The effects of sexual selection and ecology on adaptation and diversification in *Drosophila melanogaster*

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Abstract

Sexual selection is pervasive in nature and plays an important role in the evolution of biological diversity both within and among sexual species. However, while we have a good understanding of how competition for reproductive opportunities and mate choice can drive the evolution of exaggerated secondary sexual traits, much less is known about how sexual selection interacts with other forms of natural selection and the consequences such interactions may have for adaptation to novel environments, the purging of deleterious mutations, and population divergence/speciation. In my thesis, I carried out a series of experiments with the fruit fly *Drosophila melanogaster* to test hypotheses regarding the operation of sexual selection and to broaden our understanding of how sexual selection may influence adaptation and diversification. Theory suggests that natural and sexual selection may align to promote adaptation and the purging of deleterious mutations, although the harm imposed by sexual conflict may counter this. In two separate experiments, I find no evidence that sexual selection promotes adaptation to a novel environment and, rather than aligning with natural selection, I find that the effects of sexual conflict may cause sexual selection to hamper the purging of deleterious mutations. With respect to diversification, sexual conflict has been suggested to be an important, non-ecological driver of population divergence. However, the traits involved in sexual conflict may also affect nonsexual fitness and natural selection may therefore act to constrain diversification. Using an evolution experiment, I demonstrate ecologically-dependent parallel evolution of traits involved in sexual conflict, providing evidence for ecology's importance in divergence via sexual conflict. Overall, my work has shed light on the interaction of natural and sexual selection and the consequences this may have beyond the evolution of exaggerated sexual displays and armaments.

Résumé

La sélection sexuelle est largement répandue dans la nature et joue un rôle important dans l'évolution de la biodiversité intra- et inter-spécifique. Alors que nous avons une bonne compréhension de l'effet de la compétition pour l'accès à la reproduction et du choix du partenaire sur l'évolution de caractères sexuels secondaires extravagants, nous avons une méconnaissance de l'interaction entre la sélection sexuelle les autres formes de sélection naturelle, ainsi que de l'effet de ces interactions sur l'adaptation à un environnement nouveau, la purge des mutations délétères ou sur le processus de divergence/spéciation. Durant ma thèse, j'ai mené une série d'expérience sur la mouche du vinaigre, *Drosophila melanogaster* testant certaines hypothèses sur la sélection sexuelle afin de mieux comprendre son influence sur les processus d'adaptation et de diversification. La théorie suggère que les sélections naturelle et sexuelle peuvent agir dans la même direction en favorisant l'adaptation et la purge de mutations délétères, même si les conflits sexuels peuvent s'opposer à cette action conjointe. Lors de deux expériences indépendantes, je n'ai trouvé aucun effet favorable de la sélection sexuelle sur l'adaptation à un environnement nouveau. Contrairement à une action en conjonction avec la sélection naturelle, j'ai découvert que les conflits sexuels et la sélection sexuelle peuvent réduire l'effet de la sélection naturelle sur la purge des mutations délétères. Quant à la diversification, certaines études suggèrent que les conflits sexuels représentent des processus "non-écologiques" capables de conduire à la divergence entre populations. Les caractères impliqués dans les conflits sexuels peuvent cependant influer certains composants de l'aptitude indépendants de la sélection sexuelle, ce qui conduit la sélection naturelle à ralentir le processus de diversification. En utilisant une approche d'évolution expérimentale, j'ai montré que les caractères impliqués dans les conflits sexuels peuvent évoluer de manière parallèle, en corrélation avec l'environnement, ce qui démontre que les conflits sexuels peuvent jour un rôle écologique important en favorisant la divergence entre populations. Plus généralement, mon travail a permis d'éclairer l'interaction entre sélection sexuelle et sélection naturelle, ainsi que de mieux comprendre les conséquences évolutives de cette interaction, au delà de l'affichage d'ornements sexuels extravagants ou du développement d'armements pour la compétition entre individus du même sexe.

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Chapter 1: Introduction

Sexual selection occurs when there is competition for reproduction and particular traits convey reproductive advantages (Andersson 1994). Although sexual selection can be seen as a subcategory of natural selection (Endler 1986), it is often distinguished from nonsexual selection because its most obvious consequences involve the evolution of traits that, while increasing sexual fitness, may decrease components of nonsexual fitness; for example reducing longevity. In this respect, the outcome of sexual selection is exemplified by exaggerated secondary sexual traits such as weapons and displays used in male-male combat and/or targeted by female mate choice. Much of the sexual selection research to date has sought to understand the evolution and function of such traits, while other potentially important implications, such as its interaction with other forms of natural selection and its impacts on diversification and population persistence, have received less attention. For example, sexual selection may increase the rate of population divergence (e.g. Arnqvist 1998), thereby promoting speciation (Arnqvist et al. 2000, Barraclough et al. 1995, Panhuis et al. 2001, Van Doorn et al. 2009). In addition, sexual selection may affect population mean fitness, potentially playing a role in adaptation to novel environments (and hence niche expansion), though this is not particularly well understood (Rundle et al. 2006, Whitlock and Agrawal 2009).

In my thesis, I address two understudied aspects of sexual selection and its impacts on evolution: the alignment of natural and sexual selection, and how ecological adaptation influences sexual conflict. Chapters 2 and 3 investigate whether sexual selection complements natural selection by aiding in the purging of deleterious alleles

and whether condition-dependent sexual fitness promotes ecological adaptation and divergence. Chapters 4 and 5 explore how ecological adaptation may influence sexual conflict and sexually antagonistic evolution, and tests for possible parallel evolution in association with ecology of traits involved in sexual conflict. These two broad components of my thesis, and the concepts underlying the individual chapters, are discussed below.

The alignment of natural and sexual selection

The effects of sexual selection on population fitness are unclear and a subject of much debate. On one hand, sexual selection may decrease population mean fitness through the harmful effects of sexual conflict (Houle and Kondrashov 2002, Pischedda and Chippindale 2006, Stewart et al. 2008, Bonduriansky and Chenoweth 2009) and via the evolution of sexual displays and intrasexual competition that serve to lower nonsexual fitness. Therefore, there may be a fundamental misalignment between natural and sexual selection, meaning these two evolutionary processes work in opposition. Alternatively, sexual selection may act to increase population mean fitness by reinforcing natural selection, promoting the fixation of advantageous alleles and the purging of deleterious ones. This may occur through a good genes process in which female mate preferences evolve for males with high breeding values for fitness (Iwasa et al. 1991, Price et al. 1993, Promislow et al. 1998, Lorch et al. 2003), or more generally because of the condition-dependence of reproductive success (Whitlock & Agrawal 2009). The relative importance of these various effects, and hence sexual selection's net influence on population mean fitness, are unresolved. If sexual selection decreases population fitness through sexual conflict, then adaptation will be hindered and diversification may be

limited. If, on the other hand, natural and sexual selection align such that sexual selection increases population mean fitness, sexual selection may speed adaptation and promote diversification.

With regard to the former, sexual selection may decrease mean population fitness through sexual conflict. Sexual conflict occurs because the two sexes are often characterized by different reproductive strategies that can generate sex-specific selection on shared traits (Parker 1979, Arnqvist and Rowe 2005). Such sexually-antagonistic selection can generate two forms of intragenomic conflict: interlocus and intralocus. Interlocus sexual conflict occurs when sexually-antagonistic selection targets a shared trait, arising from male-female interactions, that involves unique loci in each sex (e.g. mating rate, offspring care). Intralocus sexual conflict occurs when males and females have different fitness optima for a shared trait that is controlled by the same loci in each sex (e.g., body size) such that an increase in trait value (and hence fitness) in one sex pulls the other sex away from their optimum (Bonduriansky and Chenoweth 2009). Both forms of sexual conflict may act to decrease population mean fitness (Pischedda and Chippindale 2006, Bonduriansky and Chenoweth 2009) because an increase in one sex's fitness results in a decrease in the other sex's fitness, either through reproductive interactions and their consequences (i.e. interlocus sexual conflict) or as a correlated effect that pulls the opposite sex away from trait values that maximize their fitness (i.e. intralocus sexual conflict).

Recent empirical work has shown that sexual conflict can have important fitness consequences. For example, Stewart et al. (2008) found that offspring resulting from free mate choice (and therefore subject to stronger sexual selection) in *Drosophila*

melanogaster were not more fecund than offspring from randomly enforced matings (reduced opportunity for sexual selection). Furthermore, interacting with multiple males, which is necessary for mate choice, greatly decreased female lifetime fecundity. In fact, the influence of male mate choice may serve to limit adaptive evolution in *D. melanogaster*: when Long et al. (2009) assessed the influence of male mate choice on sexual conflict, they found that males preferentially courted and harassed high fecundity females and that this harassment decreased female fitness. Therefore, the harm of sexual conflict decreases the variance in fitness, and may subsequently overwhelm the potential benefits of sexual selection.

Another convincing study suggesting that sexual conflict may outweigh the potential benefits of sexual selection comes from an evolution experiment by Stewart et al. (2005) in *D. melanogaster.* Here, the authors simulated the presence of an allele that both limits the harm from sexual conflict (harassment from males) as well as the potential benefits of adaptive mate choice, and then tracked the frequency of this allele. Their results showed that this allele was favoured in their experimental populations, increasing in frequency over time, suggesting that the benefits of removing male harassment exceed any cost of a reduced opportunity for mate choice. Lastly, Pischedda and Chippindale (2006) found that females with high reproductive success produced sons with low reproductive success, and males with high reproductive success produced daughters with low reproductive success in *D. melanogaster*, suggesting intralocus sexual conflict can potentially remove any fitness advantages of sexual selection within a single generation.

In contrast to the potential costs of sexual conflict, sexual selection may also increase population fitness and speed the process of adaptation if it tends to align with

natural selection, favouring alleles that increase nonsexual fitness and acting against those that decrease it. In the classic good genes model, female mate preferences evolve for males possessing elaborate sexual display traits because the cost of these traits ensures that they are honest indicators of high condition (Iwasa et al. 1991, Price et al. 1993, Lorch et al. 2003). Males achieve high condition because they can efficiently acquire and make use of resources, and if this has a genetic basis (i.e. they are of high genetic quality), females mating with them gain an indirect benefit through increased offspring fitness, driving the evolution of the preference. Female mate preferences therefore evolve to target high fitness males, the result of which can be an increase in population mean fitness from mate choice (Iwasa et al. 1991, Price et al. 1993, Rowe and Houle 1996, Proulx 1999, 2001, 2002, Kotiaho et al. 2001, Houle and Kondrashov 2002, Lorch et al. 2003).

In addition to a good genes process, two potentially more general mechanisms also exist by which sexual selection may serve to increase population mean fitness, neither of which require the evolution of costly mate preferences or exaggerated sexual display traits. First, individuals must often go through several steps to successfully reproduce, including intrasexual competition and attracting potential mates (Andersson 1994). It seems reasonable that healthy individuals may be more likely to successfully navigate these processes, and thus alleles that decrease overall health will also tend to decrease reproductive success (Whitlock and Agrawal 2009). The end result is that sexual selection will reinforce natural selection, favouring the healthiest individuals. Second, sexual selection may often lead to positive assortative mating by condition, and by extension fitness (Rice 1998). For example, if males compete more intensely for access to

high than low quality females, then only high quality males may be successful in acquiring mating with high quality females. Such positive assortative mating by fitness would increase the variance in fitness, which would subsequently increase the efficiency of selection and thereby promote adaptation (Sharp and Agrawal 2009, Whitlock and Agrawal 2009).

In the first two data chapters of my thesis, I test for the alignment of natural and sexual selection in two ways. First, I assess whether sexual selection speeds the purging of deleterious alleles from populations, as has been predicted under condition-dependent reproductive success. Second, I assess whether increases in nonsexual fitness (i.e. adaptation) also increase sexual fitness in an ecological context. If sexual selection complements natural selection in these ways, sexual selection would be predicted to facilitate adaptation and play a role in ecological diversification.

Sexual selection and the purging of deleterious alleles

Sexual selection may increase population mean fitness by aiding in the removal of deleterious alleles, thereby helping to purge a population's mutation load (Whitlock 2000, Agrawal 2001, Sharp and Agrawal 2009, Whitlock and Agrawal 2009). Theory suggests that such benefits may even be sufficiently large to contribute to the maintenance of sexual reproduction (Agrawal 2001, Siller 2001). There are few appropriate empirical tests for the effects of sexual selection on the purging of deleterious alleles from populations, though there is some evidence of sexual selection acting against several deleterious alleles in single generation mating assays (e.g., Sharp and Agrawal 2008, MacLellan et al. 2009, Whitlock and Agrawal 2009). However, though these

results are consistent with the notion that sexual selection may reduce mutation load in a population, they do not demonstrate that the net effect of sexual selection (including sexual conflict) serves to decrease deleterious allele frequency. In the first data chapter of my thesis, I test the hypothesis that sexual selection accelerates the purging of deleterious mutations from populations using experimental evolution in *D. melanogaster*.

Sexual selection and ecological adaptation

The alignment of natural and sexual selection may not only aid in the purging of deleterious mutations, but it may also facilitate niche expansion by promoting adaptation to novel environments (Bonduriansky 2011). For example, Proulx (1999, 2001, 2002) modeled a scenario in which the most locally adapted individuals are able to achieve higher condition, and thus allocate more resources to sexual traits. Sexual selection promoted adaptation in these models because potential mates found locally adapted individuals more attractive, and local adaptation therefore leads to higher mating success in a specific environment. Population adaptation to a particular environment is increased under this scenario, as alleles that increase fitness in the environment are associated with attractive individuals and are therefore passed to more offspring than if mating was random. Similarly, simulations carried out by Van Doorn et al. (2009) suggest that condition dependent sexual selection speeds the rate of adaptation to new environments, and facilitates speciation.

A fundamental assumption of sexual selection's role in niche expansion is that a gain in nonsexual fitness will facilitate an increase in sexual fitness through condition-dependent mating success (Whitlock and Agrawal 2009). However, the alignment of

natural and sexual selection is unclear and remains an open question in evolutionary biology. Several studies have found support for an association between nonsexual and sexual fitness (Promislow et al. 1998, Radwan 2004, Dolgin et al. 2006, Sharp and Agrawal 2008, Hollis et al. 2009) while others have found none (Holland and Rice 1999, Martin and Hosken 2004, Long et al. 2009, Correia et al. 2010). There are even less relevant data for this issue when placed in an ecological context, and the data that do exist are unclear. For example, sexual selection did not speed adaptation to a new environment in experimental *Drosophila serrata* populations (Rundle et al. 2006) or in *D. melanogaster* populations (Holland 2002), but it did appear to speed the response to directional selection in experimental populations of seed beetles (Fricke and Arnqvist 2007).

Past relevant studies investigating the effects of local adaptation on male mating success (Dolgin et al. 2006, Correia et al. 2010) employed a design that could not separate two different processes that could lead to an alignment of natural and sexual selection. In these experiments, an increase in male sexual fitness when tested in their adapted environment could arise through condition-dependent mating success of males, or through environmental plasticity of female mate preference. In theory, both mechanism could promote ecological adaptation and niche expansion in populations, although they have very different implications for how ecology and sexual selection may interact. Plasticity in mate preferences in particular may have important consequences for sexual selection's role in adaptation and speciation. If an environmental change leads to a change in mate preferences, this may have an immediate effect in generating a behavioural (also known as sexual) form of reproductive isolation. The resulting

divergent sexual selection may also promote population divergence, further contributing to the evolution of reproductive isolation.

If plasticity in preference is adaptive such that a preference for locally-adapted males is stronger among individuals raised in that local habitat (as opposed to individuals raised in other habitats), this could also contribute to niche expansion and adaptation (Proulx 2001, van Doorn et al. 2009). Plasticity in mate preference could also result in the patterns seen in parallel speciation, with the repeated evolution of ecologically-based reproductive isolation. West-Eberhard (2003) argued for this possibility in detail for threespine stickleback (one of the most well-established cases of parallel speciation) and Shaw et al. (2007) showed that there is plasticity in courtship behaviour among marine stickleback, mirroring the differences seen in benthic and limnetic ecotypes. This possibility stands in stark contrast to the standard interpretation of parallel evolution providing strong evidence for reproductive isolation evolving as a consequence of natural selection alone (e.g. Nagel and Schluter 1998). However, plasticity in female mate preferences with respect to ecological adaptation has received relatively little attention, though it may have large impacts on sexual selection and the process of adaptation (Price 2006, Qvarnstrom 2001). Preferences for locally adapted mates, and plasticity in sexually relevant traits as a whole, are not well established.

In chapter 3 of my thesis, I test for the effects of rearing environment on both male mating success and female mate preference among divergently adapted populations of *D. melanogaster*. This experiment allows me to infer the importance of condition-dependent mating success in males as well as environmental plasticity of female mate

preferences, and ultimately allows me to assess how ecology and sexual selection may interact to influence overall fitness and ecological adaptation.

Ecology and sexual conflict

While sexual selection may influence ecological adaptation, it has also been observed that the reverse is true, that ecology can have a large impact on the evolution of traits influencing male-female interactions (Maan and Seehausen 2011). Ecology (such as predation, diet, and signaling environment) may generate natural selection on sexual traits if these traits affect performance or survival in a given environment. For example, the ecological context of sexual traits may determine what trait values are detectable, whether pre-existing ecological preferences and attraction play a role in mate choice (sensory drive; Boughman 2002), and whether certain trait values influence mortality rates (Maan and Seehausen 2011). Ecological conditions have previously been shown to influence the evolution of male and female sexual traits (e.g. Henry and Wells 2004, Rundle et al. 2005, Ballentine 2006, Karlsson et al. 2010), and can even induce environment-dependent parallel evolution of sexual signals and preferences. For example, in Trinidadian guppies, male colouration and courtship rate increase the likelihood of detection by predators, and the exaggeration of these traits therefore increases with decreasing predation rates across populations (Endler 1980, 1987, Magurran and Seghers 1994). Such parallel evolution of sexual traits may contribute to parallel or ecological speciation (Schluter 2000), where ecology influences the evolution of sexual traits and preferences, potentially leading to reproductive isolation between ecologically divergent populations.

However, while it is recognized that ecology can influence the evolution and divergence of sexual displays and preferences, less is known about how ecology may affect sexual trait diversification when reproductive interests are not aligned (i.e. under sexual conflict). Traits involved in sexual conflict have the potential to change and diverge rapidly within species (e.g. Civetta and Singh 1995, Rice 1996), and across species (Arnqvist 1998). Because of this increased potential for divergence, it has been suggested that sexual conflict may play an important role in diversification and speciation (Parker and Partridge 1998, Chapman et al. 2003). Some models suggest that sexual conflict can lead to rapid change in male sexual traits and female mating preferences, and can thus act as an "engine of speciation" (Gavrilets et al. 2001, Gavrilets and Waxman 2002). Empirical work also supports this notion, as insect clades with greater opportunities for sexual conflict are more speciose than are monandrous sister clades (Arnqvist et al. 2000).

The proposed mechanism behind this increased divergence and speciation rate is rapid sexually antagonistic coevolution, which is commonly interpreted in a non-ecological framework. Because the sexes may differ in their optimal trait values, such as mating rate, each sex's attempt to maximize their reproductive output can come at a cost to the other sex's fitness, leading to an evolutionary arms race. This framework, verbalized by Holland and Rice (1998) with respect to mating rate, predicts that females increase their mating threshold with respect to exaggerated male sexual traits in order to maintain an optimal mating rate. In response, male sexual traits are further exaggerated over time to overcome female resistance and increase male reproductive potential, which can lead to an ongoing process of chase-away antagonistic coevolution between male

traits and female resistance. Because males and females are constantly coevolving with respect to each other, the potential for rapid and consistent evolutionary change is present even in an unchanging external environment (Long et al. 2006, Coyne and Orr 2004). Holland and Rice (1998) assumed that which particular male stimulatory trait and associated female resistance are exaggerated is initially based on random events, such as the order in which particular mutations occur (Schluter 2009), and that there are essentially limitless possible antagonistic pathways available to populations.

Based on this theoretical framework, diversification via sexual conflict has been examined among populations in the absence of any ecological context, as the primary mechanism of change is through male-female coevolution. However, as discussed above, traits involved in sexual conflict (such as mating rate) can also be a target of ecological selection that varies with environment. As such, the potential for the exaggeration of traits under sexual conflict may be dependent on the environment to which populations are exposed. In fact, natural selection has long been central to sexual conflict theory (Parker 1979; Rowe *et al.* 1994; Rice & Holland 1997; Rowe & Day 2006; Fricke *et al.* 2009; Maan & Seehausen 2011), potentially limiting the extent to which certain traits can be exaggerated due to cost within an ecological context. Therefore, ecology could determine the extent and direction of sexual trait exaggeration, as well as divergence among isolated populations in such traits. However, such considerations of ecology have largely been ignored in the sexual conflict literature investigating population divergence and speciation, though the interaction of ecology and sexual conflict could have large implications for how populations adapt to new environments, as well as for ecological speciation. In the same way that ecology can promote the parallel evolution of sexual

traits, it is possible that ecology may bring about the parallel evolution of male-female antagonistic interactions. In chapters 4 and 5 of my thesis, I investigate the role ecology may play in sexual conflict by examining male-female interactions among replicate experimental populations of *D. melanogaster* adapted to separate environments. In chapter 4, I evaluate the potential for ecologically-dependent parallel evolution of male harm and female resistance to such harm. In chapter 5, I evaluate the extent of population-specific and ecologically-dependent divergence of traits under sexual conflict (mating rate and offspring production).

Note on authorship

Chapters 2-5 are revised versions of manuscripts that have been published in, submitted to, or prepared for submission to peer-reviewed scientific journals. Because each chapter was written as a stand-alone publishable manuscript, there is some overlap in the material covered. Also, although I use the pronoun "I" throughout this thesis, the work was collaborative and the resulting papers include multiple authors (details below). However, for each chapter, I took the lead in project planning and management, data collection and analysis, as well as the writing.

Chapter 2: Arbuthnott, D. and Rundle, H. D. 2012. Sexual selection is ineffectual or inhibits the purging of deleterious mutations in *Drosophila melanogaster*. Evolution 66: 2127-2137.

Chapter 3: Arbuthnott, D. and Rundle, H. D. In Press. Misalignment of natural and sexual selection among divergently adapted *Drosophila melanogaster* populations. Animal Behaviour.

Chapter 4: Arbuthnott, D., Dutton, E. M., Agrawal, A. F., and Rundle, H. D. In Press. The ecology of sexual conflict: ecologically-dependent parallel evolution of male harm and female resistance in *Drosophila melanogaster.* Ecology Letters.

Chapter 5: Arbuthnott, D., Agrawal A. F., and Rundle H. D. Accepted. Environment and population-level divergence of sexual conflict traits in *Drosophila melanogaster*. PLoS ONE.

Chapter 2: Sexual selection is ineffectual or inhibits the purging of deleterious mutations in *Drosophila melanogaster*

Abstract

The effects of sexual selection on population mean fitness are unclear and a subject of debate. Recent models propose that, because reproductive success may be condition-dependent, much of the genome may be a target of sexual selection. Under this scenario, mutations that reduce health, and thus non-sexual fitness, may also be deleterious with respect to reproductive success, meaning that sexual selection may contribute to the purging of deleterious alleles. We tested this hypothesis directly by subjecting replicate *Drosophila melanogaster* populations to two treatments that altered the opportunity for sexual selection and then tracked changes in the frequency of six separate deleterious alleles with recessive and visible phenotypic effects. While natural selection acted to decrease the frequency of all six mutations, the addition of sexual selection did not aid in the purging of any of them, and for three of them appears to have hampered it. Courtship and mating have harmful effects in this species and mate choice assays showed that males directed more courtship and mating behavior towards wild-type over mutant females, providing a likely explanation for sexual selection's cost. Whether this cost extends to other mutations (e.g., those lacking visible phenotypic effects) is an important topic for future research.

Introduction

Two forms of natural selection are often distinguished: that arising from variation in nonsexual fitness (e.g., viability, fecundity, longevity) and sexual selection, arising from differential fertilization success among living individuals. Although Darwin's (1859) original description of sexual selection suggested that it may often increase nonsexual fitness, the focus of sexual selection research subsequently shifted to elaborate secondary sexual traits that are detrimental to nonsexual fitness and whose evolution can therefore only be explained by sexual selection. However, the past decade has seen renewed interest in the population genetic consequences of sexual selection, in particular its role in facilitating adaptation and purging deleterious mutations, thereby potentially increasing population mean fitness (Whitlock and Agrawal 2009).

The effects of sexual selection on nonsexual fitness are unclear; on the one hand, sexual selection has often been thought to reduce population mean fitness due to the evolution of costly sexual displays and preferences for them (Lande 1980; Kirkpatrick 1982; Gavrilets et al. 2001; Houle and Kondrashov 2002). Sexual selection can also generate sex-specific selection and the resulting intra- and/or inter-locus sexual conflict may further reduce population mean fitness (Pischedda and Chippindale 2006; Stewart et al. 2008; Bonduriansky and Chenoweth 2009). For example, sex-specific selection on shared traits may cause males and females to have different evolutionary optima, generating intralocus sexual conflict. Because, at least initially, males and females are assumed to have a common genetic basis for such traits, the evolution of sexual dimorphism is impeded and a gender load results (Rice and Chippindale 2001). Sexual selection can also lead to interlocus sexual conflict, a process of antagonistic coevolution

between loci that increase male sexual fitness at the expense of female nonsexual fitness, and loci that increase female resistance to these harmful male effects (Trivers 1972; Parker 1979). This process drives coevolutionary arms races that have produced many dramatic examples of traits that are beneficial to males while harmful to females (Arnqvist and Rowe 2005). Finally, in species with such male-induced female harm, male mate choice may cause this harm to be disproportionately directed toward otherwise high quality (i.e. preferred) females, thereby reducing the variance in realized female fitness and hence the strength of selection (Long et al. 2009).

On the other hand, sexual selection may also facilitate adaptation, and particularly the purging of deleterious mutations, in two main ways. First, positive assortative mating by fitness may arise as a by-product of sexual selection; for instance if high quality males compete more intensely for access to high quality females, or if costly mate preferences are expressed to a greater degree in high quality females (Fawcett and Johnstone 2003; Whitlock and Agrawal 2009; Sharp and Agrawal 2009). A positive correlation in fitness between mates will increase the variance in fitness and hence the efficiency of selection (Rice 1998). Second, sexual selection may directly favor individuals of high nonsexual fitness, aligning with selection arising from variation in non-sexual fitness (hereafter 'natural' selection for convenience) to promote the fixation of advantageous alleles and the purging of deleterious ones. This may occur via a classic good genes process in which female mate preferences evolve for males of high breeding value for fitness, driven by the indirect benefits females receive from mating with such males (Iwasa et al. 1991; Kirkpatrick and Ryan 1991; Houle and Kondrashov 2002). It may also occur more generally if fertilization success (i.e. male sexual fitness) is condition dependent.

Individuals often invest substantial time and effort in reproducing, often going through several steps to successfully reproduce including finding a potential mate, competing directly or indirectly with members of the same sex for access to that mate, courting or coercing them into mating, and finally achieving fertilization when in competition with the sperm from past and future matings (Andersson 1994). If healthier and more vigorous (i.e. higher condition) males are more likely to be successful at these various stages, then alleles that decrease overall health will also tend to decrease reproductive success (Rowe and Houle 1996; Whitlock and Agrawal 2009). Theoretical models, focusing on good genes benefits, suggest that such an alignment of natural and sexual selection may increase the rate and extent of adaptation (Proulx 1999, 2001, 2002; Lorch et al. 2003), aid in the purging of deleterious alleles (Whitlock 2000), and may even be sufficient to contribute to the maintenance of sexual reproduction (Agrawal 2001; Siller 2001).

There are few direct tests of the effects of sexual selection on nonsexual fitness. Several studies have manipulated the opportunity for sexual selection and then measured the consequences for population fitness or components thereof, either in a constant (Partridge 1980; Promislow et al. 1998; Holland and Rice 1999; Martin and Hosken 2003; Radwan et al. 2004; Crudgington et al. 2005; Tilszer et al. 2006) or a novel environment (Holland 2002; Rundle et al. 2006; Fricke and Arnqvist 2007). These studies have provided mixed results, however, and it is unclear what effect, if any, sexual selection has on nonsexual fitness. Three related studies have manipulated the opportunity for sexual selection within the context of the purging deleterious mutations, either using mutation accumulation or following mutagenesis. Results are again mixed. Consistent with a benefit, sexual selection appeared to reduce the mutation load on

productivity in *Drosophila serrata* during mutation accumulation (McGuigan et al. 2011), and embryo viability of populations of bulb mites exposed to ionizing radiation increased significantly after one generation of sexual selection (Radwan 2004). In contrast, following exposure to mutagens, *D. melanogaster* populations with reduced sexual selection showed higher reproductive output after 60 generations as compared to those maintained in the presence of sexual selection (Hollis and Houle 2011). Several of the above studies are multigenerational evolution experiments that measured only population mean fitness (or components thereof). The interpretation of the results from such experiments is hampered because changing the opportunity for sexual selection also alters the opportunity for sexual conflict, confounding their effects. In particular, over the limited time-frame of these experiments, a short-term increase in fitness when populations are released from sexual conflict may overwhelm longer-term benefits of sexual selection in reducing the mutation load, biasing such studies to finding a cost of sexual selection (Whitlock and Agrawal 2009).

An alternative approach to testing the consequences of sexual selection for nonsexual fitness is to measure the effects on male sexual fitness of individual deleterious mutations. Male reproductive success has been quantified for 17 independent deleterious mutations with visible phenotypic effects in *D. melanogaster*, with natural and sexual selection aligning on 13 of these (Whitlock and Bourguet 2000; Stewart et al. 2005; Pischedda and Chippindale 2005; Sharp and Agrawal 2008; Whitlock and Agrawal 2009; MacLellan et al. 2012). While providing some support for the idea that sexual selection may align with natural selection to reduce mutation load, these studies are not sufficient

to show that the combined effects of sexual selection (including sexual conflict) serve to decrease deleterious allele frequencies.

A more powerful and straightforward test of the net effect of sexual selection on nonsexual fitness is to track changes in the frequency of individual deleterious alleles across generations when the opportunity for sexual selection is manipulated. Such an approach allows the effects of selection on the allele to accumulate across generations, and allows all fitness components to be integrated by the evolutionary process itself. In the only application of this approach of which I am aware, Hollis et al. (2008) found that sexual selection increased the rate at which a single alcohol dehydrogenase null allele was purged from replicate experimental *D. melanogaster* populations. Our understanding of the net effects of sexual selection on nonsexual fitness would therefore benefit from additional data from a wider array of deleterious mutations.

In the current study, I manipulated the opportunity for sexual selection in replicate populations of *Drosophila melanogaster* and directly measured the change, across generations, in the frequency of six independent mutations with recessive, visible phenotypic effects. If sexual and natural selection align, populations with stronger sexual selection should purge these deleterious alleles faster than populations with weak sexual selection. Counter to this prediction, I found that, while natural selection acted against these mutant alleles, sexual selection did not aid in their purging and, for three of the mutations, even appeared to inhibit it. Given known harmful effects of courtship and mating in this species (Fowler and Partridge 1989, Partridge and Fowler 1990, Long et al. 2009), I subsequently explored the possibility that male mate preferences may

disproportionately direct this harm to wild-type females, thus hampering the response to selection.

Materials and Methods

Study populations

The experiment used six different recessive mutations with visible phenotypic effects in adult *Drosophila melanogaster*, all originally obtained from the Bloomington Stock Center. Three of these mutations are autosomal (*brown*, *sepia*, *plexus*) and three are X-linked (*yellow*, *white*, *forked*), and of these, three influence eye color (*brown*, *sepia*, *white*), one affects wing morphology (*plexus*), one affects body color (*yellow*), and one affects bristle morphology (*forked*). Each mutation was separately introgressed, via five rounds of backcrossing, into an outbred and laboratory-adapted stock population, as described in MacLellan et al. (2009). One or more rounds of introgression were initiated by a mating in each direction (i.e. stock female × mutant male and mutant female × stock male) to ensure that the mtDNA and Y chromosome from each introgressed mutant population were derived from the stock. The resulting populations were each fixed for a different recessive mutation yet shared ~97% of their outbred genetic background with the original stock.

These mutations have been previously used to investigate the importance of varying male mate search ability in generating sexual selection against them (MacLellan et al. 2009), and in quantifying the effects of dietary stress on the strength of selection against them (MacLellan et al. 2012). The mutations are presumably deleterious relative to the wild-type allele at each locus, although only one (*sepia*) significantly reduced productivity relative to the stock in a previous assay, with a second (*white*) approaching

significance (p = 0.066; MacLellan et al. 2012). Four of them (*brown*, *sepia*, *white*, and *yellow*) significantly reduced male mating success in multiple choice trials with virgin females.

Experimental evolution

For each mutation, six replicate populations at Hardy-Weinberg proportions were created by mixing homozygous mutant, heterozygote, and homozygous wild-type individuals to produce an initial mutant allele frequency of 0.7. Three replicate populations were assigned to each of two experimental treatments that manipulated the opportunity for sexual selection: sexual selection present (+S) or sexual selection greatly reduced (-S). Within each population, a female's expected contribution to the next generation was proportional to the number of adult offspring she produced, allowing natural selection to operate unhindered. This design therefore allows us to compare changes in the frequency of the mutant allele caused by natural selection alone with that caused by the combined presence of natural and sexual selection.

Populations were maintained via non-overlapping generations by allowing 100 adult females to lay eggs for 24 h in two bottles (50 females/bottle), after which the females were discarded. Offspring developed in these bottles and 120 male and 120 female adult virgins were collected at emergence, using light CO_2 anesthesia and pooling all individuals among the two bottles, and then placed in their respective mating treatments to manipulate the opportunity for sexual selection. In the +S treatment, these virgin adults were transferred to two new food bottles (60 males and 60 virgin females each) and allowed to interact and mate for three days, allowing the opportunity for

various forms of sexual selection including mate choice, male-male competition, and sperm competition. In the -S treatment, the virgin adults were randomly assigned to 110 separate male-female pairs in individual vials, enforcing monogamy and thereby greatly restricting the opportunity for sexual selection. Following the three day mating period, groups of 50 randomly chosen females were transferred to each of two new laying bottles (in the +S treatment, the 50 females in a laying bottle all originated from the same mating bottle) such that, except for the mating environment, conditions were similar across treatments throughout the rest of their life cycle. In both the mating and laying environments, enough live yeast was added to the substrate to allow flies to eat *ad libidum* throughout the experiment.

Due to a slow and variable response to selection after nine generations of the *brown* experimental trials, we attempted to reduce genetic drift by doubling the population size of all six populations for the remainder of the experimental generations (i.e. 200 females laid eggs to produce the next generation in each). Population density is a stressor that can influence the strength of selection on new mutations (Agrawal and Whitlock 2010), so as not to alter density this doubling was done by creating twice as many bottles/vials every generation in every *brown* population (both for egg laying and in the mating treatments), keeping the number of flies per bottle/vial constant. More generally, across mutations and treatments I attempted to control population egg/larval density by standardizing the number of females and their egg laying times (i.e. 50 females for 24 h), and by keeping sex ratios the same at 1:1 in both mating treatments (to reduce the possibility of differences in total harassment causing female fecundity to vary between +S and -S populations).

Each mutation is visible only when homozygous and the persistence of the deleterious allele was tracked via the frequency of visible mutants (i.e. homozygotes) every generation. At least 200 individuals per population were phenotyped every generation and these individuals were collected both before and after the individuals used to maintain the populations during the evolution experiment, thereby minimizing any bias in the frequency estimates caused by difference in emergence time of mutant vs. wild-type individuals. To determine the true frequency of the mutant allele, a set of test crosses were performed separately for each of the six populations of a given mutation on three (two for *brown*) separate occasions (i.e. generations). These crosses were initiated when the visible frequency decreased below approximately 0.3, although this varied among mutations in response to the rate and consistency of the decline. These crosses involved mating virgin wild-type individuals from an experimental population with a virgin homozygous mutant of the opposite sex. 50-55 crosses were performed per replicate population in each assay and the resulting offspring of each pair were scored for the presence of the mutant phenotype, indicating that the parent from the experimental population was heterozygous at that locus.

To determine whether natural selection acted against each of these mutations, I tested whether the true allele frequency of a particular mutant allele decreased over time in the three replicate -S populations (i.e., the treatment in which 'natural' selection acted alone). The analysis treated populations as replicates and used a repeated measures ANOVA with generation as a within-subjects factor. Huynh-Feldt-adjusted p-values were employed because the assumption of sphericity could not be confirmed due to limiting degrees of freedom (Huynh and Feldt 1976). To determine whether sexual selection

influenced the purging of each mutation, I tested whether there was a significant difference in true allele frequency between the two sexual selection treatments (i.e., -S vs. +S), separately for each mutation and again treating populations as replicates. To do this, for each population I calculated the average allele frequency across the 2-3 replicate measures and then used a two-sample *t*-test to determine whether the sexual selection treatment influenced these frequencies. Results from these analyses were qualitatively the same as those from a repeated measures ANOVA of the true allele frequencies that included treatment as a between-subjects factor (and excluded the first generation in which all populations had an initial frequency of the mutant allele of 0.7). I therefore present the former for simplicity.

Male persistence

Sexual selection appeared to hamper natural selection against several of the mutations (see Results), either overall or early during the evolution experiment when the mutant frequencies were higher. Courtship and mating in *D. melanogaster* are known to be harmful to females, so to provide insight into the contribution of male mate choice in directing such harm preferentially to wild-type females and thereby hampering natural selection, I carried out two independent male choice persistence assays, using the *plexus* and *sepia* mutations, following Long et al. (2009). In each assay, both homozygous wild-type and homozygous mutant males were separately presented with two females, one being a wild-type homozygote and the other being either a mutant homozygote or a heterozygote. Vials were scored every 40 minutes for male persistence behavior (defined as active courtship, mating, or males facing female at a distance of 5 mm or less, as in

Long et al. 2009) recording which (if any) female the behavior was directed towards. A total of seven observations were made for each of 200 vials per trial, with 50 replicates for each of the four combinations of male and female type.

Flies for use in the assays were collected as offspring of known crosses (involving individuals from the wild-type and introgressed-mutant stock populations), using light CO_2 anesthesia, as non-virgins on day 12 of their life-cycle. Males and females were held separately for 24 h in vials that lacked the usual addition of live yeast, at densities of seven individuals/vial. Females were marked by transferring them for an additional 24 h to new vials that contained abundant yeast paste formed by mixing live yeast with red or blue food coloring (McCormick, Canada). The colored yeast the females eat is visible through their abdomen, temporarily marking them either red or blue. All combinations of female color and genotype were tested in a balanced design. Extensive use of such markings in previous experiments has shown no effect on mating patterns (Rundle et al. 1998, 2007; Mooers et al. 1999; Rundle 2003), and there was no evidence of a male preference for red or blue females in the current experiment when testing either the *plexus* (paired *t*-test; wild-type males: $t_{99} = 0.29$, p = 0.773; *plexus* males: $t_{99} = 0.11$, p = 0.92) or *sepia* (wild-type males: $t_{99} = 0.78$, p = 0.44, *sepia* males: $t_{99} = 0.75$, p = 0.46) mutations. Assays were set up on day 14 by aspirating, without anesthesia, a single male and two females into a new vial for observation.

Total male persistence directed toward the two females in a vial was expressed as a difference score (persistence towards wild-type female – persistence towards mutant female); these scores were unimodal and symmetrically distributed among vials. A two-way analysis of variance (ANOVA) was used to determine whether this difference score

depended on the male type (homozygous wild-type vs. homozygous mutant) and the identity of the 'mutant' female (mutant homozygote vs. heterozygote), separately for each mutation. Paired *t*-tests were then used to determine whether male persistence was preferentially directed toward wild-type vs. mutant females, treating vials as replicates, conducted separately for those treatments (male type and female identity) that differed significantly from the ANOVA.

Results

Experimental evolution

For all six mutations, the average visible (i.e. homozygous mutant) and true allele frequencies across the three replicate -S populations decreased during experimental evolution from their initial values of 0.7, implying natural selection acted against these alleles (Figs. 2-1 and 2-2). For five of the mutations, this decrease resulted in a significant generation effect in a repeated measures analysis of the true allele frequencies (Table 2-1). Among-population variation was substantial for the sixth mutation (*yellow*), driven in large part by a decrease in allele frequency in two of the replicate populations that was absent in the third (in which both the visible and true allele frequencies varied across generations around the starting value, showing no directional trend).

In contrast to the natural selection against these mutations, there was no evidence that sexual selection promoted their purging (Figs. 2-1 and 2-2). Average allele frequency did tend to be lower in the +S as compared to the -S treatment for one mutation (*forked*), but this difference was not significant (Table 2-1). For four of the other mutations, average allele frequencies were very similar in the +S and -S populations (*brown*, *plexus*, *white* and *yellow*), generating no significant treatment effect (Table 2-1). This did not

change qualitatively for *yellow* if the outlier -S population was excluded (t_3 = -1.98, p = 0.142). A significant effect of sexual selection was present for the sixth mutation (s*epia*) but the difference was in the opposite direction, with the average allele frequency of the deleterious *sepia* allele being higher in the +S compared to the -S populations after 5-7 generations of experimental evolution (Fig. 2-1; Table 2-1).

These allele frequency data were collected via test crosses after a number of generations of experimental evolution and therefore do not directly address potential treatment effects occurring in earlier generations. Two mutations in particular (*brown* and *plexus*; Fig. 2-1) showed an initial pattern in the frequency of visible mutants (i.e. mutant homozygotes) in which sexual selection appeared to hamper purging, but this disappeared in later generations. This varying effect of sexual selection was sufficient to generate a significant treatment × generation interaction in a repeated measures ANOVA of the visible frequency for these mutations (*brown*: $F_{17.}$ = 2.07, p = 0.02; *plexus*: $F_{11.}$ = 2.24, p = 0.03). For *brown*, the disappearance of this initial hampering effect of sexual selection roughly coincided with the doubling of the population size at generation 9 (Fig. 2-1A). In particular, prior to this doubling, visible mutant frequency had declined in the -S populations, but not in the +S; after it, visible mutant frequency in the +S populations declined whereas little change occurred in the -S populations.

Male persistence

To gain insight into why sexual selection may have hampered natural selection, male persistence assays were performed for two mutations, one for which sexual selection showed a significantly harmful effect in the analysis of the true allele

frequencies (*sepia*), and one for which the effect of sexual selection appeared variable, hampering the decline in visible frequency in the early generations of experimental evolution but not in later generations (*plexus*). For the *sepia* mutation, the difference in male persistence toward wild-type vs. mutant females was independent of the identity of the mutant female (heterozygous vs. homozygous mutant female: $F_{1,196} = 0.37$, p = 0.543), but showed a borderline significant difference depending on the identity of the males (wild-type vs. mutant homozygote: $F_{1,196} = 3.84$, p = 0.051). In particular, *sepia* males were significantly more persistent toward wild-type than mutant females (paired *t*-test, $t_{99} = 2.14$, p = 0.035), whereas wild-type males showed no such difference (paired *t*-test, $t_{99} = -0.52$, p = 0.301; Fig. 2-3A). The interaction of male and female type was nonsignificant ($F_{1,196} = 0.11$, p = 0.734). *Sepia* males were also more active than wild-type males overall, displaying significantly more persistence behaviors toward the two females combined (mean number of persistence behaviors ± SE, *sepia* males = 1.38 ± 0.12, wild-type males = 0.77 ± 0.10; two-sample *t*-test, $t_{198} = -3.86$, p < 0.001).

For the *plexus* mutation, the difference in male persistence toward wild-type vs. mutant females was independent of the type of male (wild-type vs. mutant homozygote: $F_{1,196} = 1.03$, p = 0.313) but varied depending on the identity of the mutant female (heterozygous vs. homozygous mutant female: $F_{1,196} = 4.01$, p = 0.044). In particular, males were significantly more persistent toward wild-type females when choosing between these females and *plexus* homozygous females (paired *t*-test, $t_{99} = 2.17$, p = 0.032), but showed no difference when choosing between wild-type and heterozygous females (paired *t*-test, $t_{99} = -0.78$, p = 0.440). Although the interaction between male and female type was nonsignificant ($F_{1,196} = 1.03$, p = 0.313), this preferential courtship and

mating of wild-type over plexus homozygous female arose largely from wild-type (paired *t*-test: $t_{49} = 2.56$, p = 0.014) as opposed to mutant (paired *t*-test, $t_{49} = 0.47$, p = 0.636) males (Fig. 2-3B). Wild-type males were also more active than *plexus* males overall, displaying a significantly greater number of persistence behaviors toward the two females combined (mean number of persistence behaviors ± SE, *plexus* males = 0.75 ± 0.09, wild-type males = 1.05 ± 0.11; two-sample *t*-test: $t_{198} = 2.05$, p = 0.041).

Discussion

Average allele frequency of all six mutations decreased through time in the absence of sexual selection, confirming that these mutations are deleterious with respect to non-sexual fitness. This decrease was nonsignificant for one mutation (*yellow*), due in large part to a single replicate -S population in which the *yellow* allele showed no directional trend despite decreasing in the other two populations and all three +S populations. The reason for this discrepancy is unknown, although it is possible that a compensatory mutation that reduced the fitness cost of *yellow* was segregating in the original mutant stock population, or arose de novo early during experimental evolution in this particular replicate. Nevertheless, my conclusions concerning the effects of sexual selection on *yellow* are robust to the inclusion or exclusion of this single population. Only one of these six mutations (*sepia*) significantly reduced productivity in a previous assay using these same introgressed mutant stocks, with a second approaching significance (*white*; MacLellan et al. 2012). That natural selection against these alleles was detectable in the current experiment is a testament to the power of experimental evolution in integrating net fitness effects across generations.

Contrary to my prediction, sexual selection did not act to promote the purging of any of the six mutations tested. Rather, the addition of sexual selection significantly hampered the purging of the *sepia* mutation and, based on the more extensive visible (i.e. mutant homozygote) frequency data, sexual selection also appeared to slow the initial decline of *brown* and *plexus*, although this effect disappeared in later generations. Four of the tested mutations (*brown*, *sepia*, *white*, and *yellow*) were previously shown to significantly reduce one component of male sexual fitness (mating success with virgin females in multiple choice trials; MacLellan et al. 2012). That the net effects of sexual selection on these mutations, as estimated across generations in the current study, did not promote their purging despite these previous results highlights the danger of drawing general conclusions about net fitness effects from non-evolutionary assays of particular fitness components.

For the *brown* mutation, the disappearance of the hampering effect of sexual selection appears to have coincided with the doubling of the population size (Fig. 2-1A). This suggests that the initial cost of sexual selection for this mutation may have arisen from a lowering of the effective population size by sexual selection (via increased variance in male reproductive success; Whitlock & Agrawal 2009) to the point at which the *brown* allele was effectively neutral in the +S, but not the -S treatment. The subsequent doubling then reduced drift, allowing selection to act on the mutation in the +S populations. Costs of sexual selection arising from its lowering of effective population size have received little attention, yet will occur even when natural and sexual selection align. Additional, direct tests of this potential cost of sexual selection are an important topic for future work.

Given known or suggested costs to females of courtship and mating in *D. melanogaster*, mediated via male harassment, physical harm during mating, and accessory gland proteins (Fowler and Partridge 1989; Partridge and Fowler 1990; Chapman et al. 1995; Kamimura 2007; Wigby and Chapman 2005; Wolfner 2009), I also sought to determine whether sexual selection's interference in the purging of two mutations (*sepia* and *plexus*) could be explained, at least in part, by male mate choice. In particular, as originally suggested by Long et al. (2009), in species with male-induced harm (like *D. melanogaster*) male mate preferences for high condition females may cause this harm to be disproportionately allocated to high condition (in this case non-mutant) females, decreasing their fecundity and thereby reducing the variance in realized female fitness and inhibiting adaptation. Male mate preferences for larger, and hence more fecund, females have been previously shown in *D. melanogaster*, the consequence of which is a reduction in the variance of female fecundity which should hamper the purging of any deleterious allele that also reduces female body size (Long et al. 2009).

With respect to the s*epia* mutation, mutant males disproportionately courted and mated wild-type over mutant females (both homozygous and heterozygous), whereas wild-type males showed no such preference. *Sepia* males were also significantly more active overall, courting and mating females ~80% more often during the assay than wild-type males, although this apparently does not translate into increased sexual fitness of these males based on the current results. That mutant males differentiated between wild-type and heterozygous females implies that, despite its recessive phenotypic effects on eye color, this mutation is not fully recessive in its effects on one or more other traits that are targets of male mate choice. Preferential harassment of mutant females by *sepia* but

not wild-type males should, all else being equal, lead to a cost of sexual selection that diminishes across generations as the frequency of the *sepia* mutation declines and these males become less common. Although there was no evidence of such a diminishing cost in my results (Fig. 2-1B), data are limited given the rapid decline and thus short time-frame of the experiment with this mutation (seven generations). In addition, mate preferences of heterozygous males were not evaluated; a preference of these males for wild-type over mutant females, including heterozygotes, could maintain a cost of sexual selection at much lower mutant allele frequencies.

For *plexus*, mutant and wild-type males did not differ in their persistence behavior, with both preferentially courting and mating wild-type over homozygous mutant females. When choosing between wild-type and heterozygous females, however, there was no evidence of a significant preference by either type of male, suggesting that the effects of *plexus* are fully recessive in this regard. Such a pattern of male mate choice should produce a diminishing cost of sexual selection because, as the frequency of *plexus* declines, these alleles will to an increasing extent be found primarily in the heterozygous state, therefore shielding wild-type females from preferential male harassment. Changes in the visible frequency of the *plexus* mutation across generations shows precisely this pattern (Fig. 2-1C), with an initial cost of sexual selection that vanishes as allele frequency declines. Whether sexual selection may shift to facilitating the purging of this mutation at low allele frequencies is an interesting possibility for which I lack sufficient data to properly evaluate.

A failure of sexual selection to aid in the purging of these mutations could alternatively be caused by a failure of my treatments to alter sexual selection. As

discussed above, the overall cost of sexual selection in purging *sepia*, and its apparent costs early during the purging of *brown* and *plexus*, are inconsistent with this interpretation. In addition, compared to the monogamous -S treatment, the +S treatment mixed 60 males and females for three days, providing extensive opportunities for both male-male interactions, including sperm competition arising from multiple matings, and female mate choice. Indeed, benefits of sexual selection in purging an *Adh* null allele in *D. melanogaster* were previously found in a +S treatment that combined five males and five females for two days (Hollis et al. 2008). It is possible that the higher densities in the +S treatment may have increased the opportunity for male harassment and hence sexual conflict, thereby altering the balance between the costs and benefits of sexual selection, and that this might contribute to the differences between these results and those of Hollis et al. (2008). However, this density is reflective of that experienced by the stock populations during their normal life cycle, and the net effects of sexual selection under these conditions are therefore relevant to the evolution of mean fitness in these populations.

In addition to differential male harassment, another cost of sexual selection could arise via density-mediated changes in the strength of natural selection, deriving ultimately from male harassment. Increased population density is one of the few environmental stressors that appear to cause a consistent strengthening of selection against new mutations (Agrawal and Whitlock 2010). If +S females were more harassed than -S females overall, then these females may have had reduced fecundity and subsequently laid fewer eggs than their -S counterparts during the set 24 h laying period every generation. The resulting decreased density in the +S treatment bottles may therefore

have weakened natural (i.e. non-sexual) selection against the mutant alleles through decreased larval competition, potentially offsetting other benefits of sexual selection or even generating a net cost. No consistent treatment differences in larval density were observed during these experiments (D. Arbuthnott, personal observation) and sex ratios were constant between treatments at 1:1 (suggesting that the average harassment received by females should be the same). Nevertheless, I did not directly control nor measure egg or larval density, so it remains possible that such density-mediated effects contributed to the observed lack of a benefit of sexual selection.

The mutations used in the current study are not representative of a random sample of deleterious mutations. Rather, these mutations have large and visible phenotypic effects when homozygous, and natural selection against them is likely to be stronger than that acting on typical new mutations of small effect. Whether the effects of sexual selection on deleterious mutations depends on the strength of nonsexual selection against them has not been previously considered, although there is no reason to think that these mutations will behave any differently in this respect. However, the visible nature of these mutations may also provide a direct target for mate preferences, potentially causing sexual selection to act differently on them than on other new mutations. In particular, such direct effects may allow individuals to differentiate between mutant and non-mutant mates more easily than would otherwise be possible for deleterious mutations in which the phenotypic effects are visibly manifested only indirectly via changes in condition-dependent traits (e.g., body size). The opportunity for such direct choice could cause sexual selection to enhance or hamper the purging of these mutations. For example, in theory female mate preferences for non-mutant males could reduce the sexual fitness of

mutant individuals, causing sexual selection to align with natural selection. Alternatively, the visible effects of these mutations may facilitate increased male persistence of non-mutant females, thereby reducing the variance in female fitness and hampering purifying selection. The net effect of sexual selection on such mutations is therefore an empirical question, and my results suggest that it may often be costly. The effects of sexual selection on the purging of randomly occurring new mutations remains an important, albeit challenging, goal for future studies.

Past studies of sexual selection's effects on non-sexual fitness have used a diversity of approaches and have provided conflicting results. This is true specifically with respect to the purging of deleterious alleles, with several studies suggesting a benefit of sexual selection (Hollis et al. 2008; Radwan 2004; McGuigan et al. 2011) while others, including this study, suggest a cost (Hollis and Houle 2011). These studies differ in the nature of the genetic variance (e.g., individual deleterious alleles vs. those introduced by mutagenesis or mutation accumulation) and the response variable measured, with some addressing the net effect of sexual selection on mean fitness and others addressing the frequency of individual alleles. While it is clear from past results that sexual selection in *D. melanogaster* reduces population mean fitness via sexual conflict (Chippindale et al. 2001; Pischedda and Chippindale 2006; Stewart et al. 2005, 2008, Long et al. 2009; Hollis and Houle 2011), its potential benefits in promoting the purging of deleterious mutations may accrue over much longer timescales, making them hard to detect via short term changes in fitness (Whitlock and Agrawal 2009) or possibly even via effects on the frequency of individual mutations.

In conclusion, theory suggests that sexual selection may increase population mean fitness (e.g. Rowe and Houle 1996; Lorch et al. 2003) and some empirical evidence suggests that sexual selection may increase non-sexual fitness (Promislow et al. 1998; Radwan 2004) and speed adaptation under directional selection (Fricke and Arnqvist 2007). However, my results agree with a number of other studies that failed to find a net benefit of sexual selection (e.g., Holland and Rice 1999; Rundle et al. 2006; Hollis and Houle 2011). Furthermore, direct evidence of preferential male harassment of wild-type over mutant females is consistent with previous results showing a substantial cost of sexual conflict (e.g. Chippindale et al. 2001; Stewart et al. 2005, 2008; Pischedda and Chippindale 2006; Fricke and Arnqvist 2007; Long et al. 2009). A detailed understanding of the contribution of sexual selection to population mean fitness will require consideration of its short and long term effects under constant and novel environmental conditions, and with respect to various types of genetic variance (e.g., segregating vs. new mutations) in a wide variety of organisms, and this remains an important goal in evolutionary genetics.

Table 2-1 Effects of natural and sexual selection on allele frequencies for six replicate mutations

	Natural selection[1]			Sexual selection[2]	
Mutation	*F*	*d.f.*	p	t_4	p
brown	11.52	2,4	0.022	-0.11	0.917
sepia	92.55	3,6	0.002	4.35	0.012
plexus	20.09	3,6	0.022	-0.64	0.556
white	27.27	3,6	0.019	-0.37	0.731
forked	5.85	3,6	0.033	-1.29	0.277
yellow	2.47	3,6	0.240	0.32	0.767

[1]Generation effects from repeated measures ANOVA of -SS populations. Significance values (p) are Huynh-Feldt adjusted.

[2]Two-sample *t*-tests comparing final allele frequencies between sexual selection treatments.

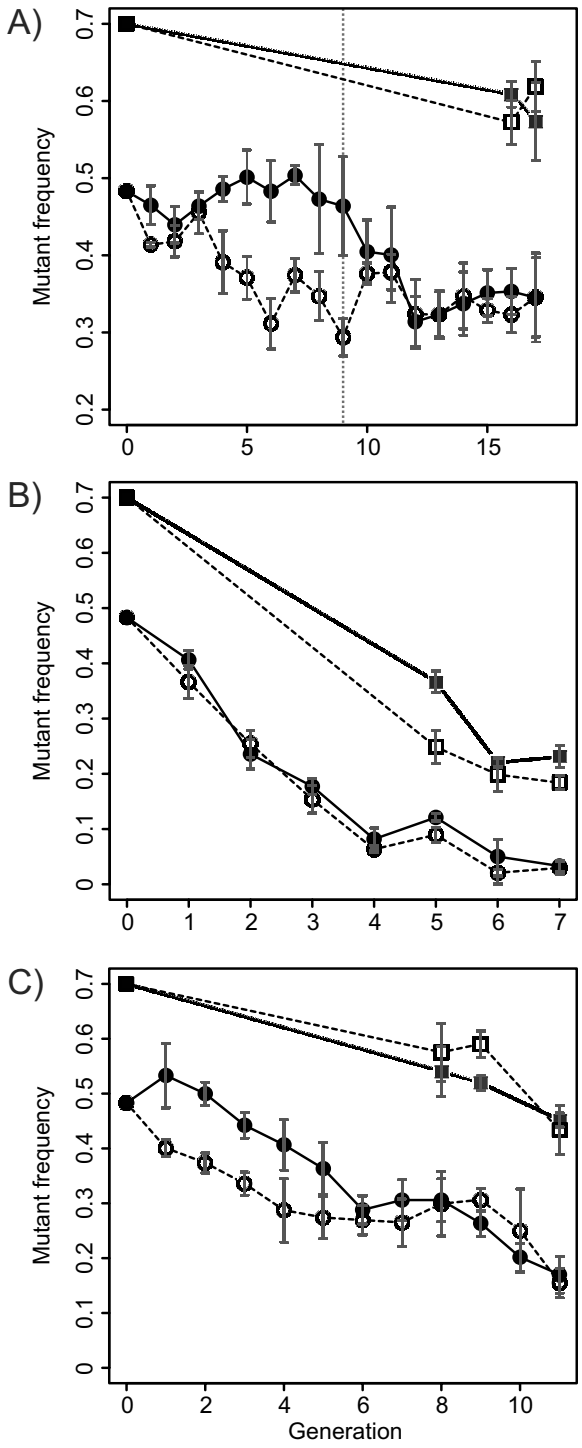

Figure 2-1 Allelic frequency changes through time for autosomal mutations

Average (±SE) visible (i.e. homozygous mutant; circles) and true allele (squares) mutant frequencies from the three replicate populations in the +S (solid lines, closed symbols) and -S (dashed lines, open symbols) treatments for the autosomal mutations A) brown, B)

sepia, and C) plexus. The vertical dotted line in A) indicates when population size was doubled (see Methods).

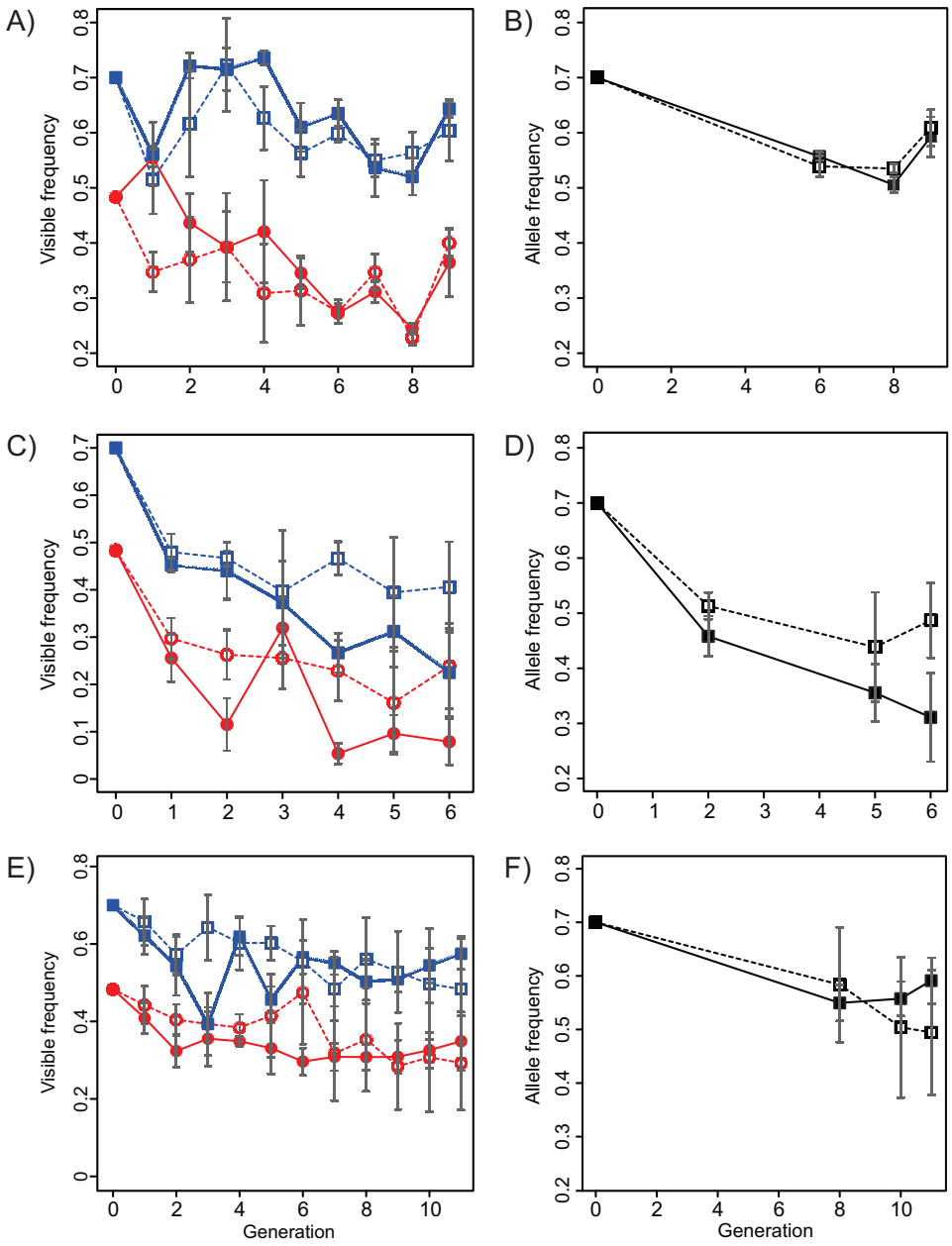

Figure 2-2 Allelic frequency changes through time for X-linked mutations

Average (±SE) visible frequency of male (blue) and female (red) mutant individuals (left column, panels A, C, and E), and true mutant allele frequencies (right column, panels B, D, and F), from the three replicate populations in the +S (solid lines, closed symbols) and -S (dashed lines, open symbols) treatments for the X-linked mutations A) and B) *white*, C) and D) *forked*, and E) and F) *yellow*.

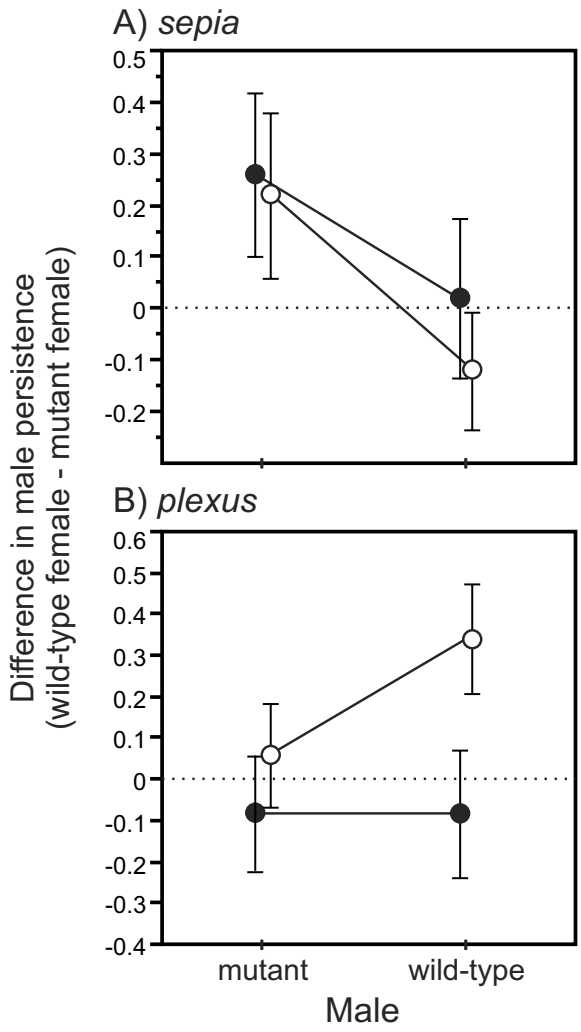

Figure 2-3 Male persistence toward mutant vs. wild-type females

Difference in male persistence behavior toward wild-type and mutant females for the A) *sepia* and B) *plexus* mutations. Single males, either homozygous mutant or wild-type, were given a choice between a single wild-type female and either a single homozygous mutant (open circles) or a single heterozygous mutant (closed circles) female. Persistence was quantified as the difference (±SE) in the total number of observed male courtship behaviors and matings involving the two types of females (wild-type – mutant). Positive values indicate that males disproportionately courted and mated wild-type over mutant females.

Chapter 3: Misalignment of natural and sexual selection among divergently adapted Drosophila melanogaster populations

Abstract

The effect of sexual selection on nonsexual fitness is a major unanswered question in evolutionary biology that may have important implications for adaptation, diversification, and the evolution of mate preferences. If reproductive success is condition-dependent, the resulting sexual selection will tend to align with natural selection, promoting adaptation. One prediction under such a scenario is that adaptation to a novel environment should increase male mating success and and hence sexual fitness. Environmentally-induced plasticity in mate preferences could also contribute to an alignment of natural and sexual selection if the changes cause females to prefer locally adapted males as mates. I tested for both forms of alignment using a set of 10 independent populations of *Drosophila melanogaster* that were adapted to one of two different environments. Competitive mating trials were performed between pairs of populations adapted to these two environments, with the trials designed to separate the effects of local adaptation on male mating success from plasticity of female mate preferences in response to these environments. Contrary to expectations under an alignment of natural and sexual selection, males did not have higher mating success when competing in the environment to which they were adapted. Furthermore, there was no evidence that females altered their mate choice based on their rearing environment, indicating the absence of any adaptive plasticity in mate preferences. Overall, despite previous evidence of reciprocal adaptation to these different environments, increased nonsexual fitness did not translate

into higher mating success, indicating a lack of any alignment with natural selection of this component of male sexual fitness.

Introduction

Natural selection can arise both from variation in nonsexual fitness (e.g., longevity, fecundity) and from variation in sexual fitness (i.e. the differential success in mating and fertilization among individuals that are otherwise capable of reproducing), with the latter process commonly known as sexual selection. In his original description of sexual selection, Darwin (1859) hypothesized that, because the most vigorous and best adapted males will tend to acquire the most mates, sexual selection may act to promote adaptation. More recently, theory suggests that the extent to which these two forms of selection align may also have major implications for population mean fitness (Lorch et al. 2003; Whitlock & Agrawal 2009), niche diversification (Proulx 1999, 2001, 2002; van Doorn et al. 2009), and the evolution of sex (Agrawal 2001; Siller 2001), among other things.

Building on Darwin's (1859) original idea, it has been suggested that sexual selection may align with selection arising from variation in nonsexual fitness (hereafter natural selection for simplicity) because successful reproduction requires a substantial investment of time and effort, and includes direct and/or indirect competition with other members of the same sex (Andersson 1994). Therefore, an individual's sexual fitness is likely to depend on their condition, as is expected for all major fitness components (Rowe & Houle 1996; Whitlock & Agrawal 2009). Individuals of high genetic quality (i.e. individuals well adapted to their environment) should acquire and use resources more efficiently to achieve a higher condition, and should therefore have higher sexual fitness

as well (Whitlock & Agrawal 2009). As a consequence, condition-dependent sexual fitness should promote the transmission of locally-adapted alleles.

Despite this expectation, data on the potential alignment of natural and sexual selection are mixed, with some studies finding evidence for alignment (Promislow et al. 1998; Radwan 2004; Dolgin et al. 2006; Sharp & Agrawal 2008; Hollis et al. 2009), while others do not (Holland & Rice 1999; Martin & Hosken 2004; Rundle et al. 2006; Long et al. 2009; Correia et al. 2010; Arbuthnott & Rundle 2012). Sexual selection also generates the opportunity for sexual conflict which arises from the divergent reproductive interests of the sexes and can lead to the evolution of traits with sexually antagonistic fitness effects (Arnqvist & Rowe 2005). The costs associated with such conflict may therefore reduce or even outweigh the benefits of mate choice and/or intrasexual competition in some systems (e.g. Stewart et al. 2005, 2008; Long et al. 2009), potentially explaining some of the variation among studies (Rowe & Day 2006; Whitlock & Agrawal 2009).

Here I address the effects of adaptation on male mating success. While much attention has been given to studying adaptation to novel environments with respect to nonsexual fitness (e.g., Schluter 1993; Sandoval 1994; Losos et al. 1998), its effects on male reproductive success are poorly understood. In many species, mating success is a major component of male sexual fitness that is likely to depend on the health or vigour of the males (i.e. is likely to be condition-dependent). If sexual selection arising from variation in mating success tends to align with natural selection, one simple prediction is that individuals should have higher mating success in the environment to which they are adapted (i.e. in which they have higher nonsexual fitness; Dolgin et al. 2006).

Surprisingly, there are few direct tests of this. Dolgin et al. (2006) and Correia et al. (2010) tested this prediction using independent sets of *Drosophila melanogaster* populations from two separate long-term selection experiments. In both cases, half of the populations were adapted to a cold temperature (18°C or 16°C respectively) and the other half a hot temperature (25°C in both cases). Results were mixed, with increased mating success of males in the environment to which they were adapted in one set of populations (Dolgin et al. 2006) but not in the other (Correia et al. 2010). The interpretation of these data were also complicated by the choice of environments (i.e. divergent temperatures) because, during mating trials, females must necessarily be exposed to the environment to which one of the two types of males are adapted. If female mate preferences are plastic with respect to these environments, increased mating success of males in their adapted environment could have arisen from divergent sexual selection generated by these different preferences rather than the higher condition of locally-adapted males, as recognized by Dolgin et al. (2006). More generally, plasticity of mate preferences may cause sexual selection to differ among environments, potentially altering its alignment with natural selection.

Female mate preferences can be affected by a diversity of factors including age and reproductive experience (Bateman et al. 2001; Moore & Moore 2001; Mautz & Sakaluk 2008; Judge 2010), season (Qvarnström et al. 2000; Borg et al. 2006; Milner et al. 2010), a female's condition (Hunt et al. 2005; Cotton et al. 2006), predation risk (Hendrick & Dill 1993; Csada & Neudorf 1995), and learning (Amcoffe et al. 2013). While such plasticity has been observed under various conditions, its impacts on offspring fitness among environments have rarely been considered. This is surprising

given the potential impact of plasticity on adaption to new environments and the alignment of natural and sexual selection (Qvarnström 2001; Price 2006). If female preferences are consistent across environments and target condition-dependent male displays, then the preferences themselves will be adaptive to females, causing them to mate with the best adapted males in any given environment. Under such a scenario, the preferred male may vary among environments due to changes in their underlying sexual displays as a consequence of their nonsexual fitness in a given environment. Alternatively, if female preferences change across environments, targeting different traits or different values of the same trait(s), then such plasticity will only be adaptive to females if it results in an increased preference for traits reflecting local adaptation in males. Importantly, such adaptive plasticity in mate preferences could contribute to the alignment of natural and sexual selection independently of condition-dependent male mating success (West-Eberhard 2003). In addition, whether adaptive to females or not, males may evolve increased mating success during adaptation to a particular environment in response to the divergent sexual selection created by such plastic changes in female mate preferences, rather than because of their higher nonsexual fitness. Therefore, changes in male mating success and female preference in response to novel environments may have important implications for our understanding of the alignment of natural and sexual selection.

I evaluated the potential alignment of natural and sexual selection by examining male mating success across two novel environments in a way that allowed us to separate the effects of local adaptation of the males (i.e. their nonsexual fitness) and plasticity of female mate preferences in these environments. This experiment took advantage of a

long-term evolution experiment involving ten replicate *D. melanogaster* populations, with five being adapted to the presence of salt in their food medium and the other five to the addition of the heavy metal cadmium. When assayed after 20 generations, these populations showed evidence of reciprocal adaptation such that nonsexual fitness was always higher for populations when tested in the environment in which they were evolved as compared to the other environment (Long et al. 2013). Using males from these different populations, I performed mating trials in a common garden experiment conducted separately within each environment. In this design, salt- and cadmium-adapted males competed for mates when both were raised in a salt environment and when both were raised in a cadmium environment, with females always being raised in a consistent (ancestral food) environment. If sexual selection arising from variation in male mating success aligns with natural selection, then males should have higher mating success when raised and tested in the environment to which they are adapted. In addition, to gain insight into plasticity of female mate preferences, I also used ancestral, non-adapted females in mate choice trials to estimate their preference for salt vs. cadmium adapted males (each raised in their own environment) when these females were raised for a single generation in each of the two environments. If female mate choice is plastic, the outcome of these trials will vary based on the females' rearing environment, and if adaptive, females will more strongly prefer males raised in the same environment as them. Overall, I found no evidence for the alignment of natural and sexual selection, as neither male mating success nor female mate preference varied consistently based on the test environment. The lack of such alignment suggests that sexual selection, at least that

arising from variation in mating success and the female preferences underlying this, will not necessarily enhance natural selection nor promote adaptation to new environments.

Materials and methods

Drosophila populations

This experiment used a set of replicate *Drosophila melanogaster* populations from an evolution experiment begun by A. Wang, C. C. Spencer, Y. Huang, and A. F. Agrawal at the University of Toronto. For a detailed description of the history of these populations, see Long et al. (2013). These populations were established from a laboratory stock population, originally collected in 2005 in the Similkameen Valley, British Columbia, Canada (Yeaman et al., 2010), that has been maintained at large population size on standard cornmeal/sugar/killed-yeast/agar medium. Following transfer to the University of Toronto, separate populations were exposed to experimental selection, with some raised on food with added salt (8% NaCl, mass by volume), and others raised on food that included the heavy metal cadmium (70 ug/mL $CdCl_2$) for 36-56 generations. In 2009, these separate populations were mixed and ten replicate populations were then established from the pooled stock and assigned to one of the two environments, with five populations raised on the salt food and five populations raised on the cadmium food. This divergence, pooling, and then reestablishment of new populations in the two environments was performed as part of a separate study. While the initial response to selection of any population in a novel environment will often involve the sorting of existing genetic variation, this was likely particularly important in these populations given their evolutionary history. However, I can think of no reason why these populations

would not be representative of others with respect to inferences concerning the alignment of natural and sexual selection during adaptation.

Following their reestablishment, the new experimental populations were maintained on a two-week cycle with non-overlapping generations in vials. Throughout the entire time, the stock population was maintained on a standard medium at a large census size in cages. After 20 generations in their respective environments, a reciprocal transplant experiment measuring egg-to-adult survival showed strong evidence of divergence and reciprocal adaptation of these ten populations to their respective environments in both survival and fecundity. Cadmium populations showed approximately 15% higher survival than salt populations in cadmium medium, and the salt populations showed approximately 45% higher survival than cadmium adapted populations in the salt medium (Long et al. 2013). Similarly, cadmium-adapted females produced significantly more eggs than the salt-adapted females when both were raised in the cadmium environment, while the reverse was true in the salt environment (Long et al. 2013).

In September of 2012, a large sample of each of these populations was transferred to the University of Ottawa and maintained under the same conditions (25 C, 12:12 L:D) on their respective media. Because cadmium-adapted populations have very low survival in the 8% salt medium, I decreased the salt concentration to 6% when rearing individuals from all populations (cadmium and salt) for use in the assays below. Salt-adapted populations were therefore not raised in the same conditions to which they were adapted, although previous survival assays using a 6% salt concentration found reciprocal adaptation among these populations (Y. Huang and A. F. Agrawal, unpublished data),

demonstrating that 8% salt-adapted populations outperformed cadmium-adapted populations when tested in 6% salt. When our assays were conducted, the experimental populations had been evolving in their respective environments for 80-90 generations.

Male mating success

I first sought to determine whether males have higher mating success in the environment to which they were adapted as compared to the other environment. To do this, I conducted mating trials in which multiple cadmium and salt-adapted males competed for matings with stock females within a small cage. Trials were performed when both types of males were raised in cadmium and when both types were raised in salt, with females always raised on the standard food. Each of the five cadmium and salt-adapted populations were paired randomly such that males from one cadmium adapted population were always competing for females against males from one salt adapted population, resulting in five independent cadmium-salt population pairs. Because female population and rearing environment are the same for all trials, any differences in mating success can be attributed directly to the males.

To collect males raised in each environment, 20-30 adults from each experimental population (reared in their normal selective environment) were allowed to lay eggs for 24 h in vials containing either salt or cadmium food. Stock females were collected by placing food-containing vials in population cages and allowing females to lay eggs for 1-2 h. All vials were controlled for egg density such that no vial contained more than 50 individuals. Virgin females/males were collected from their respective vials using light CO_2 anesthesia within 8 h of emergence. Males were held in vials containing food

matching their rearing environment at a density of seven per vial, without edible yeast, for 2-3 d. Stock females were held at a density of ten females per vial, with excess live yeast, for 3 d prior to the mating trials. Virgin flies were used to ensure that variance in reproductive history did not influence mating behaviour, including mate choice. Four hours prior to the mating trials, males were transferred to vials containing yeast paste coloured with either red or blue food colouring (McCormick, Canada). Males ate this coloured yeast and the colour was visible through their abdomen, temporarily marking them and making it possible to identify their population of origin. Males were coloured in a balanced design such that each population was blue for half of the trials and red for the other half. Previous use of such markings in mating trials has shown no effects on mate choice (Rundle et al. 1998, 2007; Mooers et al. 1999; Rundle 2003), and I found no evidence for differential mating by males of one colour in this assay (one sample t-test: $t_{96} = 0.0168$, $P = 0.99$) nor the female mate preference assay below ($t_{77} = 1.32$, $P = 0.19$).

Twenty replicate mating trials were performed for each population pair: ten when both types of males were raised in 6% salt and ten when both types were raised in cadmium. The only exception was population pair 4, for which I performed 14 trials rather than 20 due to a limited number of males. Within each trial, 21 cadmium-adapted males and 21 salt-adapted males were placed together with 20 stock females in a translucent plastic cage (14 × 14 × 14 cm). Both types of males were introduced first and females were subsequently released. Cages were covered with white paper to limit external disturbances and then left for 8 min, after which the first ten mating pairs were removed by aspiration. If fewer than ten pairs were collected, this procedure was repeated at 8 min intervals until 10 mating pairs were sampled. Males from the mating pairs were

scored for colour using a dissecting microscope under CO_2 anesthesia. If a male's colour could not be identified, it was discarded (approximately 6% of males overall). To determine whether the rearing environment of males influences their mating success, I used a linear mixed model with the proportion of matings achieved by cadmium-adapted males in a given cage as the response variable, male rearing environment as a fixed effect, and population pair as a random effect. In this model, population pair, rather than the number of trials, is used as the unit of replication. The model was fit via restricted maximum likelihood and significance of the random effect of population pair was tested using a likelihood ratio test.

Female mating preference

I next sought to determine whether female mate preference changed with rearing environment by raising stock females in both cadmium and salt media and then assaying their preference using mating trials like those above. The same population pairs were used such that within a given trial, stock females raised in one of the two environments had the choice of mating with either a cadmium-adapted male raised in cadmium or a salt-adapted male raised in salt. Because male populations and rearing environment are constant across trials, an effect of female rearing environment on the difference in the mating success of the two types of males cannot be attributed to changes in male competitive ability. Rather, an effect of female rearing environment would be consistent with plasticity in female mate preferences, although in theory it could also arise from an interaction between female rearing environment and male mate choice (e.g., if cadmium-adapted males were more attracted to females reared in cadmium as opposed to ethanol).

While male mate preferences have been demonstrated in *D. melanogaster*, they have been found to target female body size, likely because of the direct fecundity benefit this provides to males (Bonduriansky 2001; Long et al. 2009), suggesting that such an interaction is unlikely. More importantly, the absence of an effect of female rearing environment (see Results) argues against this possibility.

Stock females for use in the trials were raised in both cadmium and salt by allowing females in population cages to lay eggs in vials containing one of the two food types (only one food type was presented to females at any one time), with egg density again controlled at 50/vial. Following development, emerging females were collected as virgins and subsequently held at ten individuals per vial, with excess live yeast, for 5 d. Cadmium and salt-adapted populations were raised in their adapted environment (i.e. cadmium populations in cadmium, salt populations in 6% salt) using the same egg collection protocols described above, and emerging males were collected as virgins and held at seven individuals per vial, without live yeast, for 4-5 d. Males were marked in a balanced design and trials were conducted as described above. Seven to nine replicate mating trials were performed for each population pair per female rearing environment, yielding 78 trials overall. To test whether female rearing environment changed their preference for the two types of males, I used a linear mixed model as described above but with female (instead of male) rearing environment as a fixed effect.

Results and Discussion

If condition-dependent mating success contributes to the alignment of natural and sexual selection during adaptation to new environments, then males should have higher mating success in their adapted environment than in a non-adapted environment.

Contrary to this hypothesis, the mating success of cadmium and salt-adapted males did not differ overall based on male rearing environment ($F_{1,4} = 0.04$, $P = 0.860$), providing no evidence of higher mating success of locally-adapted males (Fig. 3-1). While differences among population pairs in the relative mating success of the two types of males approached significance (Likelihood ratio test, $\chi^2 = 3.0$, $P = 0.083$), and three population pairs showed the expected pattern of males having higher mating success in their adapted environment, the other two showed the opposite pattern and in no case were any of these differences significant (two-sample *t*-test, $P > 0.08$ in separate tests of all five pairs). Therefore, despite confirmed adaptation with respect to nonsexual fitness in these populations, and strong trade-offs between environments, this did not translate into increased mating success of locally adapted males.

Natural and sexual selection may also align if mate preferences are plastic such that females raised in a given environment are more likely to mate with locally-adapted males than are females raised in a different environment. By generating divergent sexual selection, such plasticity could also result in higher mating success of locally-adapted males without any alignment of sexual and natural selection, provided males show an evolutionary response to this selection. Inconsistent with any plasticity in these populations, female mate preferences for cadmium vs. salt-adapted males did not differ significantly when ancestral, stock females were raised for a single generation in cadmium vs. salt food ($F_{1, 4} = 0.84$, $P = 0.412$; Fig. 3-2). Results did not vary significantly among the five population pairs ($\chi^2 = 1.4$, $P = 0.237$), although two showed the predicted pattern of females more strongly preferring males adapted to the female's rearing environment. The other three pairs showed the opposite pattern, however, and one

of these (pair 4) was the only one in which the difference was significant (two-sample *t*-test: $t_{13.99} = -5.13$, $P < 0.001$; $P > 0.4$ for the other four pairs). Therefore, my results provide no evidence of any consistent plasticity of female mate preferences with respect to their choice between these two types of males.

Condition-dependence of the traits underlying sexual fitness has been proposed as a general mechanism to explain the maintenance of unexpectedly high genetic variance in these traits in the face of strong directional sexual selection (Rowe & Houle 1996). The condition-dependence of sexual displays is well supported (Cotton et al. 2004; see Delcourt & Rundle 2011 for a more recent discussion), at least with respect to environmental manipulations, and this should contribute to the alignment of natural and sexual selection (Whitlock & Agrawal 2009). Increased reproductive success of high condition males has been found in some cases (e.g., Sharp & Agrawal 2008; Maclellan et al. 2009), although these studies made use of single deleterious mutations of large effect that may not be representative of the genetic basis of adaptation to new environments. There are few investigations of male mating success in particular among divergently adapted populations, despite the relevance of such studies to our understanding of the effects of sexual selection on adaptation to novel environments, and the two that have been done have provided mixed results despite employing the same species and almost identical environments (i.e. low and high temperature in *D. melanogaster*; see Dolgin et al. 2006; Correia et al. 2010). My finding that local adaptation does not necessarily result in increased male mating success suggests that at least this component of sexual selection may not reinforce natural selection, consistent with Correia et al.'s (2010) results. Given the limited number of experiments that address such alignment, as well as their

conflicting results, I echo Correia et al's (2010) call for additional studies across a range of species that use different environments to determine the generality of this result. The sensitivity of other components of sexual fitness to local adaptation also remains a key question.

The lack of alignment between sexual and nonsexual fitness is surprising, especially given strong evidence of the adaptive divergence of these populations (Long et al. 2013), and the underlying explanation is therefore of interest. Much attention of late has been given to sexual conflict's ability to obscure the benefits of condition-dependent reproductive success (Whitlock & Agrawal 2009; Pischedda & Chippindale 2006; Stewart et al. 2008; Bonduriansky & Chenoweth 2009; Long et al. 2009). However, my approach tested only for a benefit of sexual selection and did not consider its overall fitness consequences. The operation of sexual conflict itself cannot therefore explain the lack of an alignment I observed.

The nature of these environments could, in theory, contribute to the lack of an alignment. In particular, these environments altered the larval food media and this may not have impacted adults directly. One would expect that adaptation to these environments would entail not only increased survival, but also as an increased ability to gain and use resources, leading to increased condition of surviving adults. However, it is possible that, when raised in the 'other' (i.e. non-adapted) environment, the adults that do successfully emerge are of similar condition to locally-adapted males and thus perform equally well in terms of intrasexual mate competition. Inconsistent with this, differences in adult body mass mirror patterns of survival and fecundity when measured in a reciprocal transplant using the same food environments as these experiments (Y. Huang

and A. F. Agrawal, unpublished data), with adapted males being heavier on average than non-adapted males, and male mass being lower when individuals were raised on non-adapted vs. adapted food (Table 3-1). This suggests that adult males were of lower condition when raised in the environment to which they were not adapted.

Another possibility is that I may have underestimated the condition-dependence of male mating success by using a 6% salt environment in these assays, despite the salt populations being adapted to 8% salt. I used 6% salt in order to collect sufficient numbers of cadmium-adapted males as few, if any, of these males survived when raised in 8% salt. Salt-adapted males may therefore not have been of as high condition as if they were raised in their evolved environment, although as noted in the Methods, salt-adapted males were larger and had significantly higher survival than cadmium-adapted males in an assay using a 6% salt environment.

An alternative explanation for the lack on an alignment involves the differential allocation of condition. In particular, while males reared in a non-adapted environment may be of lower average condition, and thus have fewer resources overall, these males may allocate disproportionately more of these resources to mating success at the expense of other life history traits, thereby maintaining this fitness component at the cost of others. Such differential allocation has been previously demonstrated in male *D. melanogaster* (Rundle et al. 2007), but has received limited attention in sexual selection research.

Finally, the use of ancestral rather than salt- and/or cadmium-adapted females in these mating trials may have contributed to the lack of any detectable alignment. If female mate preferences evolved in each population to target well-adapted males, but

these preference changes were population and/or environment specific such that they targeted different male traits (possibly because different male traits are indicative of high condition in different environments), then local males would only have higher mating success when tested with females with the appropriate preference (i.e. that evolved in the same population or environment), and not with stock females. I used stock females to avoid confounding the effects of local adaptation of the males with plasticity of female mate preferences. My results suggest that such plasticity did not occur, so further experiments using females adapted to each of the two environments could be used to test for such population or environment-specific effects that could contribute to an alignment of natural and sexual selection.

I focused here on male mating success because it is likely a particularly important component of sexual fitness in these populations given their maintenance routine involving non-overlapping generations and severely truncated adult lifespans. Male mating success has also been the subject of past studies with respect to natural selection (e.g. Proulx 1999, 2001, van Doorn et al. 2009), including direct tests concerning the effects of local adaptation on it (Dolgin et al. 2006, Correia et al. 2010). The lack of alignment I observed with respect to mating success does not preclude the possibility that sexual selection arising from other components of male sexual fitness (e.g., postcopulartory performance) may align with natural selection and thereby promote adaptation. It is also possible that virgin females may be less discriminatory than previously mated females, and the use of them may therefore fail to detect an alignment that may occur in these populations. Like past studies (Dolgin et al. 2006, Correia et al. 2010), I used virgin females to control for female mating history. In other insects, there is

some evidence that non-virgin females show stronger mate preferences than virgin females (Bateman et al. 2001; Judge 2010), although others do not find this pattern (Ivy & Sakaluk 2007). However, in each of these studies, mate preferences were observed in virgins, and females preferred similar traits regardless of mating history, suggesting that studying mate choice in virgins should be representative of mate choice of all females. I am not aware of any evidence for decreased mate discrimination by virgin, as compared to mated, *D. melanogaster*, and given the short adult phase of these populations (4 days on average), a substantial portion of the matings in these unmanipulated populations likely involve virgin females, making mating success with such females an important component of a male's sexual fitness.

Turning to the plasticity of female mate preferences, such changes could have large impacts on sexual selection and adaptation (Qvarnström 2001; Price 2006) and yet have received little attention within the context of adaptation to new environments (Price et al. 2003). There is some evidence for the condition-dependence of mate preferences themselves (Cotton et al. 2006) as well as other forms of preference plasticity (e.g., Hendrick & Dill 1993; Qvarnström 2000; Grace & Shaw 2004; Amcoffe 2013), but the effect of environmentally-induced preference variation on adaptation to novel environments has not been directly addressed. Plastic changes in mate preferences may alter the direction and strength of sexual selection rapidly upon colonization of a new environment, and the nature of such plasticity would determine its adaptive benefit (or not) with respect to nonsexual fitness. Plasticity in preference could also cause males to have higher mating success in their local environment without requiring any alignment of natural and sexual selection, and its existence is therefore of interest in such studies. In

my experiment, no increase in mate preference for cadmium or salt-adapted males was observed when stock females were raised in each of these respective environments, indicating the absence of any adaptive plasticity that would contribute to an alignment of natural and sexual selection. Although not an issue given my results, it is worth noting that had I observed such a pattern, it would not necessarily have indicated adaptive plasticity. My experimental design used cadmium and salt-adapted males, so if males evolved higher mating success during experimental evolution in response to the divergent sexual selection generated by plasticity, these males would be expected to be more attractive to the females expressing this preference (i.e. those raised in that environment).

In conclusion, despite significant divergence among populations and reciprocal adaptation to their different environments, I found no effect of rearing environment on male mating success or female mate preferences. In particular, although the nonsexual fitness of populations differed greatly when raised in their local as opposed to the other environment, this did not serve to increase a male's ability to acquire mates, suggesting that at least this component of sexual selection does not reinforce natural selection in these environments. Other studies have also failed to find alignment between natural and sexual selection in experimental populations (Holland & Rice 1999; Martin & Hosken 2004; Correia et al. 2010), and the presence of sexual selection did not speed adaptation to a new environment in *D. serrata* (Rundle et al. 2006) nor *D. melanogaster* (Holland 2002). Sexual selection on some loci will likely be sex-specific, generating intra- and/or interlocus sexual conflict that may hamper natural selection and reduce nonsexual fitness (Pischedda & Chippindale 2006; Stewart et al. 2008; Bonduriansky & Chenoweth 2009; Long et al. 2009). Overall, the costs associated with sexual conflict may outweigh the

potential benefits of sexual selection, causing the net effect to be deleterious (Stewart et al. 2005, 2008; Rice et al. 2006; Arbuthnott & Rundle 2012). Recent work has suggested that the potential alignment of natural and sexual selection may depend on a population's location within a fitness landscape, such that alignment is more likely when the population is far from a fitness peak, where benefits are more likely to outweigh costs of sexual selection (Fricke & Arnqvist 2007; Long et al. 2012). These current results suggest that, even aside from any potential costs of sexual conflict, sexual selection arising from variation in mating success may not promote adaptation to novel environments.

Table 3-2 Effects of adapted and rearing environment on male body mass

Average male body mass (standard error) from a reciprocal transplant experiment. Masses represent the average for five populations adapted to each environment in each test environment. Three males from the same vial were combined and their mass was measured in milligrams. Differences in mass produced a significant adapted × test environment interaction (linear mixed model with population (nested within adapted environment) as a random effect; $F_{1,8} = 15.22$, $P = 0.0045$).

		Adapted environment	
		Cadmium	Salt
Test environment	Cadmium	0.60 (0.013)	0.55 (0.012)
	Salt	0.59 (0.014)	0.61 (0.011)

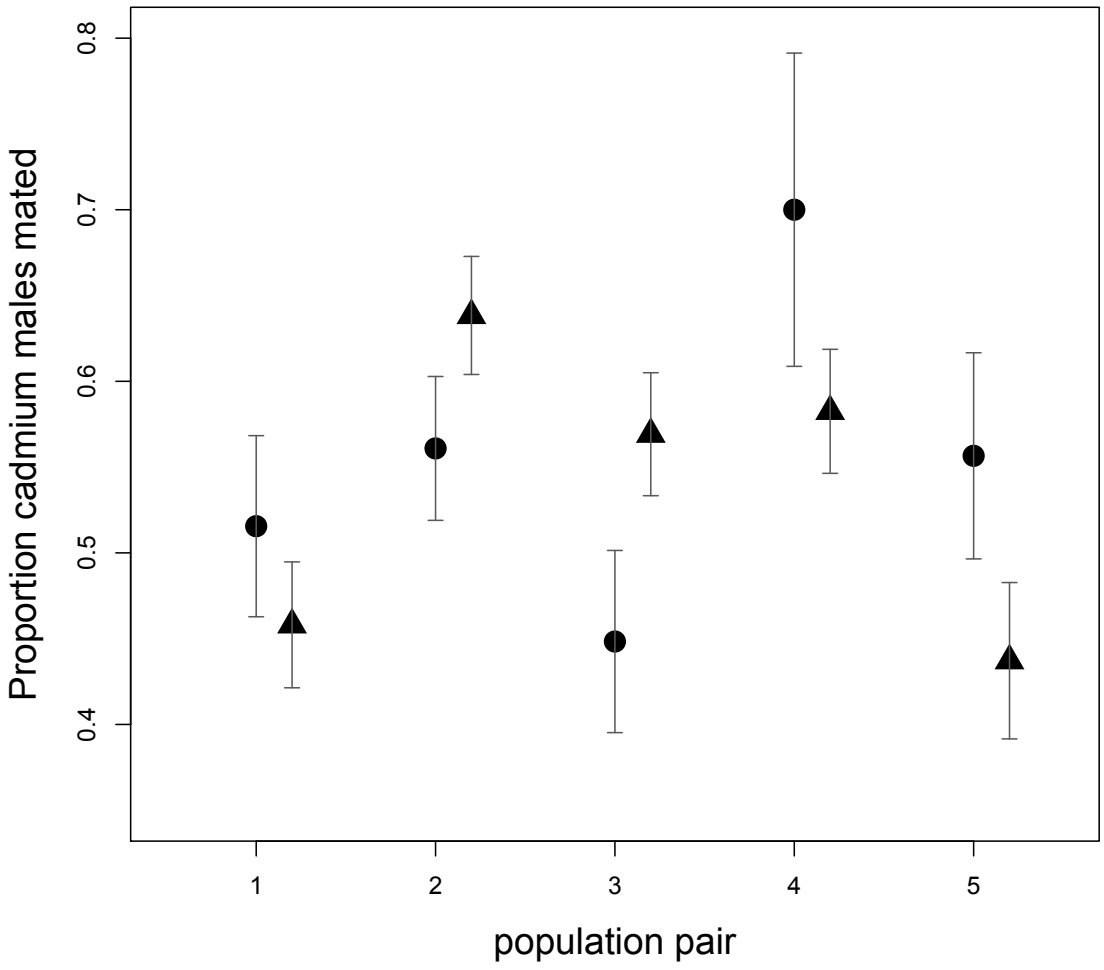

Figure 3-1 Male competitive mating success when raised in salt and cadmium environments

The proportion of cadmium-adapted males among mating pairs in trials measuring male mating success. Points represent the average (± SE) of the 10 replicate cages for each unique population pair when all males were raised either in cadmium (circle) or in salt (triangle) media. A proportion of 0.5 represents equal mating success of cadmium and salt-adapted males. A higher mating success of locally-adapted males would cause the circles to have larger values than triangles.

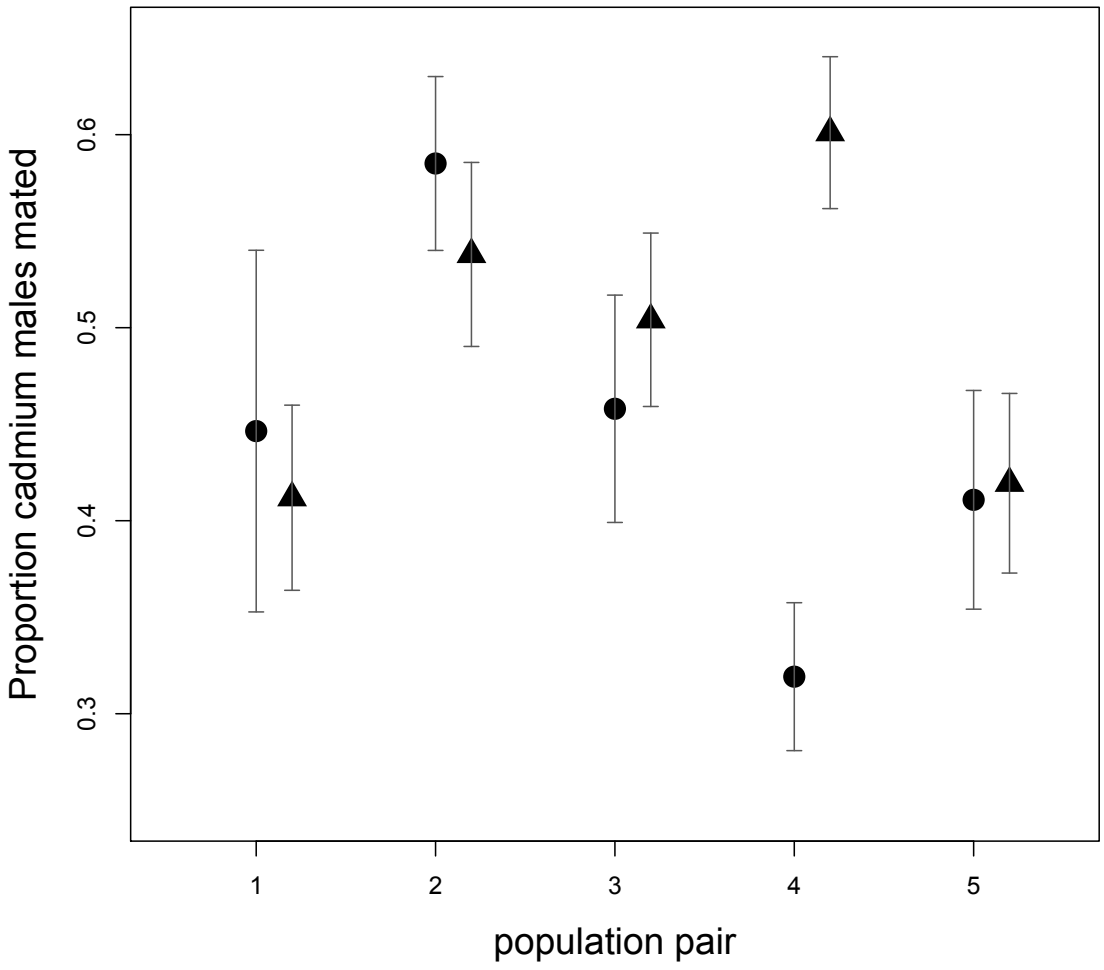

Figure 3-2 Male competitive mating success when females were raised in salt and cadmium environments

The proportion of cadmium-adapted males among mating pairs in trials measuring female mate choice. Points represent the average (±SE) of the replicate cages for each unique population pair when females were raised either in cadmium (circles) or salt (triangles) media. All males were raised in their adapted environment. If females preferred to mate with males adapted to their rearing environment, the circles would have higher values than the triangles.

Chapter 4: The ecology of sexual conflict: ecologically-dependent parallel evolution of male harm and female resistance in *Drosophila melanogaster*

Abstract

The prevalence of sexual conflict in nature, along with the potentially stochastic nature of the resulting coevolutionary trajectories, makes it an important driver of phenotypic divergence and speciation that can operate even in the absence of environmental differences. The majority of empirical work investigating sexual conflict's role in population divergence/speciation has therefore been done in uniform environments and any role of ecology has largely been ignored. However, theory often invokes natural selection as constraining phenotypes influenced by sexual conflict. I use replicate populations of *Drosophila melanogaster* adapted to alternative environments to test how ecology influences the evolution of male effects on female longevity. The extent to which males reduce female longevity, as well as female resistance to such harm, both evolved in association with adaptation to the different environments. These results demonstrate that ecology plays a central role in shaping patterns of population divergence in traits under sexual conflict.

Introduction

Sexual conflict arises when the reproductive interests of males and females are not aligned, generating sex-specific selection on shared traits such as mating rate (Parker 1979). When the genetic basis of the trait differs between the sexes, sexually antagonistic coevolution can favor loci that enhance a male's reproductive success relative to intrasexual competitors, even if males carrying such alleles reduce female fitness. Reciprocally, selection will also favor loci that increase a female's resistance to such harm, even if these come at a cost to male fitness (Rice 1996; Holland & Rice 1998). Such interlocus sexual conflict can therefore cause repeated bouts of coevolution, driving an evolutionary 'arms race' in which any advantage gained by individuals of one sex decreases the fitness of individuals of the other, generating renewed selection that can lead to an endless evolutionary chase between the sexes (Parker 1979; Civetta & Singh 1995; Rice 1996; Chapman *et al.* 2003; Arnqvist & Rowe 2005).

The integral role sexual antagonism plays in evolution has been confirmed by experimental (Rice 1996; Martin & Hosken 2003; Stewart *et al.* 2005; Long *et al.* 2009), comparative (Arnqvist 1998; Arnqvist & Rowe 2002) and theoretical studies (Gavrilets *et al.* 2001; Gavrilets & Waxman 2002; Rowe *et al.* 2003), and there are numerous examples in nature of traits that increase male reproductive success at the expense of females, and traits that enhance female resistance to specific forms of male coercion or harm (Arnqvist & Rowe 2005). The particular phenotypes that evolve via interlocus sexual conflict are thought to be in part a product of chance, subject for example to the nature of segregating variance, new mutational input, and the evolutionary history of the population in question. Consistent with this, traits enhancing male sexual fitness are

extraordinarily diverse among taxa (e.g., persistent courtship and harassment, toxic ejaculates, spiny genitalia, and means of traumatic insemination; Arnqvist & Rowe 2005). Together with the persistent and potentially strong selection such interlocus conflict can generate, the arbitrary nature of the resulting coevolutionary trajectories may contribute to rapid population divergence and ultimately speciation (Arnqvist 1998; Arnqvist *et al.* 2000; Gavrilets *et al.* 2001; Gavrilets & Waxman 2002; Martin & Hosken 2003) and there is therefore a great deal of interest in sexual conflict's role in diversification.

Because sexually antagonistic selection arises from an interaction of the sexes, divergence via sexual conflict has often been considered to be driven by chance events such as the order in which particular mutations occur, and is thus often cast as independent of ecology (Coyne & Orr 2004; Rundle & Nosil 2005; Schluter 2009). The majority of experimental work investigating sexually antagonistic coevolution and its consequences has therefore been done in a uniform environment (e.g. Rice 1996; Andrés & Arnqvist 2001; Long *et al.* 2006; Edward *et al.* 2011). Despite the emphasis in the speciation literature of sexual conflict as a non-ecological driver of diversification (Gavrilets 2000; Coyne & Orr 2004; Rundle & Nosil 2005), discussions of the evolution of traits mediating conflict have repeatedly noted that these do not evolve in an ecological vacuum, but rather are likely to be targets of natural selection as well (Parker 1979; Rowe *et al.* 1994; Rice & Holland 1997; Rowe & Day 2006; Fricke *et al.* 2009; Maan & Seehausen 2011). Natural selection on the traits involved in conflict could limit their exaggeration due to cost within an ecological context, thereby influencing the coevolutionary pathways followed (or not) by sexual conflict. Ecological selection could

also drive trait evolution on its own, and if sufficiently strong, the traits underlying sexual conflict may evolve not by sexually antagonistic coevolution, or at least not by such coevolution alone. As a consequence of such effects, there is an implicit prediction of ecologically-dependent parallel evolution in which populations adapting to similar ecological contexts should evolve along relatively similar evolutionary pathways with respect to sexual conflict, whereas populations in different environments should be more divergent (Rowe et al. 2003).

I confirmed this verbal argument concerning the potential effects of natural selection via computer simulations that extended previous models of sexual conflict (Gavrilets & Waxman 2002; Rowe *et al.* 2003; Hayashi *et al.* 2007). My model considered multiple male and female traits under sexual conflict simultaneously and permitted natural selection to vary qualitatively among populations. When environments differed in the strength and direction of natural selection on the male traits, these simulations revealed that male stimulation/coercion and female resistance often evolved in parallel among replicate populations in correlation with the environment. The result was that the evolution of traits normally considered as phenotypic manifestations of sexual conflict (e.g., male harm, female resistance) was predictable by ecology (see Appendix).

Empirically, by exposing females to males from different populations (or vice versa), it is possible to detect evolved differences among populations in male (or female) components of sexual conflict. However, much of the experimental work employing this technique has not explicitly considered ecology. Instead, focus has been given to assessing the claim that females should be more resistant to coevolved (i.e. local), as

opposed to foreign, males (Holland & Rice 1998; Andrés & Arnqvist 2001; Brown & Eady 2001; Chapman *et al*. 2003; Long *et al*. 2006; Wigby & Chapman 2006). However, this claim is not based on a robust theoretical framework and the value of population crosses for assessing sexually antagonistic coevolution has therefore been questioned (Rowe *et al*. 2003). In spite of this, such crosses do provide a powerful approach for testing ecologically-dependent parallel evolution of the traits involved in conflict. In particular, if ecology is important then (i) males from populations independently-adapted to the same type of habitat should have similar effects on females, and/or (ii) females from the same habitat type should be similarly affected by (or resistant to) males. While ecology would promote parallel evolution of such male and female sexual conflict traits within environments in this scenario, it should simultaneously promote consistent patterns of divergence in these traits among populations occupying different environments. The ecologically-dependent parallel evolution of male and/or female effects on sexual conflict would provide direct evidence of the importance of ecology in the divergence of conflict phenotypes.

A number of empirical studies have shown that the opportunity for sexual conflict, as well as its economics (i.e. costs and benefits), can depend on the ecological context under which it is assayed (Endler 1980, 1987; Magurran & Seghers 1994; Rowe *et al.* 1994; Edward & Gilburn 2007; Fricke *et al.* 2010b; Karlsson *et al.* 2010). More recently, ecology has been manipulated within the context of evolution experiments in seed beetles addressing traits involved in sexual conflict. In particular, Fricke *et al.* (2010a) showed that two food environments differed in the extent of evolutionary diversification among populations, although there was no evidence that populations from

shared environments followed similar evolutionary pathways. In a separate experiment, a manipulation of the duration of the reproductive period affected the evolution of genital and mating traits (Maklakov *et al*. 2010; Cayetano *et al*. 2011).

As a test of ecology's role in sexual conflict, I used a long-term evolution experiment to assess the consequences of male-female interactions on a key life history trait among replicate populations of *Drosophila melanogaster* that had independently adapted to one of two alternative laboratory environments. I assess how much female longevity is reduced by high levels of exposure to males from different types of populations (e.g., coevolved males, males from alternative populations evolving in the same environment, or males from alternative populations evolving in a different environment). Because of the large number of inter-population combinations, I did not measure female offspring production, and therefore do not know the impact of the observed reductions in lifespan on net fitness. Nonetheless, male-induced reductions in female longevity have classically been interpreted as an outcome of sexual conflict in fly populations (e.g. Fowler & Partridge 1989; Rice 1996) and have been shown to be associated with reductions in total fitness in other populations (Edward *et al.* 2011). Longevity is also a key life history component in populations with overlapping generations such as these. Furthermore, males with the greatest effect on female mortality tended to have the highest fitness (Rice 1996), indicating that differences in female longevity are associated with male fitness gains and thereby provide insight into ongoing sexual conflict (Chapman 2001). Therefore, male-induced reductions in female longevity are likely manifestations of sexual conflict over fitness, though further tests would be required to confirm this. I demonstrate that male harm and female resistance to such harm

both evolve in correlation with environment, providing some of the first experimental evidence that ecological adaptation may explain a substantial component of the among-population variation in traits previously thought to evolve solely via sexually antagonistic selection.

Material and Methods

Adaptation and sexual conflict in Drosophila *populations*

A stock population of *D. melanogaster* was founded in Sept. 2005 from a large collection of wild flies in the Similkameen Valley, British Columbia (Yeaman *et al.* 2010). Since its collection, this stock has been maintained at a large census size in population cages with overlapping generations. In 2007, 20 independent and isolated populations were derived from this stock, with ten of these maintained on food supplemented with 12% ethanol and the other ten on food containing 70 ug/mL cadmium chloride, while the stock continued to be maintained on their standard medium. These experimental populations (which I refer to as selection lines below) were maintained in population cages with overlapping generations like the stock, with new food added every 21/14 d in ethanol/cadmium environments respectively. (The different feeding schedules were used because the development time was initially slower in ethanol as compared to cadmium.) These environments were arbitrary, chosen only because selection was likely to differ between them and, based on past studies, adaptation was likely. All populations were kept on a 12h:12h light:dark cycle at 25°C and at 50% humidity.

My main goal was to test for the ecologically-dependent parallel evolution of traits involved in sexual conflict. Prior to this, however, in July 2011 I first confirmed that these populations had adapted to their respective environments by carrying out a

reciprocal transplant experiment to assess each line's fitness in both ethanol and cadmium-containing media. Eggs were collected on both food types from all ten ethanol populations, all ten cadmium populations, as well as the ancestral stock population. These eggs were allowed to develop and adults were collected as virgins at emergence using light CO_2 anaesthesia. The sexes were held separately for 2-4 days on the same food in which they developed.

Following this, I paired single males and females from the same selection line in vials containing the same food as they developed in, thereby generating all four combinations of selection environment and test environment, as well as the ancestral stock in both environments. Pairs were allowed to mate and lay eggs for 2 days, after which they were removed and discarded and the eggs were allowed to develop. The number of adult offspring that emerged was counted daily from days 9-17. Forty replicate male-female pairs were created per selection line per test environment. Because the assay took place over two generations, the number of adult offspring produced was an inclusive measure of fitness that depended on both the fecundity of the male-female pair (i.e. the number of offspring produced) as well as the egg-to-adult survival of their offspring in the test environment.

Productivity data were bi-modally distributed because some pairs produced very few or no offspring (19% overall). Variation in productivity was therefore tested in stages. First, for vials that produced six or more offspring (and for which productivity data were normally distributed), variation in the number of offspring produced was analyzed using a partially-nested general linear mixed model with selection environment, test environment, their interaction, and block all as fixed effects. Population nested within

selection environment, and the interaction of this with test environment, were included as random effects. Second, variation in the probability of producing offspring was tested by coding all vials as either non-productive (i.e. the pair produced five or fewer offspring) or productive (i.e. the pair produced six or more offspring) and then calculating the proportion of non-productive vials separately for each population × test environment combination. Variation in the proportion of non-productive vials was then analyzed using a general linear model with selection environment, test environment, and their interaction as fixed effects. Results were qualitatively the same if the proportions were arcsine square root transformed prior to testing. The stock population was unreplicated (i.e. there was only a single ancestral stock) and could therefore not be included in either of the above productivity analyses.

I also confirmed the presence of sexual conflict in the ancestral stock population by testing for male induced harm of females. This assay was conducted in the fall of 2012 and involved measuring the longevity of stock females when exposed to their own males either continuously or for a 4 h period once per week (high vs. low exposure respectively).

Given evidence from the above assays for the adaptive divergence of these populations, as well as ongoing sexual conflict in the stock from which they were derived (see Results), I tested for ecologically-dependent parallel evolution of traits involved in this conflict by measuring male harm and female resistance among a subset of eight of the selection lines, as described below. These populations were selected as the four with the highest fitness in each of the two environments.

Male harm

To test for differences among populations and environments in male's effect on female life history, in the fall of 2012 I measured the longevity of stock females when continuously exposed to males from each of the eight chosen selection lines (four ethanol and four cadmium). All flies were reared for one generation on the 'ancestral' food (i.e. no ethanol or cadmium added) prior to the assay, and individuals for use in the assay were collected as virgins using light CO_2 anesthesia within 8 h of emergence. Thirty replicate vials were set up for each male population, with each vial containing five stock females in the ancestral medium. Each vial also contained five selection line males, and males were replaced weekly with fresh individuals that were collected on day 14 of their life cycle from the appropriate selection line. Flies were also transferred to new vials containing fresh food weekly at this time. Extra agar was added when preparing the food for all experimental vials to slow its breakdown (i.e. liquefaction) from larval feeding. Female death was recorded daily and used to estimate average female longevity for each vial. If male mortality was observed, replacement males from the appropriate population were added such that each vial always had five males present. The assay was performed in two complete blocks separated by one day, with half the replicates from each population being performed in each block.

Variation in female longevity was tested using a general linear mixed model, fit via restricted maximum likelihood (REML), using the average longevity of the five females from each replicate vial as the response variable to avoid pseudoreplication. Male environment (ethanol vs. cadmium) and experimental block were fixed effects, and population was a random effect nested within environment. Fixed effects were evaluated

via standard type 3 tests, while significance of the random effect of population was evaluated via a likelihood ratio test (LRT) that compared the fit of models that included and excluded this term. Results were qualitatively similar if block was modeled as a random effect. Analyses were performed using the mixed procedure in SAS.

Female resistance to male harm

I next tested whether adaptation to the different environments affected female resistance to male-induced harm. The assay was conducted in the fall of 2012. Females from the same eight selection lines as the male harm assay were housed under high and low male exposure treatments using common stock males. Female resistance to male-induced harm can be quantified as the extent to which female longevity is reduced in the high as compared to low exposure treatments, with smaller differences indicating greater resistance (Friberg 2005). In the high exposure treatment, females were held together with males continuously, with the males replaced weekly with fresh individuals that were collected on day 14 of their life cycle, as in the male harm assay above. In the low exposure treatment, females were held together with stock males for a 4 h period once each week, with the timing coinciding with replacement of males in the high treatment level. Short, weekly access to males provided sufficient sperm such that female offspring production was unlikely to be sperm limited while greatly reducing female exposure to male harassment and the opportunity for multiple matings.

Virgin males and females were collected as previously described. Thirty replicate vials were created for each combination of female population and exposure treatment, with each vial again containing five females. Vials in the high exposure treatment also

held five males, and any males that died between transfers were replaced daily by similar aged stock males. In the low exposure treatment, the number of males added for the 4 h period was matched to the number of females alive in the vial at that time. All flies were anesthetized with light CO_2 during weekly addition/replacement of males, and high exposure vials were again anaesthetized 4 h later to coincide with the removal of males from the low exposure vials, thereby ensuring that both treatment levels were handled similarly. Flies were also transferred to new vials containing fresh high agar food weekly at this time. The assay was performed in two complete blocks separated by one day, with half of the replicates from each combination of treatment and population being performed in each block. Female death was recorded daily.

Variation in female longevity was tested using a partially-nested general linear mixed model, fit via REML, again using the average vial longevity as the response variable. Fixed effects included male exposure treatment (low vs. high), female environment (ethanol vs. cadmium), and their interaction, as well as block, while female population nested within environment, along with the interaction of this with male exposure treatment, were modelled as random effects.

Because similar average lifespans can be achieved via varied life history strategies involving different temporal patterns of mortality (Pletcher 1999), I also estimated mortality parameters for every combination of exposure treatment and female population (150 females/combination). For each combination, the best-fit mortality model from the Gompertz family was first determined using a series of nested likelihood ratio tests as implemented in the program WinModest (Pletcher 1999). For the majority of population × male exposure combinations (11 out of 16), female mortality was best

described by a Gompertz model (Pletcher 1999), $\mu_x = \alpha e^{\beta x}$, where μ_x is the instantaneous mortality rate at age x, α is the baseline mortality, and β is the senescence rate (i.e. the rate at which mortality increases with age). α and β were estimated separately for each population per treatment and analyzed with the linear model above excluding both terms involving the random effect of population as there was only a single value of each mortality parameter for each population × treatment combination.

Factorial assay of selection lines

All of the above assays involved interactions between evolved individuals of one sex and stock individuals of the other. Therefore, to test the consequences of male-induced harm within and between selection lines, I also performed a full factorial experiment, measuring female longevity for all 64 pairwise combinations of males and females from all eight selection lines in the fall of 2011. Populations were raised for one generation on the ancestral food prior to conducting the assay and virgins were collected as above. Ten replicate vials were established for each male × female population combination, split evenly between two blocks separated by one day. Each vial initially contained five females and five males on the ancestral food medium. Males were replaced weekly with fresh individuals that were collected on day 14 of their life cycle, as in the male harm assay above. Flies were also transferred to new vials containing fresh high agar food weekly at this time. I checked vials daily for mortality, recording the day of death for females and replacing any dead males as in the assays above.

Variation in female longevity was tested using a general linear mixed model, fit via REML, with average female longevity of a vial as the response variable. Fixed effects

included male environment (ethanol vs. cadmium), female environment (ethanol vs. cadmium), and their interaction, as well as block. Male and female population were random effects nested within male and female environment respectively. The interaction of male and female population was non-significant and was therefore excluded from further analyses.

Results

Adaptation and sexual conflict in Drosophila populations

In a reciprocal transplant fitness assay of all populations, I found that each of the twenty selection lines had higher fitness in its selected environment than the ancestor (i.e. the stock; Fig. 4-1). For productive vials (i.e. those producing six or more offspring), average productivity was higher when populations were tested in the environment in which they evolved as compared to the other environment, generating a significant selected × test environment interaction ($F_{1,17} = 23.3$, $P = 0.0002$) indicative of the adaptive divergence of these populations between environments. Within each set of ten populations that evolved within a particular environment, the cadmium-evolved populations produced more offspring in the cadmium environment than in the ethanol environment (paired *t*-test: $t_9 = 2.75$, $P = 0.022$), and the ethanol adapted populations produced more offspring in the ethanol environment than in the cadmium environment (paired *t*-test: $t_8 = 4.13$, $P = 0.003$). Variation in the proportion of productive vials followed the same pattern, revealing a significant selected environment × test environment interaction ($t_1 = 3.14$, $P = 0.004$). In particular, non-productive vials were more likely for the cadmium-evolved flies when tested in the ethanol than in the cadmium environment (mean percentage of non-productive vials ± among-population

SE: 13.3 ± 2.3% vs. 17.8 ± 2.2% respectively), and were more likely for the ethanol-evolved flies when tested in the cadmium than in the ethanol environment (30.9 ± 2.7% vs. 15.5 ± 5.0% respectively). Although adaptation to cadmium also conferred increased fitness in ethanol as a by-product (the converse did not occur), my data provide evidence for the adaptive divergence of these populations between the two environments.

Also, consistent with past studies in this species, and indicative of ongoing sexual conflict with respect to life history in the ancestor, the longevity of stock females was decreased substantially (from 47 to 25 d on average) in the high as compared to the low male exposure treatments (*t*-test: $t_{49.08} = -12.21$, $P < 0.0001$), demonstrating substantial male-induced harm.

Male harm

I tested whether adaptation to the different environments affected male-induced harm by measuring the longevity of stock females when exposed continuously to males from each of the eight selection lines. Suggestive of population-specific effects of sexual conflict, there was observable variation in female longevity induced by exposure to males from the different populations, although this was non-significant (Fig. 4-2; LRT: $\chi^2 = 2.3$, $P = 0.129$). However, a large component of this variation was associated with environment (58% overall), with females exposed to ethanol-adapted males dying an average of 3 d sooner than females exposed to cadmium-adapted males (Fig. 4-2). This generated a significant environment effect overall ($F_{1,6} = 11.96$, $P = 0.013$), demonstrating ecologically-dependent parallel evolution of male-induced harm in correlation with environment.

Female resistance to male harm

I next tested for variance in female resistance to male harm among the eight selection lines. As previously observed for stock females, increased male exposure had a large and significant effect on the longevity of evolved females overall, reducing it by 19 d on average across the eight lines ($F_{1,6}$ = 1415.6, $P < 0.0001$). Female longevity also varied significantly between environments, with ethanol-adapted females living longer than cadmium-adapted females across the male exposure treatments ($F_{1,6}$ = 27.2, P = 0.002). The reduction in female longevity between male exposure treatments did not differ significantly between females adapted to ethanol vs. cadmium when measured on an absolute scale of days (treatment × environment interaction: $F_{1,6}$ = 0.50, P = 0.501; Fig. 4-3). However, because cadmium-adapted females had significantly shorter lifespans overall, a given reduction in absolute lifespan represented a greater proportion of the maximum lifespan of these females as compared to ethanol-adapted females (Fig. 4-3), generating a difference in female resistance on a relative scale that approached significance ($t_{5.94}$ = 2.39, P = 0.055).

Mortality parameters may differ even in the absence of differences in average longevity, and I therefore also analyzed these for each female population × male treatment combination. The estimated baseline mortality (α) did not differ between male exposure treatments ($F_{1,10}$ = 1.1, P = 0.32), between females adapted to the cadmium vs. ethanol environments ($F_{1,10}$ = 0.05, P = 0.83), nor was there evidence of an exposure treatment × environment interaction ($F_{1,10}$ = 1.1, P = 0.33). In contrast, senescence rates (β) were significantly higher in the high vs. low male exposure treatments ($F_{1,10}$ = 188.2,

$P < 0.0001$), and for cadmium as compared to ethanol-adapted females ($F_{1,10} = 6.48$, $P = 0.029$), mirroring the differences in average lifespan. However, the effect of female environment depended on male exposure, generating a significant exposure treatment × environment interaction ($F_{1,10} = 5.47$, $P = 0.038$) in which senescence rates were similar for cadmium and ethanol-adapted females under low male exposure, but greater for cadmium than ethanol-adapted females under high male exposure (Fig. 4-4). Ethanol-adapted females were therefore more resistant to male-induced increases in senescence rate, on average, than were cadmium-adapted females.

Factorial assay of selection lines

Finally, to test the consequences of male-induced harm within and among selection lines, I created all 64 possible combinations of males and females among the eight selection lines. Consistent with unique evolutionary trajectories of separate populations under sexual conflict, I found significant effects of both male and female population on female longevity (LRT: $\chi^2_1 = 39.5$, $P < 0.0001$ and $\chi^2_1 = 67.3$, $P < 0.0001$, respectively). Longevity was also greater for ethanol-adapted females overall ($F_{1,6} = 93.19$, $P < 0.0001$), as previously observed when these females were exposed to stock males. There was also a trend for ethanol-adapted males to be more harmful than cadmium-adapted males, as previously observed, although this was not significant ($F_{1,6} = 1.49$, $P = 0.27$). There was no interaction between male and female environment (Fig. 4-5; $F_{1,603} = 1.3$, $P = 0.26$).

I found little indication that females were more resistant to their coevolved males among these crosses (Fig. 4-5): the male × female population interaction was non-

significant overall ($\chi^2_1 = 1.6$, $P = 0.20$) and, when considering only within-environment crosses, there was no significant difference in longevity when females were paired with their own vs. foreign males (ethanol populations: $F_{1,148} = 0.27$, $P = 0.60$; cadmium populations: $F_{1,141} = 1.10$, $P = 0.30$). Therefore, the among-population differences in male and female effects on female longevity, including those associated with environment, are relatively consistent irrespective of the identity of the other sex.

Discussion

In line with numerous past studies (Fowler & Partridge 1989; Partridge & Fowler 1990; Friberg 2005; Edward *et al.* 2011), I found that males impacted females through a substantial reduction in their longevity. Though I did not measure female offspring production and therefore do not know the impact on net female fitness, that these reductions are harmful appears likely given the magnitude of the effect (19 d on average) in populations evolving with overlapping generations. Male-induced reductions in female longevity have previously been shown to be associated with reductions in fitness (Edward *et al.* 2011), though further tests would be required to confirm such reductions. Consistent with previous theory (Holland & Rice 1998; Arnqvist & Rowe 2005), I also found significant variation in the magnitude of male harm and female resistance among independently evolved populations, including within environments (e.g., Fig. 4-2), confirming that isolated populations can follow unique evolutionary trajectories under sexual conflict. However, my results also revealed that a significant portion of this variation was predictable based on the environment to which a population was adapted, such that the magnitude of both male harm and female resistance depended to a large extent on the ecological histories of both sexes. Specifically, ethanol-adapted males were

more harmful, and ethanol-adapted females were more resistant to this harm, than were cadmium-adapted flies, demonstrating ecologically-dependent parallel evolution of these traits. The evolution of such traits in correlation with ecology demonstrates that sexual conflict should not be considered as an entirely non-ecological promoter of divergence (Coyne & Orr 2004; Rundle & Nosil 2005), and also suggests that the common division of models of diversification into ecological vs. non-ecological may be overly simplistic.

Ecology may affect sexual conflict in several (non-mutually exclusive) ways including imposing limits on trait exaggeration via antagonistic coevolution, biasing coevolutionary pathways, or even overriding sexually antagonistic selection entirely by favoring particular values of harmful or resistant traits outside of their role in sexual conflict. While I do not know the particular mechanism by which ecology caused the divergence of male harm and female resistance among these populations, my results suggest that these traits have been exaggerated to a greater extent in the ethanol environment than in the cadmium environment. In my simulations (Appendix), I found that the exaggeration of traits under sexual conflict tended to be limited in environments in which natural selection on these traits was stronger, whereas environments with weaker natural selection allowed for greater trait exaggeration (see also Rowe et al 2003). This pattern led to ecologically-dependent parallel evolution of increased male harm and female resistance in populations evolving in environments with weak natural selection on the traits mediating sexual conflict. From this perspective, one interpretation of these results is that the cadmium environment acted as a harsh and constraining habitat that limited the evolution of male harm and female resistance, while sexual conflict was less constrained in the populations evolving in ethanol. An alternative is that the ethanol

environment selects for more robust genotypes than does cadmium, resulting in males capable of causing more harm and females capable of greater resistance. Regardless, it is clear that ecological history has a marked influence, through both sexes, on sexual conflict measured as the effect of males on female survival.

Fricke et al. (2010a) measured the divergence of sexual conflict traits (including male harm and female resistance) among replicate seed beetle populations that had evolved in an ancestral vs. novel food environment. In this experiment, populations adapting to a new environment showed less trait divergence, demonstrating that the selective environment can influence sexual conflict and suggesting that stronger selection acting on populations can limit the extent of sexual conflict trait exaggeration. Similarly, male mate guarding and harassment in freshwater isopods was exaggerated to a greater extent in environments with less predation (Karlsson et al. 2010). These fly populations, which were all under directional selection, experimentally demonstrate that the particular environment to which populations are adapting can have large impacts on sexual conflict trait values, as well as the level of among-population divergence.

Contrary to previous predictions (Holland & Rice 1998; Andrés & Arnqvist 2001), I did not find that females were best defended against the harm of their own (i.e. local) males. The evidence for females having greatest resistance to harmful effects of coevolved males is mixed (Andrés & Arnqvist 2001; Brown & Eady 2001; Chapman *et al.* 2003; Long *et al.* 2006; Wigby & Chapman 2006), and as Rowe et al. (2003) mathematically demonstrated, it is just as easy to make the opposite prediction that males should be best adapted, and thus most harmful to, their own females. Obviously, both predictions cannot be simultaneously true and the realized outcome of sexually

antagonistic interactions depends on a variety of details. What is remarkable in these current results is the extent to which ecology predicted divergence among populations of both male harm and female resistance over and above such details that could have produced population-specific outcomes. My results highlight the variation that could arise in crosses among natural populations with different (and possibly poorly understood) histories of ecological selection.

Ecologically-dependent selection plays a large role in species radiations, often driving the parallel evolution and/or divergence of phenotypes and reproductive isolation (e.g., Rundle *et al.* 2000; Schluter 2000; Nosil *et al.* 2002). A role for ecology in the evolution of traits under sexual conflict could have large impacts on how populations adapt to new environments, potentially also influencing patterns of reproductive isolation. The joint influence of ecology and sexual conflict on phenotypic diversification and speciation is therefore an important topic for future work.

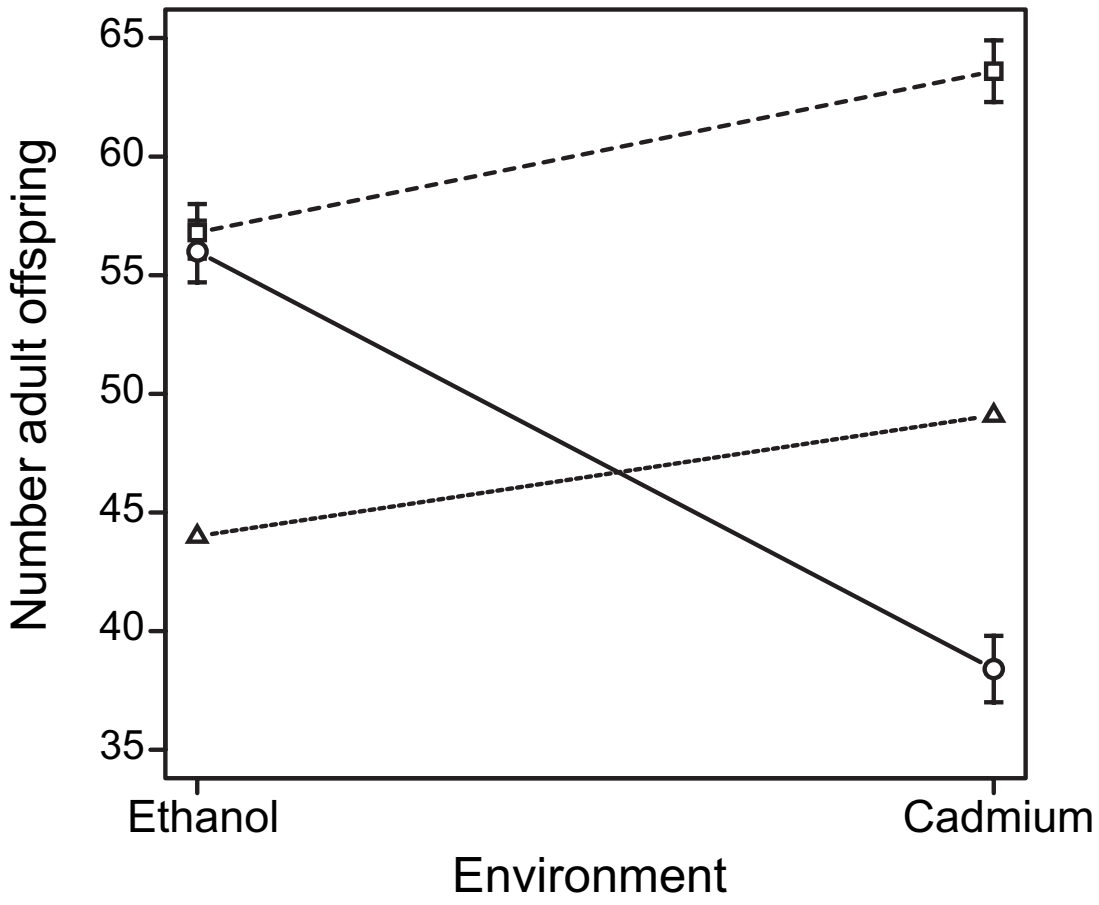

Figure 4-1 Adaptive divergence among experimental *Drosophila melanogaster* populations

Number of adult offspring produced by single *D. melanogaster* male-female pairs in each of two environments (ethanol vs. cadmium containing food) for each of ten ethanol-adapted (circles, solid line) and ten cadmium-adapted (squares, dashed line) populations. The ancestral stock population is also shown for reference (triangles, dotted line). Points represent the mean (± among-population SE) of the ten replicate populations within each environment. Forty replicate male-female pairs were created per population per test environment.

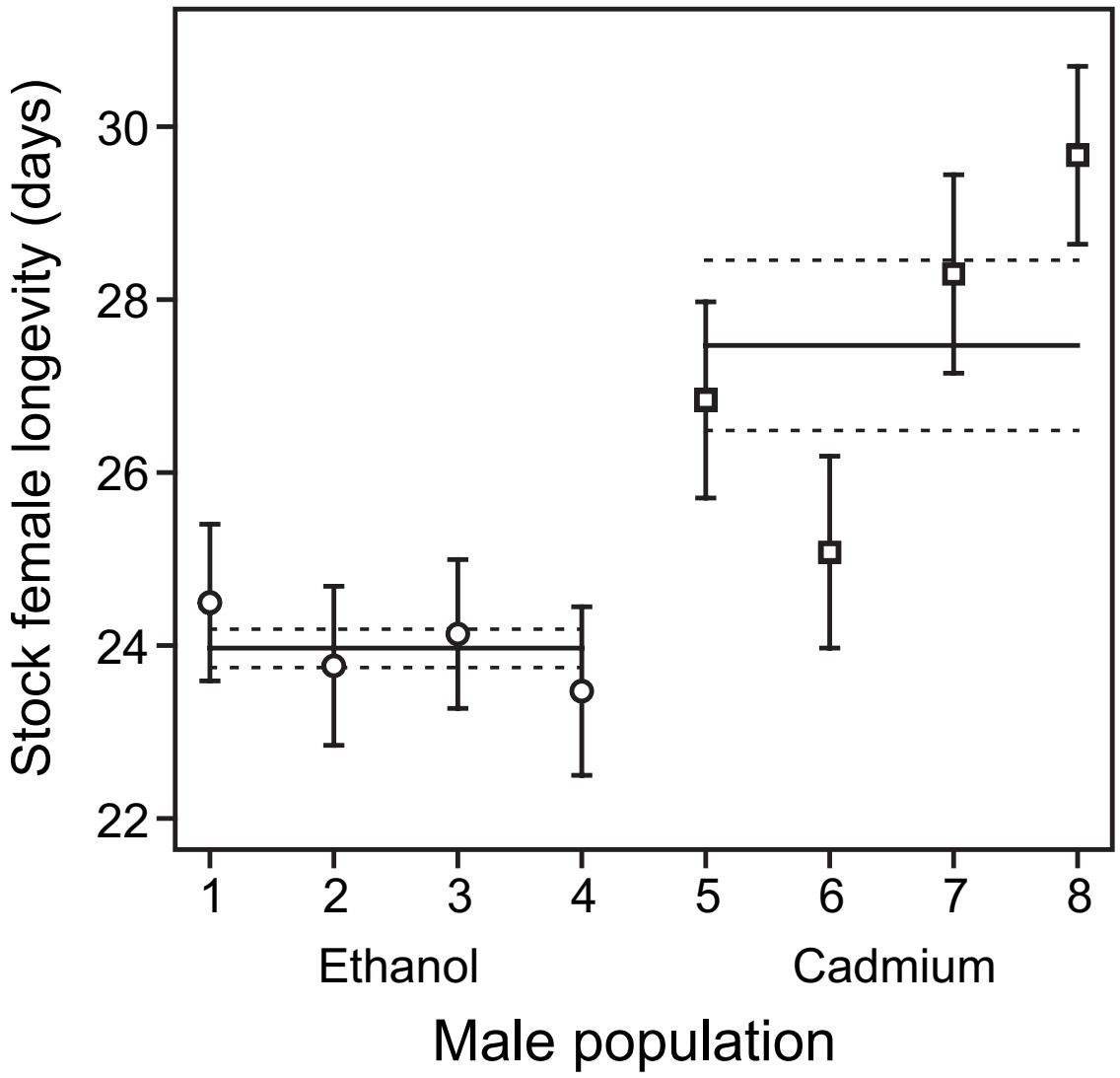

Figure 4-2 Male harm among ethanol- and cadmium-adapted *D. melanogaster* populations

Average longevity (± SE) of stock *D. melanogaster* females when exposed to evolved males adapted to either ethanol (populations 1-4, circles) or cadmium (populations 5-8, squares; 30 vials per population). Solid lines show the means (± SE, dashed lines) for the two types of males, treating populations as replicates.

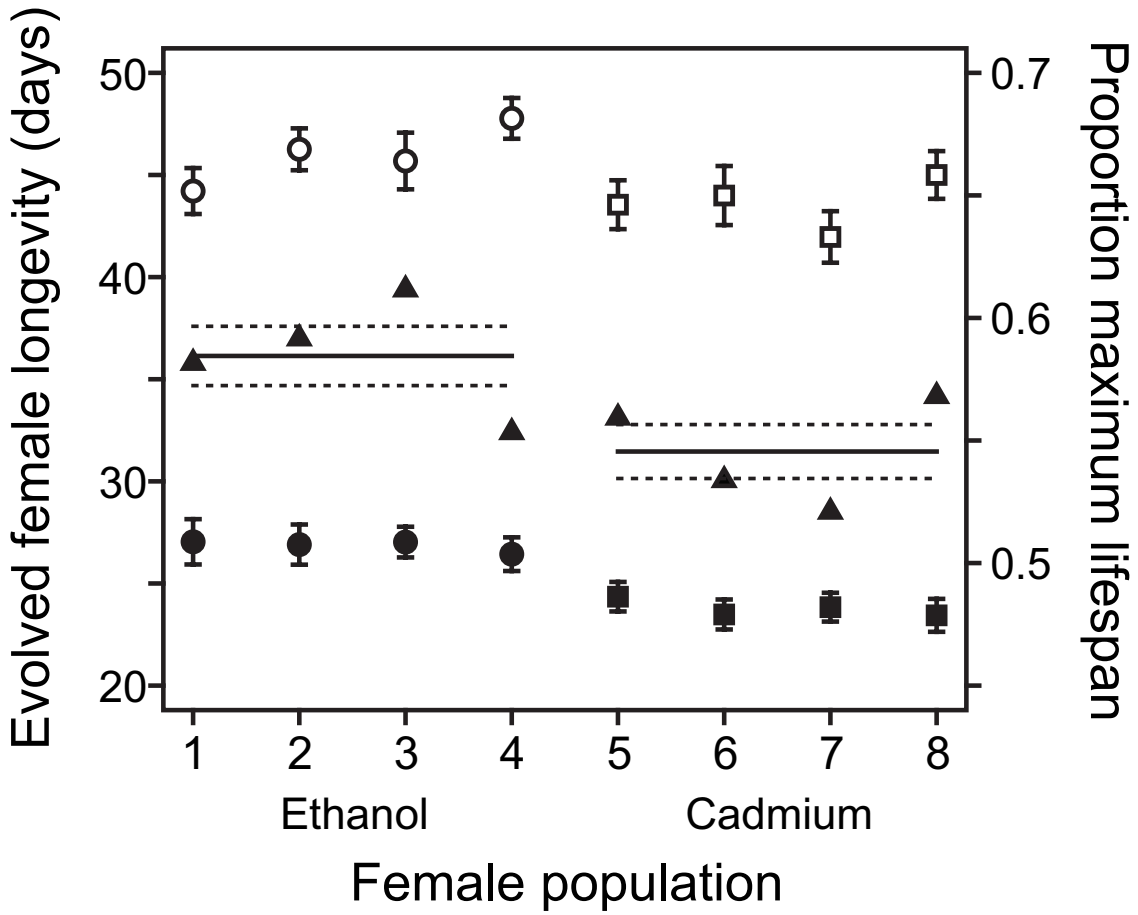

Figure 4-3 Female resistance to male harm among ethanol- and cadmium-adapted *D. melanogaster* populations

Average (±SE) longevity of *D. melanogaster* females from each of the eight selection lines (ethanol: populations 1-4, circles; cadmium: populations 5-8, squares) under treatments involving high (filled) and low (open) exposure to stock males (30 replicate vials per female population per treatment). Triangles (right axis) represent the proportion of the low exposure treatment lifespan achieved by high exposure treatment females for each population, with among-population means and SE for each environment denoted by the solid/dashed lines respectively.

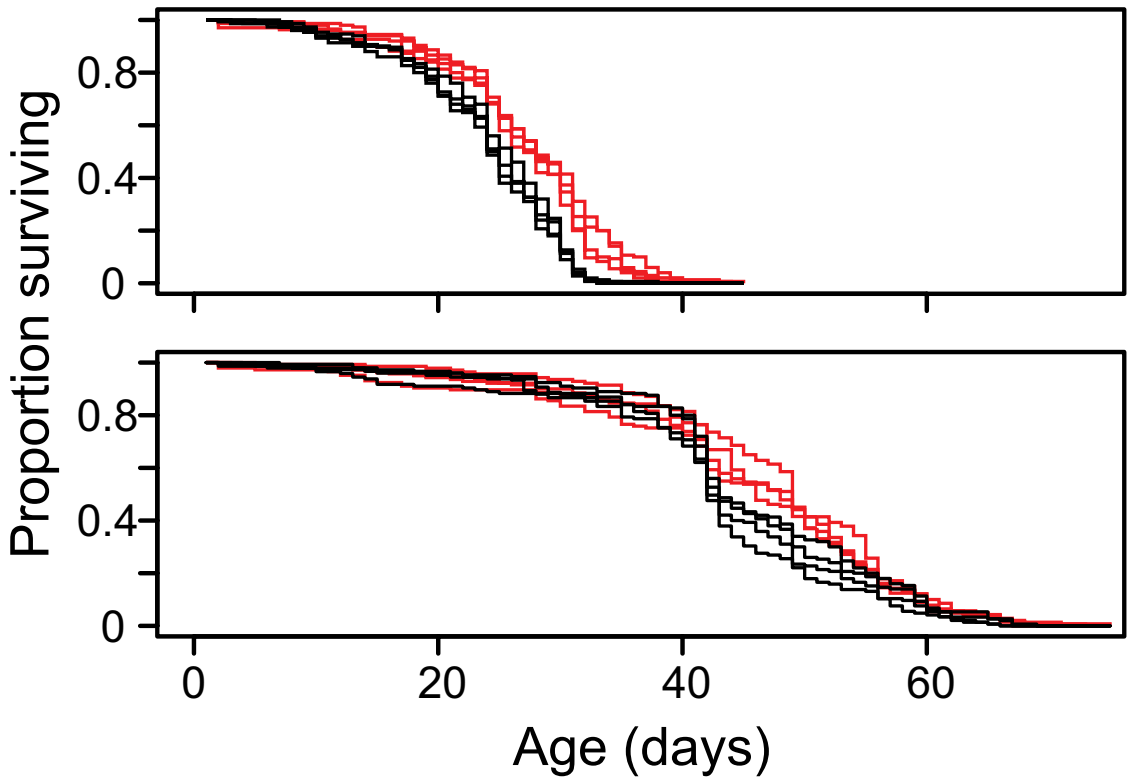

Figure 4-4 Female life history under high and low male exposure

Proportion survival over time of *D. melanogaster* females from four ethanol-adapted populations (red lines) and four cadmium-adapted populations (black lines) under treatments of high (top) and low (bottom) exposure to stock males. Each population started with 150 females.

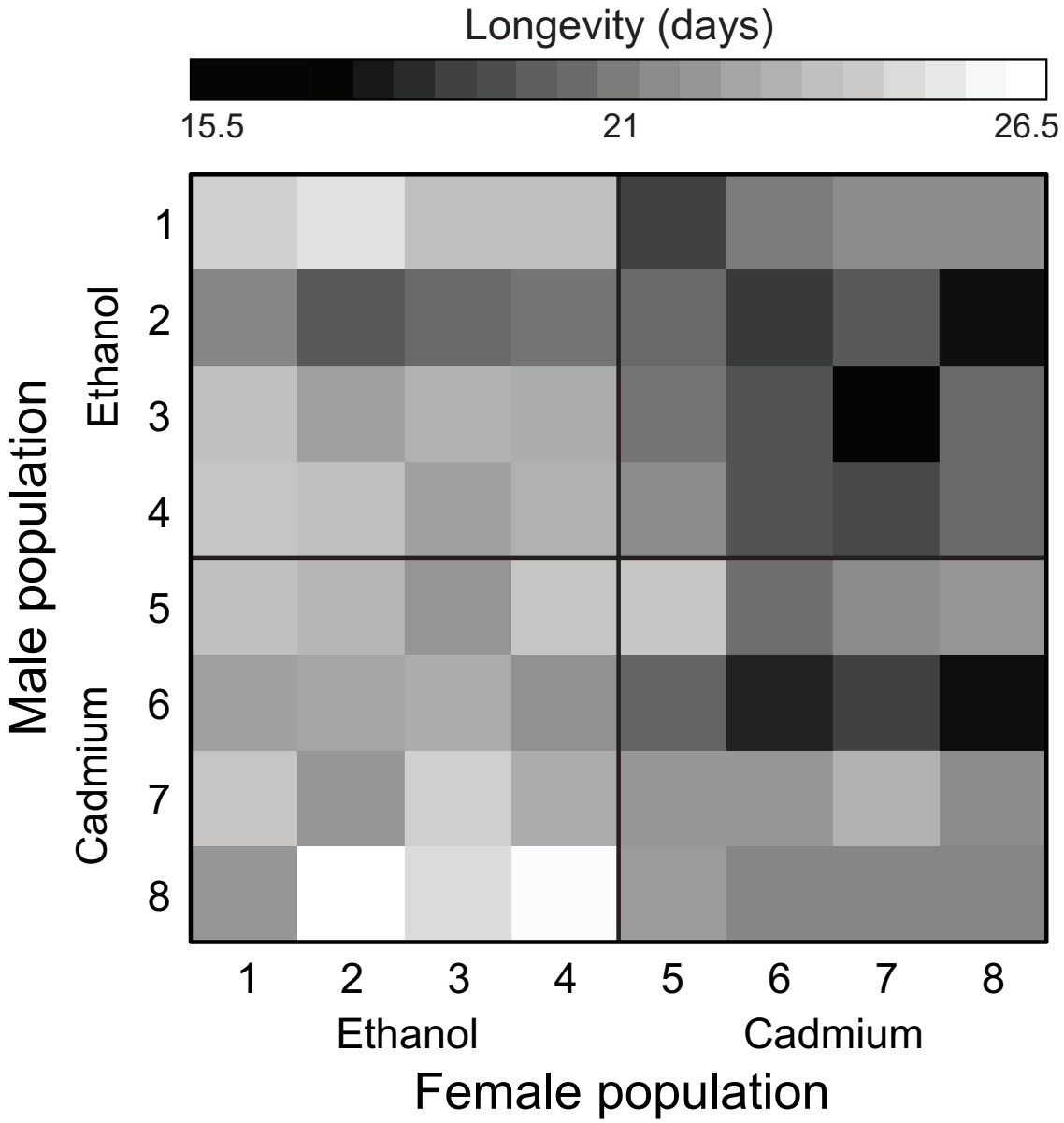

Figure 4-5 Female longevity for all experimental male x female population combinations

Average longevity of *D. melanogaster* females from each of the eight selection lines (x-axis) when paired with males from each of the eight populations (y-axis). Ten replicate vials were established for each female x male population combination. Populations 1-4 are adapted to ethanol and populations 5-8 are adapted to cadmium. Darker shades represent lower female longevity as shown in the legend.

Chapter 5: Environment and population-level divergence of sexual conflict traits in *Drosophila melanogaster*

Abstract

The prevalence of sexual conflict in nature, as well as the arbitrary nature of the resulting coevolutionary trajectories, suggests that it may be an important driver of phenotypic divergence even in a constant environment. However, natural selection is known to be central to the operation of sexual conflict within populations and may therefore constrain or otherwise direct divergence among populations. Ecological context may therefore matter with respect to the diversification of traits involved in sexual conflict, and if natural selection is sufficiently strong, such traits may evolve in correlation with environment, generating a pattern of ecologically-dependent parallel evolution. In this study I assess among-population divergence both within and between environments for several traits involved in sexual conflict. Using eight replicate populations of *Drosophila melanogaster* from a long-term evolution experiment, I measured remating rates and subsequent offspring production of females when housed with two separate males in sequence. I found no evidence of any variation in male reproductive traits (offense or defense). However, the propensity of females to remate diverged significantly among the eight populations with no evidence of any environmental effect, consistent with sexual conflict promoting diversification even in the absence of ecological differences. In addition, females adapted to one environment (ethanol) tended to produce a higher proportion of offspring sired by their first mate as compared to those adapted to the other (cadmium) environment. This difference approached significance, suggesting ecologically-based divergence of this conflict

phenotype. Because I find evidence for both stochastic population divergence operating outside of an ecological context and environment-dependent divergence of traits under sexual conflict, the interaction of these two processes is an important topic for future work.

Introduction

Sexual conflict occurs when the reproductive interests of the two sexes are not aligned, leading to sex-specific selection on shared traits (Parker, 1979). When the trait is controlled by different loci in males and females (e.g., mating rate), sexually antagonistic selection can favour alleles that increase a male's reproductive success relative to other males, even if this comes at a cost to female fitness. Similarly, selection can also favour alleles in females that increase their resistance to such male-induced harm, even if this reduces male fitness (Arnqvist & Rowe, 2005). Such interlocus sexual conflict can drive a process of ongoing antagonistic coevolution in which changes in one sex generate renewed selection on the other (Rice, 1996; Holland & Rice, 1998). The economics of the resulting conflict in terms of sex-specific costs and benefits can have large impacts on the fitness of males and females (Fowler & Partridge, 1989; Rice, 1996; Chapman, 2001; Edward *et al.*, 2011), and by extension, population mean fitness (Chippindale *et al.*, 2001; Stewart *et al.*, 2008; Long *et al.*, 2012).

Interlocus sexual conflict is common in nature and has led to a great diversity of traits that serve to increase the reproductive success of males at the expense of females (e.g., persistent male courtship and harassment, toxic ejaculates, spiny genitalia), as well as traits that provide resistance in females (e.g., escape behaviour, complicated

reproductive tracts; (Arnqvist & Rowe, 2005). The particular traits that evolve in any given case are thought to be the product of chance events such as the order in which mutations occur or the standing genetic variation in a population. Because of the persistent and potentially strong selection sexual conflict can generate, traits involved in sexual conflict may diverge rapidly among populations (Civetta & Singh, 1995; Chapman *et al.*, 2003) Consistent with this, cases of remarkable diversity in conflict-mediating traits have been documented even among recently isolated populations and species (Arnqvist, 1998; Perry & Rowe, 2012; Rowe & Arnqvist, 2012). In addition to generating phenotypic diversity, such divergence may contribute to the evolution of reproductive isolation (Parker & Partridge, 1998; Gavrilets & Waxman, 2002; Martin & Hosken, 2004), leading to the suggestion that sexual conflict may be an important engine of speciation (Arnqvist *et al.*, 2000; Bonduriansky, 2011). Given the central role of sexual conflict in the evolution of mating interactions, sex-specific and population mean fitness, and population divergence/speciation, understanding how traits involved in sexual conflict diverge among populations is an important goal in evolutionary biology.

Divergence through sexual conflict is driven by sexually antagonistic selection and can therefore occur even in the absence of ecological differences between populations. This has led some to classify sexual conflict as a non-ecological promoter of speciation (Coyne & Orr, 2004; Rundle & Nosil, 2005). However, the traits involved in sexual conflict may also affect non-sexual fitness and may thus be subject to natural selection within an ecological context (Parker, 1979; Rowe *et al.*, 1994; Rice & Holland, 1997; Rowe & Day, 2006; Fricke *et al.*, 2009). While natural selection and the environment have therefore long featured in the study of sexual conflict and mating

system evolution within populations (Rowe et al. 1994), only recently has this been extended to studies of sexual conflict's role in divergence/speciation.

An emerging appreciation of the importance of ecology is suggested by several empirical studies of sexual conflict that included different environments, either through an experimental manipulation or by exploiting naturally occurring variation. For example, among experimental populations of seed beetles, adaptation to a new food appeared to hamper the extent of diversification of traits involved in sexual conflict, relative to populations that had evolved on the same food as their ancestor (Fricke *et al.*, 2010a). Also in seed beetles, a life history manipulation altering the duration of the reproductive period was shown to affect the evolution of some genital and mating traits (Maklakov et al. 2010; Cayetano et al. 2011). In freshwater isopods, male harassment of conspecific females has been shown to differ between habitats differing in predation, demonstrating ecologically-based differences (and divergence) in the opportunity for sexual conflict (Karlsson *et al.*, 2010). Similarly, the strength of predation has been shown to influence the patterns of genital divergence in mosquitofish (Heinen-Kay & Langerhans, 2013). Nevertheless, our understanding of the impact of ecological differences on traits involved in sexual conflict is limited.

In a previous test of ecology's influence on the divergence of traits involved in sexual conflict, I demonstrated ecologically-dependent parallel evolution of male harm and female resistance among replicate populations of *Drosophila melanogaster* that had independently adapted to two different food environments containing either ethanol or cadmium (Chapter 4). Specifically, I showed that males from populations that had independently adapted to ethanol decreased female longevity more on average than did

males from replicate populations independently adapted to cadmium. Ethanol-adapted females from these populations were also more resistant to male-induced reductions in longevity, on average, as compared to females from the replicate cadmium-adapted populations. Such ecologically-dependent parallel evolution of a key life history trait demonstrates that ecology can play a central role in shaping traits involved in sexual conflict.

Here I extend work on these populations by assaying divergence in female remating rate and male reproductive offense and defense. These are traits that are commonly thought to evolve by sexual selection in general, with a strong role for sexual conflict in particular. This contrasts with longevity, a trait on which natural selection is also likely to be strong. Of particular interest is the relative importance of among-population divergence within environments as compared to ecologically-mediated divergence between environments (i.e. parallel evolution in correlation with environment). I find some evidence for divergence among populations both within and between environments in females (but not in males), suggesting roles for both environment-independent sexual conflict and ecologically-divergent natural selection in the evolution of these traits.

Materials and Methods

Experimental populations

For a detailed description of population origin and maintenance, see Chapter 4. In brief, in 2007 a laboratory-adapted *Drosophila melanogaster* stock was split into 20 independent, isolated populations, with ten of these maintained on food supplemented with 12% ethanol and the other ten on food containing 70 ug/mL cadmium chloride. Like

the stock, which continued to be maintained on standard (i.e. lacking ethanol and cadmium) food, these experimental populations were maintained in population cages with overlapping generations. After four years, a reciprocal transplant assay measuring the number of adult offspring produced by replicate male-female pairs demonstrated that each of the 20 experimental populations had increased in fitness relative to the stock when tested in its evolved environment. Although adaptation to cadmium also conferred increased fitness in ethanol as a by-product (the converse did not occur), each population also produced significantly more offspring on average in its evolved environment than in the other selective environment, demonstrating the adaptive divergence of these populations between environments (Chapter 4). For subsequent measures of reproductive traits, I selected four populations from each environment, chosen as those with the highest fitness in their evolved environment. These are the same populations as used in Chapter 4.

In each assay below, I collected flies from each of the eight experimental populations, the stock population, and a population fixed for a dominant visible mutation (brown eyes: bw^D). All flies were reared for a single generation in standard (i.e. ancestral) stock food in vials with 50 or fewer individuals. Virgins were collected within 8 h of emergence using light CO_2 anesthesia, and sexes were held separately (10 females/vial or 7 males per vial) for 3 d prior to conducting an assay. All three assays employed a design similar to that used for measuring sperm offense and defense in *Drosophila* in that a single female was held individually with two different males in sequence. One of the two males was from the bw^D stock whereas the other was not, allowing mating with each male to be inferred by presence of any offspring of the matching phenotype (i.e. mutant vs.

wild-type). Unlike a standard sperm competition assay, the female was held in the same vial throughout, ensuring that offspring from the first male would be present (provided she mated with this male) and allowing the timing of mating, as well as a male's sperm competitive ability, to contribute to the relative frequency of their offspring. The three assays separately varied the identity of the female (assay one: female remating), the first male (assay two: male reproductive defense), or the second male (assay 3: male reproductive offense), as described below.

Female remating and offspring production

This assay tested whether females from the different populations and environments varied in their propensity to remate, and also quantified their subsequent offspring production using sperm of the two males. In each trial, a single female from one of the experimental populations (i.e. cadmium or ethanol-adapted) was placed in a vial of standard food along with a single wild-type stock male and left for 6 h. After this period, the stock male was removed and replaced with a single bw^{D} male. These marker males were given the opportunity to mate the experimental female for 8 h, after which they were removed. Females were allowed to lay eggs in the same vial for an additional 17 h, after which they were discarded. Because the allelic marker in the second male is dominant, offspring sired by that male will express the brown-eye phenotype. 14 days after the first male was introduced to the experimental female, vials were checked for the presence/absence of *bw* offspring, indicating whether the female remated or not. *D. melanogaster* shows strong last male sperm precedence (Boorman & Parker, 1976;

Gromko *et al.*, 1984), so it is unlikely that I would incorrectly categorize a remated female as singly mated.

I carried out 100 trials for each experimental population (800 trials total). Trials were split evenly between two blocks, separated by one day. Because females had the opportunity to lay eggs in the same vial over the course of the entire assay (i.e. since her first mating), offspring from both sires should be present if the female remated. Vials in which wild-type offspring were absent, suggesting that the female did not mate with the first male, were discarded (71 trials). In addition to determining the presence or absence of any *bw* offspring, I also counted the number of *bw* and wild-type offspring for all females that remated (i.e. in which both types were present), providing an estimate of the relative number of offspring sired by each male. Variation in the relative number of offspring sired may arise from the time to each mating, sperm competition, and cryptic female choice. As females from each population were subjected to the same combination of stock then *bw* male, variance in remating and offspring production among female populations can be attributed to the effect of the female's population and/or the environment in which the population evolved.

Male reproductive defense

The second assay assessed whether males from the eight experimental populations varied in their influence on female remating and reproductive output. In each trial, a single stock female was placed together with a single experimental male (i.e. a male from one of the cadmium or ethanol-adapted populations) in a vial of standard food for 6 h during which the female could mate and lay eggs. The male was then replaced with a *bw*

male and the pair was left for another 8 h at which point the second male was removed. Females were then allowed to lay eggs for 17 h, as above. 14 d after the beginning of the experiment, offspring were scored for eye colour as a proxy for the occurrence of remating. If remating occurred, I again counted the number of wild-type and *bw* offspring to gain an additional measure of the first male's reproductive defense among remated females. This measurement is similar to the measurement of sperm defense (P1) except that I also counted offspring produced between the first and second mating, thereby including variation among males in their time to mating. Trials were split evenly between two blocks, separated by one day. In this assay, females and the second, competitor males were the same for all experimental males, making any variance in male remating defense and offspring production attributable to the first male's population and/or environment. As above, 100 trials were performed for each male population (800 trials total), and data from females who did not mate with the first male were discarded (75 trials).

Male reproductive offense

The third assay tested whether males from these experimental populations differed in their ability to induce mated females to remate, and their success at siring offspring of these females. In each trial, a female was first mated to a *bw* male and then given the opportunity to mate with a second male from one of the eight experimental populations. All trials were conducted within a single block. Offspring were scored to determine whether remating occurred (i.e. presence of wild-type offspring) and, for those that remated, the proportion of offspring sired by each male. This last measure is similar

to sperm offense (P2) except that it includes offspring produced between the first and second matings. 110 trials were conducted for each male population (880 in total).

Statistical analysis

For all three assays, variation in the propensity to remate was analyzed using a general linear mixed model in which the environment of the target individual was a fixed effect and population was a random effect nested within environment. Experimental block was also included as a fixed effect. The model was fit via restricted maximum likelihood (REML) via the mixed procedure in SAS v. 9.3 (SAS Institute, Inc., Cary, NC), with significance of the random effect of population determined using a likelihood ratio test (LRT). These analyses rely on the Gaussian approximation to the binomial distribution, which performs best when the probability of either outcome (i.e. remated or not) is approximately equal (Zar, 1984). This was not the case in all of my assays, so I repeated the above analyses employing a generalized linear model that specified a binomial distribution and a logistic link function, fit via maximum likelihood, as implemented in the Genmod procedure in SAS. Population was modeled as a fixed effect in this case and significance was determined via LRT's. Because results did not differ qualitatively between these two approaches, I present the mixed model results.

In each assay, variation in the proportion of offspring sired by each male was tested using the same general linear mixed model with experimental block and environment as fixed effects and population as a random effect nested within environment. Proportions were arcsine square root transformed prior to analysis.

Results

Female remating and offspring production

There was significant among-population variation in the propensity of females from the eight experimental population to remate (LRT: $\chi^2_1 = 15.2$, $p < 0.0001$; Fig. 5-1), indicating the evolutionary divergence of this trait among the eight populations. There was no evidence, however, that remating propensity differed on average between females adapted to the cadmium vs. ethanol environments ($F_{1,6} = 0.13$, $p = 0.732$). Among the females that did remate, among-population variation in the proportion of offspring sired by the first vs. second male was not significant (LRT: $\chi^2_1 = 0.98$, $p = 0.321$). However, in females from the ethanol-adapted populations, a higher proportion of offspring tended to be sired by the first male than in females from the cadmium-adapted populations (Fig. 5-2). This difference between environments approached significance ($F_{1,6} = 4.76$, $p = 0.072$).

Male reproductive defense

There was no significant among-population variation in the ability of males to prevent stock females from remating (LRT: $\chi^2_1 = 0.12$, $p = 0.727$), nor was there any evidence a male's ability to do this varied on average between populations adapted to the cadmium vs. ethanol environments ($F_{1,6} = 0.39$, $p = 0.557$; Fig. 5-3). Similarly, for those females that mated with both males, there was no significant among-population variation in the proportion of offspring sired by the first vs. second male (LRT: $\chi^2_1 = 1.53$, $p = 0.216$), nor did this vary on average when the first males were adapted to the cadmium vs. ethanol environments ($F_{1,6} = 0.63$, $p = 0.456$; Fig. 5-3).

Male reproductive offense

There was no significant among-population variation in the ability of a male to acquire a mating from a previously mated stock female (LRT: $\chi^2_1 = 0.004$, $p = 0.950$), nor was there any evidence that this varied on average between males from populations adapted to cadmium vs. ethanol ($F_{1,6} = 1.82$, $p = 0.226$; Fig. 5-4). Likewise, for those females that mated with both males, there was no significant among-population variation in the proportion of offspring sired by the first vs. second male (LRT: $\chi^2_1 = 0.001$, $p = 0.933$), nor did this vary on average between second males adapted to the cadmium vs. ethanol environments ($F_{1,6} = 0.63$, $p = 0.456$; Fig. 5-4).

Discussion

Sexual conflict is suggested to be a potentially potent engine of speciation because it can drive the rapid evolutionary divergence of traits between allopatric populations even in the absence of ecological differences (Coyne & Orr, 2004; Arnqvist & Rowe, 2005; Rundle & Nosil, 2005; Long *et al.*, 2006). Consistent with this, female propensity to remate differed significantly among these eight experimental populations, varying from as low as 38% to as high as 76% (Fig. 5-1). There was also no evidence that this divergence was associated with adaptation to the different environments, with the most divergent populations above both being adapted to ethanol. In contrast, when examining offspring production by these females, there was some evidence that the proportion sired by the first vs. second males differed between females adapted to the two environments. In particular, ethanol-adapted females tended to produce a higher proportion of offspring sired by the first male as compared to cadmium-adapted females, although this difference (means: 68% vs. 74% respectively; Fig. 5-2) was not quite

significant (P = 0.076; P = 0.065 using non-transformed data). Ecologically-dependent parallel evolution of traits involved in sexual conflict was shown previously in these populations with respect to male and female effects on female lifespan (Chapter 4). If borne out by further work, the extension of such effects to female reproductive traits involving male-male competition would further highlight a central role for ecology in the divergence of traits involved in conflict.

In contrast to these female reproductive traits, I found no evidence for the diversification of male traits involved in sexual conflict, either at the population or environment level. First, there was no significant among-population or between-environment variation in a male's ability to prevent stock females from remating, nor in their ability to acquire a mating from a previously mated stock female. Second, for those females that mated with both males, there was no significant among-population or between-environment variation in the proportion of offspring sired by the first vs. second male, independent of the mating order of the experimental male (i.e. first vs. second). The latter assay includes variation in the time to (re)mating, sperm competitive ability, and male-induced variation in female fecundity. Taken together, these results suggest that competitive male reproductive success, both defensive and offensive, did not diverge among the eight populations, nor between those adapted to ethanol vs. cadmium.

I previously found divergence among these eight populations in male harm that was associated with environment, wherein ethanol-adapted males reduced female longevity significantly more than cadmium-adapted males (Chapter 4). Ethanol-adapted females were also more resistant to this harm, on average, as compared to cadmium-adapted females. The lack of any environmentally-associated divergence in male

reproductive defense and offense, as currently measured, implies that these traits do not underlie this previously observed ecologically-dependent parallel evolution of male harm. Long *et al.* (2006) previously found significant differences in male remating defense, though not offense, among six replicate *D. melanogaster* populations independently adapted to the same environment, demonstrating divergence within a constant environment. It is unclear why no such divergence was detected here, although at more than 600 generations the Long et al. (2006) study had substantially more time for changes to accumulate. All these populations were also evolving in novel environments in which directional selection was likely strong. Previous simulation results (Appendix 1) and an empirical study (Fricke et al. 2010a) suggest that stronger natural selection may limit evolutionary exaggeration via sexual conflict, potentially explaining the lack of evolved differences in males. Finally, these assays also employed young flies and included only the first mating for the experimental males. It is therefore possible that effects could be detected in subsequent matings with older and more experienced flies. However, this design is similar to those that have been previously used and which have inferred ongoing sexual conflict (Rice, 1996; Linder & Rice, 2005; Long *et al.*, 2006).

As noted above, there was a trend in which ethanol-adapted females tended to produce a higher proportion of offspring sired by the first male as compared to cadmium-adapted females. If real, there are several potential explanations. First, ethanol-adapted females could take longer to remate, possibly because they have a longer refraction period between matings and/or because they more vigorously resist mating attempts by the second male, thereby giving them more time to lay eggs sired by the first male. More vigorous resistance could arise if the ethanol environment selects for more robust

genotypes than does cadmium, a mechanism that could also explain why these females are more resistant to male-induced reductions in their longevity and why ethanol-adapted males are more harmful on average (Chapter 4). Alternatively, ethanol-adapted females could lay eggs faster after their initial mating, either because this is adaptive in ethanol or because they are differently affected by (i.e. resistant to) male seminal proteins (Wolfner, 1997; Chapman, 2001; Liu & Kubli, 2003). Lastly, it is possible that females from different environments vary in their effects on sperm competition. Sperm competition is known to be influenced by male genotype (Clark *et al.*, 2000), female genotype (Clark & Begun, 1998), and complex interactions between the rival males and females (Clark *et al.*, 1999; Bjork *et al.*, 2007). Whether the observed differences in offspring production among these experimental populations contribute to the previously observed ecologically-dependent parallel evolution of female resistance (Chapter 4) is not known. When taken together with my previous data from these populations (Chapter 4), these current results suggest that the traits involved in sexual conflict may often not evolve by sexually antagonistic selection alone, and that divergence via sexual conflict should not be considered an entirely non-ecological process.

Mating rate has large impacts on male and female fitness and is one of the classic examples of a trait under sexual conflict, wherein male fitness increases with increasing mating rate while female fitness is optimized at an intermediate rate (Bateman, 1948; Arnqvist & Rowe, 2005). The fitness costs to females of mating beyond their optimum are well established in *D. melanogaster* (Fowler & Partridge, 1989; Chapman *et al.*, 1995; Rice, 1996; Linder & Rice, 2005; Edward *et al.*, 2011) and remating rate (measured using similar methods as mine) has been shown to be negatively correlated

with female resistance to male harm (Linder & Rice, 2005). The fact that I and others (Long *et al.*, 2006; Fricke *et al.*, 2010a) have found divergence in this key component of sexual conflict within a constant environment provides strong evidence that sexual conflict can promote diversification in the absence of ecological differences, supporting its interpretation as a potential engine of speciation. However, given evidence that traits under sexual conflict may diverge in association with ecology (Chapter 4, Fricke *et al.*, 2010a; Karlsson *et al.*, 2010), the interaction of within-environment diversification with ecologically-based divergent selection is an important topic for future work.

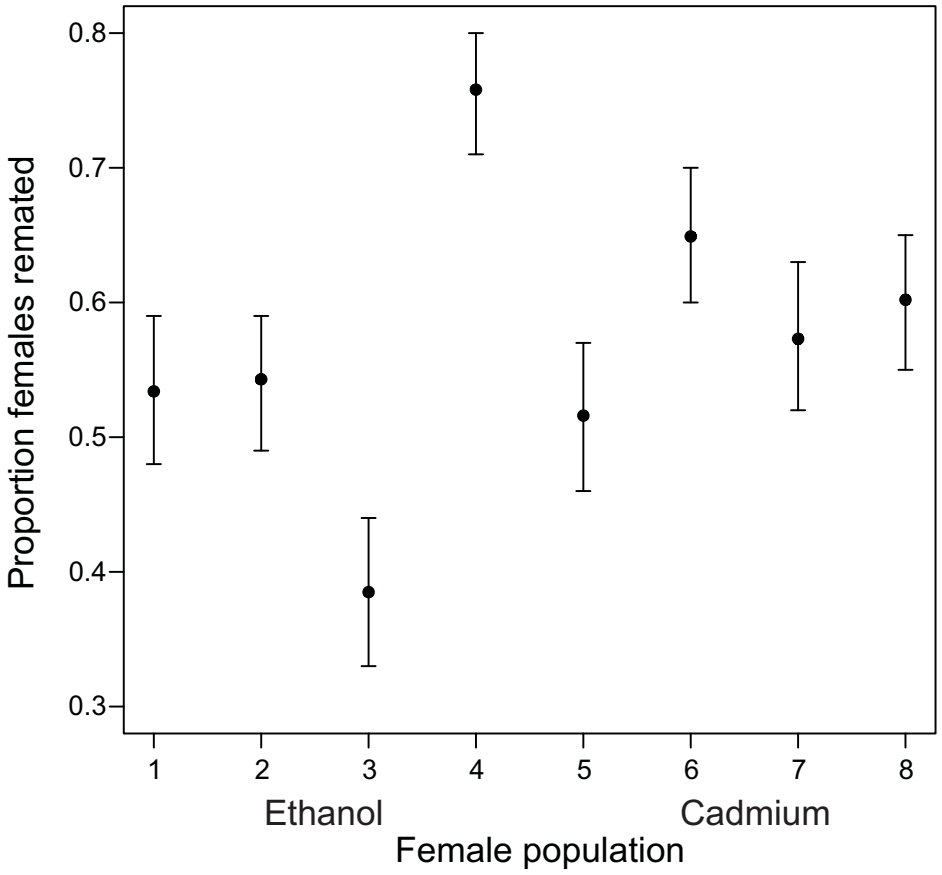

Figure 5-1 Female remating rate among ethanol- and cadmium-adapted *Drosophila melanogaster* populations

The proportion of experimental females that remated. Points are population-specific proportions (± SE) from the female remating assay. Populations 1-4 are ethanol-adapted, while populations 1-5 are cadmium-adapted.

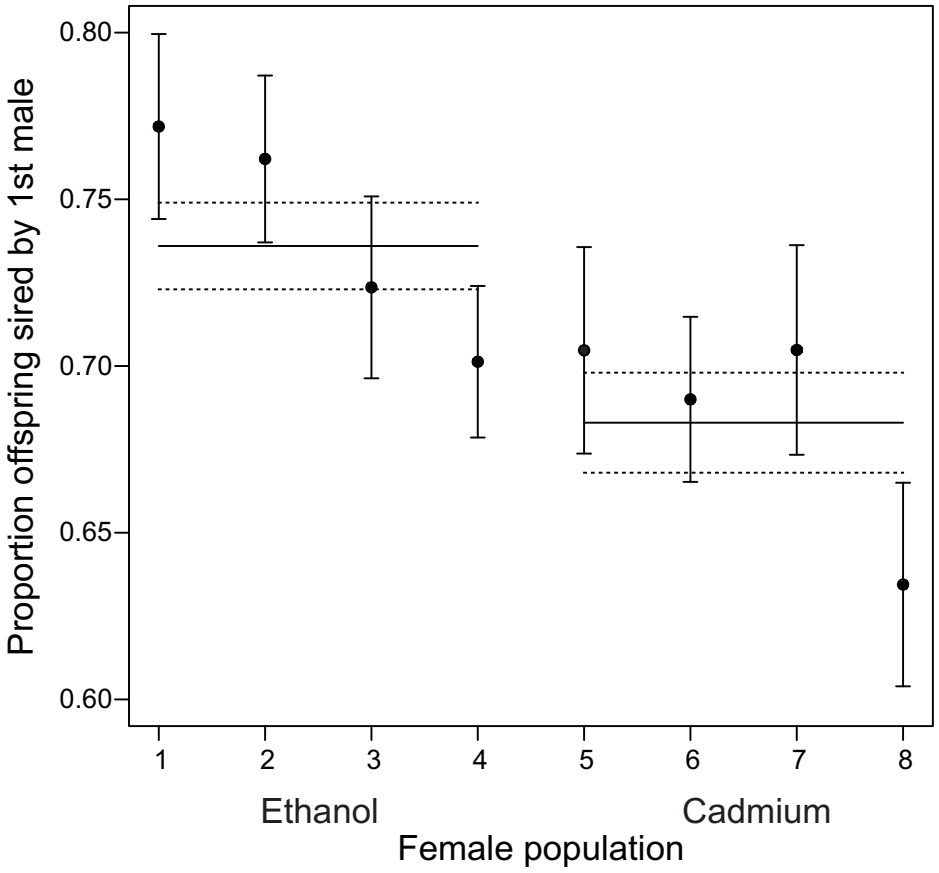

Figure 5-2 Female offspring production among ethanol- and cadmium-adapted *D. melanogaster* populations

The proportion of offspring sired by first mates of experimental females that mated twice. Points represent population means (± SE). Horizontal lines represent the environment-level means for all ethanol and cadmium-adapted populations respectively (± SE, dashed lines). Populations 1-4 are ethanol-adapted, while populations 1-5 are cadmium-adapted.

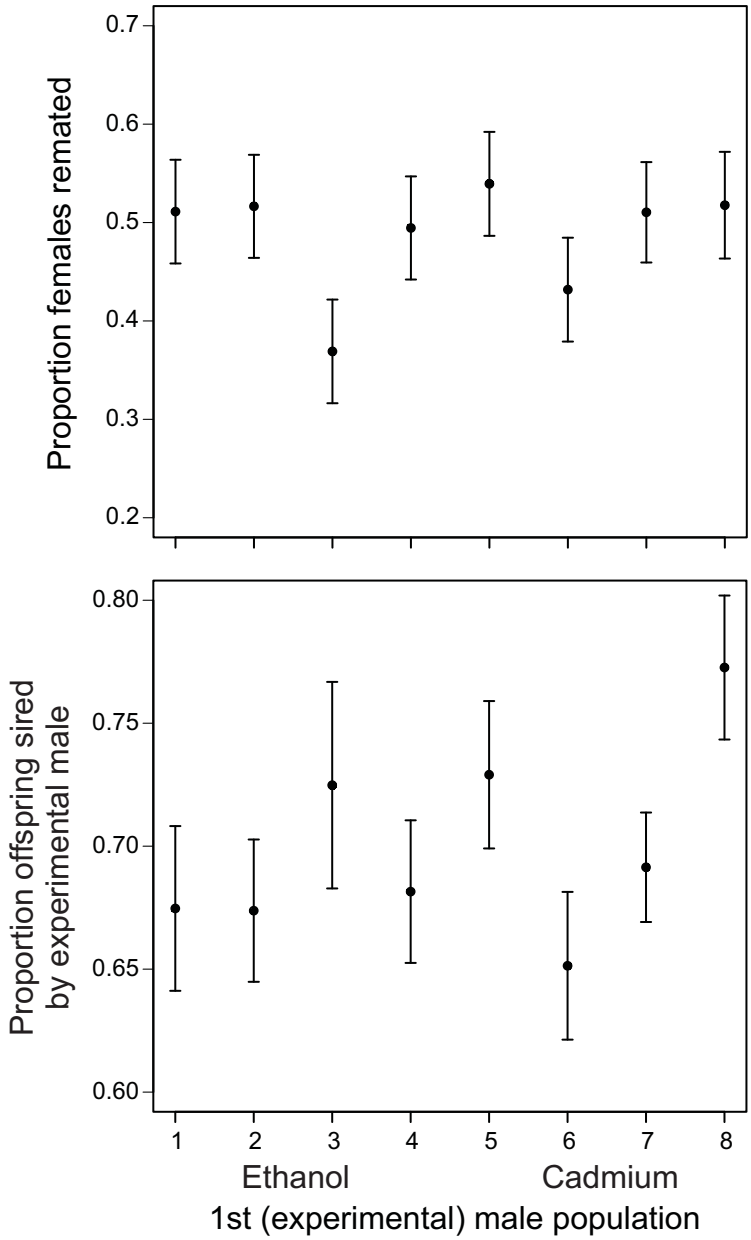

Figure 5-3 Male reproductive defense among ethanol- and cadmium-adapted *D. melanogaster* populations

Top panel: the proportion (± SE) of stock females that remated when first mated to males from each of the eight experimental populations. Bottom panel: the mean proportions (± SE) of offspring sired by the experimental males among females that first mated to an experimental male followed by a *bw* male.

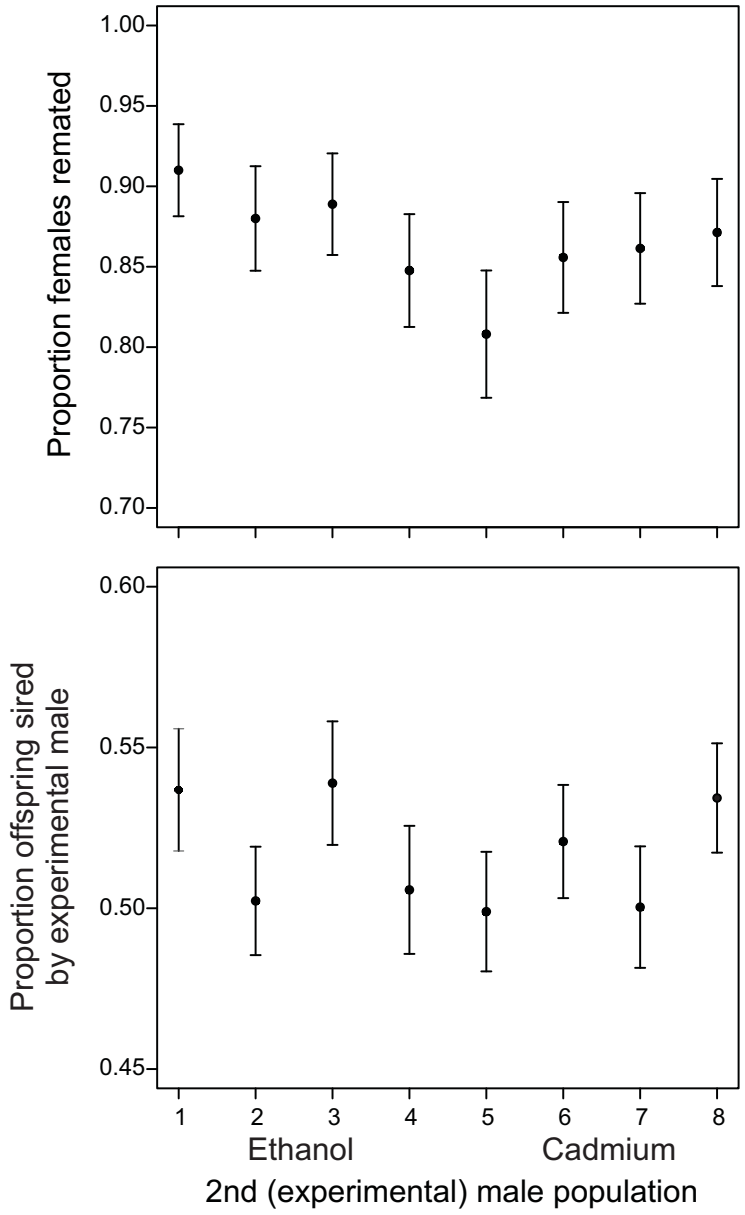

Figure 5-4 Male reproductive offense among ethanol- and cadmium-adapted *D. melanogaster* populations

Top panel: the proportion (± SE) of stock females that remated when first mated to *bw* males and were subsequently exposed to males from one of the eight experimental populations. Bottom panel: the mean proportions (± SE) of offspring sired by the experimental males among females that first mated to a *bw* male followed by an experimental male.

Chapter 6: Discussion and Conclusions

Sexual selection is responsible for some of the most diverse and spectacular structures seen in nature, from brightly coloured plumage and elaborate courtship rituals to intimidating armaments and oddly structured genitalia. Not surprisingly, these traits have captured the interest and imaginations of biologists for generations. While a great deal of progress has been made towards understanding how such exaggerated traits evolve by quantifying their costs and benefits to the individual, sexual selection may also have other important evolutionary consequences that have been less appreciated. For example, it is unclear whether sexual selection is aligned with natural selection, a situation that could potentially accelerate adaptation and niche diversification (Proulx 1999; Lorch et al. 2003; Whitlock and Agrawal 2009; Bonduriansky 2011), or if sexual selection, and specifically sexual conflict, often act against natural selection and thus inhibit adaptation (Chippindale et al. 2001; Stewart et al. 2008; Bonduriansky and Chenoweth 2009; Long et al. 2009). A related, but similarly unexplored question, is how sexual selection and ecology interact to influence adaptation and trait divergence (Maan and Seehausen 2011). In my thesis I used populations of *Drosophila melanogaster* to address some of these important questions and to gain insight into the evolutionary impacts of sexual selection and sexual conflict.

First, I tested the hypothesis that sexual selection, through condition-dependent reproductive success (Whitlock and Agrawal 2009), would accelerate the purging of deleterious alleles from populations, thereby increasing population mean fitness and speeding adaptation (Chapter 2). To do this, I manipulated the opportunity for sexual selection in experimental fruit fly populations, with known initial frequencies of six

different deleterious alleles, and then measured changes in the frequency of each allele through time. Counter to my prediction, sexual selection did not accelerate the decline in deleterious allele frequency for any of the six, and for three of the tested alleles, there was some evidence that it inhibited it. Follow-up experiments showed that males preferentially court and mate non-mutant females in these populations, consistent with previous work in another *D. melanogaster* population (Long et al. 2009). Because male courtship and/or mating are harmful in this species, the costs of this are disproportionately directed towards females of high genetic quality, thereby decreasing their fecundity. The combination of male mate preferences and sexual conflict therefore appears to reduce the genetic variance in female fitness and thus hamper the response to natural selection (Long et al. 2009). In summary, in a manipulative, evolutionary test, sexual selection did not align with natural selection and this was due at least in part due to the harmful effects of sexual conflict.

While my experimental approach was able to address changes in deleterious allele frequencies more directly than studies measuring net fitness, this approach limited me to using mutations of large phenotypic effect. Such mutations are a non-random subset of all mutations, and are likely a poor representative of the majority of deleterious mutations arising in natural populations. Therefore, future work should aim to measure the impacts of sexual selection on more natural genetic variation, for example taking advantage of genomic tools to measure genetic changes through time for populations under different sexual selection treatments, and relating these changes to fitness. Furthermore, results vary among related studies with respect how manipulations of sexual selection impact population fitness and adaptation (e.g., Radwan 2004; Rundle et al. 2006), even within

the same species (e.g. Chapter 2, Hollis et al. 2008). Some of this within-species variation likely originates in methodological differences between studies, such as the density at which populations and treatments are held, which subsequently alters the strength of aspects of sexual selection and conflict. Controlled manipulations of such maintenance procedures could be used to determine the extent to which population characteristics (e.g., density and sex ratio) influence trade-offs between potential fitness gains via sexual selection and losses via sexual conflict. Along the same lines, one could also manipulate the ecological context in which sexual selection occurs, such as by altering the extent to which females can resist male harassment by providing male-free refugia (Byrne et al. 2008), and thus limiting the potential harm of male mate choice and sexual conflict. Such experiments would address questions on how population demographics and the environment may directly influence sexual selection and its relationship with natural selection, and how the alignment of natural and sexual selection could vary within and among populations based on environmental variation.

I next tested for the alignment of natural and sexual selection in an ecological context by examining whether local adaptation and rearing environment influenced male mating success and female mate choice (Chapter 3). It has been suggested that locally adapted individuals should be of higher condition and therefore better able to locate and acquire mates (Rowe and Houle 1996; Maclellan et al. 2009; Whitlock and Agrawal 2009). As a consequence, when adapting to a new environment, those individuals best suited to their surroundings should have higher reproductive success, which would speed adaptation and niche expansion (Proulx 1999, 2001, 2002; Lorch et al. 2003; van Doorn et al. 2009; Bonduriansky 2011). To address this hypothesis, I subjected divergently

adapted fruit fly populations to competitive mating trials in each of two environments, to determine whether males have higher mating success in their adapted environment, and whether females alter their mate choice based on rearing environment. Again, I found no evidence for any alignment between natural and sexual selection, as locally-adapted males did not have higher mating success than non-adapted males, and female mate preference was unchanged across rearing environments. Condition-dependent reproductive success is an important component of the theoretical alignment of natural and sexual selection, and the absence of such condition dependence in our populations suggests that sexual selection does not aid in niche expansion. Taken together, these data further imply that sexual selection does not aid in the purging of deleterious alleles (Chapter 2), nor does it aid in ecological adaptation and diversification (Chapter 3), such that natural and sexual selection do not act in concert, and may even oppose one another.

My failure to detect condition-dependent mating success among divergently adapted males is surprising, as such a pattern is commonly assumed and thought to contribute to the alignment of natural and sexual selection (e.g. Rowe & Houle 1996; Proulx 1999; van Doorn et al. 2009; Whitlock & Agrawal 2009). The particular mechanism underlying my negative results is unclear, as individuals showed increased condition (size) when raised in their adapted environment among these independent populations. An interesting possibility is that males may have differentially allocated their limited resources to reproduction success at the expense of other life history traits, which has been suggested theoretically (Roff and Fairbairn 2007) and is consistent with some previous empirical results in this species (Rundle et al. 2007). Future work on these populations could quantify such trade-offs between reproduction and other life history

components, as well as the condition-dependence of potential trade-offs. More broadly, there are only limited data from a small number of species with respect to environmental adaptation, condition, and reproductive success, and results vary even within species and environments (e.g. Dolgin et al. 2006, Correia et al. 2010, Chapter 3). More environments and species must be examined if we are to gain a general understanding of sexual selection's role in adaptation, and to determine if the assumption of condition-dependent reproductive success, central to many theoretical models of adaptation, is realized.

Though I found no evidence that sexual selection impacts ecological adaptation and diversification, it is possible that the reverse, that ecological adaptation may impact sexual selection, could have important consequences on evolution. Ecology has been central to certain aspects of sexual selection theory, playing a key role for example in limiting the exaggeration of sexual displays (Endler 1980; Henry & Wells 2004; Ballentine 2006), in the origin of mate preferences (i.e. sensory bias; Ryan 1990; Endler & Basolo 1998), in the divergence of communication systems and the evolution of reproductive isolation (i.e. sensory drive; Boughman 2002), in the extent of among-population diversification of sexual traits (Fricke *et al.* 2010a), and in the parallel evolution of sexually-selected traits (Endler 1980, 1987; Magurran & Seghers 1994; Maan & Seehausen 2011). Furthermore, theoretical work has long noted that traits under sexual selection and sexual conflict are likely to also be targets of nonsexual ('natural' for simplicity) selection, and that ecology should thus play an important role in their evolution within populations (Parker 1979; Rowe *et al.* 1994; Rice & Holland 1997; Rowe & Day 2006; Fricke *et al.* 2009; Maan & Seehausen 2011). In spite of this theoretical framework, ecology has largely been overlooked when studying sexual

conflict's role in population divergence and speciation. In fact, sexual conflict has often been classified as a "non-ecological" promoter of diversification (Coyne and Orr 2004; Rundle and Nosil 2005), due to the fact that sexually antagonistic selection can occur even in a constant environment, and different populations can follow separate coevolutionary pathways in response.

I explored the role of ecology in the divergence of traits involved in sexual conflict via a long-term selection experiment. In Chapter 4, I tested for parallel evolution of male harm and female resistance to such harm among replicate populations that had independently adapted to either an ethanol or a cadmium environment by measuring male and female effects on female longevity. I found strong evidence for the importance of ecology in sexual conflict, as ethanol-adapted males reduced female longevity to a greater extent than cadmium-adapted males, while ethanol-adapted females were more resistant to such male-induced harm. Therefore, environmental selection and ecological divergence induced the parallel evolution of traits under sexual conflict, and I conclude that divergence via sexual conflict should not be considered an entirely non-ecological process, and that ecology should be integrated into models and tests of conflict driven population diversification.

Expanding on this work, in Chapter 5 I examined the relative importance of stochastic population-level change and predictable environmental selection on the divergence of several additional traits involved in sexual conflict. While changes in female longevity (Chapter 4) are a classic manifestation of sexual conflict, longevity is a trait on which natural selection is also likely to be strong. In contrast, the traits I examined in Chapter 5, including mating offense and defense, are thought to evolve by

sexual selection in general, with sexual conflict likely to be of particular importance. I measured mating rate and male reproductive success among the experimental populations from Chapter 4 to determine the patterns of divergence of these traits among populations both within and between environments. Specifically, I estimated female propensity to remate, male effects on female remating, and relative reproductive success among competing males when females mated multiply. While these traits did not differ for males among these populations either within or between environments, I found that female propensity to remate showed significant among population divergence within environments, consistent with the classic model of divergence among populations even in the absence of ecological differences. However, there was also some evidence that offspring production differed between females adapted to the alternative environments, such that ethanol-adapted females tended to produce more offspring sired by their first mate than did cadmium-adapted females. Therefore, among these different traits involved in sexual conflict, divergence appeared essentially random in one case yet ecologically-constrained or otherwise directed in another. This provides evidence consistent with sexual conflict being an engine of speciation, but also suggests that it does not necessarily do so outside of an ecological context.

My work, both theoretical and empirical, on the ecology of sexual conflict serves as a proof of principle that ecology may have important impacts for the diversification of traits under sexual conflict. However, the prevalence and importance of such processes in natural populations remains unknown, and future work should focus on determining how much the interaction of ecology and sexual conflict influence trait diversification in nature. Furthermore, such interactions could potentially play a role in the evolution of

reproductive isolation and hence speciation, and may even contribute to patterns of isolation normally attributed solely to ecological speciation. If ecology promotes divergence in sexual conflict such that isolated populations display environment-specific male harm and female resistance traits, females may produce fewer offspring when paired with an ecologically divergent male. Therefore, this reproductive isolation would be the product of sexual conflict, rather than divergence in mate preference or postzygotic isolation, which are often cited as potential causes of ecological speciation. For example, when females of *Timema cristinae* are paired with ecologically divergent males, they produce fewer eggs than when they are paired with ecologically similar males (Nosil et al. 2003), even though such inter-environment hybrids are viable and fertile. This pattern is consistent with ecologically-dependent parallel evolution of sexual conflict traits promoting reproductive isolation, as in my computer simulations (Appendix 1), though this data was not interpreted in this way. Further studies on sexual conflict among divergently adapted populations in the wild, as well as environmental manipulations of laboratory populations, would clarify how different environments influence the extent and direction of sexual conflict trait diversification, and the relationship between sexual conflict and speciation.

While sexual selection is pervasive in nature, it is unclear whether it complements or counters natural selection. In my thesis, I show that for experimental populations of fruit flies, sexual selection does not appear to be the helpful process it has been hypothesized to be, as it does not speed adaptation nor aid in ecological diversification. Rather, sexual selection in these populations appears to act separately or counter to the components of natural selection acting on nonsexual fitness to promote adaptation. Yet

sexual selection is not completely removed from the processes of natural selection, as I have shown that ecological divergence can promote the parallel evolution of traits under sexual conflict. In fact, in Chapters 2, 4, and 5, I found that natural and sexual selection are intricately linked and act simultaneously to influence population-level change, such as through sexual selection's inhibition of adaptation or natural selection's role in altering sexual traits through time. Though natural and sexual selection may not work in concert, and may even oppose one another at times, they are both important processes acting on sexual populations, and for evolutionary change to be understood, both must be considered.

Appendix 1: Ecology of sexual conflict computer simulations

We performed individual-based simulations that followed a similar framework to previous models of sexual conflict (Gavrilets et al. 2001; Gavrilets & Waxman 2002; Hayashi et al. 2007), but that added multiple male and female traits under sexual conflict and permitted natural selection on these traits to vary between different environments. Individuals in these simulations are diploid and there are 12 loci coding for six traits (two diploid loci for each trait). The traits are sex specific in that females only express sensitivity traits F_1, F_2, and F_3, while males only express stimulation traits M_1, M_2, and M_3, although all individuals carry the alleles for all traits. There are 20 allelic values for each locus (ranging from 1 to 20) that interact additively to express each trait quantitatively such that individual trait values are proportional to the sum of the four allelic values coding the trait (ranging from 0.2 to 4) (Eq. 1):

$$F_1 = \frac{\sum F_{1ij}}{20}, \tag{1}$$

where i represents the locus and j represents the allele within that locus.

An initial population was generated with 200 individuals in which all female sensitivity traits and male stimulation traits had an intermediate value of two. Each female interacted with five randomly selected males and mating occurred for each interaction with a probability (ψ) based on the female's F trait values and the male's M trait values, determined as follows (Eq. 2):

$$\psi = 1 - \exp\frac{-(F_1 M_1)^2 + (F_2 M_2)^2 + (F_3 M_3)^2}{4L}, \quad (2)$$

where L represents the number of loci (here $L = 12$). Therefore, it is the interactions of the corresponding female sensitivity traits and male stimulation traits that determine the probability of mating, and these three trait pairs can be thought of as three different potential male-female coevolutionary pathways. For example, if males have an increased trait value for M_1, they increase their likelihood of mating with a given female. The most effective way for females to counteract this is to have a decreased specific sensitivity to M_1, denoted by a lower value of trait F_1. While decreasing female sensitivity in the other two traits (F_2 and F_3) would also decrease the probability of mating, it would do so less effectively than decreasing sensitivity trait F_1.

The number of offspring a female generates is dependent on her mating rate (P), which is equal to the proportion of interacting males with which she mated (Eq. 3):

$$\text{offspring} = B_{max} \exp(-s\,(P-P_{opt})^2). \quad (3)$$

Here $B_{\max}$ is the maximum number of offspring a female can produce (set at ten), P_{opt} is the optimum mating rate for females (set at 0.4, or mating with two out of five potential mates), and s is the strength of sexual selection (how steeply the number of offspring decreases as P departs from P_{opt}, set at ten). Therefore, females decrease their fitness if they mate more or less than their optimum rate. Like other models (Rowe et al. 2003; Hayashi et al. 2007), sexual conflict here is over mating rate; males increase their fitness by increasing their mating probability and hence mating rate (by increasing the values of

their stimulation traits), while females maximize their fitness by ensuring that their mating rate is at its optimum.

After females have interacted with five males, they produce the number of offspring determined by Eq. 3. If a female did not mate with any males, she produces no offspring. For each offspring produced, a male which mated the female is randomly selected as the offspring's father. One allele is randomly chosen from each parent for each locus and assigned to the offspring, such that it inherits one maternal and one paternal allele for each of the 12 loci. Sex is then randomly assigned to the offspring with equal probability of male or female. Mutation also occurs at this stage, following a step-wise mutation model (Nei et al. 1983), such that the allelic value can increase or decrease by a value of one with the probability μ (set at 0.01) for each allele. If the allelic value is 1, it can only increase with a probability of $\mu/2$. Similarly, if the allelic value is 20, it can only decrease with the probability $\mu/2$.

Once the genotype is determined, the offspring is subject to natural selection (mortality) such that it is removed with a probability (Eq. 4a):

$$P(mortality) = 1 - \exp\left(-s_{F1}\left(F_1 - F_{1opt}\right)^2 - s_{F2}\left(F_2 - F_{2opt}\right)^2 - s_{F3}\left(F_3 - F_{3opt}\right)^2\right) \quad (4a)$$

for females, and (Eq. 4b):

$$P(mortality) = 1 - \exp\left(-s_{M1}\left(M_1 - M_{1opt}\right)^2 - s_{M2}\left(M_2 - M_{2opt}\right)^2 - s_{M3}\left(M_3 - M_{3opt}\right)^2\right) \quad (4b)$$

for males. s_{F1}, s_{F2}, $s_{F3,}$ s_{M1}, s_{M2}, and s_{M3} are coefficients specifying the strength of selection on each of the traits, with the probability of mortality increasing as a function of the deviation of the trait from its natural selection optimum (set at the initial intermediate value of two). Natural selection is sufficiently strong such that males and females cannot exaggerate all three traits to a great extent. Rather, sexual conflict can cause moderately altered values for all three traits, or cause one or two traits to be exaggerated to a greater extent while leaving at least one trait relatively unchanged. The response to sexual conflict is therefore constrained by the effects the underlying traits have on an individual's probability of dying.

The above reproduction and mortality processes are repeated for all females in a population, with the next generation generated by randomly sampling 200 of the surviving offspring. The entire process was iterated 2000 times (i.e. generations) to produce a single selection line.

Four replicate selection lines were simulated for each of four separate environments (A, B, C, and D) which differed only in the strength of natural selection acting on male stimulation traits. Environments A and B differed with respect to which traits were under strong natural selection. In environment A, natural selection acting on the male manipulatory trait M_1 was ten times stronger than that acting on traits M_2 and M_3 (i.e. increased s_{M1}, see eq. 4b) such that exaggeration of trait M_1 was more likely to lead to mortality than was the exaggeration of the other traits. In environment B, the selection coefficient acting on trait M_2 was greater. Therefore, the overall selection acting on male traits between environments A and B was equal, but the cost of trait exaggeration on M_1 or M_2 differed greatly. Environments C and D, in contrast, differed in the overall strength

of selection, where the strength of natural selection acting on all male manipulatory traits in environment D was three times that of environment C. Therefore, trait exaggeration of any of the male traits was much more costly in environment D than in environment C. Because these two pairs of environments differed fundamentally how natural selection varied, we considered the results of environments A and B (differing traits under strong selection), and C and D (strength of selection), separately. Parameter values of the four environments are given in Table A1.

After simulating all populations, male and female trait values were compared among populations and between the pairs of environments. All trait values were analyzed separately, using a general linear mixed model, with environment as a fixed effect and population (nested within environment) as a random effect. The random effect of population was tested using likelihood ratio tests. Furthermore, to investigate population interactions (mating rate and offspring production), we exposed all females from each population to 10 randomly selected males from each of the eight populations within each set (i.e. A and B, C and D), in effect performing two separate full factorial sets of population crosses. Each set of crosses included all 64 combinations of males and females from the eight populations (four from each environment), including the eight intrapopulation interactions. For each female, the number of matings and offspring produced were recorded.

Variation in female mating rate and offspring production were analyzed separately using a general linear mixed model:

mates/offspring = female environment + male environment + female × male environment
+ female population (female environment) + male population (male environment),

in which female and male environments are fixed effects, and female and male population are random effects nested within their respective environments. The models were fit via REML and random effects were tested using likelihood ratio tests. In all cases, the female × male population interaction was found to be non-significant and was therefore excluded from the model.

Differing traits under strong selection (environments A and B)

After 2000 generations, there was significant among-population variation for all male stimulation and female sensitivity traits ($P < 0.0001$ in all cases) among the eight replicate A and B populations. However, a significant component of this variation was associated with environment, where, as expected, populations adapted to environment A (strong selection against exaggerated M_1 values) tended to have lower average values of this trait ($F_{1,6} = 56.7$, $P = 0.0003$) that were therefore closer to their natural selection optimum. In fact, the exaggeration of M_1 was on average twelve times greater for populations adapted to environment B than populations adapted to environment A (Fig. A-1a). Correspondingly, reduction in the value of trait F_1 was 1.5 times greater for populations adapted to environment B (Fig. A-1b, $F_{1,6} = 17.02$ $P = 0.0062$). Similarly, the exaggeration of trait M_2 among populations adapted to environment A was nine times that for populations adapted to environment B, where exaggeration of M_2 was more costly ($F_{1,6} = 564.32$, $P < 0.0001$), while the reduction of sensitivity trait F_2 was 1.5 times

greater for populations adapted to environment A ($F_{1,6} = 15.35$ $P = 0.008$). Traits M_3 and F_3 ($P > 0.10$ for both) did not differ significantly between these environments (Fig. A-1a,b).

When we carried out full factorial crosses, exposing males and females from all populations in all possible combinations, there was a significant effect of male population on number of matings and offspring produced ($P < 0.0001$ for both), meaning that some populations' males achieved higher mating success overall, and also caused more harm to females. There was also a significant effect of female population on number of matings and offspring production ($P < 0.0001$ for both), meaning that some populations' females had greater overall defense against male manipulation of mating rate and harm.

Sexual interactions also showed a significant male × female environment effect on the number of matings ($F_{1,48} = 21.28$, $P < 0.0001$) and offspring production (Fig. A-2, $F_{1,48} = 20.66$, $P < 0.0001$). That is, even though there was no overall difference in male harm (mating rate: $F_{1,6} < 0.01$ $P = 0.95$; offspring production: $F_{1,6} < 0.01$, $P = 0.96$) or female resistance (mating rate: $F_{1,6} = 0.39$, $P = 0.55$; offspring production: $F_{1,6} = 0.52$, $P = 0.50$) between environments, the particular male × female combination had significant impacts on reproductive output (Fig. A-2). Specifically, females had lower resistance against the manipulation of ecologically divergent males, resulting in an average three percent increase in female mating rate in inter-environment pairings. Because mating beyond the female optimum is costly, females produced five per cent fewer offspring on average if they were exposed to males adapted to the other environment. Females were not most resistant to their coevolved (same population) males, as the mating rate of females was rarely lowest when exposed to these males. However, females tended to

have higher defense against males adapted to the same environment as them, as compared to males adapted to the other environment. Therefore, there was parallel evolution in both male manipulation traits, and corresponding female sensitivity traits, and this led to females being more resistant to same-environment males, meaning that females tend to produce more offspring when exposed to ecologically similar males. These results therefore show very weak reproductive isolation between divergently adapted populations resulting from the interaction of natural selection and sexual conflict.

Strength of selection (environments C and D)

There was significant among-population variation in all male stimulation and female sensitivity traits ($P < 0.0001$ in all cases) among the eight C and D populations. A significant component of this variation was associated with environment for male stimulation traits M_1 ($F_{1,6} = 7.14$, $P = 0.037$) and M_3 ($F_{1,6} = 16.26$, $P = 0.007$), where populations in the less harsh environment C had more exaggerated traits, though this trend was not significant for trait M_2 ($F_{1,6} = 3.04$, $P = 0.13$). Overall, the exaggeration of male stimulatory traits M_1, M_2, *and* M_3 was 2.3, 2.1, and 3.3 times greater, respectively, for populations adapted to environment C compared to populations adapted to environment D (Fig. A-1c). Similarly, the female sensitivity trait F_1 was reduced in environment C ($F_{1,6} = 8.1$, $P = 0.029$). While this trend was also present in traits F_2 and F_3, the effect of environment was not significant ($P = 0.10$ and 0.19, respectively; Fig. A-1c,d). The reduction in female sensitivity traits F_1, F_2, *and* F_3 was 1.3, 1.6, and 1.2 times that of populations adapted to environment C (Fig A-1 d).

When males and females were exposed to one another in all possible combinations of the eight populations, there were again significant effects of both male and female population on the number of matings and offspring produced ($P < 0.0001$ in all cases), suggesting among-population variation in male harm and female resistance. However, in contrast to results from environments A and B, there were significant differences between environments in the male's influence on both female mating rate ($F_{1,6} = 46.75$, $P = 0.0006$) and offspring production ($F_{1,6} = 47.19$, $P = 0.0006$) such that males from the less harsh environment C achieved an average 0.9 more matings than males from environment D, and subsequently decreased female productivity by an average of 1.4 offspring compared to females paired with males from environment D (Fig. A-3). Female environment also had a significant influence on mating rate ($F_{1,6} = 24.76$, $P = 0.0025$) and offspring production ($F_{1,6} = 19.73$, $P = 0.0044$), where females from the less harsh environment C had greater defense overall, reducing their mating rate by an average of 0.6 matings, and increasing their offspring production by an average of 0.9 offspring. The male × female environmental effect was non-significant in both cases (mating rate: $F_{1,48} = 0.53$, $P = 0.46$; offspring production: $F_{1,48} = 2.5$, $P = 0.11$), indicating that the main effect of each sex was consistent across environments for the other sex. Therefore, when environments differed in their overall strength of selection on traits under sexual conflict, we observed parallel evolution of all male stimulation and female sensitivity traits, such that these traits are less exaggerated in the harsh environment. This caused significant asymmetry in the reproductive output of individuals when exposed to potential mates from the two environments.

Table A-1 Parameter values specifying the strength of natural selection on each of the male and female traits in the four environments in computer simulations.

environment	A	B	C	D
s_{M1}	1	0.1	0.2	0.6
s_{M2}	0.1	1	0.2	0.6
s_{M3}	0.1	0.1	0.2	0.6
s_{F1}	0.3	0.3	0.3	0.3
s_{F2}	0.3	0.3	0.3	0.3
s_{F3}	0.3	0.3	0.3	0.3

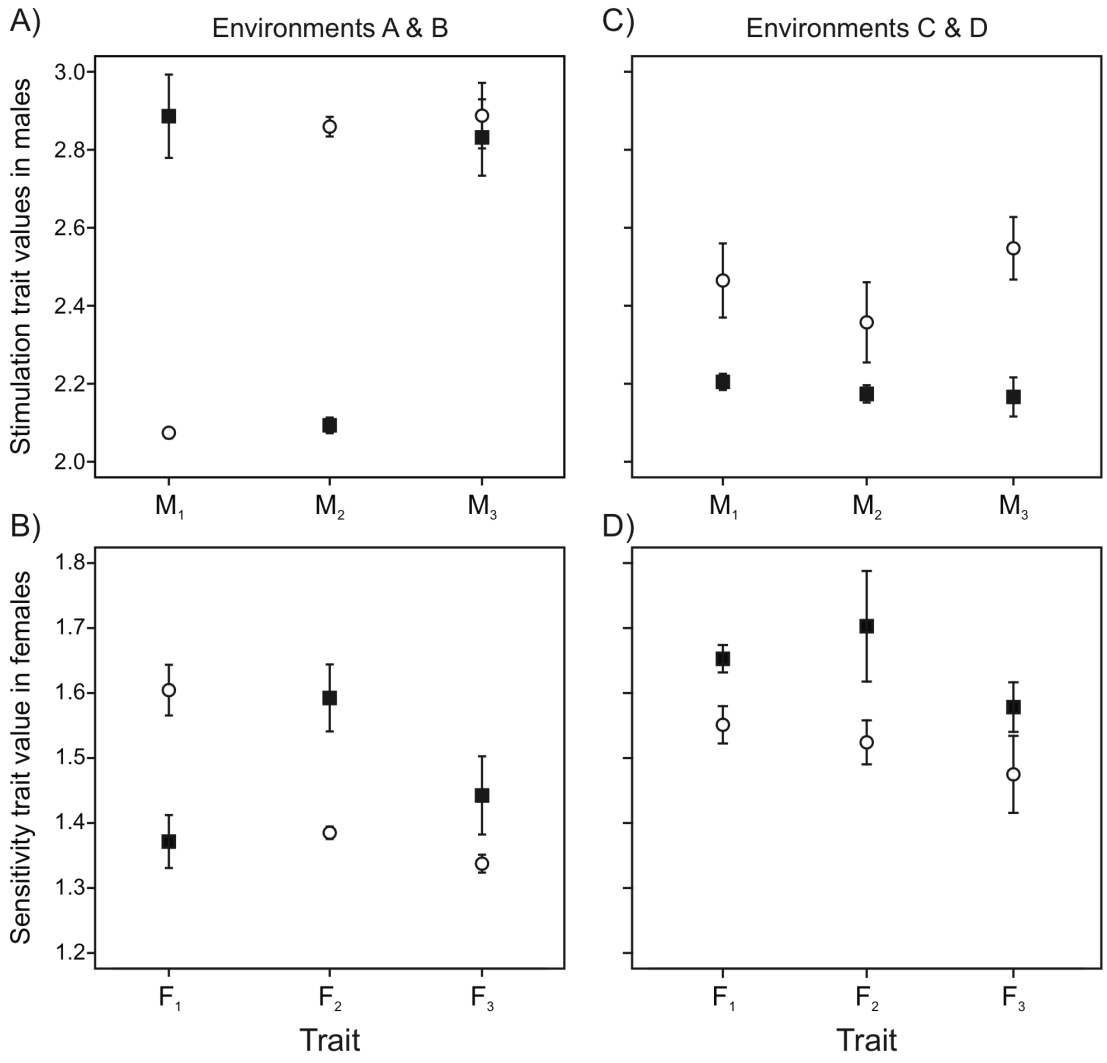

Figure A-1 Divergence of male stimulation and female sensitivity traits between environments in 2000 generation simulations.

Points represent the mean (± among-population SE) of the four replicate populations within each environment. Environments A and B differ in the identity of the traits under strong natural selection (left column), while environments C and D differ in the overall strength of natural selection (right column). A) Male stimulation and B) female sensitivity traits for populations adapted to environments A (circles) and B (squares). C) Male stimulation and D) female sensitivity traits for populations adapted to environments C (circles) and D (squares).

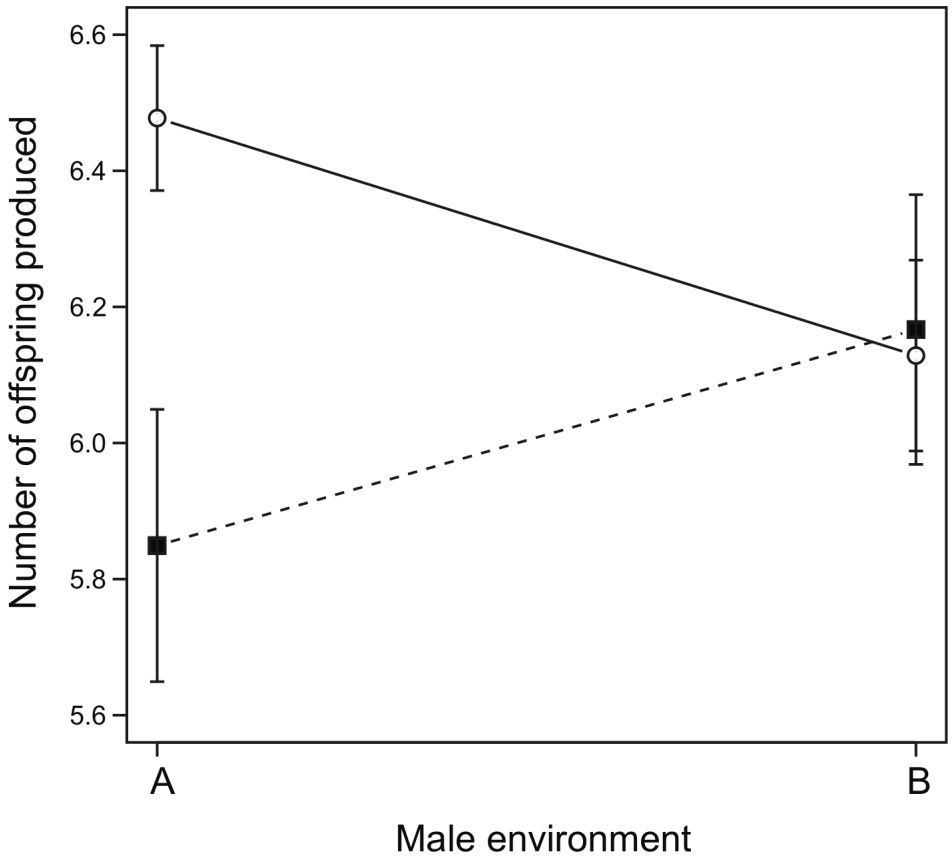

Figure A-2 Male x female interactions among simulated populations when environments differ in traits under selection

Simulation results of number of offspring produced by females adapted to environments A (circles, solid line) and B (squares, dashed line) when exposed to males adapted environments A or B. Points represent the mean (± SE) of the four replicate combinations of male and female population.

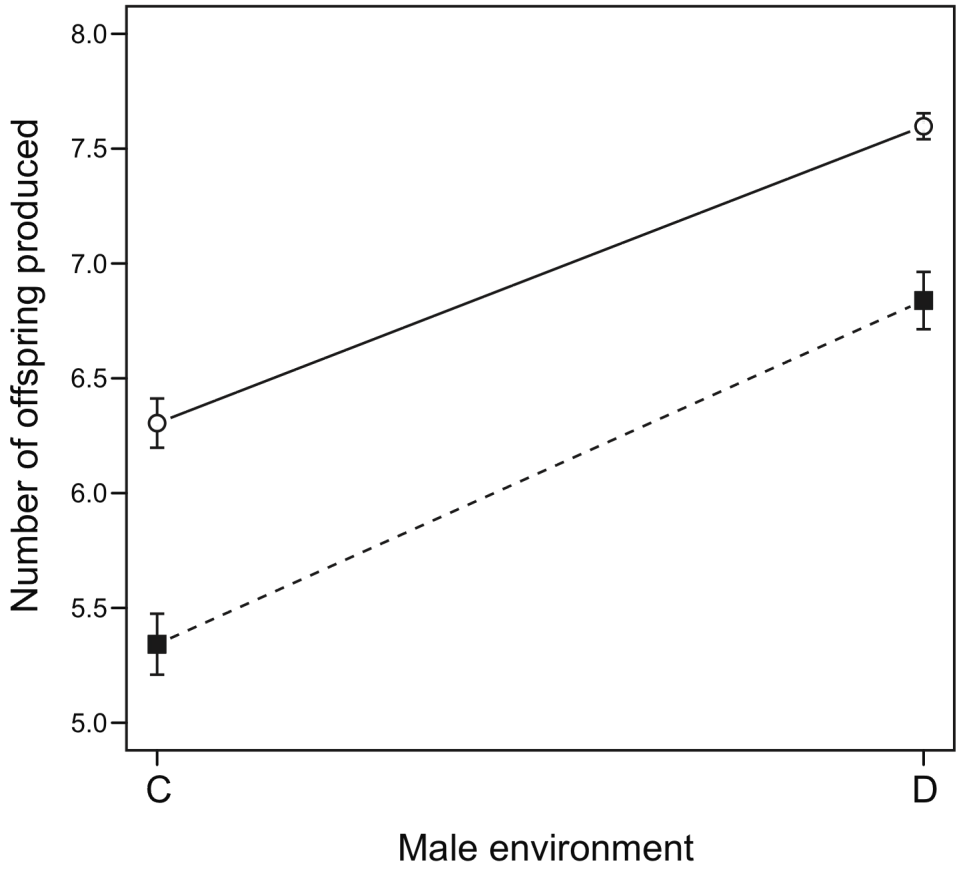

Figure A-3 Male x female interactions among simulated populations when environments differ in the strength of selection

Simulation results of the number of offspring produced by females adapted to environments C (circles, solid line) and D (squares, dashed line) when exposed to males adapted to environments C or D. Points represent the mean (± SE) of the four replicate combinations of male and female population.

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