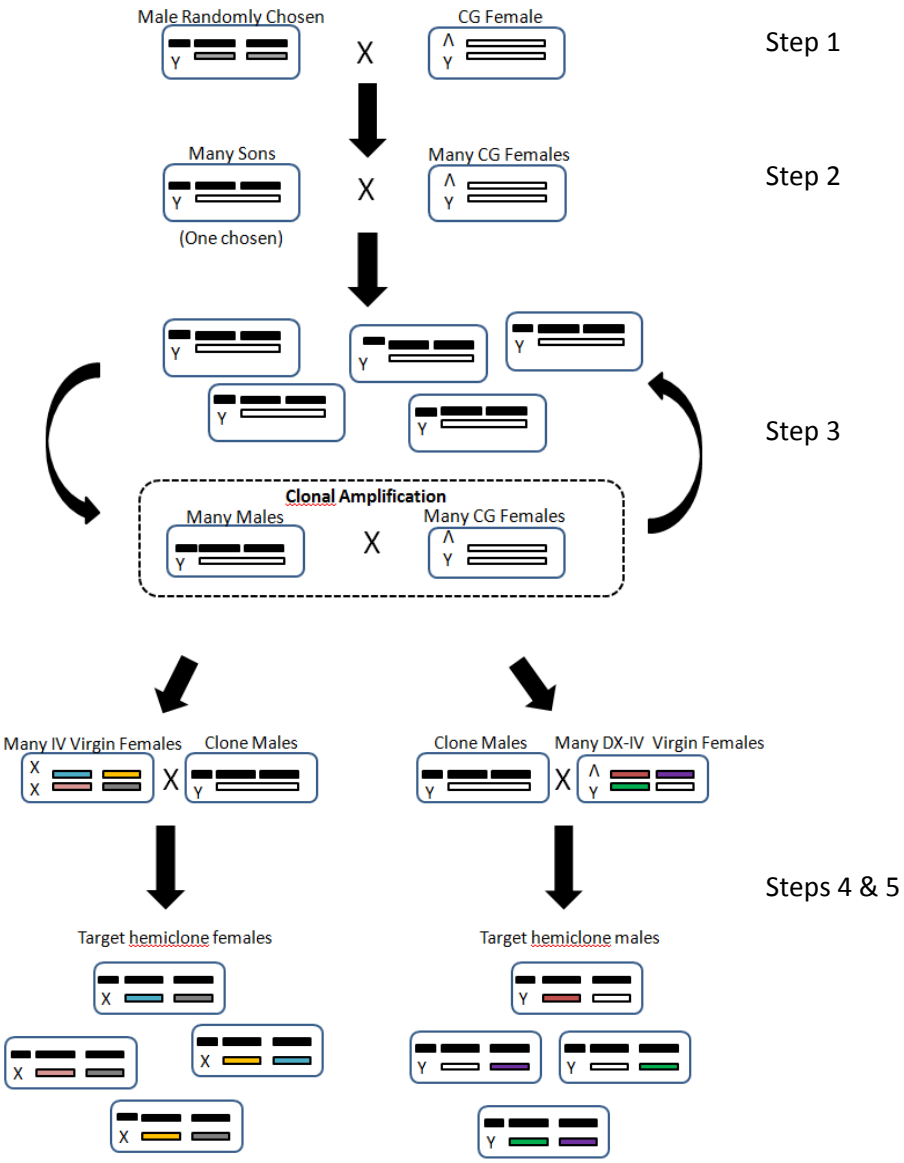

Figure 1.1: Experimental hemiclone development procedure in laboratory. The female double X is represented by the Greek letter Lambda, and the translocated autosomes by the long white bars. The short black or gray bars represent the wild-type chromosomes from the source populations, either IV or DX-IV. Clone generator (CG) females are first crossed with a variation of IV males. The male offspring produced will have one wild-type haploid genotype and one GC genotype. A single F1 male is then crossed with several of the CG females, resulting in amplification of the wild-type genome. In this study, cross 2 was performed for 31 males. Clonal amplification continues propagation of the lines in the lab. Crossing the clone males from each line to IV females and DX-IV females produces the target male and target female flies for analysis. It is important to note that the hemiclonal genome can be expressed in a random genetic background in either sex. For every generation of clone males new CG females are taken from a separate stock population (Adapted from Abbott & Morrow, 2011).

CHAPTER 2:

HEMICLONAL ANALYSIS OF INTERACTING PHENOTYPES IN MALE AND FEMALE *DROSOPHILA MELANOGASTER*

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Abstract

Background: Identifying the sources of variation in mating interactions between males and females is important because this variation influences the strength and/or the direction of sexual selection that populations experience. While the origins and effects of variation in male attractiveness and ornamentation have received much scrutiny, the causes and consequences of intraspecific variation in females have been relatively overlooked. We used cytogenetic cloning techniques developed for *Drosophila melanogaster* to create "hemiclonal" males and females with whom we directly observed sexual interaction between individuals of different known genetic backgrounds and measured subsequent reproductive outcomes. Using this approach, we were able to quantify the genetic contribution of each mate to the observed phenotypic variation in biologically important traits including mating speed, copulation duration, and subsequent offspring production, as well as measure the magnitude and direction of intersexual genetic correlation between female choosiness and male attractiveness.

Results: We found significant additive genetic variation contributing to mating speed that can be attributed to male genetic identity, female genetic identity, but not their interaction. Furthermore we found that phenotypic variation in copulation duration had a significant male-associated genetic component. Female genetic identity and the interaction between male and female genetic identity accounted for a substantial amount of the observed phenotypic variation in egg size. Although previous research predicts a trade-off between egg size and fecundity, this was not evident in our results. We found a strong *negative* genetic correlation between female choosiness and male attractiveness, a

result that suggests a potentially important role for sexually antagonistic alleles in sexual selection processes in our population.

Conclusion: These results further our understanding of sexual selection because they identify that genetic identity plays a significant role in phenotypic variation in female behaviour and fecundity. This variation may be potentially due to ongoing sexual conflict found between the sexes for interacting phenotypes. Our unexpected observation of a negative correlation between female choosiness and male attractiveness highlights the need for more explicit theoretical models of genetic covariance to investigate the coevolution of female choosiness and male attractiveness.

Keywords: sexual selection, mate choice, female choosiness, male attractiveness, *Drosophila melanogaster*, hemiclonal analysis, interacting phenotypes, mating speed.

Background

Females often differ in their response to male courtship. This difference in female "responsiveness" (the likelihood that a female will respond to a potential mate) may be influenced by a number of factors including her prior mating experience, social experience, and environmental/developmental conditions (Jennions & Petrie, 1997; Widemo & Sæther, 1999). Similarly, variation in female "choosiness" (the degree to which females discriminate amongst potential mates) may arise from the relative costs and benefits associated with female mate choice (i.e. time and energy costs) (Widemo & Sæther, 1999; Andersson, 1994). Theoretical and empirical work on sexual selection has shown considerable variation, both phenotypic and genetic, among females in their responses to sexually selected male traits (Ritchie *et al.*, 2005). Female responsiveness has been shown to exhibit additive genetic variation (Hedrick & Weber, 1998; Gray &

Cade, 1999; Brooks & Endler, 2001) and it is widely accepted that genetic variation in female choosiness is necessary for species to evolve via sexual selection (Andersson, 1994; Jennions & Petrie, 1997; Widemo & Sæther, 1999). However, despite its importance in understanding models of sexual selection, there is little information about the extent and nature of heritable genetic variation in female mating behaviours (Gray & Cade, 1999). The difficulty in studying this suite of traits stems in part from the complexity of quantifying the genetic basis of female choosiness. Of the numerous empirical studies on variation in female choosiness (Jennions & Petrie, 1997; Hedrick & Weber, 1998; Gray & Cade, 1999; Brooks & Endler, 2001), only a few have emerged with clear generalities about within population levels of genetic variation in female choosiness (see Ritchie *et al.,* 2005; Ratterman *et al.*, 2014). These studies often involve comparing females from genetically isolated populations (Hedrick & Weber, 1998; Brooks & Endler, 2001), whereas investigating the sources of this variation *within* populations is ultimately important to understanding variation in female choosiness and its role as a selective force.

Variation in female choosiness may be attributed to "innate preferences" which reflect the heritable genetic component in sensory organ development (Widemo & Sæther, 1999). For example, individual female guppies, *Poecilia reticulata,* may respond differently to male orange spots because of the level of sensitivity to that signal in the retina (Houde, 1997). Female preference and the preferred male trait (the orange spot) are then maintained by sexual selection as they are coevolving through a positive genetic correlation (Fisher, 1931). Fisher's runaway selection predicts a positive genetic correlation between female preference and male attractiveness, with the genetic

correlation arising through pleiotropy or linkage disequilibrium (Fisher, 1931; Fisher, 1958; Lande, 1981; Bakker & Pomiankowski, 1995; Bakker, 1999). Despite this predicted positive genetic correlation between female preference and male attractiveness, the ambiguity of empirical studies makes it hard to identify the sources of observed covariance (Zhou *et al.*, 2011). Ultimately, variation in female choosiness can affect the strength, direction, and nature of sexual selection acting on sexually selected male traits (usually decreasing the overall strength), which can affect male courtship displays and, indirectly, the female's responses to them (Jennions & Petrie, 1997; Widemo & Sæther, 1999).

Not only can female responsiveness to male signals determine whether or not mating occurs, but it may be manifested in post-copulatory phenotypes, such as maternal investment patterns into offspring. In species that are polyandrous, a female might adaptively alter her investment strategy depending on the specific qualities (i.e. the direct/indirect costs and benefits) associated with her most recent mate in order to maximize her lifetime reproductive success (Sheldon, 2000). According to the differential allocation hypothesis, differences in investment may be manifested in the total amount and/or quality of parental care provided, as well as by altering the number and/or size of offspring produced (Harris & Uller, 2009). For example, female Australian Rainbow fish, *Melanotaenia australis*, will produce twice as many eggs when they mate with more "attractive" (i.e. larger) males than with less "attractive" (i.e. smaller) males (Evans *et al.*, 2010). Adjusting patterns of investment into offspring can have direct consequences for the future success of those offspring. For instance, in the fruit fly, *Drosophila melanogaster*, egg size is positively correlated with variance in egg hatchability, pre-

adult size, juvenile survival, and adult starvation resistance (Azevedo *et al.,* 1997), and as such may be strongly influenced by specific maternal investment strategies. Such investment strategies may differ between species depending on the patterns of parental care. For example, in species with bi-parental care, females are more likely to invest more into clutch size rather than egg size, simply due to the fact that a highly attractive male may signal high-quality parental care (Horváthová *et al.,* 2011). Conversely, in species which lack parental care maternal investment in egg size rather than egg number is likely, often to compensate for poor egg viability (Horváthová *et al.,* 2011).

Although there is considerable evidence supporting differences in allocation in relation to phenotypic traits of males (such as body size, male ornamentation, etc.) (Hill, 1991; Petrie & Williams, 1993; Palokangas *et al.,* 1994; Cunningham & Russell, 2000; Evans *et al.,* 2010), there is scant evidence regarding whether there is genetic variation for this ability in females. Recently, an attempt was made to address this issue by measuring differences in allocation in assays where the genetic identity of male *D. melanogaster* was experimentally varied across numerous mating pairs (Pischedda *et al.,* 2011). It was found that male genotype appeared to influence both the number and size of the eggs produced after a mating. Additionally, a negative trade-off between female fecundity and egg size was also demonstrated, consistent with earlier findings (Schwarzkopf *et al.,* 1999). However, in this experiment, the genetic identity of all the females was uniform; thus the potential for female genetic identity and the interaction between males' genotypes with *different* females was not explored. Thus, only a fraction of the total genetic variation for any interacting phenotype may be determined when testing each sex independently, potentially ignoring genetic contributions from the

mating partner as well as interactions between both individuals' genotypes (Wolf, 2000). There is increasing evidence that phenotypic traits in one individual may be influenced by another individual's genotype (Moore *et al.,* 1997; Wolf, 2000); these effects are known as indirect genetic effects (IGEs). IGEs likely modify genetic architecture therefore resulting in genetic variance components in interactions between conspecifics (Wolf, 2000). Hemiclonal analysis (Rice *et al.,* 2005; Abbott & Morrow, 2011) allows us to partition out the effect a conspecific genotype has on another individual's genotype.

Previous work on genetic variation in female preference has primarily focused on varying the genetic identity of one sex (typically the male) and holding female genetic identity static (Gray & Cade, 1999; Ritchie *et al.,* 2005; Pischedda *et al.,* 2011; Pischedda *et al.,* 2012). To our knowledge, no previous study has examined female choosiness (the degree to which females discriminate among potential mates), female responsiveness (the likelihood a female will respond to a potential mate), *and* maternal investment patterns while simultaneously varying *both* male and female genetic identity. Additionally, studies examining the genetic covariance between female choosiness and male attractiveness are mixed; some have found a transient positive correlation that disappears after one generation of random mating (Bakker, 1999; Gray & Cade, 1999; Blows, 1999), others have found no correlation at all (Hall *et al.,* 2004; Zhou *et al.,* 2011; Ingleby *et al.,* 2013). While the prediction of a positive genetic correlation between male attractiveness and female choosiness is a central element of Fisherian runaway selection (Fisher, 1958) it is not essential to other models of sexual selection. For instance, sensory bias (Ryan, 1998) does not predict any particular genetic correlation between male attractiveness and female choosiness, leading many to incorrectly assume that in the absence of a genetic

correlation, sensory bias must be occurring (Ryan, 1998; Fuller *et al.*, 2005). Other models (indirect benefits, (Kirkpatrick & Ryan, 1991), good genes, (Houle & Kondrashov, 2002), or sexual conflict, (Gavrilets, 2000)) do not depend on a positive genetic correlation and have been modelled without any correlation between female choosiness and male attractiveness. Interestingly, other models, such as sexual conflict, might predict a negative genetic correlation between female choosiness and male attractiveness due to interlocus sexual conflict between sex-specific fitness-optimizing strategies (Chippindale *et al.*, 2001; Hine *et al.*, 2002; Arnqvist & Rowe, 2005). Further empirical estimates of genetic correlation may allow for clearer interpretations of models in order to make better predictions for how species evolve via sexual selection.

In this study we set out to investigate the roles of male and female genetic identity on mating behaviour in *Drosophila melanogaster*; a species with a polyandrous mating system where males do not provide any obvious post-fertilization parental care (Brown *et al.*, 2004). By creating hemiclonal lines, we are able to investigate the causes and consequences of genetic variation in both pre- and post-copulatory traits, using two aspects of female preference: female choosiness (*sensu* Jennions & Petrie, 1997; Narraway *et al.*, 2010) and female responsiveness (*sensu* Ritchie *et al.*, 2005). From measurements of females' behaviours, we are able to quantify female choosiness, female responsiveness, male attractiveness, female investment into her offspring, and determine how these phenotypes are related to her genotype, the genotype of her mate, and the interaction between them.

Methods

Experimental populations

The ultimate source of the genetic variation in our assays were *D. melanogaster* obtained from the *Ives* (hereafter "IV") population; a large, (N~2800 adults), outbred wild-type population initially derived from South Amherst, MA, USA in 1975, which has been maintained under standardized culture condition since 1980 (Rose, 1981). The IV population has previously been shown to exhibit considerable genetic variation for a variety of adult life history traits (Rose & Charlesworth, 1981a; Rose & Charlesworth, 1981b). This population, like all others used in this assay, is maintained in vials on a discrete 14-day culture cycle. Flies are reared at a controlled density (~100 eggs per vial), on a banana/agar/killed-yeast medium at 25º C, with a 12L:12D diurnal light cycle. A replicate population, IV-*bw*, is maintained under similar conditions and was created by repeatedly backcrossing the recessive brown-eyed allele, *bw*-, into the IV genetic background for 10 consecutive generations. Subsequent backcrossing is periodically done to ensure the IV-*bw* population is sound.

Hemiclonal Analysis

In order to determine whether phenotypic variation in pre and post-copulatory behaviours could be attributable to additive genetic variation in males and/or females, we used a hemiclonal analysis approach (*see* Rice *et al.,* 2005; Abbott & Morrow, 2011). This quantitative genetic technique is available in *D. melanogaster* due to a natural lack of recombination in males of this species, and the availability of phenotypically-marked artificial cytogenetic constructs (described below), which together can be used to isolate, replicate and propagate nearly-complete haploid genomes (*for details see* Chippindale *et*

al., 2001; Abbott & Morrow, 2011). These cloned haploid genomes can then be expressed in a "hemiclonal" state in either a male or a female genetic background (consisting of a random sample of wild-type haplotypes sampled from the base IV population). This technique has been used to quantify genetic variation in a variety of behavioural and morphological traits (Abbott & Morrow, 2011) but has never before been used to explore female mate choice or egg production.

For this assay, we randomly chose 12 clone lines from a larger collection of 31 that had been sampled from the IV population in May 2012. Each clone line is propagated with the use of females from a "clone-generator" population (Rice, 1996), who possess a random Y chomosome, a conjoined "double X" chromosome [C(1)DX, *y, f*], and are homozygous for translocated autosomes [T(2;3) *rdg*C *st in ri* p^{P} bw^{D}]. Creation of male hemiclones was obtained by mating clone males to virgin females from a population ("DX-IV") possessing the "double-X" chromosome, but otherwise possess a random sample of autosomes originating from the IV population. Creation of hemiclonal females involved mating clone males to virgin females obtained from the IV population. Many of the eggs produced via these crosses are inviable due to chromosomal imbalances (50% mortality of eggs laid by IV females mated to clone males, and 75% mortality of eggs laid by DX-IV females mated to clone males). As larval density has important consequences for adult phenotypes and life histories (Ashburner, 1989) great care was taken to ensure that the developmental conditions of vials containing developing hemiclones resembled the conditions typically experienced in the IV population. Thus, we added eggs (of the same age) from the IV-*bw* population to each of our experimental hemiclone-producing vials in order to ensure a desirable density of 100 viable larvae per

vial. Specifically, each vial that would yield male hemiclones received 100 eggs laid by clone-mated DX-IV females, and 75 IV-*bw* eggs, while each vial that would yield female hemiclones received 100 eggs laid by clone-mated IV females, and 50 IV-*bw* eggs. These vials were then reared under standard environmental conditions. Nine days later, wild-type virgin hemiclonal females were collected within 6 hours of eclosion from their pupae. Wild-type male hemiclones were collected on the 11th day, to ensure they had experience courting receptive females (Dukas, 2010). All hemiclones were kept in individual vials prior to the mating assay, which was conducted on the 13th day of the flies' life (i.e. 3-4 days post-eclosion).

Behavioural assays

Standard no-choice preference tests (*see* Ingleby *et al.,* 2013; Shackleton *et al.,* 2005) were conducted to conveniently measure a female's latency to mating when placed with a single male as an indication of male attractiveness and avoid the potential confounds of male-male competition. Since we were primarily interested in global male attractiveness, rather than what trait(s) were preferred, we measured *all* traits that confer male attractiveness (Narraway *et al.,* 2010; Head *et al.,* 2005). Additionally, we point out that identical outcomes were found when assessing female preference in both choice *vs*. no-choice experiments using other species of *Drosophila* (Avent *et al.,* 2008; Taylor *et al.,* 2008), but to our knowledge none have been done with *D. melanogaster*. An individual non-virgin hemiclone male was placed in a vial with an individual virgin hemiclone female from a different hemiclone line. This was repeated for all 12 lines, resulting in 132/144 possible combinations of individual mating pairs (excluding the intercrosses), with 3 replicates per block, resulting in a total of 396 vials to observe. We

deliberately avoided creating crosses where males and females were of the same hemiclone origin because there is evidence that related individuals may behave differently in mate preference than between unrelated mating pairs (*see* Tregenza & Wedell, 2002).

Assays began at 9:00am EST, which corresponds to the time when the incubator lights turn on, and flies become sexually active (H.T. Obsv). Assays were run in the same environmentally controlled room where the flies were cultured and stored prior to the assay. We recorded the date and time for each assay to control for any experimental block effects, which were then accounted for in statistical analysis (see below).

Female responsiveness was quantified using the mean mating speed (or latency to copulation, including courtship) and was measured as the time the vials from each female hemiclone line were placed in view of the observer to the moment copulation began. Since all female genotypes were exposed to essentially the same 11 multiple male genotypes (because of excluded intercrosses) acceptance of a male by female after taking time to assess the potential mate reflected female choosiness. Thus, female choosiness was quantified as the standard deviation (within genotype) in female responsiveness across male hemiclone line (see statistical analysis). Male attractiveness was defined as the average responsiveness for each female genotype to the 11 other male genotypes (*sensu* Ratterman *et al.*, 2014) Quantifying all phenotypes influencing male attractiveness allowed us to determine whether or not male attractiveness has a genetic basis. Copulation duration was measured as the time the male mounted the female to when the pair disentangled. Each individual mating pair was observed for a period of 90 minutes until copulation was observed. If copulation was ongoing at the 90 minute mark, the

mating pair was observed until copulation ended. Our conservative analysis excluded any non-mating pairs, where our complete analysis reflected the latency to mating as 90min.

Measurement of maternal investment: Volume and number of eggs laid

Immediately following the preference assays, all males were removed from the vials using light CO_2 anesthesia. The vials containing only females were placed in the incubator for 24 hours to allow the females to lay eggs. The next morning, the number of eggs laid by each female were counted using a stereo light microscope to determine any immediate post-copulatory effects of male genetic identity on fecundity. At this time, the 3 females from the replicate crosses were placed together into a small egg laying chamber outfitted with a disc of coloured media (Sullivan *et al.,* 2000), and left to lay eggs for an additional 24 hours, as the effects of males on egg size may not be detectable until 24 hours after mating occurs (Pischedda *et al.,* 2011). The following morning, all of the chambers were immediately placed into the refrigerator for 24h to ensure there were no changes in egg sizes due to further egg development. A pilot study confirmed that this short-term refrigeration had no significant effect on egg size measurements (E. Sonser, unpublished data). Upon retrieval from the refrigerator, the eggs that had been laid were counted and then photographed using a microscope-mounted camera. All eggs were placed in the same orientation (i.e. ventrally or dorsally; not laterally) to control for any variation in measurements that could arise from different orientations. ObjectJ (Vischer & Nastasa, University of Amsterdam), a plug in for ImageJ 1.46n (Rasband, National Institute for Mental Health), was used to measure the eggs' lengths and widths to the nearest thousandth of a millimeter. Length was defined as the measurement of the polar axis, while width was the diameter of the egg, orthogonal to the length and at the widest

point. From these values, the volume of the eggs was calculated using the formula for a prolate spheroid: $V=1/6\pi W^2L$ (*as per* Pitnick *et al.,* 2003; Markow *et al.,* 2008; Pischedda *et al.,* 2011). From previous studies (Markow *et al.,* 2008) it is known that there is considerable variation in egg volume as well as in length and width, which is why it is important to consider absolute size (i.e. volume) when investigating maternal investment patterns. Repeatability scores were calculated for measurements of both egg length (96%) and egg width (91%) indicating that one measurement per egg would give us precise measurements.

Statistical analysis

Statistical analysis was completed using JMP 8.0.0 (SAS Institute, Cary, NC) and R version 2.13.1 (The R Foundation for Statistical Computing) to determine the role of genetic identity in *D. melanogaster* mating behaviours. Sources of variation in behavioural, morphological, and fecundity data were analysed using a restricted maximum likelihood (REML) approach because it gave an accurate estimate of variance components when sample sizes were not perfectly balanced (Searle *et al.,* 1992). The genetic variation for mating speed, copulation duration, egg length, and egg width was estimated using a random effects variance component estimate. Female genetic identity, male genetic identity, and the interaction of male and female genetic identities were nested within experimental block and modelled as random effects. Mating speed and copulation duration was square root transformed to obtain normality of distributions and differences in average blocks was accounted for by multiplying data from each block by the inverse of the ratio of the block mean to the global mean across all blocks. To estimate the additive genetic variation seen among all 12 of our hemiclone lines we

partitioned the variance of mating speed, copulation duration, and egg size for block effect, male genetic identity, female genetic identity, and the interaction of the two. Significance was determined by examining the lower 95% confidence interval of the estimate to see if it included zero. Data for non-mating pairs was excluded from this statistical analysis.

To represent genetic variance in female responsiveness, female responsiveness was measured as the mean mating speed of each female hemiclone line across mean male hemiclone lines. Since mating speed is thought to be controlled primarily by female genotype (Fulker, 1966), this variable was used to quantify male attractiveness (i.e. average response of female genotype to the male genotype).

To determine the genetic correlation between male attractiveness and female choosiness we followed established procedures (Hedrick & Weber, 1998; Gray & Cade, 1999; Brooks & Endler, 2001). Female choosiness was calculated as the coefficient of variance (CV) and was obtained by calculating the standard deviation of the mean mating speed for female hemiclone lines (calculated by obtaining the mean mating speed value for each female hemiclone line mated with each male hemiclone line and averaged across experimental block) (Brooks & Endler, 2001). To ensure independence of male and female genotypes (which could cause a positive correlation by influencing the x and y values) the experiment did not include intercrosses between males and females of the same hemiclone line. We then regressed female choosiness on male global attractiveness for all 12 hemiclone lines.

To determine if any trade-off existed between provisioning (i.e. egg size) and production (i.e. egg number) we performed correlation tests and plotted regression lines

representing the relationship between provisioning and production for each female hemiclone line. Data for non-mating pairs was excluded from all statistical analyses (except see results).

Results

Partitioning of variance: genetic identity and pre-copulatory interacting phenotypes

Of a total of 1967 pairs of flies that were observed, 1667 pairs initiated copulation within the 90min observation time frame. For all possible male-female mating combinations we have data on the proportion of pairs that successfully mated, including the latency to mating, and the copulation duration for these successful mating pairs. We decided to exclude the pairs that did not mate from subsequent analysis as we did not want to inflate our estimate of variance components. This did not have any effect on the analyses of our results, as non-mating was randomly distributed across all mating pairs so that excluding them was not statistically biasing any combination (χ^2=126; p=0.32). If we included those non-mating pairs (substituted a value of 90min for mating latency – the maximum duration of observation), we found, for the most part, the same results as in our more conservative data set. Using an REML approach we were able to quantify the extent to which phenotypic variation in mating speed was dependent on genetic identity of one or both sexes. We found a small, but significant amount of the variance in mating speed could be attributed to differences in female genetic identity (7.96%) and to differences in male genetic identity (7.56%), but there was no statistically detectable interaction between the two (Table 2.1). Copulation duration (CD) also varied between the 12 hemiclone lines (Table 2.1). Male genetic identity had a significant effect on the amount of CD variance (4.06%), while female genetic identity accounted for a non-

significant 1.75% of the observed variation. The notable difference when including all non-mating pairs in the statistical analysis is a significant effect of male and female interaction on mating speed (5.1%; SI Table 2.1).

Partitioning of variance: genetic identity and post-copulatory interacting phenotypes

REML results (Table 2.1) indicated that female genetic identity (F) and the interaction between female and male identities (FxM) both accounted for a sizeable amount of the observed phenotypic variation in both egg length (F=8.15%; FxM=25.29%, Table 2.1) and width (F=8.58%; FxM=23.18%, Table 2.1). Similarly, female genetic identity accounted for 40.40% of the observed variation in egg volume and female x male genetic identities accounted for an additional 18.86% of the variance. The number of eggs laid in the first 24 hour period following the behavioural assay were significantly influenced by female genetic identity (17.67%, Table 2.1), the specific interaction of male and female genetic identities (6.13%), but not significantly by male genetic identity (0.94%).

Trade-offs between fecundity and egg size

By examining the relationship between the number of eggs and the size of eggs laid by each female hemiclone line when mated to males from the other 11 hemiclone lines we were able to look for evidence of trade-offs. Only 2 of the 12 female genotypes assayed exhibited a significant negative relationship, suggestive of a trade-off between egg size and number (Fig 2.1). Overall the mean of the 12 regression lines was not significantly different from zero ($\bar{x}$=-5.585x10^{-6}, t_{11}=0.8801, p=0.3976). Interestingly, the slope of the regression lines was more negative in hemiclone lines of low fecundity ($F_{(1,10)}$=13.42, corr=0.76, p=0.0044, slopes: G=-5.81x10^{-5}, I=-2.44x10^{-5}). Furthermore,

we found that only one of the male genotypes exhibited a significant negative relationship (Fig 2.2) between female fecundity and egg size. The same significant male genotype also led to the lowest fecundity**.**

Genetic correlation between attractiveness and choosiness

From the variation in mean mating speed for each female hemiclone measured with each of her 11 possible hemiclone males (Fig 2.3), we calculated the coefficient of variance (CV) as an index of the degree of female choosiness (Brooks & Endler, 2001). The mean mating speed of each male hemiclone line (based on mating speed obtained with each of the other 11 female hemiclone lines) was used to calculate male attractiveness (with longer times to mate indicating "less attractive" males (Fulker, 1966)). Our estimates of female choosiness and male attractiveness between the two analyses (non-mating pairs included and excluded) are significantly positively correlated (female choosiness: $t=3.44$, $df=11$, $p=0.0063$; male attractiveness: $t=10.26$, $df=11$, $p=0.0001$). We examined the genetic correlation between the two variables and found a strong negative correlation between male attractiveness and female choosiness ($r=-0.836$, $p=0.0006$, $n=12$; Fig 2.4). The complete analysis including all non-mating pairs also demonstrates a significant negative correlation ($r=-0.584$, $p=0.0458$, $n=12$; SI Fig 2.1). The haploid genome that produced the most choosey females also yielded the least attractive males, while the genotype producing the least choosey females yielded the most attractive males.

Discussion

Influence of genetic identity on pre-copulatory interacting phenotypes

The relationship between female preferences and male display traits is central to the function of inter-sexual selection, and understanding the causes and consequences of its variation is of great importance to the fields of behavioural genetics and evolutionary biology (Ritchie *et al.,* 2005). Using hemiclonal analysis we clearly demonstrate the underlying genetic basis for variation in several interacting phenotypes (mating speed, copulation duration, and fecundity) present in this population and how these traits are affected by the genetic identity of each sex.

Differences in the specific genetic identity of males and females both individually (but not jointly) had a significant effect on the variation in mating speed. This indicates that females varied genetically in their receptivity to the available male, and males differed genetically in their attractiveness. However, a lack of a significant male x female interaction suggests that these factors acted independently of each other. Previously (Ratterman *et al.,* 2014; Pischedda *et al.,* 2012) it was found that female genotype (but not male genotype) strongly influenced the variance in mating speed, which is consistent with the theory that this trait is controlled primarily by the female (Fulker, 1966). This may also have been due to the willingness of females to mate simply because of an association with the ability to produce eggs, but no significant association between mating speed and female fecundity was found ($t=-0.7373$, $df=10$, $p=0.4779$; SI Fig 2.2). Females also appeared to rank male phenotypes the same (i.e. females tended to "agree" on male attractiveness).

It was somewhat surprising that we found no evidence for a significant male x female genotype interaction for mating speed, as previous work has demonstrated within population genetic variation for this trait in male and female *D. melanogaster* (Casares *et al.*, 1993; Mackay *et al.*, 2005). By mating males to two different female genotypes (low receptivity *vs.* high receptivity), it appeared that the expression of mating speed in both absolute and relative performance of male genotypes in *D. melanogaster* was strongly influenced by the female genotype (Mackay *et al.*, 2005). In this study, the interaction between genotypes was so dramatic that a given male genotype could be among the quickest to mate with one female genotype, yet among the slowest when presented with another female genotype. In a similar study, the male x female genotype interaction contributed to 38.1% of the variance observed in mating speed, suggesting that the mating speed of males was strongly influenced by the genetic identity of the female they courted (Pischedda *et al.*, 2012). Therefore, variation in mating speed among females may be determined by female responsiveness, varying according to female genotype, and the effectiveness of male courtship may depend on the genotype of the female being courted. The significant MxF interaction for mating speed from our estimates of variance components using the complete analysis is likely due to our data set, and not experimental design.

Compared to previous research, there may be some differences in the amount of genetic variation present in the current study system and those used by others (Pischedda *et al.*, 2012; Mackay *et al.*, 2005). For example, studies have used isofemale lines (inbred lines of the same population) and therefore have low genetic variation (Falconer, 1981) and low potential for G x E interaction within isofemale lines. The covariance of

interacting phenotypes may also be affected by relatedness of individuals. Relatedness produces a predictable covariance between phenotypes of interacting individuals (Wolf, 2000). Since related individuals share genes, a covariance is due to phenotypic similarity. In our assays we used hemiclonal analysis, which allows for genetic variation and natural selection to act on the male and female hemiclones (Gavrilets, 2000), increasing the potential for GxE interactions. The use of different source populations of *D. melanogaster* can also strongly influence the composition of genetic variation present (Pischedda *et al.*, 2012). Genetic incompatibilities as a result of outbreeding may lead to variance in mating speed and other pre-copulatory traits. Differentially adapted genotypes can also result in low genetic diversity, as divided populations may have evolved different co-adapted gene complexes, resulting in reduced fitness of hybrids when individuals from different populations mate (Tregenza & Wedell, 2002). There is strong evidence that geographically distinct populations of *D. melanogaster* have genetic variation in pre-copulatory traits due to differences in selection history and genetic architecture (Widemo & Sæther, 1999; Long *et al.*, 2006) that may not be present within each population; future studies should consider this.

Male genotype significantly contributed to the amount of variation in copulation duration, a result which is consistent with theory and previous evidence that this trait is primarily under male control (Friberg, 2006). Increasing the duration of copulation may potentially be associated with direct fitness benefits for males (i.e. ensuring paternity in competitive environments) via transfer of increased number of sperm in the presence of rival males (Price *et al.*, 2012), and/or transferring products that are (indirectly) harmful to females by reducing their lifespan (Pitnick & Garcia-González, 2002; Friberg &

Arnqvist, 2003), subsequent reproductive success (Pitnick & Garcia-González, 2002; Friberg & Arnqvist, 2003; Wigby & Chapman, 2005), and female remating rate (Pitnick, 1991). Reducing the risk of sperm competition by prolonged copulation duration allows males to achieve high fertilization success (Friberg, 2006).

We found no significant interaction between male and female genetic identities for phenotypic variation in copulation duration. Previous work also reported no significant interaction between male and female genotypes in *D. mojavensis*, suggesting that genotypic differences did not account for behavioural interactions (Krebs, 1991). This is somewhat surprising since recent studies have determined that females exert at least some control over copulation duration in *Drosophila* species (Hirai *et al.,* 1999; Mazzi *et al.,* 2009; Edwards *et al.,* 2014). It may be in the best interest of both sexes that sperm transfer is successful because both individuals have made the choice to mate with each other. A lack of a significant interaction between male and female genotype suggests that there may be limited opportunity for coevolution for copulation duration (Hall *et al.,* 2013), male and female *D. melanogaster* may be dealing with different suites of traits associated with copulation duration, or selection pressures may differ between the sexes for this trait, varying copulation duration optima (Rice, 1998; Chippindale *et al.,* 2001). From the male's perspective, selection may favour longer copulation for transferring accessory seminal proteins (Acps), increasing the likelihood of siring a female's clutch (Wigby & Chapman, 2005; Friberg, 2006) and succeeding in sperm competition (Bretman *et al.,* 2009) (although factors other than copulation duration may contribute to the allocation of Acps (Sirot *et al.*, 2011)). On the other hand, females may suffer physical harm during copulation (Kamimura, 2007) and/or the contents of male

ejaculate may be detrimental to female fitness (see below), thus selection may favour shorter copulation. Further investigation of copulation duration as an interacting phenotype and whether or not it is subject to sexual selection is warranted.

A negative correlation between female choosiness and male attractiveness

We found a significant negative genetic correlation between female choosiness and male attractiveness (Fig 4). This association indicates that the genotypes which produce highly attractive males also produce females of low choosiness, and vice versa. According to predictions of the Fisherian model of sexual selection, a positive genetic correlation between male attractiveness and female choosiness would result in both attractive males and choosey females [2,3,8,12]. While previous empirical tests of genetic correlations between male attractiveness and female choosiness have yielded mixed results (*see* Gilburn *et al.,* 1993; Gray & Cade, 1999; Brooks & Endler, 2001; Zhou *et al.,* 2011; Ingleby *et al.,* 2013), this is, to the best of our knowledge, the first instance where a negative correlation has been reported. Our results show that the production of choosey female genotypes also yields unattractive male genotypes, and vice versa, consistent with sexual conflict theory (Chippindale *et al.,* 2001; Arnqvist & Rowe, 2005; Foerster *et al.,* 2007). This negative correlation may reflect the effect of sexually antagonistic genetic variation in our population.

The adaptive benefit of female choosiness is a component of almost all models of sexual selection – whereby females exhibiting non-random mating patterns gain a direct and/or indirect fitness advantage (Andersson, 1994; Jennions & Petrie, 1997). It follows, therefore, that females of high fitness would be more choosey than those that were less choosey, and that the two traits should be positively genetically correlated. Similarly, the

evolution of elaborate display traits in males is viewed as being adaptive, as those who possess them are viewed as more attractive, and will be at a selective advantage in acquiring mates and/or post-copulatory success (Andersson, 1994). However, it is becoming increasingly evident that the fitness maximizing strategies of males and females are often incompatible, and traits that increase fitness in one sex, decrease fitness in the other sex (Rice, 1998; Chippindale *et al.,* 2001). This sexual conflict can arise either via the evolution of antagonistic adaptations in males and females under sex-specific expression (inter-locus sexual conflict) or on traits with a common genetic basis in both sexes (intra-locus sexual conflict) (Rice & Chippindale, 2001; Pischedda & Chippindale, 2006; Prasad *et al.,* 2007). One of the consequences of intra-locus sexual conflict is that the fitness consequences of alleles will depend on the sexual genetic background in which it is expressed. Genotypes resulting in high male fitness will yield low female fitness (and vice versa) (Chippindale *et al.,* 2001; Foerster *et al.* 2007). Here, we suggest that the presence of sexually-antagonistic alleles in our laboratory population (a common observation in *D. melanogaster* stocks – see (Rice, 1998; Chippindale *et al.*, 2001)) may be the root cause of our observed negative genetic correlation between female choosiness and male attractiveness. As stated above, each of these traits is likely to be genetically correlated with fitness-related traits (in their respective sexes), and if some of these fitness-related traits have a genetic architecture that is the subject of intra-locus sexual conflict, then as a result, female choosiness and male attractiveness will ultimately show a negative genetic correlation.

Whether or not this pattern is limited to our laboratory population or may be more widespread is unclear and is deserving of further investigation. However, there is

increasing evidence that traits (and fitness) in wild populations show the signs of being subject to genetic tug-of-war between the sexes (Rice & Chippindale, 2001; Pischedda & Chippindale, 2006). Furthermore, the absence of many clear examples of positive genetic correlations between choosiness and attractiveness may be in part due to a wide-spread role of this co-evolutionary conflict. Our experimental results will hopefully stimulate theoretical models to further consider the implications of negative genetic correlations in shaping species' evolutionary trajectories via sexual selection.

Trade-offs between fecundity and egg size

Our examination of a potential trade-off between egg provisioning and production found that only 2 out of 12 female hemiclone lines surveyed displayed a significant negative relationship between fecundity and average egg size. When viewed from the male hemiclone perspective, only 1 genotype out of 12 exhibited a significant negative trade-off, suggesting that males were able to influence females similarly in egg production and provisioning, possibly due to experimental design (lack of male-male competition, no-choice assay). Genetic models of life history evolution predict a negative correlation between egg size and fecundity (Schwarzkopf *et al.,* 1999), and thus it is of interest to investigate the reasons why the majority of hemiclone females did not show a trade-off between fecundity and egg volume.

A negative correlation between egg size and egg number is expected when clutch size (=egg volume x egg number) is constant (Ebert, 1993) and a change in egg size is associated with a concomitant change in egg number (Schwarzkopf *et al.,* 1999). The lack of a relationship suggests that the phenotypic trade-off between egg size and number may evolve independently without a direct genetic trade-off (Schwarzkopf *et al.,* 1999). Non-significant correlations between egg size and number may also be due to variation in

reproductive investment between male and female genotypes, and physical condition. Since environmental conditions and resource availability were constant for all aspects of our study, we can probably rule out environmental variation as a factor (trade-offs allow a female to optimize fitness by maximizing resource potential (Smith & Fretwell, 1974); when resources are in abundance, a trade off may not exist (see Semenchenko, 1989; Ebert, 1993). Reproductive investment often increases with female body size (Berrigan, 1991; Ebert, 1993; Czesak & Fox, 2003). Larger females are predicted to produce more eggs, therefore the fitness gain in terms of eggs fertilized will be greater in large females (Czesak & Fox, 2003; Pitnick *et al.,* 2009) than with small females of low fecundity (Lefranc & Bundgaard, 2005; Long *et al.,* 2009). Natural variation in female body size could influence clutch size and result in large variation in egg number, therefore producing non-negative correlations between egg size and number (Ebert, 1993).

Genetic variation among female genotypes in the provisioning and production of eggs and genetic variation among male genotypes in their ability to stimulate both egg production and provisioning in females could lead to differences in clutch size. The use of hemiclonal lines allowed us to create many individuals of a consistent haplotype expressed in either a male or a female genetic background in an outbred state (Abbott & Morrow, 2011). Cross-mating these individuals enabled us to examine the effect of *both* maternal and paternal genotype, while also considering sex-specific effects within and among hemiclone lines. Depending on the female genotype, certain male genotypes may only be successful in stimulating either egg size or female fecundity in their mates, but not both traits simultaneously. Attractive males may stimulate short-term female fecundity by transferring accessory seminal proteins (Acps) in the ejaculate to females

during copulation. These Acps stimulate oogenesis and ovulation in females after mating when there is sperm available to fertilize the eggs, increasing the egg laying rate (Long *et al.,* 2009). Males differ genetically in their stimulatory capacity towards females (Tennant *et al.*, 2014) and females vary genetically in their seminal receptors (Pitnick, 1991; Long *et al.,* 2009). This is reflected in our REML analysis which shows a significant interaction between male and female genotypes in terms of female fecundity and egg size.

Sexual conflict theory predicts that there is genetic variation among males for harm imposed upon females and genetic variation among females for resistance to males (Linder & Rice, 2005), which is consistent with the theory of sexually antagonistic coevolution (Holland & Rice, 2002). Female *D. melanogaster* suffer direct costs when mated with attractive males (Chapman, 2001), and may attempt to reduce these costs by "resisting" copulation with attractive (and presumably harmful) males (Friberg, 2005). Females stimulated into mating with attractive males have an increased short-term fecundity, but decreased overall lifetime reproductive success (Arnqvist & Nilsson, 2000; Wigby & Chapman, 2005), whereas females stimulated into mating with unattractive males may suffer immediate fitness costs, but benefit long term by reduced personal harm and potentially higher quality offspring (Moore *et al.,* 2001). The effect of male harm to females is reflected in female egg laying patterns. In *D. melanogaster,* large males are presumed to be more attractive because they may be better at stimulating/coercing potential mates (Bangham *et al.*, 2002; Pitnick & Garcia-González, 2002; Friberg & Arnqvist, 2003). The larger the male, the bigger the accessory glands (Bangham *et al.*, 2002; South & Lewis, 2011), and thus the more Acps can potentially be transferred in the

ejaculate during copulation, depending on female mating status and the risk of sperm competition (Wigby *et al.,* 2009; Sirot *et al.,* 2011). However, in addition to boosting female short-term fecundity, Acps also reduce female longevity (Chapman, 2001), alter feeding behaviour (Caralho *et al.,* 2006), and induce a refractory period (Chapman, 2001; Pitnick *et al.,* 2009). Choosey females who avoid mating with harmful males may resist the negative effects of male courtship via better control over their own reproductive physiology. By "controlling" who they mate with (i.e. avoiding the largest, most attractive males via pre-copulatory mate choice (Moore *et al.,* 2001)), these females may mediate the dosage of short-term fecundity-stimulating seminal fluid they receive, resulting in lower short-term fecundity (Friberg & Arnqvist, 2003). Non-choosey females may be unable to resist/distinguish harmful (attractive) males as effectively as choosey females, resulting in an increase in their short-term fecundity (Chapman, 2001; Pitnick & Garcia-González, 2002; Friberg & Arnqvist, 2003).

We did not see a consistent significant relationship between provisioning and production of eggs when varying both parental genotypes (in contrast to previous studies varying only male genetic identity (Pischedda *et al.,* 2011)). Our study suggests that these patterns are a result of a female's genetic identity, and not necessarily dependent on her mate. Our results also demonstrate how genotype x genotype interactions and resource availability may play a significant role in maternal investment patterns.

Influence of parental genotype on egg size and number

In *D. melanogaster,* both male and female genotype influenced the number and size of eggs produced from mating pairs. Using a REML approach we were able to determine that ~60% of the observed phenotypic variation seen in egg size could be

collectively attributed to the genetic identities of one (the female) or both of the individuals in a mating pair (Table 1). Female genotype accounted for the largest amount of the variation seen in egg size. As mentioned previously, egg size can be a proxy of female maternal investment strategies and is important to the future success of offspring in many animals (Czesak & Fox, 2003). Offspring genotype may play a role in determining nutrient usage as maternal investment nutrient-wise can be a limiting factor for offspring development (Czesak & Fox, 2003). Studies of maternal effects have shown that maternal genotype accounts for approximately half of the variance in offspring phenotype (Cheverud & Moore, 1994) while the direct effect of the offspring's genotype accounts for between 10-50% of the phenotypic variance (Cheverud & Moore, 1994), suggesting that paternal genotype may also influence offspring phenotypic variance. This creates a "multi-layered" indirect genetic effect (IGE) wherein the maternal genotype's "environment" is influenced by variation in the paternal genotype, subsequently influencing the fitness variance in future offspring (Moore *et al.*, 1997; Wolf, 2000).

We found significant differences in egg size variation due to the interaction of male and female genetic identity, suggesting that some contribution from the ejaculate may influence egg production. Some contents of a male's ejaculate may be allocated as nutrients for the eggs (e.g. Markow *et al.*, 2001), or more importantly, act as stimulants for egg production/investment (Wigby & Chapman, 2005; Pitnick *et al.*, 2009) resulting in various egg sizes (i.e. females who receive larger amounts of seminal product may lay larger eggs than those females who receive less (Czesak & Fox, 2003)). In *D. melanogaster*, larger eggs have higher viability and greater successful larval development rates (Azevedo, 1997), therefore it is of interest to both the male and female that

offspring viability is successful. However, since the interactions of male and female genotypes had such a significant effect on egg size, this highlights the importance for *both* males and females to be choosey in their mate selection.

Female genotype significantly influenced the number of eggs laid 24 hours after post-mating, suggesting that females vary genetically in their oviposition rates (Andrés & Anrqvist, 2001). A significant interaction between male and female genotypes for this trait suggests that females also differ genetically in response to male seminal products (Andrés & Anrqvist, 2001). The number of eggs sired by a male may be due to the composition and/or amount of his ejaculate which might reflect differences in types and/or amounts of components. Since accessory protein composition exhibits genetic variation among males in *D. melanogaster* for oogenesis and oviposition stimulation (Holland & Rice, 2005), females may not only differ in responsiveness, but may receive different kinds of bioactive components from male ejaculate to incorporate into their eggs (Czesak & Fox, 2003) resulting in variation in the number of eggs laid. Male accessory proteins may also affect female behaviour and physiology by increasing the rate of eggs produced, resulting in a short-term increase in the number of eggs laid (Long *et al.,* 2010; Tennant *et al.,* 2014). This would also increase male reproductive success, suggesting that it may rely on both male and female genotype.

Male genetic identity alone did not account for a significant amount of the variation seen in egg size or egg number. The eggs measured in our study represented the females' 2nd clutch (see Materials and Methods), and therefore developed in the presence of male seminal products. Males may benefit female fecundity in the short-term by transferring accessory seminal proteins (Acps) to females during mating (Tennant *et al.*,

2014). These Acps stimulate oogenesis and ovulation in females after mating when there is sperm available to fertilize the eggs, increasing the egg laying rate (Pitnick *et al.,* 2009). Variation in egg size and number in a female's 2nd clutch attributed to male genotype has been found (Pischedda *et al.,* 2011), suggesting that a male's genotype influences a female's fecundity and the size of eggs she produces. However, only the effects of male genotype on maternal investment patterns were previously tested as the genetic identity of the females was held constant, limiting their ability to draw conclusions about the effects of both parental identities on maternal investment patterns or their interactions (Pischedda *et al.,* 2011). Our results suggest that the interaction of genetic identity plays a significant role in maternal investment patterns, as females from the same hemiclone line (i.e. carrying the same haploid genome, and therefore of similar size) invested differently when mated with different male hemiclonal lines.

Conclusion

In conclusion, we demonstrated the genetic basis for variation in female choosiness and female responsiveness. When mated with non-related individuals, males and females differed genetically in their sexual responsiveness but did not differentially respond to their mate's genetic identity. We also discovered a strong negative correlation between female choosiness and male attractiveness. The combined genetic identities of mating pairs had a significant effect on the amount or quality of resources a female will invest into her offspring. The interaction of male and female genotypes influencing fecundity and/or offspring size can result in a coevolution between males and females for investment into reproductive success.

Our results indicate that whether or not sex-limited interacting phenotype development extinguishes intralocus sexual conflict may depend on a population's genetic architecture and selective history (Harano *et al.,* 2010). Intralocus sexual conflict may be interfering with adaptive evolution in our population because of evidence that sexually antagonistic selection can lead to a trade-off between the optimal genotypes for males and females, biasing the reproductive outcome towards one sex, influencing the maintenance of genetic variation, and ultimately the evolutionary trajectory in a population. Our results confirming MxF genetic variation for mating speed and maternal investment support the prediction that indirect genetic effects act on pre- and post-copulatory traits in *D. melanogaster*.

Further studies on the plasticity of female choosiness, body size, and the correlation between choosiness and lifetime reproductive success could offer insight into whether or not condition-dependence influences genetic variation in the interacting phenotypes studied. More empirical studies investigating genotype x genotype interactions in genetically different individuals for both pre- and post-copulatory behaviours should support the above findings.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

HMET and TAFL conceived of the study. All authors helped design and conduct the experiment. TAFL performed the statistical analyses. HMET and EES drafted the manuscript. All authors read and approved the final manuscript.

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Tables and Figures

Table 2.1: Decomposition of variance components of interacting phenotypes for 12 hemiclone lines using REML.

Interacting phenotype	Source of variation	Variance component	SE	95% Lower	95% Upper	% of Total
Mating Speed	Female	18.9	4.86	9.32	28.48	7.96
	Male	17.97	4.65	8.85	27.09	7.56
	Female x Male	0.95	5.95	-10.72	12.63	0.4
	Residual	199.71	8.84	183.44	218.25	84.07
	Total	237.54				100.00
Copulation Duration	Female	0.43	0.25	-0.05	0.91	1.75
	Male	0.99	0.34	0.32	1.66	4.06
	Female x Male	-0.02	0.67	-1.32	1.29	-0.07
	Residual	23.07	1.01	21.21	25.18	94.27
	Total	24.47				100.00
Number of Eggs Laid in 1^{st} 24hrs	Female	0.31	0.07	0.16	0.45	12.18
	Male	-0.01	0.02	-0.05	0.31	-0.33
	Female x Male	0.23	0.69	0.09	0.36	9.17
	Residual	1.99	0.87	1.83	2.17	78.98
	Total	2.52				100.00
Egg Length	Female	$5.6x10^{-5}$	$1.5x10^{-5}$	$2.6x10^{-5}$	$8.6x10^{-5}$	8.15
	Male	$-6.09x10^{-6}$	$4.4x10^{-6}$	$-1.5x10^{-5}$	$2.5x10^{-6}$	0.00
	Female x Male	0.00017	$1.6x10^{-5}$	0.00014	0.00020	25.29
	Residual	0.00046	$8.1x10^{-6}$	0.00044	0.00047	66.56
	Total	0.00069				100.00

Egg Width	Female	$5.05x10^{-6}$	$1.35x10^{-6}$	$2.40x10^{-6}$	$7.71x10^{-6}$	8.58
	Male	$3.65x10^{-7}$	$4.83x10^{-7}$	$-5.82x10^{-7}$	$1.31x10^{-6}$	0.62
	Female x Male	$1.36x10^{-5}$	$1.25x10^{-6}$	0.000011	$1.61x10^{-5}$	23.18
	Residual	$3.98x10^{-5}$	$7.03x10^{-7}$	$3.85x10^{-5}$	$4.13x10^{-5}$	67.63
	Total	$5.89x10^{-5}$				100.00
Egg Volume	Female	$7.3x10^{-7}$	$1.4x10^{-7}$	$4.5x10^{-7}$	$1.0x10^{-6}$	40.40
	Male	$1.0x10^{-8}$	$1.3ex10^{-8}$	$-1.6x10^{-8}$	$3.6x10^{-8}$	0.55
	Female x Male	$3.4x10^{-7}$	$2.9x10^{-8}$	$2.8x10^{-7}$	$3.9x10^{-7}$	18.86
	Residual	$7.2x10^{-7}$	$1.3x10^{-8}$	$6.9x10^{-7}$	$7.5x10^{-7}$	40.18
	Total	$1.8x10^{-6}$				100.00

SI Table 2.1: Inclusive estimates of variance components of mating speed for 12 hemiclone lines using REML.

Interacting phenotype	Source of variation	Variance component	SE	95% Lower	95% Upper	% of Total
Mating speed	Female	15.70	4.89	6.10	25.28	4.53
	Male	22.42	6.00	10.66	34.19	6.48
	Female x Male	17.65	8.17	1.63	33.65	5.10
	Residual	290.80				83.19
	Total	346.56				100.00

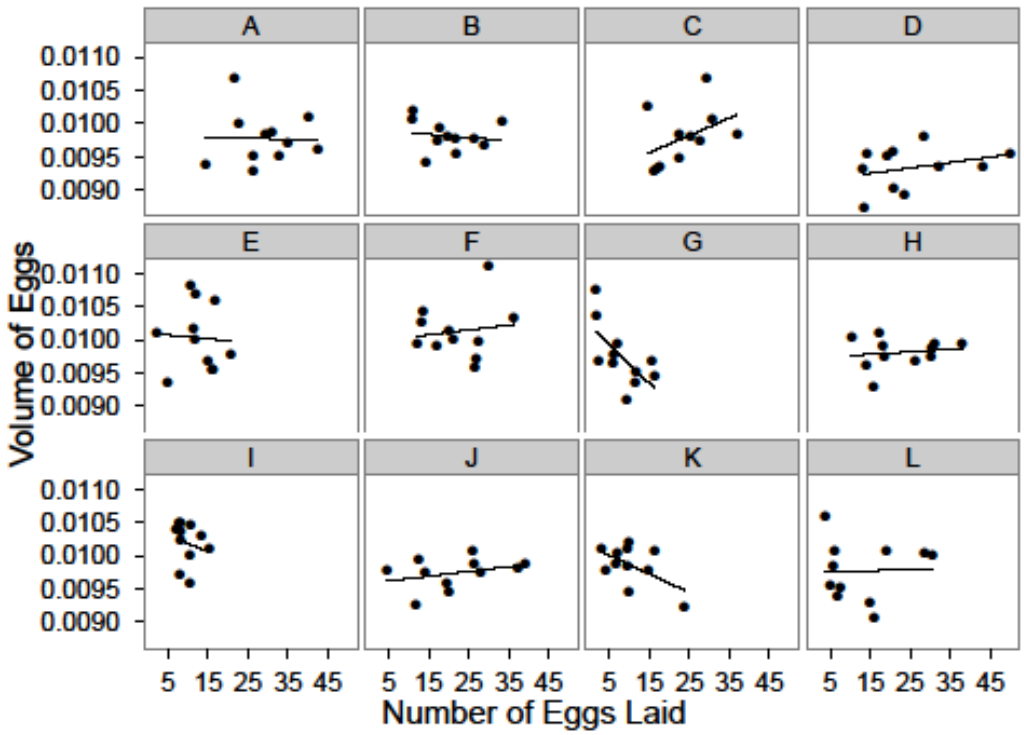


Figure 2.1: Trade-off between egg size and egg number among 12 female hemiclone lines.

Individual plots each represent one female hemiclone line (A-L) and each point on the graph represents an average for both the number and volume of eggs laid when a hemiclone female mated with one of the 11 other male genotypes. Regression lines indicate only 2 of 12 female hemiclone lines (G and I) show a significant negative trade-off between egg volume and egg number.

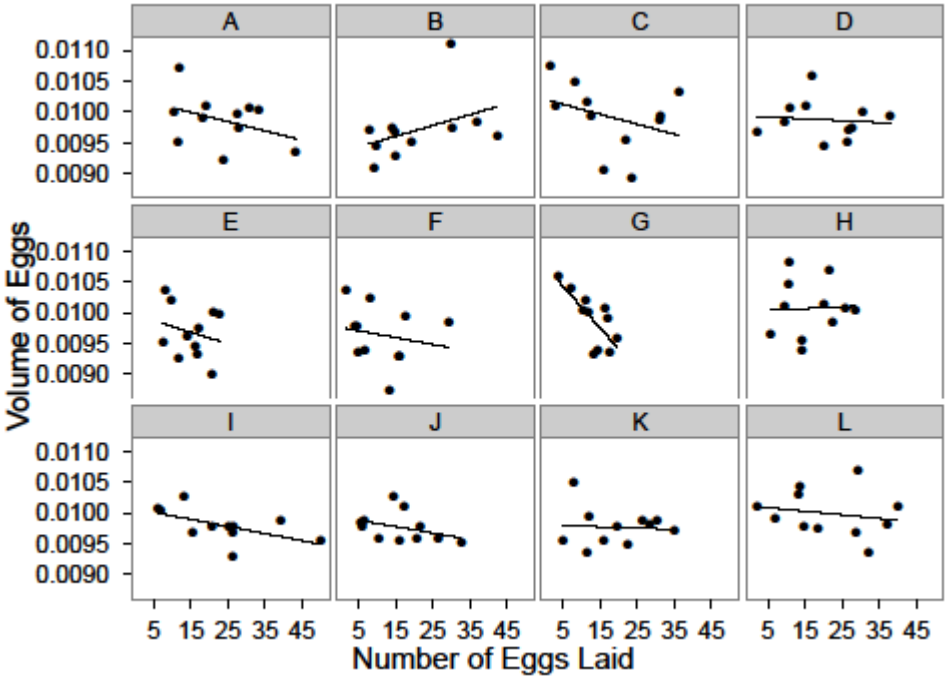


Figure 2.2: Trade-off between egg size and egg number among 12 male hemiclone lines.

Individual plots each represent one male hemiclone line (A-L) and each point on the graph represents an average for both the number and volume of eggs laid when a hemiclone male mated with one of the 11 other female genotypes. Regression lines indicate only 1 of 12 male hemiclone lines (G) show a significant negative trade-off between egg volume and egg number.

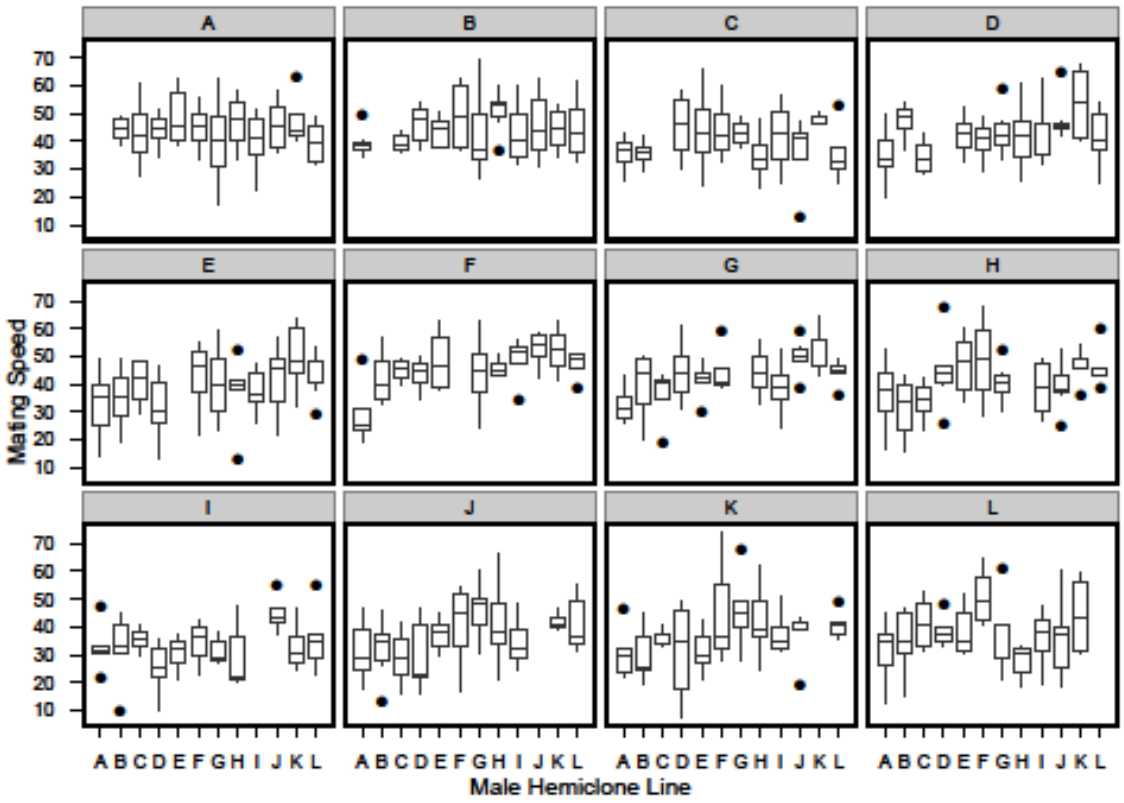


Figure 2.3: Female responsiveness and female choosiness for male attractiveness. Each individual plot represents data collected from one female hemiclone line for the time to mating with 11 different male hemiclones (excluding the intercrosses). Female responsiveness is measured as the mean mating speed among female hemiclone lines and is evident in the variation among lines in the height of the means. Female choosiness is measured as the variance of that mean (responsiveness) with the choosiest females having the most variance in responses. Differences in the height of mating speed indicate male attractiveness, i.e. the faster the mating speed (lower y-values), the more attractive the male. Male hemiclone lines are ordered from the most attractive (A) to the least attractive (L), left to right, along the x- axis.

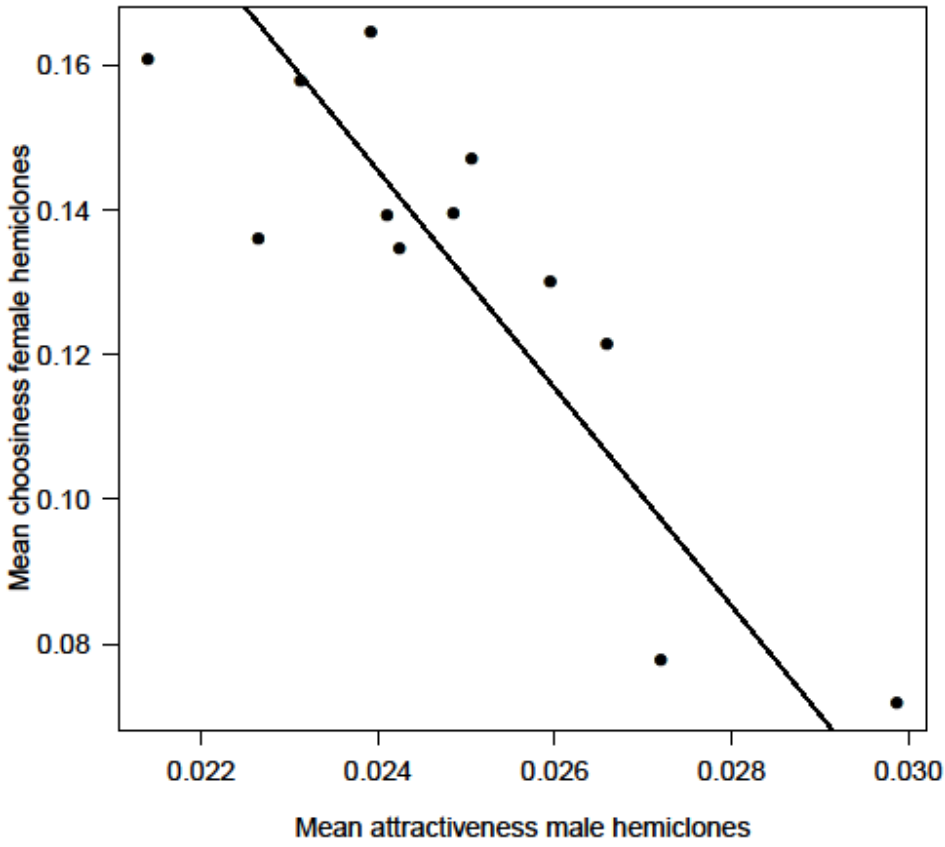
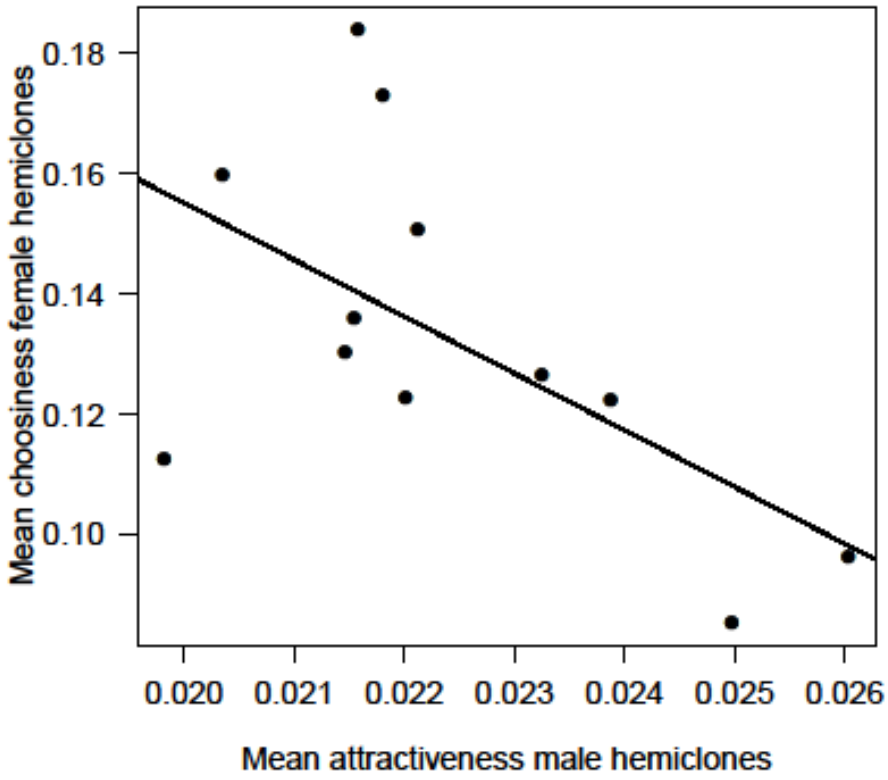
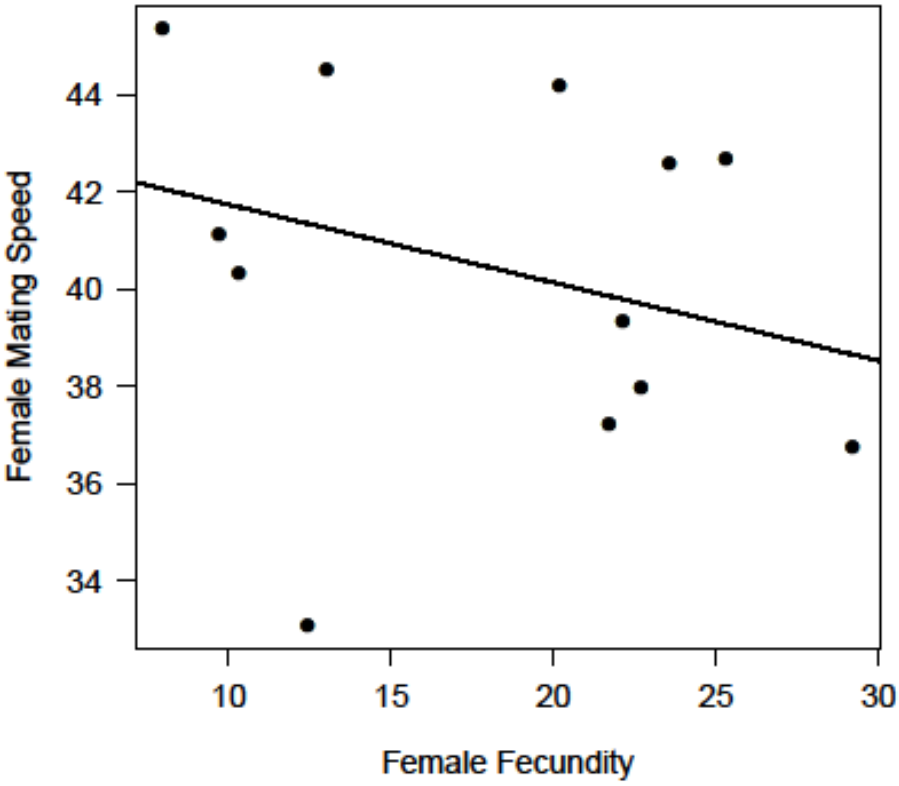


Figure 2.4: A negative genetic correlation between male attractiveness and female choosiness. We estimated genetic correlation by regressing mean male mating speed (attractiveness) on the coefficient of variance (CV) (choosiness) for all 12 hemiclone lines. This association indicates that the genotypes which produce highly attractive males also produce non-choosey females, and vice versa (P=0.0006, r=-0.836, n=12). We used the inverse of mean male mating speed to demonstrate the negative genetic correlation so that the larger x-values corresponded to attractive males.



SI Figure 2.1: A negative genetic correlation between male attractiveness and female choosiness. Our estimates of female choosiness and male attractiveness incorporated non-mating pairs with a latency of 90mins. This association indicates that the genotypes which produce highly attractive males also produce non-choosey females, and vice versa (P=0.0006, r=-0.836, n=12). We used the inverse of mean male mating speed to demonstrate the negative genetic correlation so that the larger x-values corresponded to attractive males.



SI Figure 2.2: No correlation between latency to mating and female fecundity.

We estimated the correlation between latency to mating and female fecundity for each of the 12 female hemiclone lines (t=-0.7373, df=11, p=0.4779). The phenotypic variation for female mating speed was not due to an association between female's willingness to mate and the ability to produce eggs.

CHAPTER 3:

WHEN THE GOING GETS TOUGH, DO THE TOUGH GET CHOOSY?

CONDITION-DEPENDENCY OF FEMALE CHOOSINESS IN *DROSOPHILA MELANOGASTER*

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Abstract

Individuals often vary considerably in physical condition, resulting from both genetic and environmental sources, potentially stemming from differences in their ability to assess potential mates and express their preferences. Consequently, condition-dependence may be an important source of variation in female responsiveness and female choosiness with respect to sexually selected male traits. It is possible that condition-dependent GxE interactions maintain genetic variation in female choosiness and female responsiveness; however, few studies have examined the influence of GxEs in this context despite their potential importance to sexual selection. Here, we use cytogenetic cloning techniques developed in *Drosophila melanogaster* to assess how female choosiness and other female mating and reproductive behaviours are influenced by genetic identity and/or larval density conditions. Our results do not indicate a significant GxE interaction for female choosiness or any of the other mating and reproductive behaviours we examined. We discuss potential reasons for the lack of a GxE interaction and the potential consequences to the study of sexual selection.

Introduction

Female mate preference (the combined processes of perception of signals from potential mates, and the assessment and response to those signals (Chenoweth & Blows, 2006)) is central to much of the variation in sexual selection pressures. As genetic variation in female preference implies variation in the strength of the selection exerted on male traits (Rodríguez & Greenfield 2003) it has important consequences for a population's genetic structure and evolutionary trajectory. Genetic variation in female preference is not only important from the perspective of understanding the selection acting on male traits, but also for the evolution of female preference itself (Chaine & Lyon, 2008). Female preference can be subdivided into two components, both important conceptually and empirically. Female *responsiveness* (the likelihood that a female will respond to the courtship signal of a potential mate) has been shown to exhibit additive genetic variation in several species (Hedrick & Weber, 1998; Gray & Cade, 1999; Brooks & Endler, 2001), and it is widely accepted that genetic variation in female *choosiness* (the degree to which a female can discriminate among potential mates) is necessary for species to evolve via sexual selection (Jennions & Petrie, 1997; Widemo & Sæther, 1999; Andersson, 1994). Thus, individual-level variation in female choosiness and responsiveness can arise due to genetic differences, environmental factors, or a combination of genetic and environmental interactions.

Genotype x environment interactions (GxEs) influence trait expression so that individuals with identical genotypes can have different phenotypes when exposed to different environments (Ingleby *et al.,* 2010). GxEs may explain some of the phenotypic variation in female choosiness (Tomkins *et al.,* 2004; Hunt *et al.,* 2004; Narraway *et al.*,

2010) and may provide one mechanism for the maintenance of genetic variation in female choosiness that is needed for it to ultimately evolve (Narraway *et al.*, 2010). GxEs may also be important in influencing the expression of both sexually selected male traits and female preferences, ultimately shaping how these traits co-evolve (Ingleby *et al.*, 2010). If the phenotypic expression of genetic variation in female preference is dependent on the specific characteristics of a female's developmental environment, the strength of genetic covariance between male attractiveness and female choosiness may vary across environments as well (Narraway *et al.*, 2010). Changes in the strength of a genetic covariance are also likely if there is a GxE for male attractiveness (e.g. Jia *et al.*, 2000). As preferred male phenotypes change across environmental conditions, linkage disequilibrium (likely responsible for the covariance) between female preference and the preferred male trait is disrupted. This may interfere with the operation of both Fisher's runway process and the "good genes" model of sexual selection (Jia *et al.*, 2000). Recently, a study examining female mate choice across two different post-eclosion temperatures did not detect significant GxE variation in female preference for male attractiveness across rearing temperature (Ingleby *et al.*, 2013). To our knowledge, no other study has examined GxE variance for *both* female preference *and* male attractiveness, suggesting that the ultimate outcome of mate choice may be fairly robust (Ingleby *et al.*, 2013) or that the phenomenon has not been sufficiently studied (Ingleby *et al.*, 2010).

The extent to which female preference is plastic – that is, a phenotypic trait whose expression depends on the specific biotic and/or abiotic environment – is also unclear (Chaine & Lyon, 2008). We currently have limited knowledge regarding the genetics

underlying plasticity in female mate choice, yet it has been argued that it is unlikely that female preference is static and that all females prefer the same males in every environment (Jennions & Petrie, 1997; Ingleby *et al.*, 2013). Since both social and physical environments are constantly changing, flexibility in female mate preferences may allow females to choose the most appropriate male to meet their current needs. Detecting a GxE for female choosiness would, in fact, suggest there is genetic variation for plasticity in female choosiness across environments. To our knowledge, only two studies have found a GxE for female mate preferences: Rodríguez & Greenfield (2003) found a GxE for female responsiveness to male pulse rate thresholds in the wax moth, *Achroia grisella*, reared at two different temperatures, while Narraway *et al.*, (2010) discovered a significant GxE for female choosiness in *Drosophila melanogaster* by manipulating larval developmental conditions. Both studies reported a significant effect of female genetic identity on measures of female preference, suggesting a genetic basis for variation in this trait. There was evidence of significant ecological crossovers in both studies for females of good condition and poor condition, indicating that the relative choosiness for females reared in poor environmental conditions did not always predict their relative choosiness when reared at standard conditions. Therefore, GxE interactions may contribute to the maintenance of genetic variation for female choosiness that is required for it to evolve.

One of the reasons why the expression of genetic variation in female choosiness may differ depending on environmental condition is that there may be differences in the magnitude of the costs that an individual is prepared to invest in the assessment of potential mates (Jennions and Petrie, 1997). If being choosey is costly, and expression of

this trait depends on physical condition, then condition dependence should restrict female mate choice (Jennions and Petrie, 1997; Bakker *et al*, 1999). It has been specifically predicted that females who find themselves in poor physical condition may be less choosey in their mate choice decision than individuals in good condition. (Bakker et al 1999; Cotton et al 2006). Even though condition-dependent female mate choice may dramatically influence the shape, direction, and strength of sexual selection both within and among populations (Wagner 1998; Jennions and Petrie 1997; Widemo and Sæther, 1999), ultimately shaping evolutionary trajectories, few studies have examined the relationship between physical condition and female choosiness (Jennions and Petrie 1997, Widemo and Sæther, 1999)

In many species, body size is considered an important factor in the outcome of mate choice because of the positive correlation between physical condition and lifetime reproductive success, i.e. "fitness" (Andersson 1994; Arnqvist *et al.*, 1996). Poor condition may reduce fitness via reduced survival and/or reproductive success (Lindström, 1999). For example, adult *D. melanogaster* in poor physical condition have been shown to exhibit reduced body size and a decrease in fat free dry weight (Baldal *et al* 2010). Fat content is a measure of the amount of energy available per unit of body mass (Baldal *et al*, 2010) and higher fat content is required for reproduction purposes. Lipid and glycogen content is known to be genetically correlated in adult flies (Clark and Keith, 1988; Clark, 1989) and since lipids are more abundant than carbohydrates in adults (Chippindale *et al* 1998) absolute fat and relative fat content can become a proxy for condition (Rode and Morrow, 2009). Little is known about the genetic basis of "condition" itself (Tomkins et al, 2004), despite a number of sexually selected traits

known to be influenced by the nutritional state of organisms during development (Andersson, 1994). In many insects, fitness is primarily influenced during larval development by resource availability and acquisition (Boggs and Freeman, 2005). Previous work on larval density in *Drosophila melanogaster* has demonstrated negative effects on the expression of several adult life-history traits. Flies reared at high larval density are typically smaller in size, have reduced fat content, and reduced fecundity/reproductive success (in females and males, respectively) due to the increased competition for limited resources (Byrne and Rice 2006; Amitin and Pitnick 2007; Rode and Morrow 2009). Generally, adult body size in insects is genetically determined and can be modified by larval rearing environments (Honěk, 1993). Females of varying body size may have different physiological and environmental constraints acting on the ability to produce eggs. The amount of resources a female can allocate to egg production may be limited due to the inability to gain resources, or the allocation of resources to other metabolically costly functions (i.e. somatic maintenance, growth, finding food, etc.) (Honěk, 1993; Bernardo, 1996). In general, body lipids accumulated during insect larval development are known to be important as sources of nutrients for egg production (Arrese & Soulages, 2010), but the relative importance of lipid materials and dietary nutrients is unknown. In *Drosophila* species, larval environment influences adult body size, but egg production may be largely dependent on resource availability as adults (Edward & Chapman, 2012).

Hemiclonal analysis is a modern cytogenetic cloning technique that is used to provide direct estimates of additive genetic variation by measuring the total phenotypic composition of numerous groups of individuals who share a common haplotype (Rice,

1996; Abbott & Morrow, 2011; Morrow *et al.*, 2008). Hemiclonal analysis has been used to examine the genetic basis of both fitness and fitness-related traits in *D. melanogaster* (Chippindale *et al.*, 2001; Rice *et al.*, 2005; Friberg *et al.*, 2005; Long & Rice, 2007; Tennant *et al.*, 2014) and has proven to be useful in studying genetic variation and GxEs in sexually selected male traits (e.g. Morrow *et al.*, 2008). Using hemiclonal analysis, we investigated the effects of manipulating larval density on two aspects of female preference in *D. melanogaster*: female choosiness (*sensu* Narraway *et al.*, 2010; Ratterman *et al.*, 2014) and female responsiveness (*sensu* Rodríguez & Greenfield 2003; Ritchie *et al.*, 2005). In a heterogenous environment, the optimal genotypes may also be constantly changing so that selection may maintain genetic variation for condition and mate choice in a population (Tomkins *et al.*, 2004). Since larval density is known to influence both adult life-history traits (Mueller *et al.*, 1993) and post-copulatory traits (McGraw *et al.*, 2007), we manipulated developmental environment to quantify the standing genetic variation for condition, plasticity in female mate choice, and potential GxE interactions for female choosiness. The choosiness of a given female genotype may depend on whether females developed under high or low larval densities. Based on *a priori* information, we predicted that females reared at high larval densities would be more responsive to male courtship and in theory, less choosey compared to females reared at low larval densities. In addition, we also predicted that the changes in a female hemiclone's choosiness between high and low density environments would be proportional to the changes in the female hemiclone's condition. We also predicted that females reared at high larval densities would experience lower fecundity than females reared at low larval densities.

Materials and Methods

Experimental Populations

The ultimate source of genetic variation in the assay were *D. melanogaster* obtained from the IV (Ives) population; a large (~2800 adults/generation), outbred wild-type population which has been maintained under standardized lab conditions since 1980 (Rose, 1984). This population is maintained in vials on a discrete 14-day culture cycle. Flies are reared at a controlled density (~100 eggs/vial) with a banana/agar/killed yeast medium at 25°C, 60% humidity, on a 12L:12D diurnal light cycle. A replicate population, IV-*bw*, that is maintained under indentical conditions as the IV population, was created by repeatedly backcrossing the recessive brown eyed allele, *bw*, into the IV genetic background 10 times. Finally, the DX-IV population, possessing the "double-X" chromosome but otherwise containing a random sample of autosomes originating from the IV population, is also cultured at similar conditions as the previous two populations and was used in creation of hemiclonal males (*see below*).

Hemiclonal analysis

The contribution of genetic effects, environmental factors, and potential GxEs for phenotypic variation in female choosiness were investigated using hemiclonal analysis. Hemiclonal analysis allows for genetic variation and natural selection to act on the male and female hemiclones (Abbott & Morrow, 2011), increasing the potential for GxE interactions to be manifested. Genetic variation in mating behaviour and female body condition was measured for 11 hemiclone lines which were initially created for a previous experiment that examined genetic variation in female choosiness in a standard developmental environment (*for details see* Tennant *et al.*, 2014). Hemiclonal individuals share a nearly complete (99.5%) haploid genome (Abbott & Morrow, 2011) and these

clone haploid genomes can then be expressed in a "hemiclonal" state in either a male or a female genetic background (consisting of a random sample of wild-type haplotypes sampled from the base IV population; for details see Rice, 1996; Rice *et al.,* 2005; Chippindale *et al.*, 2001; Abbott & Morrow 2011; Tennant *et al.*, 2014).

A previous experiment (Chapter 2; Tennant *et al.,* 2014) quantified the relative "attractiveness" of 12 male hemiclone lines. Males with relatively fast mating speeds were classified as being more "attractive" because it took less time for them (on average) to achieve copulation with a wide range of females (Fulker, 1966) than "unattractive" males, and mating success is an important component of male lifetime reproductive success (Edward *et al.,* 2014). Because it was assumed that all hemiclone females would readily mate with the *most* attractive male genotype, we chose to use males derived from the *least* attractive male hemiclone line in the previous assay to test whether or not condition affected female choosiness and female responsiveness. Responsiveness reflects the likelihood a female will respond to a potential mate, and was quantified as the mean mating speed for female hemiclone line (Ratterman *et al.*, 2014). Choosiness describes the time and effort a female takes to evaluate potential mates, and was interpreted as the standard deviation of the average mating speed or the coefficient of variance (CV) (Gray & Cade, 1999; Brooks & Endler, 2001; Ratterman *et al.*, 2014). Females that are not very choosey will mate with all males in approximately the same amount of time, whereas choosier females will show large variation in mating speed. We therefore examined female choosiness and female responsiveness for 11 different haploid genotypes (female hemiclones from the least attractive male line were excluded) by subjecting all 11 female hemiclone lines to the same male genotype and observed mating speed, copulation

duration, and subsequent egg production. This was done using female hemiclones that experienced one of two different developmental environments (high or low larval density) to determine genetic variation for the aforementioned interacting phenotypes and a potential GxE for female choosiness.

High vs. Low larval density treatments

In *D. melanogaster* developmental environment is associated with competitive larval density. Development at high larval density is known to have substantial consequences on juvenile survivorship (Boggs & Freeman, 2005) and adult life-history traits (Mueller *et al.*, 1993), including reproductive traits (Edward & Chapman, 2012; Morrow *et al.,* 2008; Rode & Morrow, 2009, McGraw *et al.,* 2013). Before initiating this experiment, we explored the effect of larval densities on body size to determine the appropriate larval densities as treatments for the experiment. To prepare the pilot density vials, approximately 100 adult IV flies were each placed into a half-pint egg laying chamber outfitted with a grape juice cookie (Sullivan *et al.,* 2000) and a drop of yeast paste. The following day (day 0), adult flies were discarded and sets of eggs laid by the females were collected from the surface of the grape juice cookies using isotonic egg wash solution (Sullivan *et al.,* 2000) and transferred into the following: 5 vials each containing 300 eggs, 7 vials each containing 200 eggs, and 10 vials each containing 100 eggs, which were then incubated. When the majority of the flies had eclosed from their pupae, flies were sorted by sex from each density treatment. The flies were mechanically separated based on their body size according to their ability to pass through a series of sieves using the Gilson Company Inc. Perfomer III model SS-3 sieve shaker (see Long *et al.*, 2009; Long *et al.*, 2010). Males and females were lightly anaesthetized with CO_2 and

placed in the top of the sieve column. The shaker was run for three, 2-minute intervals. Vibrations reverberated up the chambers ensuring the flies' downward movements through the 12 chambers, each with holes differing in diameter by 5%. In this manner, flies were sorted by sex from large body size to small body size (1420µg - <998µg). Flies that could fit through the electroformed holes fell to lower sieves while larger flies were retained in the higher sieves. The distribution of female body sizes were compared from each larval density and were significantly different from each other ($F=139.1702$, $df=2$, $p<0.0001$; SI Fig 1); larval densities of 200 eggs/vial (mean body size (±SE)=1284.52µg ±5.022) and 300 eggs/vial (mean body size (±SE)=1180.66µg ±6.15) significantly reduced body size in both female *D. melanogaster* adults compared to densities of 100 eggs/vial (mean body size (±SE)=1311.80µg ±5.38). In males, larval densities of 100 eggs/vial (mean body size (±SE)=1201µg ±5.63) and 200 eggs/vial (mean body size (±SE)=1201µg ±5.10) were not significantly different from each other but both were significantly different from 300 eggs/vial (mean body size (±SE)=1153.30µg ±6.05; $F=22.2883$; $df=2$, $p<0.0001$; SI Fig 3.1). This suggests that males were not as sensitive to larval density as females were and is consistent with a previous study (Edward & Chapman, 2012).

To test for condition dependence in mating speed, copulation duration, and egg production, we reared each of the 11 female hemiclone lines in both high (~200 viable eggs/vial) and low (~100 viable eggs/vial) larval density environments. Male hemiclone flies were reared under normal larval density environment (100 viable eggs/vial). In order to ensure a desirable density of 100 viable larvae (low larval density) per vial for hemiclone males, each vial that would yield male hemiclones was created by combining

100 eggs laid by clone-mated DX-IV females (which, due to chromosomal imbalance, experience a 75% egg to larval mortality) and 75 IV-*bw* eggs of the same age. We set up vials so that hemiclone females would experience either a high or low larval density environment (200 viable larvae/vial or 100 viable larvae/vial, respectively). In the low larval density treatment, each vial was created by combining 100 eggs laid by clone-mated IV females (which, due to chromosomal imbalance, experience a 50% egg to larval mortality) with 50 IV-*bw* eggs of the same age. For high larval density treatment, each vial was created by combining 200 eggs laid by clone-mated IV females with 100 IV-*bw* eggs of the same age. These vials were then placed in an incubator where the eggs developed under standard laboratory conditions. Starting 9 days later, approximately 30 individual females from each hemiclone line were collected as virgins (within 8 hours of eclosion from pupae) and held in individual vials before the experiment. Eighteen females from each hemiclone line and treatment were used in no-choice mating assays (see Shackleton *et al.*, 2005; Tennant *et al.*, 2014) while 10 individuals were frozen for a body condition assay (described below). At the same time as virgin female collection, approximately 400 non-virgin hemiclone males (to ensure prior mating experience (Dukas, 2010)), all from the same hemiclone line, were collected and held individually until the assay.

Behavioural assay

The assay began at 9:00am EST, which corresponds to when the incubator lights turn on and sexual activity of the flies increases (H. Tennant, pers. obsv) and was conducted in a well-lit, humidified room at 25°C. Individual pairs of male and female hemiclones were combined without anaesthesia in vials containing a small amount of

media, which were then placed on their side for observation. Mating pairs were observed for 90 min (5400 seconds) and mating speed (the time to begin mating, including courtship) and copulation duration (the length of copulation) were recorded to the nearest second. If copulation was ongoing at the 90 min mark, the mating pair was observed until copulation ended. If no mating was observed within 90 min, we substituted that time (5400 seconds) as the latency to mate for the pair. We quantified female receptivity by including a 1 if mating occurred within the 90min observation period and a 0 if no mating was observed. We assayed mating behaviour with individual males and females (i.e. no choice assay (Shackleton *et al.*, 2005)) which allowed us to avoid confounds of male-male competition in mate choice. In studies using other *Drosophila* species, no-choice vs. choice assays often produced identical results (Taylor *et al.*, 2008; Avent *et al.*, 2008)

Female fecundity

Immediately following the behaviour assay, all males were removed from the vials using light CO_2 anaesthesia. The vials containing only females were placed in the incubator for 24 hours to allow the females to lay eggs before being discarded. The following day, the number of eggs laid by each individual female were counted using a compound light microscope to determine any immediate post-copulatory effects of genetic identity and female body condition on fecundity.

Body condition assay

To determine physical condition of hemiclone females, absolute fat content and relative fat content (RFC) were measured for samples of flies reared under high larval density and low larval density for each of the 11 hemiclone lines. Ten females were collected from each hemiclone line/treatment and individually placed in microcentrifuge

tubes and frozen overnight. The microcentrifuge tubes were then placed in a drying oven with the caps off for 17 hrs at 60°C to remove any excess moisture from the flies. Subsequently, the dry mass of individual females was determined using a Sartoris M5 ultramicrobalance (Gottinger, Germany) to the nearest 0.0001mg.

Fat extraction, following the protocol described in Rode & Morrow (2009), was performed to determine fat content of the female hemiclones. Female hemiclones were individually placed into 4mL glass vials and 2mL of dichloromethane/methanol solvent (2:1) was added into the vial. The vials were capped with Teflon-sealed screw caps and horizontally agitated at a low speed with no heat for a period of 48 hrs. At this point, 95% of the fat was expected to be extracted (Rode & Morrow, 2009; Fischer, 2006). Any remaining solvent was removed using glass eyedroppers and another 2mL of the dichloromethane/methanol solvent was added to the vials. The vials were horizontally agitated for an additional 48 hours before removing all solvent with glass eyedroppers and then placed in the drying oven at 60° for 48hrs. Flies were then individually reweighed (as previously described) to determine the fatless dry mass. The absolute fat content was then determined by subtracting the fatless dry mass from the initial dry mass. The RFC was calculated by dividing the absolute fat content by the dry mass as per Rode & Morrow (2009).

Statistical analysis

All analyses were carried out using JMP version 11.0 (SAS Institute, Cary, NC) and R version 3.0.1 (The R Foundation for Statistical Computing). Weight variables were analysed using a restricted maximum likelihood (REML) approach to construct models in which larval density treatment was treated as a fixed effect, while hemiclone line and the

interaction between hemiclone line and larval density treatment were treated as random effects. Additional models that examined the contribution to hemiclone line (a random effect) on weight variables separately by larval density treatment were also created. The same analysis was performed for mating speed, copulation duration, and egg production. REMLs were created using the lmer function in the lme4 package (Bates *et al*., 2011). Statistical significance of each variable was determined using Log Likelihood Ratio (LLR) tests, implemented by comparing the fit (measured as the deviance) for models with and without the variable being examined.

Female responsiveness was estimated as the mean mating speed of each female hemiclone line from each larval density treatment. We also calculated the standard deviation (SD) of female responsiveness to see if larval density influenced the variance for this trait. Since the data was non-normal, we used Kruskal-Wallis tests to determine whether there was any difference in median between the hemiclone lines for female responsiveness. We then used paired t-tests to assess whether there were differences between high larval density-reared females and low larval density-reared females in the SD of responsiveness and copulation duration. A general linearized model (GLM) with a quasipoisson distribution was used to examine the effects of larval density, female genotype, and their interaction on female egg production. Finally, since mate acceptance is binary (mated=1, unmated=0) we examined the effects of larval density environment, female genotype, and their interaction on female receptivity (mated/unmated) using a GLM with binomial distribution.

Female choosiness for each of the 11 female hemiclone lines was estimated as the coefficient of variance (CV; Brooks & Endler, 2001; Tennant *et al.*, 2014) and was

calculated as the standard deviation of the mean mating speed for female hemiclone line from each larval density treatment. To determine whether female choosiness differed among females between the two larval densities, we conducted a paired t-test on our estimates of choosiness (CV) for each hemiclone line measured under each larval density. We also estimated the linear regression and the Pearson's product-moment correlation for female choosiness measured under both larval densities. All non-mating pairs were included in the statistical analysis with a substituted value of 5400 seconds. For simplicity, we refer to female responsiveness and female choosiness in combination as female preference in the results and discussion.

Results

Larval density effect

The variance components calculated among the 11 hemiclone lines for dry mass, fatless dry mass, and absolute fat content were significantly different from zero (Table 3.1). Larval density had a significant effect on female dry body mass (LLR χ^2=10.6, df=1, p=0.0011), as females developing under high densities weighed significantly less than females developing under low larval densities (mean mg ±SE; high: 0.273 ±0.006; low: 0.305 ±0.009). Larval density had a significant effect on female fatless dry body mass (LLR χ^2=5.9, df=1, p=0.0147), as females developing under high densities weighed significantly less than females developing under low larval densities (mean mg ±SE; high: 0.206 ±0.0047; low: 0.227 ±0.0077). Larval density had a significant effect on the absolute amount of fat in females (LLR χ^2= 9.8, df=1, p=0.0017), as females developing under high densities weighed significantly less than females developing under low larval densities (mean mg ±SE; high: 0.066 ±0.0035; low: 0.078 ±0.0039). There was no

significant difference in the relative fat content (RFC) of females developing at high and low larval densities (LLR χ^2= 2.17, df=1, p=0.1407), (mean mg ±SE; high: 0.245 ±0.01029; low: 0.264 ±0.0121).

Female mating behaviour

We found no significant difference among the 11 hemiclone lines in the proportion of female genotypes that successfully mated between the two larval densities, except in one hemiclone line (# 5). This line had a high number of non-mating individuals, which were assigned a value of 5400 seconds (corresponding to the period of observation), and may explain why female genotype significantly influenced female mating speed (K-W χ^2 = 37.2169, df = 10, p<0.0001) and the high average mating speed for females of this particular genotype reared at high larval densities (Fig 3.1). REML analysis did not reveal that female genotype or the interaction between female genotype and larval density significantly contributed to phenotypic variation for female responsiveness (Table 3.2). Larval density also had no significant effect on mating speed (LLR χ^2= 0.0395, df=1, p= 0.8425), (mean ±SE; high: 3464.1s ±117.8; low: 3401.2s ±121.7) and we found no evidence of a GxE for female responsiveness (t = 1.5279, df = 10, p= 0.1575).

Larval density had a significant effect on copulation duration (LLR χ^2=10.6, df=1, p=0.0011; Table 3.2) as females who developed under high larval densities copulated for a significantly shorter period of time than females who developed under low larval densities (mean ±SE; high: 1042.43s ±277.28; low: 1129.93s±290.00; Fig 3.2). However, we found no GxE interaction for copulation duration and female genotype did not contribute to the phenotypic variation for this mating behaviour (Table 3.2).

GxE for female choosiness

We did not find a statistically significant correlation for female choosiness (CV) between female hemiclones from high larval density vials and low larval density vials (n=11, r=0.506, p= 0.112; Fig 3.3), suggesting that the degree of choosiness exhibited by female genotype was robust compared to the larval densities. A paired t test of the two sets of CV's indicated that there was no significant difference between estimates of female choosiness between hemiclone females in the two larval density treatments (t = -0.5799, df = 10, p= 0.5748).

Female fecundity

Larval density had no significant effect on the number of eggs laid by females after 1 mating (LLR χ^2= 0.0395, df=1, p= 0.8425), (mean ±SE; high: 40.06±15.10; low: 40.42±14.97; Table 3.2). When treated as a fixed effect, the interaction between female genotype and larval density significantly influenced egg number (F=1.954, df=10, p= 0.0384). Large individual variation for 1 hemiclone line (#1) likely influenced this result since the interaction between female genotype and larval density environment explained only 6.62% of the variance in egg production after 1 mating (Table 3.2).

Discussion

It is perhaps inevitable that individuals will differ in their condition, resulting from heterogeny in their genetic and environmental backgrounds, which will potentially create differences in their ability to assess potential mates and express any preferences (Widemo & Sæther, 1999). This ultimately leads to phenotypic variation in female choosiness and female preference functions (Jennions & Petrie, 1997; Widemo & Sæther, 1999; Brooks & Endler, 2001). It has been hypothesized that females in poor condition

will be less choosey than females in good condition due to the decreased ability to withstand the (perhaps substantial) costs associated with mate choice (Cotton *et al.*, 2006). However, some studies have recently found that females in poor condition have stronger mate preferences (Fisher & Rosenthal, 2006; Griggio & Hoi, 2010; Tobler *et al.*, 2011) than those in good condition, suggesting that the relationship between condition-dependence and female mate choice is much more complex than initially thought. Our results indicate that some components of female preference may be insensitive to variation in individual condition and female choosiness may be canalized with female genotype.

Larval density effects

Previously, it has been demonstrated that laboratory-bred populations of fruit flies reared at high larval densities experienced greater intraspecific competition for limited resources, adversely affecting their condition – a result that is consistent with those of our study (Mueller *et al.*, 1993; Byrne & Rice, 2006; McGraw *et al.*, 2007; Rode & Morrow, 2009). We found a significant effect of larval density treatment on female dry mass, fatless dry mass, and absolute fat content. The decrease in these indices of female condition from flies reared at high larval density is not altogether surprising, as a decrease in female body size may be a consequence of the nutritional constraints to larval development in high larval density conditions (Honěk, 1993). These females may also experience faster development time, which is correlated with smaller body size. Faster development, and therefore possible early eclosion rate, gives small-bodied females the opportunity to avoid expected competition from high larval density conditions and are able to acquire resources while large-bodied individuals experience a slower eclosion rate

(Honěk 1993). Additionally, while larval survivorship was not examined in this study, it has been shown to be positively correlated to fat stores in *Drosophila* (Clark, 1989). The effects of larval density and thus, larval resource availability, on female body condition highlights the importance that environmental condition has on shaping adult life-history traits and fitness – possibly greater than any genetic effects (*see* Rode & Morrow, 2009). Despite the sizeable effect of density on dry mass, fatless dry mass, and absolute fat content, RFC (an index derived by dividing dry mass by absolute fat content) did *not* differ between females reared under different larval density environments, suggesting that RFC may be an inappropriate index of condition or that female hemiclones from each larval density treatment did not differ in RFC.

No effect of density or a GxE for female responsiveness

We were able to test for the effects of female condition on female responsiveness using the average mating speed for female genotype from each larval density. While in our study larval density did not influence female responsiveness, others have demonstrated that female responsiveness can be strongly influenced by environmental conditions (Narraway *et al.,* 2010; Syriatowicz & Brooks, 2004; Hunt *et al.,* 2005). We found that female genotype only contributed to a small percentage of the total phenotypic variation (5.84%) in female responsiveness in hemiclonal *D. melanogaster,* indicating this mating behaviour may not be primarily under female control (as previously suggested by Heisler (1984) and Pischedda *et al.,* (2012)). The lack of any significant influence of female genotype on female responsiveness in our study contradicts previous studies whose authors found a significant effect of genotype on mating speed (Pischedda *et al.,* 2012; Tennant *et al.,* 2014). In the present study this is likely explained by the large

amount of individual variation we found in our female hemiclones in one assay, as opposed to average values based on multiple individuals in replicate blocks (*see* Tennant *et al.*, 2014).

To the best of our knowledge, only one study has found a GxE interaction for female responsiveness (Rodríguez & Greenfield, 2003), and the authors indicate that divergent selection may act on sexually selected male and female traits if the level of environmental change is high. Thus, population differentiation may be influenced by GxE if it maintains genetic variation in female preference and sexually selected male traits. On the other hand, a lack of a GxE interaction for female responsiveness suggests that individual variation in male courtship may also be reducing the variance in mating speed (Casares *et al.,* 1992; Narraway *et al.,* 2010) so that the variation in mating speed between female genotypes reared at the two different larval densities is not significantly different. We predicted that low larval density-reared females would have much more variation in mating speed (i.e. show greater choosiness than high larval density-reared females). This was not the case and suggests that GxE interactions may not provide a mechanism to maintain genetic variation in female responsiveness (*but see* Jia *et al.*, 2000; Rodríguez & Greenfield, 2003; Narraway *et al.,* 2010) Furthermore, this female mating behaviour may be independent of condition.

Female condition influenced copulation duration

Copulation duration varied with female condition in a manner consistent with previous studies (Lefranc & Bundgaard 2000; Byrne & Rice, 2006; Friberg, 2006). When experimentally manipulating body size using high larval density vials, Lefranc & Bundgaard (2000) found that small females had the shortest mean copulation duration

compared to both medium- and large-bodied females, irrespective of male size. Similarly, Byrne & Rice (2006) found that in no-choice assays small bodied females (also created by manipulating larval density) copulated more rapidly. These results suggest that males may prolong their copulation with large, fecund females in order to ensure greater mating success (i.e. transfer more sperm and/or accessory proteins (ACPs) to ensure paternity and reduce female remating (Wigby & Chapman, 2005; Bretman *et al.,* 2009)). Males may also be exerting their own mate choice and may not be as "vigorous" when courting females in poor condition (Long *et al.,* 2009). Consequently, they copulate faster with small-bodied females, potentially due to lower fecundity than large-bodied females (Byrne and Rice, 2006; Long *et al,* 2009). This may not result in observed changes in female choosiness (*see below*); the present study does not take male mate choice into consideration and an absence of a GxE interaction for female choosiness may suggest that differences in male courtship efforts are potentially confounding our estimates of CV (choosiness).

No GxE for female choosiness

GxEs influence trait expression so that individuals with identical genotypes may exhibit different phenotypes when exposed to different environments (Ingleby *et al.,* 2010). Our experimental manipulation of larval density did not alter either female choosiness (CV) or female receptivity (mated/not mated) for the 11 hemiclone lines surveyed, and as such, we were unable to reject our null hypothesis. There are a number of possible explanations for why we did not see females become more receptive/less choosey when reared at high larval density conditions. The first possibility is that the female hemiclones reared at high larval density were not actually in 'worse' condition

than the female hemiclones reared at low larval density. While it is true that they were smaller in body size and had less fat, the fixed effect of larval density on RFC was non-significant and the interaction between larval density and hemiclone line was marginally significant, indicating that RFC may not be the best index for quantifying condition *or* alternatively, that all female hemiclones were equally influenced by the larval density treatments. Furthermore, even if fat content is an essential indicator of condition and/or female fecundity, there may be associated costs with life-history traits which may complicate the relationship between fecundity and fitness, such as age, longevity, nutrition etc. (*but see* Barnes *et al.,* 2008). The lack of empirical evidence linking female fat content and female choosiness in *D. melanogaster* and the challenge of finding an appropriate proxy for condition (Rode & Morrow, 2009) makes it difficult to make any clear explanation for why no GxE interaction was discovered.

Secondly, it is also worth considering the (reasonable) possibility that female choosiness is not actually condition-dependent. Others have demonstrated that condition-dependence arises when females in good condition are better able to withstand the potential costs associated with mate choice (Cotton *et al.,* 2006). Our results elucidate that even in the face of significant differences in female condition associated with their developmental environment females are still quite capable of being choosey. Delcourt *et al.,* (2010) examined female preference for male CHCs using choice trials in *Drosophila serrata* females exposed to a yeast diet (which the population was adapted to) and a novel corn diet. The authors found that although the corn diet decreased female productivity, preference expression in *D. serrata* was independent of female condition; the combination of male CHCs that a female prefers did not depend on her condition or

resource availability. Similarly, Syriatowicz & Brooks (2004) did not find any evidence of condition-dependence for female preference functions in guppies, *Poecilia reticulata*, when manipulating adult diet. Not only did females in good and poor condition prefer the same male ornaments, they preferred the same individual males. Similarly, our results suggest that females in poor condition appeared just as capable in assessing and accepting male courtship as females in good condition.

Another plausible explanation is that our measurement of choosiness was inadequate. As this was a no-choice assay, females may not have had the opportunity to truly express their choosiness, as there were no competing males to choose between. Since there was only one (unattractive) male to mate with, there may have been low costs to mate assessment, giving both good and poor condition females the same threshold in terms of assessing and responding to male courtship. It is also possible that females did not incur any great costs for being choosey in our study. Previously, Narraway *et al.*, (2010) used no-choice assays including both attractive and unattractive males and found a strong GxE for female choosiness. Conversely, Byrne & Rice (2006) discovered that although males copulated more with large bodied females than small bodied females, when there was no choice between the two body sizes, the difference was not statistically significant. Furthermore, females may vary their choosiness based on variation in male courtship behaviour directed towards them (Burley & Foster, 2006). We designed the experiment to minimize male courtship variation and used males of the same "unattractiveness" to measure only GxE effects on female choosiness. It is possible that, as previously mentioned, the significant effect of larval density on variation in copulation duration (a trait primarily under male control (Friberg, 2006)) suggests a large

unidentified effect of pre-copulatory male mate choice subsequently confounding our estimates of female choosiness. A final possibility is that the study lacked statistical power. We only investigated a GxE for female choosiness using 11 hemiclone lines, and only used 18 females per larval density treatment and hemiclone line to investigate the effect of larval density on body condition. These samples may be too small to demonstrate a sizeable GxE interaction for female choosiness.

The absence of a GxE for female choosiness was somewhat surprising as it stands in contrast to the results of other studies that found variation in the strength of choosiness or preference functions when female condition was manipulated (*reviewed in* Cotton *et al.,* 2006; also see Hunt *et al.,* 2005; Rodríguez & Greenfield, 2003; Narraway *et al.,* 2010; Ingleby *et al.,* 2013). Theoretical models highlight the potential importance of GxEs for female mate preference to evolution via sexual selection. For example, a GxE interaction for female choosiness may influence the direction of sexual selection acting within a population if there is high genetic variation among individual females for choosiness by delaying the loss of genetic variation (Ingleby *et al.,* 2010; Syriatowicz & Brooks, 2010). Furthermore, the importance of female preference GxEs and sexually selected male trait GxEs should be considered in the process of maintaining genetic variation even in the face of strong sexual selection. GxEs may influence variation in selection acting on both males and females in a population (Rodríguez & Greenfield, 2003) and by assessing genetic associations between male and female sexually selected traits, empiricists may determine how GxEs influence the coevolutionary dynamics between female preference and sexually selected male traits (Ingleby *et al.,* 2010).

Female fecundity is not condition-dependent

Female body size and fecundity are often predicted to be positively correlated under constant environmental conditions (Evans, 1982; Gilbert, 1984). This positive relationship between body size and egg production is predicted as eggs are energetically costly to produce and larger females may be better able to overcome this cost (Partridge, 1986). For example, in *D. melanogaster*, Lefranc & Bundgaard (2000) found that fecundity was dependent upon female body size (larger females were more fecund and contained more ovarioles). Since female body size and fecundity are influenced by genetic factors and environmental conditions and fecundity is also dependent on the environmental conditions during oviposition, (Honěk, 1993), it is possible to test the general relationship between female size and fecundity in experimental conditions. We predicted that female genotypes reared at high larval density would produce fewer eggs after a single mating than female genotypes reared at low larval density, due to the differences in female body size/condition. In laboratory conditions, female *D. melanogaster* allocate greater energy to reproduction than somatic maintenance (Baldal *et al.,* 2010) and given the fact that stored lipids from fat bodies are important for egg production in insects (Arrese & Soulages, 2010), as female condition decreased (i.e. changes in fat content due to larval crowding at high density) the number of eggs produced should also decrease. Our results however, did not indicate a significant difference in the number off eggs produced by females reared at the two different larval densities.

There are several reasons why female fecundity after a single mating may show little correlation with female body size/condition when the risk of egg limitation is low. Smith and Fretwell (1974) proposed that females within populations that experience

similar environmental conditions all produce the same size/number of eggs, independent of female body size. In our experiment, the females were not subjected to the crowded environments during or post-mating, and they may have had the opportunity to replenish or overcome any fitness costs of reproduction. The oviposition environments were those of standard laboratory conditions and the females were alone for their oviposition (the males were removed), thus there was no competition for resources during oviposition and this could explain why there was no significant difference in egg production. When oviposition sites are limited (i.e. larval crowding), offspring competition may impose an upper limit to clutch size and variation in egg production may occur. For example, Edward & Chapman (2012) found that female fecundity was the most sensitive reproductive trait when females were reared at various larval densities. Female fecundity followed a nonlinear pattern; it significantly increased as larval density increased from 50 to 200 larvae per vial but then declined at densities above 300 larvae per vial (up to 1000 larvae per vial). This suggests that when females experienced a competitive environment (high larval density) and the amount of resources was limited, the ability to allocate resources to egg production decreased. This has been reported in other insects and indicates potential facilitation between larvae during development (Fletcher, 2009; Ronnâs *et al.*, 2010; Edward & Chapman, 2012). Intraspecific interactions may therefore shift with changing environmental conditions (i.e. larval crowding, competition for oviposition sites).

Conclusion

Theoretical and empirical work suggests that GxEs in the context of sexual selection may be fundamental in maintaining variation in sexually selected male traits,

female choosiness, and the potential coevolution between them (Ingleby *et al.*, 2010). Contrary to our *a priori* predictions, we did not find a GxE interaction for female choosiness; the choosiness of a given female genotype did not depend on whether females were reared in high larval density or low larval density environments. Female choosiness may not be as plastic as previously predicted; rather genotypes may experience canalized female choosiness for *D. melanogaster* in fluctuating environmental conditions. On the other hand, species encountering highly variable social environments may display adaptive plasticity in mate choice (Kokko & Heubel, 2008). This is especially likely when mate choice confers direct benefits. When there are only indirect genetic benefits to mate choice, which is the case with *D. melanogaster*, the situation is more complicated because the influences of male mate choice are not considered in GxE interactions for female mating behaviour.

The fitness consequences of a female's mating decision may drive the evolution of mate choice and therefore it is likely that GxEs in components of female preference could influence mate choice evolution (Ingleby *et al.*, 2010). The next step is to determine whether female preference GxEs alter the fitness consequences of female mate choice. Further studies examining GxE for female mate choice under a variety of conditions, including integrating GxEs for sexually selected male traits and male mate choice, will be beneficial in demonstrating possible existing patterns of condition-dependent genetic variation, environmental variation, and a combination of the two, for mating behaviours and other sexually selected traits.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

HMET and TAFL conceived of the study. All authors helped design and conduct the experiment. TAFL performed the statistical analysis. HMET and EES carried out the body condition assay. HMET drafted the manuscript. All authors read and approved the final manuscript.

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Tables and Figures

Table 3.1: Decomposition of the phenotypic variation using REML for dry mass, fatless dry mass, absolute fat content, and relative fat content (RFC) for 11 female hemiclone lines reared at two different larval densities.

Response Variable	Model	Random Effect	Variance	SD	% Variance Explained	LLR χ^2	p
Dry mass	Combined	Genotype	0.00027	0.016334	12.310	2.2448	0.1341
		Density x Genotype	0.00016	0.012829	7.591	1.6738	0.1958
		Residual	0.00174	0.041667	80.099		
	High Density	Genotype	0.00023	0.015237	11.241	3.3853	0.0657
		Residual	0.001833	0.042815	88.759		
	Low Density	Genotype	0.0006313	0.025126	27.774	15.579	7.909×10^{-5}
		Residual	0.001641	0.040517	72.226		
Fatless dry mass	Combined	Genotype	0.0001555	0.012472	8.943	5.9884	0.0500
		Density x Genotype	0.0001427	0.011947	8.206	14.945	0.0057
		Residual	0.001440				
	High Density	Genotype	1.1685×10^{-13}	3.4183×10^{-7}	2.278	2.657404×10^{-7}	0.9996
		Residual	1.6692×10^{-3}	4.0856×10^{-2}	97.722		
	Low Density	Genotype	0.00052994	0.023021	29.473	17.96343	2.2518×10^{-5}
		Residual	0.00126810	0.035610	70.527		
Absolute fat	Combined	Genotype	2.7286×10^{-5}	0.0052236	21.634	5.1186	0.0236
		Density x Genotype	2.7286×10^{-5}	0.0052236	6.281	1.4982	0.2209
		Residual	3.1311×10^{-4}	0.0176950			

	High Density	Genotype	0.00011956	0.010934	35.194	24.09105	$9.1886x10^{-7}$
		Residual	0.00022015	0.014837	64.806		
	Low Density	Genotype	0.00012394	0.011133	23.596	12.06106	0.0005
		Residual	0.0004013	0.020032	76.404		
RFC	Combined	Genotype	0.00047558	0.021808	10.570	1.3802	0.2401
		Density x Genotype	0.00054644	0.023376	12.147	4.4577	0.0347
		Residual	0.00347741	0.058970	77.286		
	High Density	Genotype	0.00011956	0.010934	22.167	10.81058	0.0010
		Residual	0.00022015	0.014837	77.833		
	Low Density	Genotype	0.00012394	0.011133	23.294	12.17083	0.0005
		Residual	0.00040130	0.020032	76.706		

Table 3.2: Decomposition of variance components of REML analysis models examining the sources of phenotypic variation.

Response Variable	Model	Random Effect	Variance	SD	% Variance Explained	LLR χ^2	p
Mating Latency	Combined	Genotype	166725	408.32	5.84	1.7619	0.1844
		Density x Genotype	124060	352.22	4.34	1.7318	0.1882
		Residual	2566141	1601.92	89.82		
Copulation Duration	Combined	Genotype	0.00	0.00	1.12	>0.001	0.9997
		Density x Genotype	0.00	0.00	0.00	0.00	1.000
		Residual	80428	283.6	98.88		
Eggs laid	Combined	Genotype	0.000	0.000	0.00	0.00	1.000
		Density x Genotype	10.003	3.1628	6.62	1.4605	0.2269
		Residual	216.947	14.7291	93.38		

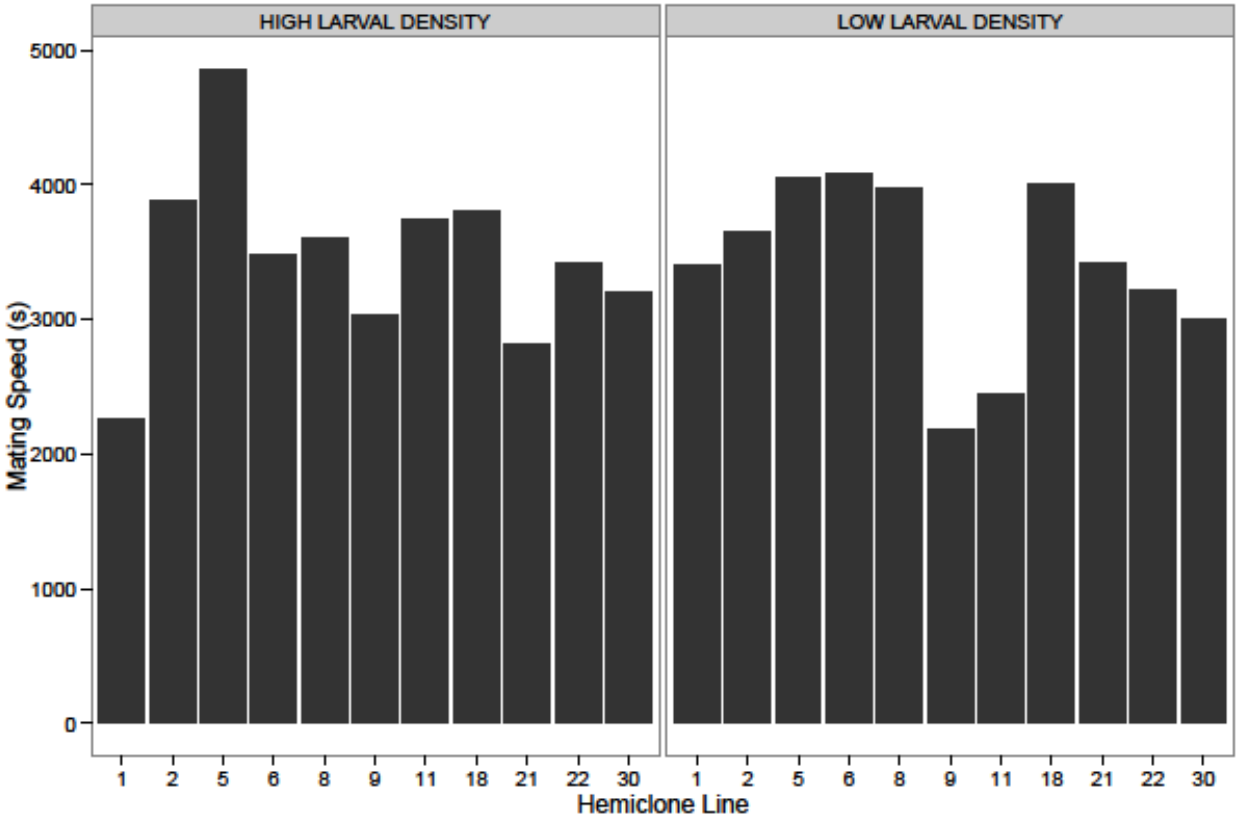


Figure 3.1: No GxE interaction for female responsiveness. Larval density did not have a significant effect on female responsiveness (t = 1.5279, df = 10, p= 0.1575). Although female genotype was found to significantly influence variation in female responsiveness (χ^2 = 37.2169, df = 10, p<0.0001), this was likely due to the large amount of individual variation seen in hemiclone line 5.

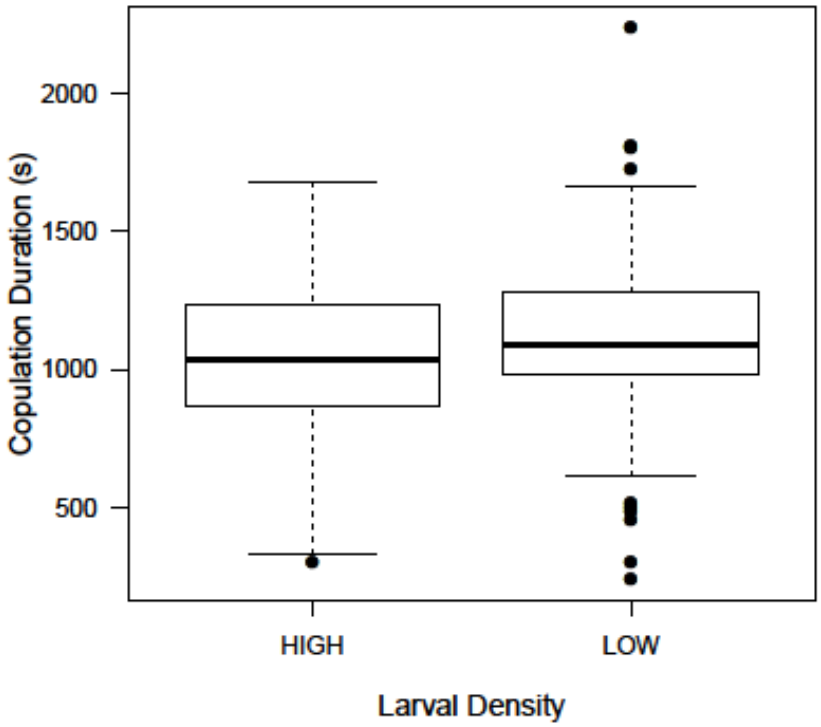


Figure 3.2: Larval density influenced copulation duration. Female hemiclones reared at low larval densities copulated for significantly longer than female hemiclones reared at high larval densities (F=5.2740, df=1, p=0.0224). This suggests that larval rearing environment influences female condition and that males can potentially "tell" the difference between females in good and poor condition and mate longer with more fecund (attractive) females.

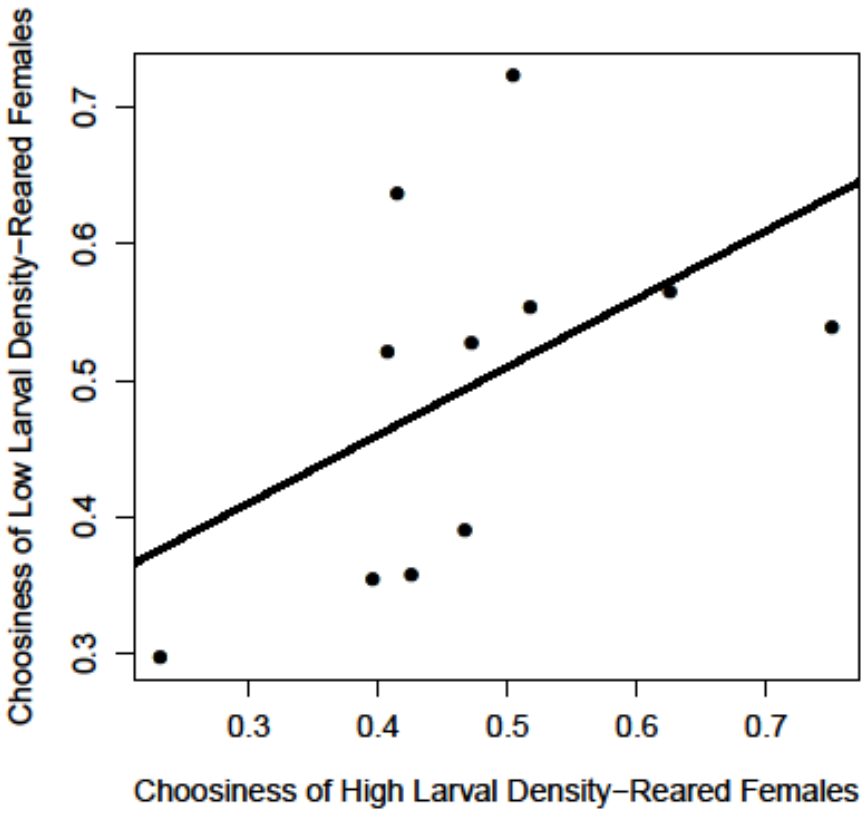
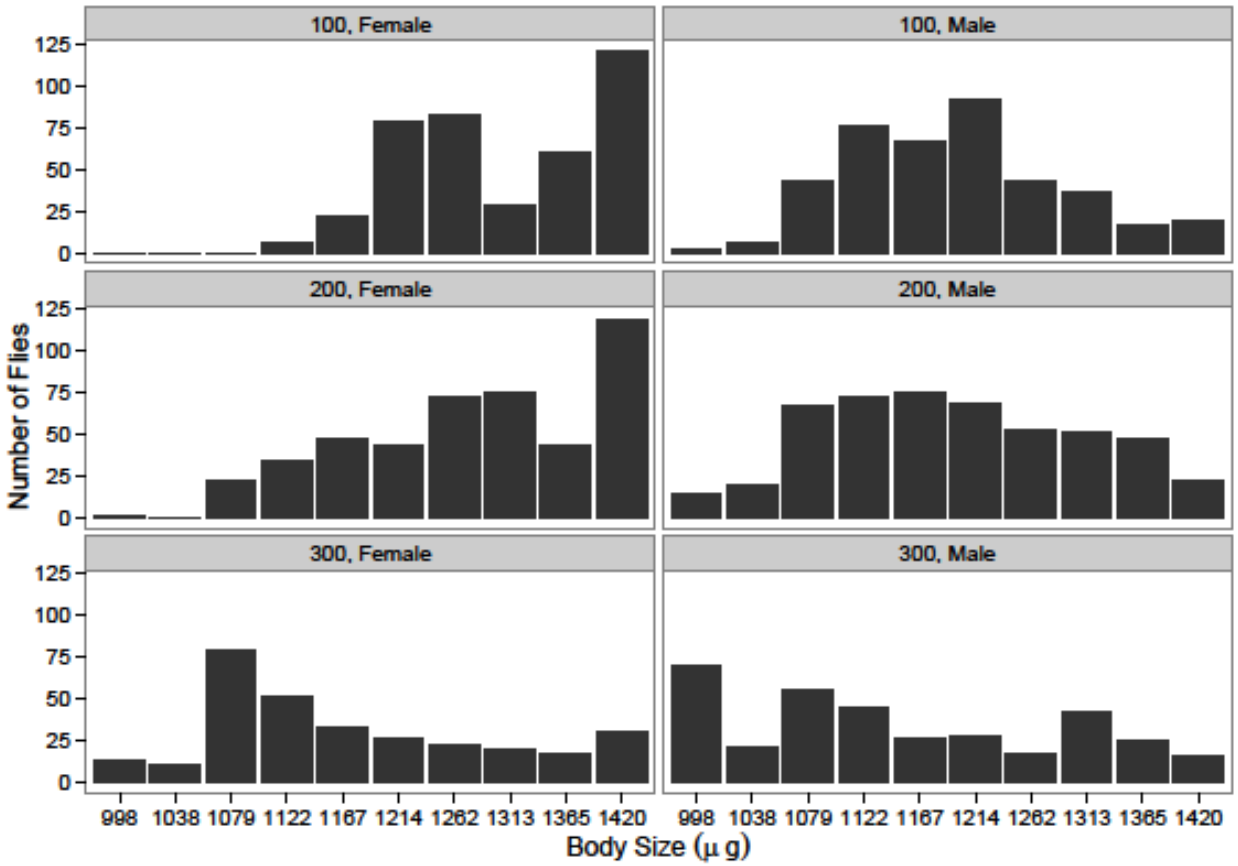


Figure 3.3: Female genotypes reared at two different larval densities did not differ in levels of choosiness. We found a positive, but not statistically significant, correlation for our estimates of choosiness (coefficient of variance; CV) between low larval density-reared females and high larval density-reared females for all 11 hemiclone lines. This relationship indicates that larval density conditions did not influence levels of female choosiness (n=11, r=0.506, p=0.112); choosey female genotypes that were choosey when reared at high larval densities were also choosey when reared at low larval densities, and vice versa.



SI Figure 3.1: High larval densities significantly reduced adult body size. Larval densities of 200 viable eggs/vial and 300 eggs/vial produced significantly greater numbers of small-bodied females than larval densities of 100 viable eggs/vial ($F=139.1702$, $df=2$, $p<0.0001$). Larval densities of 200 and 300 eggs/vial both produced significantly smaller bodied males than larval densities of 100 eggs/vial ($F=22.2883$, $df=2$, $p<0.0001$).

CHAPTER 4:

MATE CHOICE AS AN INTERACTING PHENOTYPE

The primary goal of this study was to examine the causes and consequences of variation in female mate choice and the coevolutionary dynamics between female preferences and heritable male attractiveness. The study also sought to determine whether female choosiness, one component of female preference, exhibits phenotypic plasticity. While predictions surrounding the maintenance of genetic variation in female preference are theoretically abundant, consistent empirical data is sorely lacking. Part of this scarcity stems from the difficulty in accurately and meaningfully measuring female mate preferences. Using hemiclonal analysis, we were able to observe mating patterns and behavioural interactions of particular mating pairs. Exposing the same female genotype to multiple male genotypes allowed us to determine consistency and/or genetic variability in female preference.

We were able to quantify genetic variation underlying phenotypic variation for female choosiness and female responsiveness due to male and female genotype (individually, but no interaction) in Chapter 2. The lack of an interaction between male and female genotypes for mating speed variation in the first experiment suggests that these factors operate independently of each other, supporting the prediction that this trait is primarily controlled by female genotype. Genetic variation in female responsiveness and female choosiness may be maintained via a positive genetic covariance (when two phenotypes are affected by the same gene) between male attractiveness and female choosiness. In order to have a genetic correlation, sexually selected male traits and female choosiness both need to be heritable and repeatable. In contrast to our *a priori*

prediction, we found a negative genetic correlation between female choosiness and male attractiveness. As a side effect of their constant selective environment, lab-adapted populations may only have sexually antagonistic allelic effects acting on them instead of any outside genetic variation, which suggests sexual conflict is acting on our hemiclonal male and female *D. melanogaster*.

Our Chapter 2 results also did not find any significant negative trade-offs between female fecundity and egg size. Genetic variation among female genotypes for egg production and provisioning and genetic variation among male genotypes for stimulation of female egg production and provisioning suggests that certain genotypes may only be able to stimulate either egg production or provisioning, but not both traits simultaneously. The combined genetic identities of mating pairs (genotype-by-genotype interaction) had a significant effect on the amount and/or quality of resources a female will invest into her offspring. The interaction of male and female genotypes influencing fecundity and/or offspring size can result in coevolution between males and females for investment into shared reproductive success. Furthermore, there was no significant GxE for female fecundity in Chapter 3, suggesting that this trait may either be largely independent of female body size, or that female fecundity does not vary when oviposition conditions are good (i.e. no males present, absence of competition). These results may again highlight the importance of social factors over developmental factors for reproductive success.

We found little evidence that female choosiness was condition dependent in Chapter 3. A negative correlation between coefficient of variance (CV) for females reared at low and high larval densities would have indicated differences in levels of choosiness. Instead, a positive (but not statistically significant) genetic correlation suggests that

female choosiness is independent of female condition. Furthermore, the lack of a GxE interaction suggests that differences in larval densities do not change the expression of this mating behaviour. It was more likely that social environment (i.e. mating partners) had a greater influence on female choosiness than developmental environment. Copulation duration, a trait primarily controlled by male genotype, was the only trait significantly influenced by larval density. This suggests that even though larval density may initially contribute to variation in female condition, social factors experienced in adult life have more of an impact on shaping female choosiness and female responsiveness than developmental conditions. There was also no significant difference for female responsiveness between individual hemiclones from different larval densities, suggesting that individuals were much more variable in responsiveness than the mean variation measured in Chapter 2. More empirical work on female condition and the potential effects on female choosiness are needed to strengthen predictions of GxEs for sexually selected traits.

Limitations

It is important to address the inconsistency in the terminology used in the literature to define aspects of female mate choice. This becomes a problem when references which are describing one aspect of mate choice or phenotype are cited as describing something completely different. Similarly, two studies will often examine the same behavioural architecture of female mate choice but the general conclusions may be obscured when discussed in different manors. In this thesis I used strict operational definitions that identified the measurable components of female mate choice, namely female choosiness. However, using mating speed alone may not have fully demonstrated

how females rank male phenotypes or variation in female choosiness since it did not take into account courtship latency (time to initiate courtship) or the courtship duration separate from each other. Some female genotypes may spend only a fraction of the total mating speed being courted by males (non-choosey females) while others may be courted for the majority of the time before accepting a mate (choosey females). Partitioning the time to courtship and then courtship duration until mating might improve future estimates of female choosiness.

Limitations may also exist in our experimental no-choice design. While using a no-choice assay was a starting point for this type of labour-intensive experiment, it is the best method possible (to our knowledge) to avoid potential confounds from male-male competition when examining causes of variation in female preference. Future experimental designs should consider alternative ways of conducting choice trials while avoiding male-male competition.

We had originally conducted the experiment to determine whether or not a positive genetic correlation exists between female choosiness and male attractiveness, as predicted by the Fisherian model of sexual selection. By avoiding any inbreeding effects i.e. not mating individual males and females from the same hemiclone line, we may have inadvertently ruled out Fisherian trends if a female did not have the opportunity to mate with a male that she found attractive (preference alleles are for specific male traits). There is also the possibility that female choosiness was overriding male attractiveness in this case and may be another reason we found a strong negative correlation between female choosiness and male attractiveness.

Future directions

Some of the work in this thesis highlights intriguing possibilities for follow up studies. I can envision 5 lines of investigation that build on the work already completed.

1. Choice trials to examine causes and consequences of variation in female preference. Male-male competition may influence variation in female choosiness differently than with no-choice trials (likely increasing choosiness). These results may support the findings in this thesis or provide an alternative route for investigating female choosiness.
2. GxE interaction study for female choosiness with more statistical power. By increasing the amount of individuals sampled for each hemiclone line and treatment in replicate experimental blocks we may be able to achieve a clear picture of how condition dependence affects female choosiness, as opposed to using only individuals from one experiment.
3. Condition dependent study for female preference using choice trials. By increasing the power of the study and examining male-male competition and allowing females to choose between attractive and unattractive males we can determine how social interactions (environment) *and* genotype influence female choosiness.
4. Condition-dependence of female fecundity. Similar to what was done in Chapter 2, measure length and width of eggs from a single mating to determine whether or not negative trade-offs exist for egg size (volume) and number.
5. Have a control environment for females in varying condition (i.e. poor vs. good) to lay eggs, and a competitive environment for females in both conditions to lay eggs to determine differences due to resource availability during oviposition.

Another consideration is the use of hemiclones vs. other lab populations i.e. IV or DX-IV in terms of "healthiness" in mate choice trials. It may be interesting to expand the results from hemiclonal analysis to standard lab-cultured *D. melanogaster* by using no-choice trials first with female hemiclones mated to IV males, and then reversing and using IV females with hemiclone males (or more likely, DX-IV females).

Integrative summary

This project involved integrating genetic techniques with behavioural assays for a more complete picture of genetic variation in female mate choice. I examined genetic variation for female choosiness at both the individual-level (Chapter 3) and population-level (Chapter 2) in addition to examining individual condition and potential condition-dependent GxE interactions in female choosiness as a possible mechanism for the maintenance of genetic variation at the individual level. Ultimately, variation in female choosiness can affect the strength, direction, and nature of sexual selection acting on sexually selected male traits (usually decreasing the overall strength), which can affect male courtship displays and, indirectly, the female's responses to them. The variance and covariance between these traits of interest determines the extent and nature of sexual selection and may influence population divergence and speciation. These chapters examining the causes and consequences of female mate choice will contribute to the knowledge of the genetic basis of female mate choice, potential fitness consequences, and its role in the evolutionary process of sexual selection.