The quantitative genetics of good genes: fitness, male display, and female preference

Matthieu Delcourt

Thesis submitted to the

Faculty of Graduate and Postdoctoral Studies

In partial fulfillment of the requirements

For the PhD degree in Biology

Department of Biology

Faculty of Science

University of Ottawa

Thesis Summary

The ultimate goal of my thesis is to develop a better understanding of the contribution of indirect benefits (i.e. good genes) to the evolution of female mate preferences. It is genetic variance in, and genetic correlations (covariances) among, male sexual displays, female preferences for them, and fitness that in part determine the degree to which females preferring certain male displays over others will gain an indirect benefit by having higher fitness offspring. Recent advances in quantitative genetic theory provide the mathematical means for quantifying the strength of indirect selection for female mate preferences (Kirkpatrick and Hall 2004), at least under certain conditions, but there are few empirical systems for which such data exist (Brooks and Endler 2001; Qvarnström et al. 2006). I have undertaken a classic half-sibling breeding design with the ultimate goal of estimating the specific parameters of this model in a population of the Australian fruit fly *Drosophila serrata*. The breeding design was performed across two environments - one to which the population was well adapted and a novel environment to which it was not - thereby also providing insight into genotype-by-environment interactions for this suite of traits and their effects on good genes indirect benefits in a novel environment. General insight is also gained into the genetic covariance of male and female fitness and the prevalence of intralocus sexual conflict, the quantitative genetic basis of female mate preferences for multiple male traits, the condition-dependence of these traits, and the genetic association between sexual displays and fitness when mutation-selection balance is inferred. My results advocate caution in the application of existing theory to quantify the strength of indirect selection, suggesting that a good genes process may be fundamentally different when the exaggeration of sexual displays is eventually halted and an equilibrium is reached between opposing selection.

Résumé de la thèse

L'objectif de ma thèse est d'établir une meilleure compréhension de l'importance de la théorie des bons gènes (ou bénéfices indirects) dans l'évolution des préférences sexuelles des femelles. Ce sont les variances et les corrélations (covariances) génétiques des caractères sexuels, des préférences qui leurs sont associées ainsi que de la valeur sélective qui déterminent si les femelles préférant certains traits sexuels mâles plutôt que d'autres obtiendront des bénéfices indirects par le biais d'une descendance à plus haute valeur sélective. De récentes avancées en génétique quantitative fournissent des outils mathématiques pour quantifier l'intensité de la sélection indirecte sur les préférences sexuelles des femelles (Kirkpatrick and Hall 2004), au moins dans certaines conditions, mais il n'existe que très peu de systèmes empiriques pour lesquels de telles données existent (Brooks and Endler 2001; Qvarnström et al. 2006). J'ai réalisé un élevage de demi-parents afin d'estimer les paramètres de ce modèle chez une population de mouche du vinaigre australienne *Drosophila serrata*. L'élevage a été effectué dans deux environnements différents - un auquel la population était adapté et un auquel elle ne l'était pas - permettant l'étude des interactions génotype-par-environnement de traits quantitatifs ainsi que leurs effets sur les bénéfices indirects dans un nouvel environnement. Aussi, sont analysées les covariances génétiques entre les valeurs sélectives mâles et femelles, la prépondérance du conflit sexuel intralocus, la condition-dépendance des caractères mâles, la base génétique des préférences ainsi que l'association génétique entre les traits sexuels et la valeur sélective en balance de mutation-sélection. Mes résultats mettent en garde contre l'application de la théorie des bons gènes qui peut être radicalement différente lorsque l'exagération des caractères sexuels est arrêtée à l'approche d'un équilibre généré par des forces de sélection opposées.

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Acknowledgements

I want to thank my supervisor Howard Rundle for his tremendous help during the last four years. His constant support and advices at every stage of my PhD were critical in making this project possible. Learning quantitative genetics in Howard's lab was one of the most challenging yet fascinating things I have done in my early career. Howard taught me how to address big questions in evolution using trivial, elegant and ambitious science. Thank you for your availability and enthusiasm about the project from day one. I was proud to be your first student and I will always remember the full days spent in your office brain storming, analyzing data, and troubleshooting.

I would like to thank Mark Blows for his expertise in quantitative genetics, his many advices at various stages of my PhD especially in multivariate analyses. I would like to thank the many students who were closely related to this project: Gina Schroeder, Marc Charette, Christina Carnovale, Catherine Millar who spent months sorting, counting, transferring, watching flies in the summer of 2007. I want to thank Steve Chenoweth and Emma Hine for technical issues and comments on some versions of manuscripts. Many thanks to my committee members Dr. Rees Kassen, Dr. Susan Bertram and Dr. Gabriel Blouin-Demers who provided constructive comments to successfully carry this experiment. I want to thank Linda Kimpe for her incredible help troubleshooting the moody gas chromatograph and Harvinder Singh, Alana Plummer, Kathleen Duncan, Ronald Pringadi and Stacey Shim for helping me count flies in the lab at critical times. Funding for this research project was provided by the Australian Research Council (ARC), NSERC (Natural Sciences and Engineering Research Council) and the University of Ottawa.

I especially want to thank David Punzalan for his support, teaching of matrix algebra, eigenvectors and eigenvalues during the many discussions (and even more beers) we had! Dave helped me a lot in growing as a scientist, finding confidence in myself and helping me putting words on the important evolutionary questions that I was always interested in.
I also want to thank Jacquie Sztepanacz, Lucia Kwan, Kelsie MacLellan, Devin Arburthnott, Alex Wong and the Kassen lab members for their help in the lab and their support throughout the years.

I would like to thank David Duneau and Alexandre Courtiol for their friendship, their support from far away and for the long discussions we had about evolution and sexual selection. I want to thank my cycling buddies Adam Algar, Patrick Audet and Andrew Waye with who I spent hours riding, climbing, attacking, racing and trash-talking in the Gatineau Park. This PhD would not have been possible without you guys; you helped me find the right balance between work and fun times on the bike.

Finally, I would like to thank my family for always being supportive, for their presence in the good and the bad days. Merci à vous sept ! Vous étiez la bulle d'air qui m'a aidé à respirer un peu durant ces 5 ans. Merci à mon père pour m'avoir transmis sa passion pour la nature et la biologie. Comme tu l'auras compris, ce travail n'essaie qu'en vain de comprendre cette « science aussi merveilleuse, au-dessus de ma portée » et qui semble bien « trop élevée pour que je puisse la saisir ».

Chapter 1: Good genes sexual selection and the evolution of female mate preferences

Sexual selection occurs whenever individuals vary in a trait and a non-random relationship exists between this trait and reproductive success. Two main forms of sexual selection are often recognized (Darwin 1859; Andersson 1994). First, intrasexual sexual selection occurs when individuals of one sex (generally males) compete with one another, directly and/or indirectly, for access to the other sex. Intrasexual selection may result, for example, in the evolution of weapons of combat used in overt aggressive encounters among individuals, as well as in traits that enhance an individual's success during scramble competition for access to the other sex. Second, intersexual selection occurs when individuals of one sex (usually females) exhibit particular preferences, choosing mates non-randomly among individuals of the opposite sex. Such preferences are often based, at least in part, on particular sexual displays in the other sex and can thereby be responsible for the evolutionary exaggeration of such traits.

Darwin was the first to describe both intra- and intersexual selection and highlighted their role in the evolution of traits that otherwise appeared to be maladaptive with respect to survival or fecundity. Since then, many studies on both natural and laboratory populations have demonstrated sexual selection acting on particular traits in animals and plants (Andersson 1994) and more recently in microorganisms (Rogers and Greig 2009). A recent meta-analysis of the strength of selection in the wild revealed that directional selection arising from variation in mating success was stronger on average than that arising from variation in survival or fecundity (Kingsolver et al. 2001), suggesting that sexual selection may be strong in nature relative to other forms of natural selection.

The evolution of mate preferences

My focus here is on the evolution of mate preferences that generate intersexual selection. Mate preferences exist when individuals of one sex, hereafter females, choose their mates nonrandomly with respect to values for one or more male traits. Mate preferences are extremely common in natural populations and their phenotypic consequences have been the subject of much study (Andersson 1994; Arnqvist and Rowe 2005). In addition to being responsible for the evolution of extravagant and diverse sexual display traits that characterize many species, the divergence of mate preferences among populations will also eventually generate sexual (behavioural) isolation between them, a form of premating reproductive isolation that appears to be key in the initial stages of speciation (Coyne and Orr 2004).

As first pointed out by Fisher (1930), the existence of a mate preference generates sexual selection for the preferred trait, and the assortative mating that results generates a genetic correlation between the male display and female preference. This correlation arises because females with the preference mate with males possessing the trait, causing their offspring to carry alleles for both traits. The non-random genetic association among loci (i.e. linkage disequilibrium) that results generates indirect selection for the preference (due to selection on a genetically correlated trait, here male attractiveness), causing a self-reinforcing coevolutionary process termed 'Fisher's runaway'. This coevolutionary process will occur whenever mate preferences and the display traits they act upon have a genetic basis. Theory suggests that such indirect benefits alone cannot maintain costly female preferences at equilibrium (Cameron et al. 2003). This is because at equilibrium (i.e. when the male

display is maintained at a balance between natural and sexual selection), females exercising directional preference do not gain any benefit compared to females that mate randomly, and costly female preferences are therefore selected against.

Although difficult to estimate empirically, costs of mate preferences have been inferred in a handful of cases (Reynolds and Gross 1990; Byers et al. 2005; Cotton et al. 2006; Rundle et al. 2009). These costs imply that preferences must be maintained within populations ultimately as a consequence of the direct benefits gained by females, and/or by the indirect benefits that accrue as a consequence of the genes that the males carry (e.g., good genes mate choice); (Kirkpatrick and Barton 1997; Houle and Kondrashov 2002; Cameron et al. 2003; Kokko et al. 2003). Direct benefits present little controversy – it is straightforward to understand the evolution and maintenance of costly female preferences when males provide the females with resources (e.g., nuptial gifts), parental care, or protection from predators, and examples abound of such situations (Andersson 1994; Arnqvist and Rowe 2005). Benefits may also come in the form of cost avoidance, for example if preferred males carry fewer parasites or diseases that could be transmitted to the female.

Differential resistance (equivalent to varying preferences) of females may also be directly beneficial if it minimizes male-imposed costs arising from sexual conflict (Chapman et al. 2003; Arnqvist and Rowe 2005). Such costs can arise because, although males and females share the same genome, their evolutionary interests in reproduction generally differ (Parker 1979). This can generate sexually antagonistic selection on shared traits because the values that maximize fitness in one sex are different from those in the other sex. This conflict can

occur with respect to a shared trait (e.g., body size; termed intralocus sexual conflict), or a trait arising from male-female interactions (e.g. mating rate, parental care; termed interlocus sexual conflict). In the specific case of intralocus sexual conflict, the conflict can be resolved through the evolution of sexual dimorphism via a number of mechanisms including sex-specific gene expression, thereby allowing each sex to reach its own fitness optima (Cox and Calsbeek 2009).

Benefits outside of the context of mating may also contribute to the maintenance of a costly female preference. If female sensory systems are shaped by natural selection due to their involvement in other tasks such as foraging or predator avoidance, mate preferences may arise as a side-effect of these changes (Ryan 1998). Under such a scenario, mate preferences themselves are not adaptive, but are the pleiotropic effect of adaptive traits. The existence of such preferences may create pre-existing sensory biases in females and males that exploit their existence may have a selective advantage, giving rise to sexual conflict (Arnqvist and Rowe 2005).

Indirect benefits of female mate preference exist when preferred males are of higher genetic quality (i.e. have higher breeding values for fitness) than non-preferred males (Zahavi 1975; Kirkpatrick and Ryan 1991). This is suggested to be true whenever there is a positive genetic correlation between male sexual display and genetic quality (Iwasa and Pomiankowski 1991; Rowe and Houle 1996; Lorch et al. 2003). By mating with such males, females pass these good genes on to their offspring. The contribution of good genes indirect benefits is key to understanding mate preference evolution and has therefore been the

subject of much theoretical and empirical research. As I outline below, however, results are mixed and its prevalence remains unclear.

Good genes indirect benefits

My research focuses on the contribution of good genes to the evolution of female mate preferences. While the evolution of mate preferences is easy to understand when individuals gain a direct fitness benefit from their partner (i.e. parental care, nuptial gifts, resource access, etc.), in many species there appears to be little opportunity for direct benefits because males contribute nothing to their partner beyond their genes. Why mate preferences evolve in such a situation is a central and unresolved question in sexual selection and behavioural research. As mentioned previously, a longstanding hypothesis to explain the evolution of female preferences for such exaggerated display traits is that they signal high genetic quality in males, and that by mating with these males, females gain an indirect benefit in terms of higher fitness offspring. This idea dates back to Darwin (1859) and is now known as the good genes model of sexual selection. Good genes requires that male display traits must be honest indicators of male genetic quality (Zahavi 1975; Iwasa and Pomiankowski 1991). This is suggested to be true whenever display traits are costly to produce and their exaggeration increases male mating success. This is because, once genetic variation in the display trait is exhausted, genetic variance in overall condition will be recruited because only males in high condition can allocate the resources to pay the costs of trait exaggeration (Rowe and Houle 1996). Since condition depends on many loci, it is a large target for mutation, suggesting that genetic variation in display traits may be maintained despite continued directional selection. This process, called “genic capture”, is

predicted to create a positive genetic correlation between male display traits and male genetic quality.

When good genes mate choice occurs, males with high non-sexual fitness (e.g., viability) also have high sexual fitness (e.g., mating success), meaning that natural and sexual selection should reinforce one another, thereby promoting adaptation to a given environment. This was summarized by Darwin as follows: “Amongst many animals, sexual selection will have given its aid to ordinary selection, by assuring to the most vigourous and best adapted males the greatest number of offspring.” (Darwin 1859). Recent theoretical models have confirmed that good genes sexual selection can benefit non-sexual fitness by increasing the rate and extent of adaptation (Lorch et al. 2003), purging mutation load (Whitlock 2000), and promoting the spread of advantageous alleles in structured populations (Proulx 2001, 2002).

Despite considerable attention over the past decades, evidence for good genes indirect benefits is mixed. Many of the empirical studies are observational, consisting of tests for phenotypic correlations between male sexual display and fitness (Norris 1993; Moller 1994; Petrie 1994; Welch et al. 1998; Cotton et al. 2004; Evans et al. 2004). Mating with attractive males has been shown to benefit certain components of offspring fitness, although other components sometimes appeared to suffer (reviewed in Rundle et al. 2007). Only a handful of studies have attempted to include all the main components of offspring fitness (e.g., larval competition, male attractiveness, adult survival, etc.), revealing weak indirect benefits that are overwhelmed by direct costs in some cases.

Manipulative tests of good genes are few and results are again variable. One approach has been to manipulate the opportunity for female mate choice by either enforcing monogamy or permitting polygamy, and then measuring the fitness consequences. Holland and Rice (1999) showed that the removal of mate choice increased the net reproductive rate after 47 generations in *D. melanogaster*, revealing a cost of sexual selection. In another manipulative experiment, indirect benefits were also found to be rather weak and did not counterbalance the direct costs imposed by sexual conflict associated with male-induced harm (Stewart et al. 2008). Larval competitive success has been shown to increase in the offspring when mate choice is allowed (Partridge 1980). However, this was not the case after a number of generations of experimental evolution (Promislow et al. 1998).

Experimental evolution is a powerful technique to determine the role of sexual selection on fitness because it allows fitness to be integrated by the organisms themselves. After 26 generations of sex-specific selection in *Drosophila melanogaster* (Morrow et al. 2008), lifetime fitness was found to be asymmetrical between the sexes, suggesting that sexually antagonistic mechanisms are predominant. Other cross-generational studies suggest more substantial indirect benefits from mate choice (Head et al. 2005; Rundle et al. 2007). Particular attention has also been given to testing whether sexual selection promotes adaptation to novel environments. In two cases, sexual selection failed to speed adaptation in two species of fruit flies (Holland 2002; Rundle et al. 2006), whereas a third experiment using seed beetles (Fricke and Arnqvist 2007) found a small beneficial effect of sexual selection.

Results from theory are likewise mixed, with some models suggesting that the indirect benefits are unlikely to overcome direct costs of mate choice (Kirkpatrick 1996; Kirkpatrick and Barton 1997; Cameron et al. 2003), and others suggesting the opposite (Houle and Kondrashov 2002).

Quantitative genetics of good genes sexual selection

Quantitative genetic studies of the strength of indirect selection favouring female mate preferences provide an alternative approach to quantifying the role of indirect benefits in preference evolution that has received limited attention to date (but see Hine et al. 2002; Brommer et al. 2007). Using a general framework for multilocus modeling developed by Barton and Turelli (1991) and Kirkpatrick et al. (2002), an equation predicting the quantitative change in mean mate preference caused by good genes indirect benefits has been derived that uses parameters that can be empirically estimated (Kirkpatrick and Barton 1997; Brommer et al. 2007; Kirkpatrick 2009):

$$\Delta\bar{P} = \frac{1}{2}\rho_{PT}\ h_P^2\ h_T\ r_{TW}\left(\frac{\sqrt{G_W^m} + r_W^{mf}\sqrt{G_W^f}}{2}\right). \qquad (1)$$

ΔP is the change in the mean preference (measured in units of phenotypic standard deviations), ρ_{PT} is the phenotypic correlation between female preference and male display among mated pairs, h_P^2 is the heritability of the preference, h_T is the square root of the

heritability of the display trait, r_{TW} is the genetic correlation between the display trait and total fitness, $\sqrt{G_W^m}$ and $\sqrt{G_W^f}$ are the genetic standard deviations of male and female fitness respectively, and r_W^{mf} is the genetic correlation between male fitness and female fitness. These parameters can be estimated using classic quantitative genetic techniques in laboratory populations, or employing the animal model from pedigree data from the wild (Kruuk 2004). To the best of my knowledge, the estimation of these parameters has been attempted only once (Qvarnström et al. 2006; Brommer et al. 2007).

My PhD research employs classic quantitative genetic methods to estimate the key parameters of equation (1) in a laboratory population of *Drosophila serrata*, with the ultimate goal of quantifying the strength of indirect selection for female mate preference. One of the key features of this model is the existence of a positive genetic correlation between the display trait(s) and total male fitness (r_{TW}), or in other words, the expectation that males producing the best display also have the highest fitness. The extent to which this is true in unmanipulated populations is unclear, however. Fisher (1930) described the dynamics of male display trait evolution. In a first phase, the male sexual display trait undergoes a rapid exaggeration in which the trait is positively correlated with total male fitness. This exaggeration is eventually halted as the sexual benefits (e.g. attractiveness) of the trait are counter-balanced by a trade-off with some other components of fitness (e.g. survival). In this second phase, while the covariance between the display trait and sexual fitness remains positive, the display trait is subject to stabilizing selection with respect to total male fitness. A positive covariance between sexual displays and fitness is therefore not

expected under equilibrium conditions. The extent to which male sexual display traits are undergoing continued exaggeration (e.g. in the runaway phase) or are at a selective limit in contemporary populations is therefore crucial to quantifying the indirect genetic benefits of female mate preferences, yet has received little attention.

Drosophila serrata

Mate preferences in *D. serrata* (an Australian fruit fly) are based on a suite of nonvolatile contact pheromones, composed of long-chain cuticular hydrocarbons (CHCs). These preferences are important in both species recognition (Blows and Allan 1998; Higgie et al. 2000; Higgie and Blows 2007) and mate choice within populations, and both CHCs and preferences for them have been shown to evolve in the laboratory during adaptation to a novel environment (Rundle et al. 2006). A potential contribution of good genes indirect benefits was suggested by a quantitative genetic study that found a positive genetic correlation between female preference for male CHCs and offspring fitness (Hine et al. 2002). In addition, four individual CHCs were found to be positively correlated with an index of body size in a field population, suggesting that their expression may be condition dependent (Hine et al. 2004). Contrary to expectation under a good genes process, however, the genetic correlation between male attractiveness (as determined by CHCs) and offspring fitness in the same study was negative. In addition, a manipulative evolution experiment revealed that sexual selection failed to promote adaptation to a novel environment as predicted by good genes (Rundle et al. 2006). Such conflicting evidence indicates that our understanding of the contributions of good genes indirect benefits to preference evolution in *D. serrata* is incomplete.

Of particular interest with regards to past results are the effects of a novel environment. As discussed above, the operation of good genes requires a genetic correlation between display traits and condition such that individuals choosing attractive mates also get mates of high genetic quality. However, if the genetic basis of the display traits used in mate choice, or of condition, vary among environments, the genetic correlation between them that was originally present in an ancestral environment may be weakened or eliminated in a novel environment, preventing any indirect genetic benefits of mate choice in one environment from translating immediately into another (Hunt et al. 2004). Despite the fact that such genotype-by-environment (G×E) interactions for fitness components (e.g., performance) are common (Lynch and Walsh 1998; Hunt et al. 2004), their effect on a good genes process has never been explored.

Thesis Overview

My thesis is organized as follows. Empirically, I have conducted a large half-sibling breeding design across two environments (the population's 'ancestral' laboratory environment to which it is well adapted and a novel food environment) that includes data on male display traits (CHCs), female preferences for them, and the fitness of male and female offspring. In chapters 2-4, I develop the somewhat complex analyses of these data. In particular, the second chapter presents results on the genetic (co)variance structure of male and female fitness from this breeding design. This provides fundamental insight into the prevalence of intralocus sexual conflict and how it varies between the two environments, a topic of broader interest beyond good genes mate choice. It is also an essential component

affecting the strength of indirect selection favouring mate preferences (represented by the parameters in parentheses in equation 1). In chapter 3 I undertakes a comprehensive investigation of the quantitative genetic basis of female mating preferences for male CHCs and how they vary between these environments, employing recent advances in the statistical analysis of function-valued traits. In chapter 4 I investigates the genetics of condition-dependence of male CHCs, a prerequisite for the evolution of male display traits as honest indicator of male genetic quality. Finally, in chapter 5 I estimate the genetic covariance of CHCs and fitness. The original goal was also to put together the data from chapters 2, 3 and 4 in this chapter in an attempt to parameterize equation 1 and therefore provide a direct estimate of the strength of indirect selection favouring female mate preferences in this population. However, the relationship between CHCs and fitness was found to be more complex and, taking advantage of recent methods for characterizing fitness optima under equilibrium conditions, I provide evidence for the importance of pleiotropy in mutation-selection balance of male sexual display trait. I end the thesis with some general conclusions on the evolution of female preference and male sexual display traits addressing what we have learned from the application of a multivariate quantitative genetics approach.

Chapters 2, 3, 4, and 5 were all written as stand-alone manuscripts for publication in peer-reviewed scientific journals. While this results in some replication of the background material, I attempt to keep this to a minimum. The format of each chapter also follows the guidelines for the specific journal in question, although referencing styles have been standardized for this thesis. Chapters 2, 3 and 4 have already been published (the full citation is given at the start of each chapter), and Chapter 5 is in preparation for submission

to a high profile journal (explaining the format of this chapter). Chapters 2 to 5 were collaborative projects involving my supervisor, Howard Rundle and, with the exception of Chapter 4, Mark Blows (School of Biological Sciences, University of Queensland, Australia). I took the lead role in conducting the study, analyzing the results, and writing the manuscripts and this thesis, with input from these collaborators at all of these stages.

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Chapter 2: Sexually-antagonistic genetic variance for fitness in an ancestral and a novel environment

The work in this chapter is a slightly modified version of the following article, coauthored by Mark Blows (School of Biological Sciences, University of Queensland, Australia) and Howard Rundle (Department of Biology, University of Ottawa):

Delcourt, M., Blows, M.W. and Rundle, H.D. 2009. Sexually-antagonistic genetic variance for fitness in an ancestral and a novel environment. *Proceedings of the Royal Society of London Biological Sciences*: 276: 2009-2014.

Abstract

The intersex genetic correlation for fitness (r_w^{fm}), a standardized measure of the degree to which male and female fitness covary genetically, has consequences for important evolutionary processes but few estimates are available and none have explored how it changes with environment. Using a half-sibling breeding design, I estimated the genetic (co)variance matrix (**G**) for male and female fitness, and the resulting r_w^{fm}, in *Drosophila serrata*. My estimates were obtained in two environments: the laboratory yeast-food to which the population was well adapted and a novel corn-food. The major axis of genetic variation for fitness in the two environments, accounting for 51.3% of the total genetic variation, was significant and revealed a strong signal of sexual antagonism, loading negatively in both environments on males but positively on females. Consequently, estimates of r_w^{fm} were negative in both environments (−0.34 and −0.73 respectively), indicating that the majority of genetic variance segregating in this population has contrasting effects on male and female fitness. The possible strengthening of the negative r_w^{fm} in this novel environment may be a consequence of no history of selection for amelioration of sexual conflict. Additional studies from a diverse range of novel environments will be needed to determine the generality of this finding.

Introduction

Sexually antagonistic genetic variation for fitness exists when alleles segregating within a population tend to have opposite effects on the fitness of the two sexes. Such variation arises when different values of a shared trait maximize male and female fitness, generating intralocus sexual conflict in which the adaptive evolution of one sex is impeded by the other (Lande 1980; Rice 1984; Parker & Partridge 1998; Rice & Chippindale 2001; Bonduriansky & Chenoweth 2009). The extent of this evolutionary constraint is indicated by the intersex genetic correlation for fitness (r_w^{fm}), a standardized measure of the genetic covariance of male and female fitness. In the absence of sexual conflict, r_w^{fm} is expected to be large and positive (Lynch & Walsh 1998). Sexually antagonistic fitness variation will cause a negative covariance between male and female fitness, thereby lowering r_w^{fm}. Negative correlations can severely constrain adaptive evolution, even in the presence of substantial genetic variation for lifetime fitness (Lande 1982), and if common, could explain why genetic variation in fitness appears to be higher than expected under mutation-selection balance (Turelli & Barton 2004). Negative correlations may also affect the evolution of mate choice, impeding the evolution of female preferences for males of high breeding value for fitness (i.e. 'good genes' theories of sexual selection) (Chippindale et al. 2001; Pischedda & Chippindale 2006; Kirkpatrick 2009).

A number of studies have demonstrated the existence of sexually antagonistic genetic variation for specific traits or components of fitness (Meagher 1992; Rice 1992; Gibson et al. 2002; Fedorka & Mousseau 2004; Calsbeek & Sinervo 2004; Pischedda & Chippindale 2006; Prasad et al. 2007). Fewer studies have reported estimates of the intersex genetic

correlation for lifetime fitness: −0.85 in collared flycatchers (Qvarnström et al. 2006), −0.48 in red deer (Foerster et al. 2007), and −0.16 in a laboratory population of *Drosophila melanogaster* (Chippindale et al. 2001). General conclusions are tentative, however, because direct estimates of r_w^{fm} are few, standard errors on them are large, and measures of fitness vary (Kirkpatrick 2009). Nevertheless, the available data suggest that sexually antagonistic fitness variation may be more common than previously thought.

An important aspect of the nature of sexually-antagonistic genetic variation that has yet to be investigated is the extent to which r_w^{fm} may vary among environments. Genotype-by-environment interactions are common for many traits (Lynch & Walsh 1998), including measures of performance (Hunt et al. 2004), and it is therefore likely that the genetic basis of fitness will vary with environment. Theory suggests that there is little reason to expect genetic variances and covariance to remain constant when environments change (Via & Lande 1987), and consistent with this, genetic correlations for numerous traits, including morphology and life history, have been shown to vary across environments (Sgrò & Hoffmann 2004). How r_w^{fm} in particular may change in novel environments is difficult to predict and alternative scenarios are possible depending on changes in sex-specific selection and the nature of any genotype-by-environment interactions for the traits determining fitness.

One possible scenario for changes in r_w^{fm} builds upon the idea that sustained sexually antagonistic selection in an environment is ultimately expected to favour mutations that allow males and females to achieve their sex-specific optima, thereby resolving sexual

conflict (Lande 1980; Lande 1987). Over the long term, any (partial) resolution of sexual conflict via the evolution of sexual dimorphism will cause r_w^{fm} to become less negative (reviewed in Bonduriansky & Chenoweth 2009). The timescale over which this occurs may be exceedingly slow, however, if the intersex genetic correlations for the phenotypic traits experiencing sexually antagonistic selection are high (Lande 1987). In a novel environment with unique sex-specific optima for the traits determining fitness and no history of selection to accommodate this, r_w^{fm} may therefore be expected to be strongly negative. This is of particular relevance to the recent observation that sexual selection may often fail to promote adaptation to novel environments in evolution experiments (Holland 2002; Rundle et al. 2006). One possible explanation for why sexual selection fails in this regard is that a strong negative r_w^{fm} in the novel environments hampers a good genes process, impeding or even preventing sexual selection from contributing to adaptation.

Here I estimate genetic variances for male and female fitness, and the resulting r_w^{fm}, using a laboratory population of the Australian fruit fly, *Drosophila serrata*. I obtain these measures in each of two environments, one to which the population is long adapted ('ancestral' yeast-food environment) and a novel environment (corn food) to which the population is not well adapted. The addition of the novel environment permits insight into how the genetic covariance of male and female fitness varies across environments in response to any changes in the genetic basis of fitness, and is of direct relevance to the question of whether sexual selection promotes adaptation to novel environments as predicted by good genes models (Lorch et al. 2003). The inclusion of a novel environment also allows me to address directly the possibility that the importance of sexually antagonistic fitness variation is exaggerated in

laboratory populations due to long term adaptation to unusually consistent environments (Chapman et al. 2003).

I estimate genetic variances and covariances for male and female fitness in both environments within the context of a large quantitative genetic paternal half-sibling breeding design that involved 81 sires mated to 326 dams. Fitness assays were conducted in the ancestral and novel environments on a total of 2,173 female and 2,005 male offspring from the breeding design. *D. serrata* is an ideal candidate for such experiments because fitness measures can integrate the major components relevant for the population in the environment to which it is adapted, including mating success of males within a competitive context. In addition, good genes mate choice has been well studied in this species (Hine et al. 2004), including a manipulative evolution experiment testing the contribution of sexual selection during adaptation of this same population to the same novel corn-food environment (Rundle et al. 2006).

Materials and methods

Half-sib breeding design

A paternal half-sibling breeding design was conducted using a previously described laboratory population of *Drosophila serrata* (Rundle et al. 2006; Chenoweth et al. 2008). Eighty-one sires were each mated to four virgin females (dams) and these females were subsequently allowed to oviposit for 20h in one environment (yeast or corn) and then 48h in the other. The order of oviposition environments was alternated among females. The breeding design was conducted in three blocks consisting of 22, 30 and 29 sires that spanned

five generations of the laboratory population. In all cases, fitness assays were performed on 3 day-old virgin sons and daughters from each family collected at emergence and separated by sex using light CO_2 anaesthesia.

Male fitness assays

A competitive measure of male fitness was conducted by placing together in a vial one virgin son from the breeding design along with two virgin competitor males of similar age randomly chosen from a stock population into which a recessive orange-eye mutation had been introgressed. These males competed to fertilize a randomly chosen virgin orange-eye female that was free to oviposit in the same vial. After 72h all flies were discarded and vials were retained for offspring development. Vials contained food that matched the environment in which the son was raised (i.e., yeast or corn). Five replicate trials using five separate sons were conducted using the offspring of each dam in each environment. Male fitness was calculated as the ratio of wild-type:orange-eye individuals in the adult offspring produced by the female. This measure of male fitness included his mating success, the subsequent productivity of the female he mated, and the survival to emergence of his male and female offspring in competition with half-sib offspring sired by the orange-eye competitors. Sons that did not produce any offspring were excluded from the analysis. Prior to analyses, data were ln-transformed (after the addition of a constant to both mutant and wild-type offspring counts) and standardized to a mean of zero and unit variance.

Female fitness assays

Female fitness was measured by placing together in a vial one virgin daughter from the breeding design along with one randomly chosen virgin male of similar age from the stock population. The pair was allowed to mate freely and the female could oviposit on the food. After 48h both flies were discarded and the vial was retained for offspring development. Vials contained food that matched the environment in which the daughter was raised (i.e., yeast or corn). Five replicate trials using five separate daughters were conducted using the offspring of each dam in each environment. Female fitness was calculated as the total number of adult offspring emerging in each vial. Daughters that did not produce any offspring were not included in the analysis. Data were square-root transformed and standardized to a mean of zero and unit variance prior to analysis.

This measure of female fitness included her fecundity along with the survival to emergence of her male and female offspring. This differed from my measure of male fitness in that it lacked any contribution of mating success arising from mate choice by the opposite sex (i.e. male choice), and competition from half-sibling offspring was absent during larval development. However, fitness is a different trait in the two sexes and, unlike in males, variance in female mating success due to male mate choice is likely a small component of female fitness. It is likely that a single male would have mated all three females within the time provided had I mirrored the design used to measure male fitness. The strength of larval competition overall was likely similar in both fitness assays as well because larval rearing densities did not differ drastically in the two assays (mean total number adults emerging per

vial ± SE for females and males respectively; yeast: 62.9 ± 1.2 and 79.9 ± 1.3; corn: 40.8 ± 0.7 and 45.2 ± 0.7).

Statistical analysis

The additive genetic variance-covariance (i.e., **G**) matrix for male and female fitness in the two environments was constructed at the sire-level by REML using the factor-analytic modelling approach implemented in the Mixed procedure of SAS version 9.1 (Hine & Blows 2006; McGuigan & Blows 2007). The model was:

$$Y_{ijklm} = \mu + S_j + D_{k(j)} + B_l + O_m + B_l O_m + \varepsilon_{ijklm}, \quad (1)$$

where Y_{ijklm} is the observed fitness of the *i*th offspring of the *k*th dam nested within the *j*th sire. Fixed effects include the intercept (μ), experimental block (B), the order of oviposition environments (O), and their interaction.

The factor-analytic approach has several advantages including that, by directly estimating the principal components of **G**, the resulting covariance matrix is guaranteed to be positive semidefinite (all eigenvalues ≥ zero; Kirkpatrick & Meyer 2004). This approach also provides a powerful method for directly testing the dimensionality of **G**, or in other words, the number of genetically independent traits underlying male and female fitness in these two environments (Hine & Blows 2006). For this analysis, the covariance matrix was constrained to be from four through zero dimensions and a series of nested likelihood ratio tests were used to determine whether excluding each dimension significantly worsened the

fit of the model (Hine & Blows 2006). Significance of specific covariances (correlations) from an unconstrained covariance matrix were determined using likelihood ratio tests in which the parameters of interest were fixed at specific values (either 0 or 1) and the fit of the model was compared to its unconstrained counterpart (Shaw 1991; Fry 2004).

To test whether the genetic basis of fitness differed overall between the two environments, male and female fitnesses were pooled across the environments and a single unconstrained 2×2 covariance matrix was constructed at the sire-level using eqn. (1). A fixed effect of environment was included in this model because fitnesses were standardized to a mean of zero and a unit variance by sex only in this analysis. The fit of this model was compared to one in which separate unconstrained 2×2 covariance matrices were constructed in each environment by employing the 'group' statement at the sire-level in the SAS Mixed procedure, with significance determined using a likelihood ratio test.

Results

The genetic basis of male and female fitness varied significantly between the two environments ($\chi^2_3 = 117.5$, $p < 10^{-5}$), so the additive genetic (co)variances matrix (**G**) for male and female fitness was estimated by treating the fitness of each sex in each environment as separate traits (Table 2.1). Factor-analytic modelling revealed support for one significant genetic dimension underlying this suite of traits ($\chi^2_2 = 10.60$; $p = 0.031$; Table 2.2) that explained 51.3% of the total genetic variance in fitness. This major axis of genetic variance loaded negatively in both environments in males and positively in females

(Table 2.1), thus revealing a strong signal of sexual antagonism for the majority of genetic variance in fitness across these two environments.

Within each sex, the genetic correlation in fitness between the two environments was positive (Table 2.1; males: r = 0.2734; females: r = 0.5581). Thus, sires producing high fitness daughters (sons) in one environment tended also to produce high fitness daughters (sons) in the other environment, indicating that the genetic basis of fitness in the two environments is at least partially shared within each sex. In contrast, intersex genetic correlations for fitness were negative in both environments (Table 2.1; yeast: $r_w^{fm} = -0.34$; corn: $r_w^{fm} = -0.73$), indicating that in the ancestral and the novel environments, sires producing high fitness sons tended to produce low fitness daughters and vice versa (Fig. 2.1). In both environments, r_w^{fm} was significantly less than one (yeast: $\chi^2_1 = 5.87$, p = 0.015; corn: $\chi^2_1 = 5.63$, p = 0.018) and the associated intersex covariance was significantly less than zero in corn ($\chi^2_1 = 4.43$; p = 0.035), although not in yeast ($\chi^2_1 = 1.22$, p = 0.270).

The extent to which the negative r_w^{fm} would constrain the evolution of fitness in these environments can be illustrated by applying the multivariate breeders equation using the 2×2 **G** matrices for each environment (Table 2.1) and a unit vector of linear selection gradients that seeks to increase fitness in both sexes to the same extent (i.e. $\boldsymbol{\beta}^T = [0.5, 0.5]$). In yeast, there remains a small amount of genetic variance available to increase fitness in both sexes, with fitness predicted to increase by 6% and 4% of a phenotypic standard deviation in females and males respectively. In corn, in contrast, female fitness is predicted to decrease by 0.2% while male fitness would increase by 3%.

Discussion

Sexually antagonistic genetic variance arises when the fitness optima for shared traits differ between males and females, generating intralocus sexual conflict in which the adaptive evolution of each sex impedes the other (Lande 1980; Rice 1984; Parker & Partridge 1998; Rice & Chippindale 2001; Chapman et al. 2003; Bonduriansky & Chenoweth 2009). Sexually antagonistic genetic variance will result in a negative r_w^{fm} that can have important consequences for the maintenance of genetic variation, the evolution of recombination, and rates of adaptation. It may also reduce or even eliminate the genetic benefits that accrue to females from mating with high fitness males (Chippindale et al. 2001; Pischedda & Chippindale 2006; Kirkpatrick 2009).

Using a laboratory population of *D. serrata*, I estimated a negative r_w^{fm} in a yeast-food environment to which the population was well adapted, as well as in a novel corn-food environment (r_w^{fm} = −0.34 and −0.73 respectively). This novel environment displaced the population from its adaptive peak: female fitness was initially reduced by 35 % on average, yet was shown previously to rapidly increase (52% in 16 generations) during adaptation to this environment (Rundle et al. 2006). Determining whether the persistence and apparent strengthening of the negative r_w^{fm} is a general outcome of colonization of a novel environment, as opposed to an effect specific to the novel environment I used, will require additional studies across a range of environments. Nevertheless, my results indicate that the importance of sexually antagonistic fitness variation is not necessarily an artefact of an unusually consistent laboratory environment, as has been previously suggested for laboratory populations (Chapman et al. 2003).

My estimates of negative r_w^{fm} in both environments may be conservative. Because the fitness of both sexes was measured by counting the number of adult offspring produced, egg to adult survival of male and female offspring was included in the fitness estimates of both sexes. These shared fitness components will covary positively, biasing r_w^{fm} to be more positive than it otherwise would. This bias could be substantial if sexually antagonistic genetic variance was prevalent for larval fitness. Estimates from *Drosophila melanogaster* suggest that this is not the case (Chippindale et al. 2001), although this has not been determined for *D. serrata*.

My current quantitative genetic analysis of fitness is consistent with a recent evolution experiment that independently manipulated the opportunities for both natural and sexual selection during adaptation of the same *D. serrata* population to the same novel corn-food environment. After 16 generations in the novel environment, female fitness increased by 52% on average due to natural selection alone. In contrast, and despite strong sexual selection on display traits (Chenoweth et al. 2008), sexual selection failed to promote adaptation either on its own or in combination with natural selection (Rundle et al. 2006). The strong negative intersex genetic correlation for fitness estimated here in the corn environment provides a potential explanation for this failure: genetic variance for fitness in corn is predominately sexually antagonistic in this population and is therefore not conducive to a good genes process. The negative intersex correlation for fitness also makes a clear prediction: given the dramatic increase in female fitness that occurred over 16 generations in the corn environment, male fitness should have decreased substantially as a correlated

response. Unfortunately male fitness was not tracked during that experiment, but this will be an important focus of future studies.

A negative r_w^{fm} in yeast is perhaps not unexpected: given a constant environment with moderate population size, mutations that are unconditionally beneficial (deleterious) in both sexes should be fixed (eliminated) fairly efficiently. The majority of genetic variation left segregating may therefore arise from alleles that persist at intermediate frequencies for longer because they are under conflicting selection. Sexual antagonism, in which an allele is favoured in one sex but selected against in the other, is a primary source of such conflict because other processes (e.g., spatial and temporal variation in selection) are minimized under laboratory conditions. That r_w^{fm} remained negative, potentially even strengthening, in the novel environment, is perhaps less intuitive, although it is consistent with one possible scenario concerning the resolution of sexual conflict via the evolution of sexual dimorphism after long-term adaptation to consistent selection (Lande 1980; Lande 1987). There are at least two potential processes that could be contributing.

First, the average strength of sexual antagonism may vary between novel mutations overall and the small subset of these that are left segregating after long-term adaptation. Standing genetic variation for fitness in yeast has never been subjected to selection in the novel corn environment, so in the majority of cases its effects should be deleterious in both sexes, resembling that expected for novel mutations in this environment. The strong negative r_w^{fm} in corn implies that the majority of this variation has a much higher fitness cost in one sex than the other. This could occur, for example, due to variation on the sex chromosomes (i.e.

in males, all mutations on the X would be expressed, whereas in females they would be largely masked).

Second, long term evolution under consistent sexual conflict could favour the evolution of mechanisms that allow males and females to express their sex-specific optima for the traits determining fitness, thereby generating sexual dimorphism that ameliorates sexual conflict and makes r_w^{fm} less negative (Lande 1980; Lande 1987). Such mechanisms include: 1) the physical relocation of loci to the sex chromosomes (Fisher 1930; Rice 1984); 2) the evolution of modifiers that permit the sex-limited expression of loci (Chenoweth et al. 2008); 3) the duplication of autosomal loci followed by their sex-limited expression (Rhen 2000; Rice & Chippindale 2002); and 4) the evolution of parent-of-origin effects via genomic imprinting (Day & Bonduriansky 2004). If any of these mechanisms are environment-dependent in their action, they could break-down in the novel corn environment, causing sexual conflict to increase. Environment-dependent expression levels of a modifier permitting sex-limited expression are plausible, and recent experimental work suggests the possibility of environment-dependent genomic imprinting (Bonduriansky & Head 2007).

The above two processes are not mutually exclusive and determining their contributions to changes r_w^{fm} in novel environments will be a difficult task. An important first step will be detailed studies of how r_w^{fm} changes upon colonization of a range of novel environments and its subsequent evolution as populations adapt to these environments.

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Table 2.1. Full-rank additive genetic (co)variance matrix (**G** matrix) for male and female fitness in the ancestral (yeast) and novel (corn) environments. Genetic variances are given along the diagonal (in bold) and covariances below. The corresponding genetic correlations are given above the diagonal.

	♀ yeast	♂ yeast	♀ corn	♂ corn	PC1[1]
♀ yeast	**0.1704**	-0.3429	0.5581	-0.0701	0.7902
♂ yeast	-0.0518	**0.1339**	0.0556	0.2734	-0.4493
♀ corn	0.0460	0.0041	**0.0399**	-0.7260	0.2892
♂ corn	-0.0090	0.0313	-0.0453	**0.0978**	-0.3001

[1]The first eigenvector of **G**, accounting for 51.3% of the genetic variance.

Table 2.2. Model fit statistics of the number of effective dimensions of the sire-level covariance matrix, **G**.

No. dimensions	No. parameters	-2 log likelihood	AIC[1]	p-value[2]
fa0(4)	10	11801.69472	11845.70	1.000
fa0(3)	9	11801.69471	11845.70	0.248
fa0(2)	7	11804.48596	11844.50	0.269
fa0(1)	4	11808.41856	11844.40	0.031
fa0(0)	0	11819.01405	11847.00	-

[1] Akaike information criterion.

[2] Results of a likelihood ratio test of whether the fit of a model that excludes one factor (i.e. the model in the row below) is significantly worse than the fit of the current model.

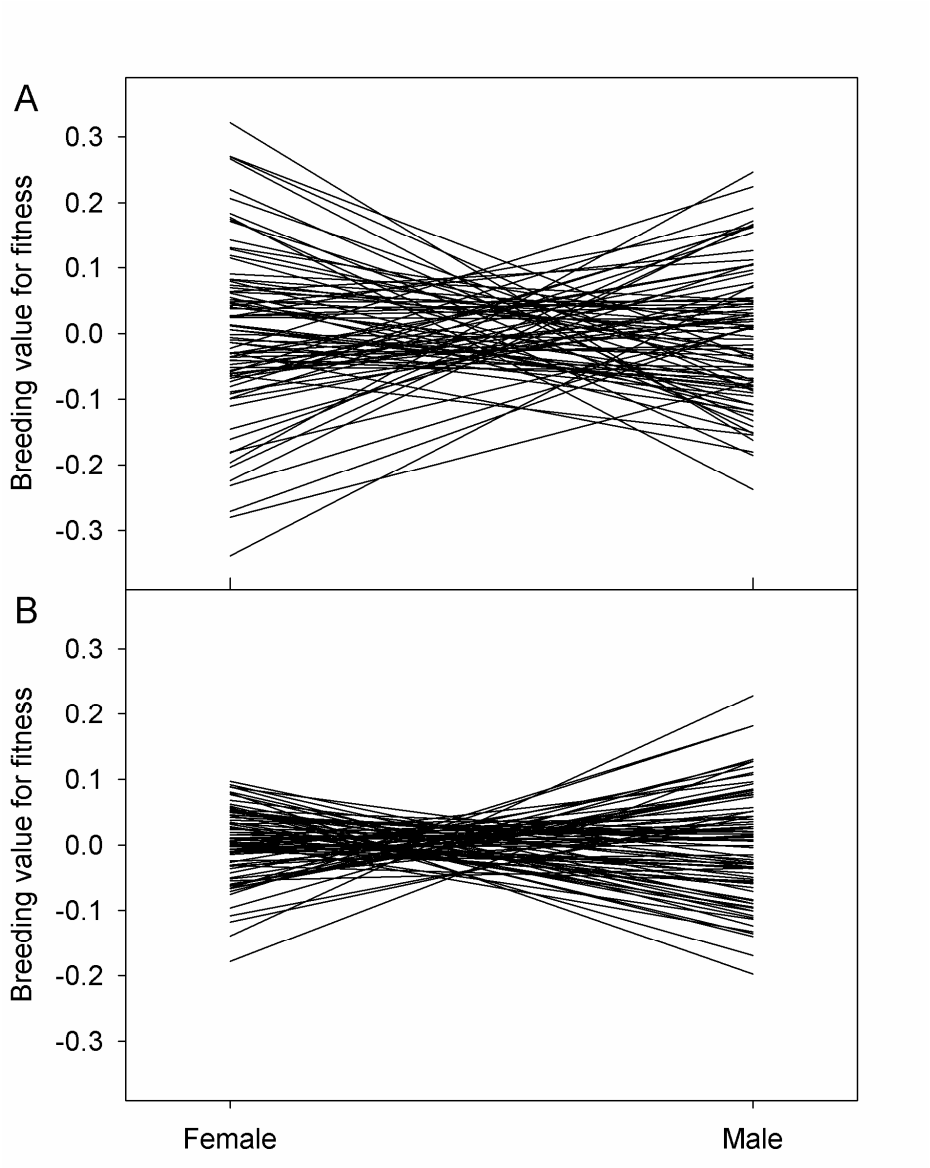

Fig. 2.1. Interaction plot of the breeding values of female and male fitness in yeast (A) and corn (B). Lines join breeding values for the same sire, estimated as best linear unbiased predictors from eqn. 1 using the factor-analytic approach with four dimensions for the sire-level covariance matrix.

Chapter 3: Quantitative genetics of female mate preferences in an ancestral and a novel environment

The work in this chapter is a slightly modified version of the following article, coauthored by Mark Blows (School of Biological Sciences, University of Queensland, Australia) and Howard Rundle (Department of Biology, University of Ottawa):

Delcourt, M., M.W. Blows and H.D. Rundle. 2010. Quantitative genetics of female mate preferences in an ancestral and a novel environment. *Evolution* 64: 2758-2766.

Abstract

A female's mate preference is a potentially complex function relating variation in multiple male phenotypes with her probability of accepting individual males as a mate. Estimating the quantitative genetic basis of preference functions within a population is empirically challenging yet key to understanding preference evolution. I employed a recently described approach that uses random-coefficient mixed models in the analysis of function-valued traits. Using a half-sib breeding design in a laboratory-adapted *Drosophila serrata* population, I estimated the genetic (co)variance function of female preference for male sexual displays composed of nine contact pheromones. The breeding design was performed across two environments: the food to which the population was well adapted and a novel food that reduced average female productivity by 35%. Significant genetic variance in female preference was detected and the majority (64.2%) was attributable to a single genetic dimension (eigenfunction), suggesting that preferences for different pheromones are not genetically independent. The second eigenfunction, accounting for 24% of the total genetic variance, approached significance in a conservative test, suggesting the existence of a second, independent genetic dimension. There was no evidence that the genetic basis of female preference differed between the two environments, suggesting the absence of genotype-by-environment interactions and hence a lack of condition-dependent preference expression.

Introduction

Female mate preferences describe the relationship between the phenotype of males and their probability of being accepted as a mate. Females often assess multiple traits when choosing mates, including various sexual signals and displays (Candolin 2003; Chenoweth and Blows 2006), and many of these traits vary continuously among males. Therefore, in contrast to scalar-valued traits that take on a single value for individuals, mate preferences constitute potentially complex functions describing the quantitative relationship between multiple male traits and a female's acceptance.

Since Darwin (1871) first recognized that female mate preferences could be responsible for the evolution of the extravagant male sexual displays that characterize many species, the evolutionary consequences of mate preferences have received much attention (Andersson 1994). The typical approach involves measuring the mating success of a range of male phenotypes across a group of females, providing information on the average female preference within the population (Wagner 1998; Kingsolver et al. 2001; Chenoweth and Blows 2006). Such population-level mate preferences are useful because they quantify sexual selection on male traits arising from female mate choice in a currency appropriate to the microevolutionary equations for predicting the short-term response to selection (Lande 1979; Lande and Arnold 1983). This approach has contributed to our understanding of the evolution of extravagant male displays that cannot be explained by natural selection alone. However, mate preferences are predicted to evolve via a coevolutionary process between males and females that involves both direct and indirect selection; knowledge of the genetic basis of preferences is therefore key to understanding this process. Population-level

preferences obscure individual preference variation and therefore provide little insight into its genetic basis (Wagner 1998; Chenoweth and Blows 2006).

Estimating individual mate preferences can present a significant empirical challenge because it is often difficult to evaluate the responses of individual females to a range of male phenotypes under controlled conditions (Wagner 1998; Chenoweth and Blows 2006). In many species, for example, male display traits cannot be synthesized artificially and measuring preferences requires copulation, an outcome that may alter a female's preference with respect to subsequent males. These difficulties were overcome recently by the application by McGuigan et al. (2008) of quantitative genetic methods for function-valued traits (Kirkpatrick and Heckman 1989; De Jong 1990; Gomulkiewicz and Kirkpatrick 1992; Kingsolver et al. 2001; Kirkpatrick and Meyer 2004; Meyer and Kirkpatrick 2005; Kirkpatrick 2009). Function-valued traits describe continuous variation of phenotypes over a range of values as opposed to scalar traits which describe discrete variation of phenotypes. This approach uses random-coefficient mixed models to quantify the genetic basis of female preference within the context of a classic quantitative genetic breeding design, even when individual females are used in only a single mate choice trial. The basis of the approach is to calculate preference functions not for individual females, but rather for groups of related females within the breeding design for which multiple observations (i.e. mate-choice trials) are available. Variance in preference among appropriate groups of females, such as offspring of separate sires, provides an estimate of the additive genetic basis of preference. When preferences are modeled as a function of multiple male traits, genetic variation in

them is represented by a covariance function (**G**) in place of the standard covariance matrix (**G**) for scalar-valued traits (Kirkpatrick and Meyer 2004).

The nature of individual variation in mate preferences, and in particular, whether female mate preferences are condition-dependent, has received increased attention of late (Hingle et al. 2001; Bro-Jørgensen 2002; Mazzi 2004; Owens et al. 1994; Hunt et al. 2005; Baugh and Ryan 2009; Holveck and Riebel 2010; reviewed in Cotton et al. 2006). Mate preferences are thought to be costly (Pomiankowski 1987; Reynolds and Gross 1990; Byers et al. 2005; Rundle et al. 2009) and if these costs, or the fitness benefits the preferences provide, vary with female quality then the strength of the preferences should evolve to depend on condition for reasons analogous to the evolution of condition-dependence for male sexual displays (Cotton et al. 2006). Preferences are expected to be weaker in low condition females and stronger in high condition females. Condition-dependent expression of mate preferences is of interest because it may alter the strength and direction of sexual selection within an environment due to variation in female condition, and it may affect the degree to which sexual selection may promote adaptation to a novel environment (Lorch et al. 2003). Condition-dependent mate preferences may also contribute to the maintenance of genetic variation in male display traits. The preferred approach for demonstrating condition-dependence has been to estimate changes in preference when female condition is experimentally manipulated, often through alterations in diet quality (Cotton et al. 2004a; Cotton et al. 2006). While studies of the phenotypic consequences of such manipulations are accumulating, the genetic basis of condition-dependent mate preferences is generally unknown (but see Bakker *et al.* 1999; Rodriguez & Greenfield 2003).

Here I conduct a standard diet manipulation of female condition within the context of a quantitative genetic half-sibling breeding design, allowing me to estimate the genetic basis of female preference and providing insight into its condition-dependence. I used *Drosophila serrata*, an Australian species in which population-level mate preferences and the sexual selection they generate have been extensively studied via a series of quantitative genetic and evolution experiments. Females use a suite of contact pheromones, composed of long-chain cuticular hydrocarbons (CHCs), in mate choice within populations (Chenoweth and Blows 2003, 2005; Petfield et al. 2005) and species recognition (Blows and Allan 1998; Higgie et al. 2000; Higgie and Blows 2007). As in past studies (Hine et al. 2002; 2004; Chenoweth and Blows 2003; 2005; Petfield et al. 2005; Skroblin and Blows 2006; Rundle et al. 2009), I quantify sexual selection by female choice on male CHCs using replicate laboratory mating trials in which individual females are allowed to choose between two or more males which subsequently have their CHCs extracted and quantified using gas chromatography. Selection gradients are estimated using a standard first-order multiple regression (Lande and Arnold 1983) that models the mating success of males as a function of their CHCs. Selection gradients estimated in this way are equivalent to the population-level female mate preference for these traits (Wagner 1998; Chenoweth and Blows 2006).

A number of lines of evidence in *D. serrata* demonstrate that male CHCs are the direct target of sexual selection arising from female mate preferences, and that the quantification of such preferences via laboratory mate choice trials is biologically relevant to our understanding of preferences in nature. Male CHCs exhibit a pattern of reproductive

character displacement along the Australian east coast that corresponds with the presence vs. absence of the related species, *D. birchii* (Higgie 2000; Higgie and Blows 2007). Laboratory mate choice trials reveal genetically-based differences in female mate preferences for male CHCs between sympatry and allopatry, and the resulting divergent sexual selection estimated in the laboratory corresponds with the pattern of character displacement in nature (Higgie and Blows 2007). In addition, in a laboratory evolution experiment that manipulated the opportunity for sexual selection, male CHCs and female preferences for them evolved to match the observed character displacement (Higgie and Blows 2008). This effect was attributable to female choice alone (the additional opportunity for both male-male competition and sperm competition had no significant effect), providing strong manipulative evidence that the measure of preference, and the experimental design for the mate choice trials, are relevant to the field. Finally, female preferences for male CHCs have also been shown to evolve in other contexts, for example in response to altered natural selection (Rundle et al. 2005; Higgie and Blows 2008; Rundle et al. 2009), indicating that females exercise choice during laboratory trials and revealing the existence of genetic variation in this choice.

I estimated the linear genetic co-variance function describing female mate preferences for eight CHCs in males using a paternal half-sib breeding design involving a laboratory-adapted *D. serrata* population. This population was created almost four years earlier by pooling collections from multiple geographic population (potential effects on the genetic basis of preference are addressed in the Discussion). I performed the breeding design across two environments, one involving a larval diet to which the population was well adapted (its

'ancestral' yeast food) and the other involving a novel larval diet (corn food). The corn-food diet reduces female productivity by 35% on average (Delcourt et al. 2009), creating an experimental manipulation of condition. If preference expression is condition-dependent, it will vary between the diet treatments; such phenotypic changes would be manifested as a genotype × environment interaction in a quantitative genetic analysis of preference.

Materials and methods

Half-sib breeding design

A paternal half-sibling breeding design was conducted using a previously described laboratory stock population of *D. serrata* (Rundle et al. 2006; Chenoweth et al. 2008). Ninety-two sires were each mated to four virgin females (dams) and these females were subsequently allowed to oviposit for 20h in one environment (yeast or corn) and then 48h in the other. The order of oviposition environments was alternated among females and the difference in oviposition time ensured similar larval rearing densities given a decline in female egg laying rate with time. The breeding design was conducted in three blocks consisting of 30, 31 and 31 sires that spanned five generations of the laboratory population. The results of fitness assays conducted on male and female offspring from this breeding designed are presented in Delcourt et al. (2009). The number of sires in the current data set differs slightly from Delcourt et al. (2009) due to incomplete data records. Unique offspring (i.e. different individuals from the fitness assays) were used in the mate preference assays described below.

Female mate preference assays

Preference assays were performed using 3 day-old virgin daughters from each half-sib family, raised in each environment, that were collected at emergence and separated by sex using light CO_2 anaesthesia. These females were held in vials in groups of ten on their respective larval food media with abundant live yeast sprinkled on top. Female preference was estimated using replicate mate choice trials in which a single daughter was placed in a vial along with five virgin males of similar age randomly chosen from the stock population and raised in the yeast environment. Five males were used to give females a greater opportunity to express their preference by increasing the range of male phenotypes among which she could choose. Vials were observed until the female successfully mated with one of the five males, at which point the chosen male, along with one of the four rejected males (randomly selected), were removed using CO_2 anesthesia for CHC extraction. The remaining three rejected males were discarded, along with the female. Mating vials contained food that matched the environment in which the daughter was raised (i.e., yeast or corn). Five replicate mating trials using five separate daughters were conducted using the offspring of each dam raised in each environment, generating more than 7000 males for CHC extraction.

CHC extraction and analysis

CHCs were extracted from single flies by washing individuals in 100 µL of hexane for approximately 3 min and then vortexing for 1 min. Individuals were then removed and samples were analyzed using an Agilent Technologies 6890N gas chromatograph fitted with

a HP5 column of 50 m×0.32 mm internal diameter, pulsed splitless inlet (at 200 ˚C), and a flame ionization detector (at 250 ˚C), using the temperature program given in Rundle et al. (2005). Individual CHC profiles were determined by integration of the area under nine peaks, corresponding to those used in past studies, and identified in order of their retention times as: (Z,Z)-5,9-$C_{24:2}$; (Z,Z)-5,9-$C_{25:2}$; (Z)-9-$C_{25:1}$; (Z)-9-$C_{26:1}$; 2-Me-C_{26}; (Z,Z)-5,9-$C_{27:2}$; 2-Me-C_{28}; (Z,Z)-5,9-$C_{29:2}$; and 2-Me-C_{30} (Howard et al. 2003). After integration, relative abundances were calculated separately for each individual by dividing the area integrated for each of their CHCs by the total area for all nine CHCs. Expressing the abundances of each CHC as a proportion of the total corrects for technical error associated with quantifying the absolute CHC abundance. Proportions were then transformed to logcontrasts, using (Z,Z)-5,9-$C_{24:2}$ as the divisor, to break the unit-sum constraint and thereby permit multivariate analyses to be performed (Atchison 1986). Logcontrast CHC values were standardized (mean = 0, standard deviation = 1) prior to statistical analyses.

Statistical analyses

Linear sexual selection gradients on the eight logcontrast CHCs, equivalent to the population-level female mate preference for these traits, were estimated separately for each environment using a standard first-order multiple regression model (Lande and Arnold 1983):

$$w = \alpha + B + O + BO + \sum_{i=1}^{8} \beta_i z_1 + \varepsilon, \quad (1)$$

in which w is the mating success score (0 = rejected, 1 = chosen) of individual males, z_i are the eight logcontrast CHC values for a given male, and ε is unexplained error. Fixed effects include the intercept (α), experimental block (B), the order of oviposition environments (O), and their interaction (BO). This regression yielded a column vector of linear selection gradients (β_i) characterizing directional selection on each of the eight log-contrast CHCs. Because these gradients arise from female mate choice, they provide an estimate of population-level linear preference functions of females for these male traits (Wagner 1998; Chenoweth and Blows 2006). Although the selection gradients were estimated using standard least squares, significance was determined using logistic multiple regression because mating success is binomially distributed (Fairbairn and Preziosi 1996; Rundle et al. 2009). This was done using a generalized linear model with a logistic link function, fit via maximum likelihood, as implemented in the GENMOD procedure in SAS v. 9. 2 (SAS Institute, Cary, NC, USA). To determine whether sexual selection, and hence population-level mate preferences, differed between the two environments, data from both environments were combined in a single analysis using the multiple regression described in equation 1 with the inclusion of a fixed effect term representing the main effect of environment and eight additional terms representing the interaction of each logcontrast CHC with environment. A likelihood ratio test was then used to compare the fit of this full model with a reduced one lacking the eight interaction terms.

Genetic variation in female preferences for the eight logcontrast CHCs in males was estimated using the following multivariate random-coefficient model (Meyer and Kirkpatrick 2005; Littell et al. 2006; McGuigan et al. 2008):

$$\mathbf{y}_{ijkl} = \alpha + E + B + O + BO + \mathbf{X}_{kl}\mathbf{b} + \mathbf{Z}^{(d)}_{kl}\delta^{(d)}_{kl} + \mathbf{Z}^{(s)}_{kl}\delta^{(s)}_{kl} + \varepsilon_{kl}, \quad (2)$$

in which **y** is a response vector containing the binomial mating success scores of the *i*th male from a mating trial employing the *j*th daughter of the *k*th dam within the *l*th sire. Fixed effects are as described in equation 1 with the addition of environment (*E*). The vector of population-wide linear partial-regression slopes (**b**) for the eight logcontrast CHCs, represented in the design matrix **X,** is equivalent to the vector of directional selection gradients (***β***) in equation 1 that quantifies linear sexual selection on the eight logcontrast CHCs. Although more complex (e.g., second-order) functions can be fit using random regression, consistent with previous investigations (Chenoweth and Blows 2005), population-level mate preferences in this population of *D. serrata* appear to be predominately linear (H. Rundle, unpublished results).

The genetic basis of female preference functions was estimated through the random-effect terms in equation (2). These terms treat the partial regression coefficients (i.e. the slopes, β, of the population-level linear preference functions for each logcontrast CHC) as random samples from some population of possible coefficients – here those at the dam and sire

levels of my breeding design. In particular, $\mathbf{Z}_{kl}^{(d)}\delta_{kl}^{(d)}$ represents the departure of the regression slope for the *k*th dam nested with the *l*th sire, and $\mathbf{Z}_{kl}^{(s)}\delta_{kl}^{(s)}$ represents the departure of the regression slope for the *l*th sire from the population-wide regression, $\mathbf{X}_{kl}\mathbf{b}$. $\mathbf{Z}_{kl}^{(d)}$ and $\mathbf{Z}_{kl}^{(s)}$ are the design matrices at the dam and sire levels, respectively, and the variances $\delta_{kl}^{(d)}$ and $\delta_{kl}^{(s)}$ are assumed to be normally distributed with means of zero and variances Σ_d and Σ_s respectively. The vector of residual (error) parameters was reduced because I lacked replication to estimate the covariance among female preference functions at this level (Meyer 1991; McGuigan et al. 2008). Separate errors were estimated for groups of sires that had different numbers of daughters to account for heterogeneity as a consequence of family size. Hypothesis tests assumed a normal distribution because samples sizes were large and the probability of either outcome (chosen or rejected males) was equal by design (Zar 1999; Chenoweth and Blows 2003).

Equation (2) was fit by restricted maximum likelihood, as implemented in the MIXED procedure of SAS v. 9.2 (SAS Institute, Cary, NC, USA), using the factor analytic approach to estimate the covariance matrices of the random effects at the dam and sire level. Of particular interest here is the sire-level covariance matrix because, when multiplied by four, it provides an estimate of the additive genetic covariance function (**G**) of female preference for male CHCs. Factor analytic modeling directly estimates the eigenfunctions of a covariance function, representing mutually orthogonal aspects of trait covariation within **G**, and is analogous to the direct estimation of the genetic principal components (i.e. eigenvectors) of an additive genetic covariance (**G**) matrix (Kirkpatrick and Meyer 2004;

Hine and Blows 2006). The factor-analytic approach provides a powerful method for directly testing the number of statistically supported eigenfunctions underlying **G**, or in other words, the number of independent dimensions of genetic variance for female preference (Hine and Blows 2006; Meyer and Kirkpatrick 2008). For this analysis, **G** was constrained to be from eight through zero dimensions and a series of nested likelihood ratio tests were used to determine whether excluding each dimension significantly worsened the fit of the model. The covariance function at the dam level was fixed at three dimensions for these tests, corresponding to the number of eigenfunctions with non-zero (i.e. positive) eigenvalues at this level.

To test whether the genetic basis of female preference functions differed overall between the two environments, sire × environment and dam × environment random effect terms were added to equation (2). A nested likelihood ratio test was then used to determine the significance of the first eigenfunction at the sire × environment level, thereby providing a test of whether the additive genetic basis of female preference differed between environments in an approach analogous to the standard technique for detecting genotype × environment interactions (Lynch and Walsh 1998). For this analysis, the sire, dam, and dam × environment covariance functions were fixed at the number of dimensions (i.e. eigenfunctions) with non-zero eigenvalues, corresponding to four, three, and three dimensions respectively.

Results

At the population level, female mate preferences generated significant linear sexual selection on the eight logcontrast CHCs in males in both environments (Table 3.1), explaining 21.2% (likelihood ratio test: $\chi^2 = 811.3$, *d.f.* = 8, $P < 0.0001$) and 20.4% (likelihood ratio test: $\chi^2 = 789.7$, *d.f.* = 8, $P < 0.0001$) of the variance in male mating success in the 'ancestral' yeast and novel corn environments, respectively. Although the population-level preferences differed significantly between these environments (likelihood ratio test: $\chi^2 = 16.67$, *d.f.* = 8, $P = 0.033$), the differences were minor: the vector correlation of the selection gradients in yeast vs. corn was 98.2%. The significance of this difference likely reflects the high statistical power (6,598 males were phenotyped). Consistent with numerous past studies using various populations of *D. serrata* (Blows et al. 2004; Hine et al. 2004; Rundle et al. 2006; Skroblin and Blows 2006), sexual selection was significant on the three methyl-branched alkanes, acting in opposite directions on 2-Me-C_{28} vs. 2-Me-C_{26} and 2-Me-C_{30}. Selection was also strong on the diene (Z,Z)-5,9-$C_{29:2}$, exceeding the median absolute value of 0.18 for directional sexual selection gradients across taxa in nature (Kingsolver el al. 2001). Strong sexual selection on this diene has been previously observed in other *D. serrata* populations (Blows et al. 2004; Chenoweth and Blows 2005).

The genetic basis of female mate preference for the eight logcontrast CHCs in males did not differ between the ancestral (yeast) and the novel (corn) environments, as indicated by a non-significant sire × environment interaction in the random regression model (equation 2; $\chi^2 = 0.678$, *d.f.* = 8, $P = 0.99$). Consequently, within this model the vector of population-wide regression slopes across these two environments (**b**), representing the fixed effects of

each logcontrast CHC on male mating success, was very similar to the environment-specific vectors of sexual selection (***β***; Table 3.1). A single additive genetic covariance function (**G**) of female preference was therefore estimated across the two environments.

Genetic variance in female preference is present for all eight logcontrast CHCs, although both positive and negative covariances give rise to strong genetic correlations that suggest a shared genetic basis of preference for these traits (Table 3.2). The absolute genetic correlation among the three methyl-branched alkanes (2-Me-C_{28}, 2-Me-C_{26} and 2-Me-C_{30}), for example, averages 0.83. This non-independence is reflected in the eigenfunctions of **G**, with their associated eigenvalues declining rapidly in magnitude (Table 3.3). This indicates that, overall, the genetic covariances severely constrain the multivariate patterns of genetic variation in female preferences for this suite of male CHCs.

The first eigenfunction of **G** ($\mathbf{g}_{max}$) accounts for well over half (64.6%) of the total genetic variance in female preference and is the only trait combination for which genetic variance is statistically supported by factor analytic modeling ($P = 0.0001$; Table 3.3). The three methyl-branched alkanes contributed strongly to this eigenfunction (Table 3.1), with 2-Me-C_{28} loading in opposite direction to 2-Me-C_{26} and 2-Me-C_{30}. This suggests that genes segregating in this population that strengthen female preference for some methyl-alkanes tend to weaken it for others. The second eigenfunction ($\mathbf{g}_2$) accounts for an additional 22.0% of the genetic variance in preference and approaches significance (Self and Liang 1987; McLachlan and Basford 1988), suggesting the existence of a second, independent genetic dimension underlying female preferences. This trait combination represents, to a large

degree, female preference for the diene (Z,Z)-5,9-$C_{29:2}$, although moderate loadings also contrast (Z)-9-$C_{25:1}$ with 2-Me-C_{30} (Table 3.1).

Discussion

Quantifying the genetic basis of female mate preference presents significant empirical challenges (Chenoweth and Blows 2006), in particular because they often target sexual displays that are composed of multiple components (e.g., the various properties of a visual or acoustic display, or the constituent components of a pheromone) and/or that involve traits of different types or modes (Candolin 2003). Insight may be gained, however, by treating preferences not as scalar values but rather as continuous functions of the display traits they target (McGuigan et al. 2008). Here I have used this approach in *D. serrata* to estimate the quantitative genetic basis of female preference for a multi-component sexual display in males composed of a suite of contact pheromones (CHCs), and to test for condition-dependence in the expression of these preferences. Mate preferences, estimated as the population-level sexual selection gradients on male CHCs arising from female choice, were relatively strong in this population, explaining 20-21% of the variance in male mating success. In comparison, a survey of nine natural *D. serrata* populations found that directional selection on CHCs explained on average approximately 6.1% of the variance in male mating success (Rundle et al. 2008), although values as high as 59% have been observed in other laboratory populations (Rundle et al. 2005).

My results reveal genetic variance in female preference for male CHCs, one effect of which may be to aid in the maintenance of genetic variance in the male display traits (CHCs) they target. The presence of genetic variance in preference is confirmed by the evolution of these traits when selection was manipulated in a recent laboratory experiment using this same population (Rundle et al. 2009). Strong genetic covariances among preferences for different CHCs were also detected such that the majority of the genetic variance in preference (64.6%) was accounted for by a single underlying trait ($\mathbf{g}_{max}$, the first eigenfunction of **G**, equivalent to $\mathbf{g}_{max}$ for scalar traits). This indicates that much of the genetic basis for female preference for the different CHCs is shared, similar to that observed in the related species *D. bunnanda* (McGuigan et al. 2008).

This first eigenfunction of **G** ($\mathbf{g}_{max}$) represents the combination of male traits for which there is the greatest genetic variance in female preference within this population. This eigenfunction was dominated by the three methyl-alkanes (Table 3.1), with 2-Me-C_{28} loading in opposite direction to 2-Me-C_{26} and 2-Me-C_{30}, reflecting the very strong genetic correlations estimated between preferences for these three traits (Table 3.2). Female mate preferences for CHCs in males vary substantially among natural population of *D. serrata*, with much of this variation being associated with the presence vs. absence of a related species, *D. birchii* (Higgie and Blows 2007; Rundle et al. 2008). Among nine natural populations, the major axis of variation in population-level mate preferences (termed $\boldsymbol{b}_{max}$ because it also represents the major axis of variation in sexual selection on male CHCs generated by these population-level preferences) also strongly contrasts 2-Me-C_{28} with the other two methyl-alkanes (Chenoweth et al. 2010). This similarity between $\boldsymbol{b}_{max}$ and $\mathbf{g}_{max}$

indicates that the combination of male traits for which genetic variance in female preference is greatest is also the combination of traits for which mate preferences have diverged most among these populations in nature.

The second eigenfunction of **G** ($\mathbf{g}_2$) accounted for a substantial proportion of the remaining genetic variance in preference (22%). Although this dimension was not statistically significant ($P = 0.142$), likelihood ratio tests comparing nested mixed models that differ in more than one random-effect parameter are known to be conservative (Self and Liang 1987; McLachlan and Basford 1988). This suggests the possible existence of an independent genetic dimension of preference variation. The potential existence of a second dimension of genetic variance in female preference implies genetic variance for changes in preference direction, or in other words, the combination of male CHCs that females find attractive. Consistent with this, such variation has been shown to exist in nature (Chenoweth et al. 2010) and has evolved in the lab (Rundle et al. 2009). My laboratory population was created (almost four years prior to the experiment) by pooling collections from multiple populations sympatric and allopatric with *D. birchii* (Rundle et al. 2006). The presence of two underlying genetic dimensions may therefore reflect a distinct genetic basis to preference variation within vs. among populations in nature, with the latter arising from the reinforcement of mate recognition in sympatry (Higgie and Blows 2000, 2008).

Notwithstanding the potential existence of a second independent dimension to the genetic variance in female preference, my results strongly suggest the absence of independent genetic variance in preference for all eight individual logcontrast CHCs in males. Although

very similar to preference variation for CHCs in *D. bunnanda* (McGuigan et al. 2008), this contrasts with findings in guppies in which female preferences for orange and black colouration respond individually to selection, implying an independent genetic basis (Brooks and Couldridge 1999). Additional studies are much needed from a diversity of species and a variety of traits, but if results such as mine are common, it suggests that explanations for the evolution of multi-component sexual displays may not lie in their conveying multiple messages (e.g., signaling different aspects of condition) or acting as redundant signals (Candolin 2003), but rather in part in the tendency of researchers to over-parse such displays relative to how they are perceived by female sensory systems. Whether the genetic basis of preference for multi-modal signals shows similar non-independence may appear less likely, although conclusions await direct empirical study.

Finally, I found no evidence that the genetic basis of female preference differed between the yeast and corn environments, despite the fact that the novel corn-food reduced female productivity by 35% on average compared to the yeast-food to which the population is adapted (Delcourt et al. 2009). A lack of statistical power is one possible explanation given that the random-coefficient mixed model relies on groups of individuals (in my case, offspring of different sires) to estimate preference. However, at the phenotypic level females chose mates with respect to CHCs in essentially the same way in yeast and corn, with the correlation in population-level mate preferences (i.e. the $\boldsymbol{\beta}$ vectors) between these two environments being extremely high (98.2%). My results therefore suggest that preference expression in *D. serrata* females is independent of their condition, or in other words, that the combination of male CHCs that a female prefers does not depend on her health or resources.

My results also provide no evidence that genetic variance in preference is greater overall in the more harsh corn-food environment, as has been predicted for condition-dependent preferences and shown in male display traits (Bjorksten et al. 2000; Cotton et al. 2006).

The absence of condition-dependence contrasts with both expectation and the results of a number of studies showing changes in preference strength or choosiness (the effort an individual is willing to invest in choice; Jennions and Petrie 1997) when female condition was manipulated (Cotton et al. 2006). For example, female zebra finch raised in larger broods develop into lower quality adults and prefer the mating songs of lower quality males (Holveck and Riebel 2010), and in túngara frogs, females of low body condition were more likely to reverse their initial choice as compared to females of high body condition when male call attractiveness parameters were altered (Baugh and Ryan 2009). The expectation for condition-dependence arises if preferences are costly and high condition individuals are more efficient at converting preference into fitness (Cotton et al. 2006; Getty 2006). In a recent evolution experiment in this same population, female preferences for male CHCs weakened overall when their expression was prevented by randomly creating monogamous pairs every generation for mating (Rundle et al. 2009). The selective removal of preferences when their benefits were prevented suggests that they are costly. My current results suggest that this cost does not depend on the condition of the female. Condition-dependence of other aspects of female preference (e.g., choosiness; Jennions and Petrie 1997; Hunt et al. 2005), is nevertheless possible and remains to be explored.

In summary, elucidating the quantitative genetic basis of mate preferences is a challenging endeavour. I have taken advantage of the random regression approach to the analysis of function-valued traits to explore female preferences for a sexual display trait in males that is composed of multiple contact pheromones (CHCs). My results demonstrate genetic variance in female preference, with strong covariances among preferences for different CHCs that are likely to constrain preference evolution. I find little evidence to suggest that preferences expression is condition-dependent at the phenotypic or genetic level.

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Table 3.1. Vectors of directional sexual selection gradients on eight logcontrast CHCs in males, equivalent to population-level female mate preferences, as estimated from a standard multiple regression (equation 1) conducted separately in each environment (***β***), and from a single random regression (**b**) across environments (equation 2). The first ($\mathbf{g}_{max}$) and second ($\mathbf{g}_2$) eigenfunctions of the additive genetic covariance function (**G**; Table 3.2) of female preference estimated from equation 2, accounting for 64.6% and 22% respectively of the total genetic variance in preference.

Logcontrast CHC	***β*** (yeast)	***β*** (corn)	**b**	$\mathbf{g}_{max}$	$\mathbf{g}_2$
(Z,Z)-5,9-$C_{25:2}$	0.021*	-0.019*	-0.002	0.154	-0.166
(Z)-9-$C_{25:1}$	-0.059**	-0.037*	-0.044**	-0.337	0.347
(Z)-9-$C_{26:1}$	-0.037**	-0.029*	-0.036**	-0.023	-0.020
2-Me-C_{26}	-0.066**	-0.100**	-0.086**	-0.212	0.031
(Z,Z)-5,9-$C_{27:2}$	-0.048**	-0.053**	-0.058**	0.137	0.102
2-Me-C_{28}	0.150**	0.179**	0.163**	0.645	-0.037
(Z,Z)-5,9-$C_{29:2}$	0.251**	0.243**	0.268**	-0.122	0.813
2-Me-C_{30}	-0.131**	-0.123**	-0.114**	-0.607	-0.423

$^{*}P < 0.05$; $^{**}P < 0.001$

Table 3.2 Genetic covariance function (**G**) of linear female preferences for the eight logcontrast CHCs in males, as estimated from the sire covariance matrix in a four-dimensional factor analytic model. Genetic variances are displayed along the diagonal (bold) with covariances below. Genetic correlations are given above the diagonal in italics.

Logcontrast CHC	(Z,Z)-5,9-$C_{25:2}$	(Z)-9-$C_{25:1}$	(Z)-9-$C_{26:1}$	2-Me-C_{26}	(Z,Z)-5,9-$C_{27:2}$	2-Me-C_{28}	(Z,Z)-5,9-$C_{29:2}$	2-Me-C_{30}
(Z,Z)-5,9-$C_{25:2}$	**0.00524**	*-0.160*	*-0.889*	*-0.553*	*-0.460*	*0.441*	*-0.314*	*-0.303*
(Z)-9-$C_{25:1}$	-0.00098	**0.00717**	*-0.095*	*0.747*	*-0.431*	*-0.788*	*0.610*	*0.519*
(Z)-9-$C_{26:1}$	-0.00096	-0.00012	**0.00022**	*0.526*	*0.539*	*-0.315*	*-0.144*	*0.344*
2-Me-C_{26}	-0.00195	0.00309	0.00038	**0.00238**	*-0.077*	*-0.853*	*0.183*	*0.727*
(Z,Z)-5,9-$C_{27:2}$	-0.00173	-0.0019	0.00042	-0.00020	**0.00270**	*0.500*	*-0.018*	*-0.580*
2-Me-C_{28}	0.00404	-0.00845	-0.00059	-0.00527	0.00329	**0.01603**	*-0.276*	*-0.911*
(Z,Z)-5,9-$C_{29:2}$	-0.00223	0.00506	-0.00021	0.00088	-0.00009	-0.00342	**0.00961**	*-0.121*
2-Me-C_{30}	-0.00283	0.00568	0.00066	0.00458	-0.00389	-0.01489	-0.00154	**0.01667**

Table 3.3: Model fit statistics of the number of effective dimensions of the genetic covariance function (**G**) of female preference, as determined from a factor-analytic model of the sire-level covariance matrix. The percent of the total genetic variance in female preference (% variance) was calculated from the full (i.e. eight-dimensional) factor analytic model.

no. dimensions	% variance	no. parameters	-2LL	AIC[a]	*P*-value[b]
8	0	81	-	-	-
7	0	80	-	-	-
6	0	78	-	-	-
5	0	75	8076.3	8222.3	1
4	2.8	71	8076.3	8212.3	0.989
3	10.7	66	8076.9	8204.9	0.722
2	21.8	60	8080.5	8200.5	0.142
1	64.6	53	8091.4	8197.4	0.0001
0	——	45	8122.9	8212.9	——

[a] Akaike's information criterion.

[b] Results of a likelihood ratio test of whether the fit of a model with one fewer genetic dimensions is significantly worse than the fit of the current model.

Chapter 4: Condition dependence of a multi-component sexual display trait in *Drosophila serrata*

The work in this chapter is a slightly modified version of the following article, coauthored by Howard Rundle (Department of Biology, University of Ottawa):

Delcourt M. and H.D. Rundle. Condition-dependence of a multi-component sexual display trait in *Drosophila serrata*. *American Naturalist* 177: 812 – 823.

Abstract

Theory predicts that costly sexual displays should evolve condition dependence if the marginal fitness gain from trait exaggeration is greater for high than for low condition individuals, and that the strength of condition dependence should increase with the strength of directional selection. While there is substantial support for the first prediction, evidence for the latter is much weaker. I undertook a quantitative test of this prediction for a multivariate sexual display consisting of a suite of contact pheromones, termed cuticular hydrocarbons (CHCs), in *Drosophila serrata*. I performed a dietary manipulation of condition (i.e. the pool of metabolic resources available for allocation to fitness-enhancing traits) within a half-sib breeding design, thereby also providing insight into the genetic basis of condition dependence. As predicted, the linear combination of CHCs under the strongest sexual selection from female mate preferences was unusually condition dependent relative to other CHC combinations within the population ($P = 0.049$). A significant positive correlation also existed between the strengths of condition dependence and sexual selection among different CHC blends ($r = 0.482$, $P < 0.001$). Finally, sires varied in their response to the dietary manipulation, demonstrating significant genetic variance in condition dependence. My results are consistent with the evolution of heightened condition dependence of sexual displays in response to persistent sexual selection.

Introduction

Condition can be defined as the pool of metabolic resources available to an individual for allocation to the production and maintenance of the traits that determine their fitness (Rowe and Houle 1996; Tomkins et al. 2004). Differences among individuals in condition arise from genetic and environmental variance in their acquisition and/or assimilation of resources. For traits subject to directional selection, fitness is an increasing function of investment in the trait, favouring individuals that allocate as much as they can afford to the trait (Andersson 1982; Nur and Hasson 1984). Theory predicts that the expression of such traits should evolve to be condition dependent if the marginal fitness gain of trait exaggeration is greater for high than for low condition individuals (Getty 1998; Proulx et al. 2002; Getty 2006).

With respect to condition-dependent trait expression, attention has focused in particular on male traits involved in courtship and mate acquisition (e.g., morphological, visual, chemical, and acoustic displays) because such traits are often targets of persistent and directional sexual selection. Such selection will drive their exaggeration to the point at which their benefit to sexual fitness is offset by their cost to non-sexual fitness (i.e. until natural selection balances sexual selection; Kirkpatrick and Ryan 1991; Andersson 1994), making such traits costly (Rowe and Houle 1996). If the marginal cost of trait exaggeration is lower for high condition males (or the marginal fitness gain is higher; Getty 1998; Proulx et al. 2002; Getty 2006), then these displays will be reliable indicators of a male's phenotypic and/or genetic quality (Zahavi 1975; Andersson 1986; Grafen 1990a; Grafen 1990b; Iwasa and Pomiankowski 1994; Rowe and Houle 1996). As condition dependence evolves, genetic

variance in overall condition is converted into variation in the male trait in a process called genic capture (Rowe and Houle 1996). Condition dependence therefore has implications for the maintenance of genetic variance in sexual display traits in particular and in life history traits in general (Rowe and Houle 1996; Houle 1998; Tomkins et al. 2004). Under such conditions, females can benefit by using these displays when choosing mates, making condition dependence central to the evolution of mate choice, and potentially population mean fitness (Proulx 1999; Whitlock 2000; Proulx 2001; Proulx 2002; Lorch et al. 2003) and even the evolution of sex (Agrawal 2001; Siller 2001).

In contrast to traits under persistent directional selection, traits experiencing stabilizing or otherwise weak selection are not expected to evolve condition dependence because allocating more resources to such traits does not increase fitness. In general, given that sexual display traits are common targets of persistent directional selection, such traits are expected to exhibit heightened condition dependence relative to other traits that are less closely associated with fitness (Pomiankowski 1987; Grafen 1990a; Grafen 1990b; Iwasa and Pomiankowski 1994). More specifically, the marginal gain in fitness from trait exaggeration should be greater for a trait under stronger as opposed to weaker directional selection and all else being equal, the former trait should therefore evolve to be more sensitive to condition than the latter. Among traits, therefore, the strength of condition dependent expression should increase as the strength of sexual selection increases, although the degree to which such relationships may be obscured by other differences among traits (e.g., genetic architecture, past selection) is not known.

Numerous studies have experimentally altered condition, often through the use of different quality diets, and examined the effect on one or more sexual traits. Unfortunately, many of these studies suffer from a number of shortcomings and compelling manipulative evidence that sexual traits exhibit heightened condition dependence is limited (Cotton et al. 2004). Although some of the concerns raised by Cotton et al. (2004) have been addressed in several recent studies (e.g., Bonduriansky and Rowe 2005; Kemp and Rutowski 2007; Siitari et al. 2007; Peters et al. 2008; Kemp 2008; Punzalan et al. 2008; McGuigan 2009; Wyman et al. 2010; Tibbetts 2010), conspicuous gaps in our knowledge remain. For example, the standard experimental approach compares the extent of condition dependent expression of sexual traits with that of one or more non-sexual traits. As non-sexual traits, such comparisons often employ the homologous traits in females or other non-sexual traits in males, under the presumption that directional selection is weaker or absent on them (but see Punzalan et al. 2008; McGuigan 2009). While useful, such comparisons often rest on an untested assumption concerning the strength of selection on these traits and therefore cannot be used to estimate the relationship between the strengths of condition dependence and selection.

In addition, like many signaling systems male sexual displays are often composed of multiple components that are used simultaneously to attract or court females (Candolin 2003) and female mate preference often act on multiple traits (Brooks and Endler 2001; Chenoweth and Blows 2006). The phenotypic and genetic covariances among these traits necessitate a multivariate approach to their analysis, including estimates of selection (Lande and Arnold 1983). Unfortunately, the multi-component aspect of male sexual displays has often been overlooked in studies of condition dependence, with the majority of work

focusing on single trait comparisons (Cotton et al. 2004). When multiple traits have been considered, analyses are often conducted separately or on a limited number of composite traits (but see McGuigan 2009). The importance of adopting a multivariate perspective in general, and in the study of sexual selection and condition dependence in particular, is well attested to by the apparent lack of genetic variance in the multivariate direction of selection on combinations of traits for which there is ample genetic variance individually (Walsh and Blows 2009), a result suggesting that condition dependence may not be able to maintain genetic variation in the face of persistent and strong directional selection. I return to this topic in the Discussion.

Finally, the genetic basis of condition dependence has received limited attention. Genetic variance in condition dependence is required for its evolution and the rapid divergence among species of sexual traits in particular suggests that condition dependence for particular traits may strengthen and weaken relatively rapidly. In addition, condition dependence likely evolves in response to sex-specific selection that will also favour the evolution of sexual dimorphism. The process of genic capture may therefore go hand-in-hand with the breakdown of the intersex genetic correlations that impede sex-specific trait evolution, resulting in condition dependent sexual dimorphism due to a shared genetic basis of these phenomena (Bonduriansky 2007a). Genetic variance in condition dependence has been demonstrated for male eye span in stalk-eyed flies, *Cyrtodiopsis dalmanni* (David et al. 2000), and condition dependence and sexual dimorphism have been shown to covary in two other fly species, suggesting their common genetic basis (Bonduriansky & Rowe 2005;

Bonduriansky 2007b). The genetic basis of condition dependence is a topic in need of additional investigation.

Here, using the Australian fruit fly *Drosophila serrata*, I investigate condition dependence and its genetic basis for a multi-component sexual display consisting of a suite of contact pheromones, termed cuticular hydrocarbons or CHCs. *D. serrata* use CHCs both in mate choice within populations (Chenoweth and Blows 2003; Chenoweth and Blows 2005) and in species recognition (Blows and Allan 1998; Higgie et al. 2000). Via a series of quantitative genetic and manipulative evolutionary experiments, particular combinations or blends of CHCs in males have been shown to be the direct target of female mate preferences, generating consistent and often strong directional sexual selection on these traits (Higgie et al. 2000; Chenoweth and Blows 2003; Chenoweth and Blows 2005; Higgie and Blows 2007; Higgie and Blows 2008; Rundle et al. 2009; Delcourt et al. 2010). The relative concentrations of three CHCs have been found to correlate positively with wing size, an index of body size, in field raised males, suggesting that they are condition dependent in their expression (Hine et al. 2004). Direct, manipulative tests of condition dependence are lacking in the species, although have been undertaken for both CHCs and wing shape in a close relative, *D. bunnanda* (Van Homrigh et al. 2007; McGuigan 2009). The relationship between the strength of condition dependence and the strength of sexual selection has not been explored in either species.

In my experiment I use a dietary manipulation of condition conducted within the context of a half-sibling breeding design (e.g. David et al. 2000), measuring the CHCs of sons from the

breeding design when raised in a high and low quality environment. I also quantify sexual selection on this suite of CHCs directly via a female choice mating assay using daughters from the same breeding design (see Delcourt et al. 2010), providing an estimate of the linear combination of male CHCs that females prefer most (i.e. the blend of CHCs that makes males most attractive). I then compare the strength of condition dependence for various CHCs blends that exist within the population (each representing a separate vector through the multidimensional phenotypic space of male CHCs), asking whether the particular combination of CHCs that females most prefer is more condition dependent than other possible blends among which they could choose. Using different combinations of CHCs provides an internal comparison that is preferable to the use, as a control, of another class of traits (e.g., morphology, behaviour) that may be developmentally more or less sensitive to a diet manipulation of condition for reasons other than their past history of selection. By combining the results of the selection assay and the diet manipulation, I also directly quantify the relationship between the strength of condition dependence and the strength of sexual selection among these different CHCs blends. Finally, taking advantage of the breeding design I employ a standard quantitative genetic method for quantifying genotype × environment interactions to estimates genetic variance in CHC condition dependence (Tomkins et al. 2004).

Materials and methods

Breeding design

Using a previously described laboratory population of *D. serrata* (Rundle et al. 2006), I conducted a paternal half-sib breeding design across two different larval food environments:

a standard yeast-based food on which the population had been previously maintained for more than two years and to which it was therefore presumably well adapted, and a novel corn flour-based food. The effects of these environments on male condition were evaluated in a separate assay (online Appendix A). Ninety-two sires were each mated to four virgin females (dams) and these females were allowed to oviposit for 20h in one environment (corn or yeast food) and then 48h in the alternative environment. The order of oviposition environments was alternated among females and the longer oviposition time in the second environment ensured similar larval rearing densities given a decline in female egg laying rate with time. The breeding design was conducted in three blocks consisting of 30, 31 and 31 sires that spanned five generations of the laboratory population. The genetic basis of male and female fitness has been previously quantified using sons and daughters from this breeding design (Delcourt et al. 2009), as has the genetic basis of female mate preferences for male CHCs (Delcourt et al. 2010). Here I use novel data collected from separate sons to examine the genetic basis of male CHCs as expressed during sexual encounters. These data are combined with the previous preference data (from which I derive direct estimates of sexual selection on these traits; Delcourt et al. 2010) to investigate the condition dependence of male CHCs. The number of sires in the current data set (91) differs from that in the previous fitness and preferences data sets (Delcourt et al. 2009; Delcourt et al. 2010) due to incomplete records for some sires.

Male display assay

Male CHC displays were assayed on 3-day-old virgin sons from each half-sib family, raised in each environment, that were collected at emergence and separated by sex using light CO_2

anesthesia. These males were held in vials in group of five on their respective larval food media with abundant live yeast sprinkled on top. Male CHCs were estimated within the context of a sexual encounter by presenting each son separately in a vial to two virgin females of the same age that were randomly chosen from the stock population. Vials were observed until the male successfully mated with one of the two females, at which point the male was removed for CHC extraction using CO_2 anesthesia and the females were discarded. Mating vials contained food that matched the environment in which the son was raised (i.e., yeast or corn). An average of 3.1 ± 0.8 (standard deviation) replicate mating trials were conducted using separate sons from each dam raised in each environment, generating more than 2,300 males for CHC extraction.

Cuticular hydrocarbons were extracted by immersing individuals in 100 µL of hexane for approximately 3 min and then vortexing for 1 min. Individuals were then removed and samples were analyzed using an Agilent Technologies 6890N gas chromatograph as previously described (Delcourt et al. 2010). Individual CHC profiles were determined by integration of the area under nine peaks corresponding to those used in the preference analyses (Delcourt et al. 2010) and identified in order of their retention times as: (Z,Z)-5,9-$C_{24:2}$; (Z,Z)-5,9-$C_{25:2}$; (Z)-9-$C_{25:1}$; (Z)-9-$C_{26:1}$; 2-Me-C_{26}; (Z,Z)-5,9-$C_{27:2}$; 2-Me-C_{28}; (Z,Z)-5,9-$C_{29:2}$; and 2-Me-C_{30} (Howard et al. 2003). After integration, CHCs were expressed as a proportion of the total CHC abundance for each individual to control for variation in both the extraction process and gas chromatographic analysis. Such controls are less prone to experimental error than the use of internal standards (Blows and Allan 1998; Savarit and Ferveur 2002). To break the unit-sum constraint, proportions were transformed to

logcontrasts, using (Z,Z)-5,9-$C_{24:2}$ as the divisor, thereby permitting multivariate analyses to be performed (Atchison 1986).

Genetic analyses

The additive genetic variance-covariance (i.e. **G**) matrix for the eight logcontrast CHCs in males was estimated via REML at the sire-level separately for each environment. I employed the factor-analytic modeling approach implemented in the 'mixed' procedure of SAS v. 9.2 (Hine and Blows 2006; McGuigan and Blows 2007). The model was:

$$Y_{ijklmn} = \mu + S_k + D_{l(k)} + B_m + O_n + B_m O_n + \varepsilon_{ijklmn}, \quad (1)$$

where Y_{ijklmn} is the observed standardized logcontrast value of the i^{th} CHC (i = 1 to 8) from the j^{th} offspring of the l^{th} dam (D) nested within the k^{th} sire (S). ε is unexplained error. Fixed effects include the intercept (μ), experimental block (B), the order of oviposition environments (O), and their interaction. Prior to analysis, logcontrast values of each of the eight CHCs were standardized (~N(0,1)) separately by environment. The factor-analytic approach ensures that the resulting covariance matrix (**G**) is positive-definite (i.e. all eigenvalues > zero; Kirkpatrick and Meyer 2004). It also provides a means of directly testing the dimensionality of **G** by constraining the covariance matrix to be from zero to eight dimensions and using a series of nested likelihood ratio tests (LRT) to determine whether excluding each dimension significantly worsens the fit of the model (Hine and Blows 2006). In these analyses, the dam effect was fixed at six dimensions in both corn and yeast, corresponding to their number of eigenvectors with non-zero eigenvalues.

As a direct test for genetic variance in CHC condition dependence, I employed a standard technique for detecting genotype × environment interactions (Lynch and Walsh 1998; Tomkins et al. 2004). Data were combined across environments and standardized globally (~N[0,1]). Sire × environment and dam × environment random effect terms were then added to eqn. 1, along with the fixed effect of environment, and the factor analytic modeling approach was used to conduct a series of nested LRTs of the significance of the eigenvectors at the sire × environment level. The first eigenvector of the sire × environment matrix, for example, represents the trait (i.e. linear combination of logcontrast CHCs) for which sires vary most in their responses to the two environments, or in other words for which additive genetic variance in condition dependence is greatest. For this analysis, the sire, dam and dam × environment effects were each fixed at five dimensions, corresponding to their respective number of eigenvectors with non-zero eigenvalues. The estimate of the first eigenvector presented in "Results" was obtained from a model with five dimensions at the sire × environment levels to avoid biases that can result from underfitting the number of genetic dimensions (Meyer and Kirkpatrick 2008).

Phenotypic analyses

I compared the extent of condition dependence and its relationship with the strength of directional sexual selection for various traits in males representing different linear combinations of the eight logcontrast CHCs, including the combination under the strongest directional selection (i.e. the most attractive blend of male CHCs). The latter was estimated using the previously published preference data (Delcourt et al. 2010). In brief, from the current breeding design five virgin daughters from each half-sib family were used

individually in separate mate choice trials with five randomly chosen virgin stock males. CHCs were quantified from the chosen and one of the four (randomly selected) rejected males. Although male-male competition could, in theory, affect the outcome of such trials, several lines of evidence suggest that this assay successfully measures female choice in this species (Delcourt et al. 2010). Here I ignored the fact that the females came from the breeding design and used a standard first-order multiple regression (Lande and Arnold 1983) to estimate sexual selection on males as a function of their eight logcontrast CHCs (standardized ~ $N[0,1]$), along with the fixed effects of experimental block, order of oviposition environments and their interaction. This yielded a column vector of directional sexual selection gradients ($\boldsymbol{\beta}$) representing the linear combination of CHCs that best explains variance in male mating success (i.e. male attractiveness; Blows 2007).

To generate additional traits representing different linear combinations of CHCs, I randomly reassigned mating success scores among males in the preference data from Delcourt et al. (2010). The multiple regression of a randomized set of male mating success scores against their eight standardized logcontrast CHC values yielded a vector of partial regression coefficients representing an arbitrary combination of CHCs. This approach preserves the phenotypic covariance structure inherent in the male CHCs, identifying linear combinations of CHCs that exist phenotypically within the population and that females could use in mate choice. I repeated this procedure 999 times, resulting in 1000 different traits: the one particular linear combination of CHCs that best determines male mating success ($\boldsymbol{\beta}$) and 999 other combinations. In all cases I used data only from the yeast environment (involving 3,315 chosen and rejected males), although preferences differed little when females were

raised in the two environments (the vector correlation of the sexual selection gradients in yeast vs. corn was 98.2%; Delcourt et al. 2010) and my results are qualitatively unchanged if I use females raised in corn or I pool those from both environments (unpubl. results). All trait vectors were standardized to unit length prior to their use in further analyses, generating different traits that vary only in the relative contributions of the underlying CHCs (i.e. in their direction in multivariate trait space).

The strength of condition dependence of a trait was estimated by scoring males from the display data for the trait vector in question. The mean of this trait was then calculated separately for males raised in corn and yeast and the strength of condition dependence was estimated as the difference in mean trait values between the two environments (corn minus yeast; the directionality given by the results of an absolute fitness assay; online Appendix A), with greater absolute differences indicating stronger condition dependence. To determine whether the particular combination of CHCs that females prefer most (i.e. male attractiveness as determined by $\boldsymbol{\beta}$) was unusually condition dependent, I calculated the proportion of traits for which the strength of condition dependence was equal to or greater than that observed for male attractiveness.

The strength of directional sexual selection on the same trait arising from female mate choice was calculated by scoring males from the preference data for the same trait vector. Linear sexual selection on this trait was then estimated using a standard first-order multiple regression (Lande and Arnold 1983) in which mating success of individual males (0 = rejected, 1 = chosen) was modeled as a function of their trait value. The trait was

standardized (~N[0,1]) prior to this analysis such that the resulting regression coefficient represents a selection gradient (β) that quantifies the strength and direction of linear sexual selection on it.

Finally, to determine the significance of the relationship between the strength of condition dependence and the strength of sexual selection for these traits, I used a randomization procedure that accounted for the fact that the 1,000 traits are not independent of one another because they represent different linear combinations of the same eight logcontrast CHCs. These CHCs define an eight-dimensional phenotypic space that can have at most eight independent (i.e. orthogonal) trait vectors within it. No particular set of eight orthogonal traits are of interest over another, so I employed a randomization procedure to generate unique sets of eight orthogonal traits within the phenotypic space. For each set, the strength of condition dependence and sexual selection were determined as described above and the correlation coefficient between them was calculated. The procedure was repeated for 1,000 times and a one sample *t*-test (two-tailed) was used to determine whether the average correlation coefficient differed significantly from zero.

Results

Genetic Analysis of cuticular hydrocarbon

The genetic (co)variance matrices of the eight male CHC logcontrast are presented in Tables 1 and 2 for the corn and yeast-raised males respectively. With the exception of (Z)-9-$C_{25:1}$, which was weakly correlated with the other CHCs, the remaining CHCs were strongly correlated with one another (genetic correlations range from 0.88 - 0.99 in yeast and from

0.83 - 0.97 in corn), suggesting that the same genes contribute to the majority of segregating variance among these traits. This is reflected in the principal components of the two **G** matrices (Table 4.3) with the first eigenvector ($\boldsymbol{g}_{max}$), representing the combination of CHC for which there is the most genetic variance, accounting for the majority of the total genetic variance in both corn (92.0%) and yeast (93.9%). Nevertheless, despite severe constraints on the multivariate patterns of genetic variation generated by these covariances, evidence does exist for the presence of a number of independent genetic dimensions of CHC variation in both environments, with five and four genetic principal components of **G** being significant in corn and yeast respectively (Table 4.4).

The first eigenvector of the sire × environment covariance matrix describes the combination of CHCs for which sires vary the most in their response to these two environments (Table 4.3) or in other words, the trait having the most genetic variance in condition dependence. This eigenvector was statistically supported (LRT: $\chi^2 = 56.19$, $d.f. = 8$, $P < 0.0001$), demonstrating significant genetic variance in CHC condition dependence. No other eigenvectors were significant at this level (LRT of the 2nd dimension: $\chi^2 = 2.9$, $d.f. = 7$, $P = 0.892$).

Phenotypic analyses of condition dependence

The combination of CHCs that best determines mate attractiveness (i.e. $\boldsymbol{\beta}$) was significantly more condition dependent than expected ($P = 0.049$), indicating that females discriminate among mates using an unusually condition dependent blend of male pheromones (Fig. 4.1). Among these various blends, the strength of condition dependence for a trait also tended to

increase with the strength of sexual selection on it (Fig. 4.1), generating a significant positive correlation between these variables ($r = 0.482$, $P < 0.001$). The sign of this association is consistent with the quality of these two environments as inferred from the absolute fitness assay (Appendix A): for combinations of CHCs under directional sexual selection favouring a higher trait value, males raised on the high quality corn diet tended to have higher (i.e. more attractive) values as compared to males raised on the lower quality yeast diet. Conversely, for combinations of CHCs under directional sexual selection for a lower value, males raised in corn tended to have smaller values than males raised in yeast, again making the former males more attractive than the latter.

Discussion

Condition dependence may have important implications for fundamental evolutionary processes including the evolution of mate preferences and the resolution of the lek paradox (Pomiankowski and Moller 1995; Rowe and Houle 1996; Kirkpatrick and Barton 1997; Houle 1998; Kotiaho et al. 2001; Houle and Kondrashov 2002; Tomkins et al. 2004), adaptation to novel environments (Lorch et al. 2003) and niche expansion (Proulx 1999; Proulx 2001; Proulx 2002; Lorch et al. 2003; Whitlock and Agrawal 2009), the purging of genetic load and the evolution of sex (Whitlock 2000; Agrawal 2001; Siller 2001; Whitlock and Agrawal 2009), and the evolution of sexual dimorphism and the resolution of intralocus sexual conflict (Bonduriansky 2007a; Bonduriansky 2007b). Although the condition dependence of sexual traits has received substantial empirical attention, many past studies suffer from a number of issues including few or no controls against which to compare changes in sexual traits and a failure to consider its genetic basis (Cotton et al. 2004). In

addition, few of these studies have taken a multivariate approach, despite the fact that mate preferences often target multiple traits simultaneously (Brooks and Endler 2001; Candolin 2003; Chenoweth and Blows 2006). Quantitative estimates of selection are also often lacking, restricting the insight that can be gained into condition dependence.

Here, working with a multivariate sexual display composed of contact pheromones (CHCs) for which I had direct estimates of sexual selection, I tested for condition dependence using an experimental design that provides insight at both the phenotypic and genetic levels. My results revealed that females mate preferences target a combination of CHCs in males that is unusually condition dependent relative to other possible blends females could use. By comparing among CHCs, I avoided possible issues concerning the differential sensitivity to condition of different classes of traits (e.g., morphological, behavioural, chemical) arising for reasons other than the process of genic capture. I also show a positive association between the strengths of condition dependence and sexual selection, as predicted by theory, with variance in the strength of selection explaining approximately 23% (r^2) of the variance in condition dependence. Finally, I demonstrate significant genetic variance for CHC condition dependence.

Cotton et al. (2004) recommend that condition manipulations should be realistic relative to those experienced under natural conditions. In this regard, my experiment appears appropriate in that individuals from my stock population survive and reproduce on both diets and the difference in emergence time between the flies raised in the two environments (1-2 days on average; M. Delcourt, unpubl. results) is well within the range of variation within a

single environment due to density-effects during normal culture maintenance. Nevertheless, the effect of these diets was unexpected in another way: although corn increased male fitness (online Appendix A) it is also known to decrease female fitness (Rundle et al. 2006; Delcourt et al. 2010). Such sexually-antagonistic fitness effects are not surprising given that the majority of genetic variance for fitness segregating in this population appears to be sexually-antagonistic in both environments (Delcourt et al. 2009). Such a negative inter-sex genetic correlation for fitness implies that at least one and likely both sexes are off their fitness peak in yeast, the environment to which they are adapted. Whether a sexually-antagonistic effect of an environmental manipulation of condition is unusual remains to be determined, although a similar result has been found in ambush bugs (Punzalan et al. 2008) and in another population of *D. serrata* (Gosden and Chenoweth 2011) and may be a general feature arising from intralocus sexual conflict over sex-specific diet optima in insects (Maklakov et al. 2008). In addition, the persistence of sexually antagonistic selection within populations (Cox and Calsbeek 2010) and the prevalence of negative intersex genetic correlations for fitness (Kruuk et al. 2008; Poissant et al. 2010) suggest that such effects may be common.

As also noted by Cotton el al. (2004), genetic aspects of condition dependence have received limited attention. Demonstrating genetic variance in condition dependence, for example, is consistent with sexual display traits evolving heightened condition dependence in response to persistent sexual selection, although it is also possible that female preferences may evolve to target male traits that are already good indicators of condition for other reasons (Johnstone et al. 2009). The relative role of these two processes is an important topic for

future research. In addition, experimental studies of condition dependence generally use environmental manipulations, whereas it is genetic differences in condition that are of primary interest. Inbreeding has been used in this respect (e.g., Sheridan and Pomiankowski 1997; van Oosterhout et al. 2003) although the effects of novel mutations have received little attention. Finally, the degree to which the genetic mechanisms underlying sexual dimorphism and condition dependence are shared is also largely unexplored (but see Bonduriansky and Rowe 2005; Bonduriansky 2007a), despite the expectation that sexual dimorphism will often be condition dependent. Unfortunately I did not quantify CHCs from daughters in my breeding design, preventing me from estimating intersexual genetic correlations for these traits in this population. This will also be an important avenue for future research.

Condition dependence and the maintenance of genetic variation

The paradox of the lek describes the apparent contradiction that strong selection on a male sexual display that signals genetic quality should rapidly deplete genetic variance in it, thus preventing females from receiving any indirect benefit from preferring the trait (Kirkpatrick and Ryan 1991). In some sense this paradox was seemingly resolved by the empirical observation of unexpectedly high genetic variance in sexually selected traits (Pomiankowski and Moller 1995). Condition dependence was put forward as a mechanistic resolution to this (Andersson 1994; Pomiankowski and Moller 1995; Rowe and Houle 1996). The idea is that genetic variance in condition is captured by display traits and, because condition is a large mutational target, genetic variance in display traits may be maintained despite persistent directional selection (Rowe and Houle 1996; Houle 1998). Despite increasing evidence for

the condition dependent expression of sexual traits, the abundance of genetic variance in them has been questioned of late, with the results of several quantitative genetic studies and manipulative experiments suggesting that little of this variance lies in the multivariate direction of selection (Walsh and Blows 2009).

In my population of *D. serrata*, females seem to prefer a combination of CHCs in males that is particularly condition dependent. Is this condition dependence sufficient to maintain relatively high standing genetic variance in this trait? Insight can be gained by projecting the vector of sexual selection due to female mate preferences (i.e. $\boldsymbol{\beta}$) through the space defined by the genetic covariance matrix **G**, as given in eqn. 9 of Blows & Walsh (2009). Focusing on the population's standard yeast laboratory environment, of the total genetic variance in male CHCs only 4.5% of it lines along $\boldsymbol{\beta}$, suggesting that despite condition dependence relatively little genetic variance is maintained in this sexual display. Consequently $\boldsymbol{g_{max}}$ (the first eigenvector of **G**), which accounts for 93.9% of the total genetic variance in CHCs, is oriented 78.3° away from $\boldsymbol{\beta}$ (see eqn. 6 of Blows and Walsh 2009). This mirrors previous results from two different populations of *D. serrata*, one lab-adapted and one from the field (Blows et al. 2004; Hine et al. 2004) and therefore appears to be a general result in this species. Similar results have also been found in the related species *D. bunndanda* via both a quantitative genetic study (Van Homrigh et al. 2007) and a selection experiment (McGuigan et al. 2008), and observational and manipulative evidence exists from at least two other species (Hall et al. 2004; Hunt et al. 2007). Low standing genetic variance in multivariate traits under persistent directional selection may therefore be more common than previously appreciated (Walsh and Blows 2009).

The condition dependence of sexual traits is important to theories for the evolution and maintenance of costly female mate preferences via indirect benefits (i.e. good genes). Its apparent inability to maintain high standing genetic variance in male sexual displays in *D. serrata* suggests that selection may be sufficiently strong to rapidly remove deleterious mutations as they arise. If CHCs are a sensitive indicator of genetic quality that permit females to avoid mating with males carrying new deleterious mutations, then females may benefit from their choice despite the presence of little standing genetic variance in the male display (Whitlock 2000; Tomkins et al. 2004; McGuigan and Blows 2009). Such a scenario suggests that the genetic relationship between condition and display traits may be more complex than originally suggested (e.g., McGuigan and Blows 2009). It also suggests that further insight into the good genes hypothesis may require tests of the ability of females to avoid mating with males carrying deleterious mutations (Tomkins et al. 2004; Radwan 2004; McGuigan and Blows 2009). Such tests are an important goal of future work. Also key will be determining the extent to which environmental manipulations of condition (as used herein) mirror the effects of genetic variance and thereby provide insight into the process of genic capture.

Appendix A: Diet manipulation and male condition

The standard approach in experimental studies of condition dependence involves manipulating environmental quality to generate individuals of differing condition, often via low vs. high quality diets (Cotton et al. 2004). My experiment took advantage of a breeding design that was conducted across two different larval food environments consisting of a

yeast vs. corn flour-based media (recipe for the novel media is given in Rundle et al. 2005). Although these environments were chosen for unrelated reasons concerning the alignment of natural and sexual selection during adaptation (Rundle et al. 2006), they constitute a larval diet manipulation. The effects of this manipulation on male condition are difficult to judge *a priori* because the foods are qualitatively different (as opposed to one being a dilution of the other). Therefore, to determine the effect of the diet manipulation on male condition, I conducted an assay to compare the absolute fitness of males raised in yeast vs. corn. Comparing mean absolute fitness between diets is appropriate in this regard because condition is defined as the pool of metabolic resources an individual possesses and it is the size of this pool, and the efficiency with which individuals allocate it to competing life history traits, that determine their fitness. In other words, high condition confers higher absolute fitness and an individual's condition can be thought of as their potential fitness (Bonduriansky and Rowe 2005; Cotton et al. 2004).

The assay involved virgin males from the same stock, raised in either yeast or corn, that were placed singly in vials along with two virgin competitor males of similar age randomly chosen from a stock population into which a recessive orange-eye mutation had been introgressed. A single, virgin orange-eye female was added to each of these vials for 72h, after which all flies were discarded and the vial was retained for offspring development. All vials contained yeast food and all competitor males and the females were raised in the yeast environment such that differences among treatments could be attributed solely to the environment (i.e. diet) in which the target male was raised. 120 (124) replicate vials were set up using males raised in the yeast (corn) environments respectively. Male absolute fitness

was calculated as the number of offspring (i.e. non-orange-eye flies) produced by the female. This measure of male fitness includes his reproductive success (pre- and post-copulatory), the subsequent productivity of the female he mated, and the survival to emergence of his offspring when in competition with half-sibling offspring sired by the orange-eye competitors.

Males raised in corn produced 14.4 ± 1.65 (mean ± standard error) adult offspring compared to 10.0 ±1.7 for males raised in yeast. This difference is significant (non-parametric Mann-Whitney *U* test, normal approximation: $z = -2.87$, $n_1 = 120$, $n_2 = 124$, $P = 0.004$), indicating higher absolute fitness, and hence condition, of males raised in corn as compared to yeast. The proportion of males that sired at least one offspring was significantly higher in corn as compared to yeast-raised males (62% vs. 45% respectively; Fisher's exact test: $P = 0.010$), suggesting that the higher fitness of the corn males arose at least in part from a higher mating success of these males. Of the males that sired at least one offspring (i.e. that successfully mated), corn-raised males also tended to produce more offspring on average as compared to yeast-raised males (23.3 ± 1.9 vs. 22.2 ± 3.0, mean ± standard error), although this difference was not significant (*t*-test on *ln*-transformed counts: $t = -1.44$, $d.f. = 129$, $P = 0.154$).

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Table 4.1. Genetic covariance matrix (**G**) for the eight logcontrast CHCs in males raised in corn. Genetic variances are displayed along the diagonal (bold) with covariances below and genetic correlations above (italics).

Logcontrast CHC	(Z,Z)-5,9-$C_{25:2}$	(Z)-9-$C_{25:1}$	(Z)-9-$C_{26:1}$	2-Me-C_{26}	(Z,Z)-5,9-$C_{27:2}$	2-Me-C_{28}	(Z,Z)-5,9-$C_{29:2}$	2-Me-C_{30}
(Z,Z)-5,9-$C_{25:2}$	**2.447**	*0.031*	*0.927*	*0.941*	*0.922*	*0.982*	*0.848*	*0.923*
(Z)-9-$C_{25:1}$	0.020	**0.166**	*-0.190*	*-0.160*	*-0.189*	*-0.068*	*-0.336*	*-0.204*
(Z)-9-$C_{26:1}$	2.360	-0.126	**2.646**	*0.849*	*0.933*	*0.936*	*0.859*	*0.952*
2-Me-C_{26}	2.057	-0.091	1.929	**1.952**	*0.833*	*0.938*	*0.850*	*0.864*
(Z,Z)-5,9-$C_{27:2}$	3.283	-0.175	3.456	2.651	**5.184**	*0.941*	*0.917*	*0.961*
2-Me-C_{28}	2.663	-0.048	2.641	2.273	3.714	**3.006**	*0.928*	*0.973*
(Z,Z)-5,9-$C_{29:2}$	3.422	-0.353	3.605	3.065	5.388	4.151	**6.657**	*0.967*
2-Me-C_{30}	3.258	-0.187	3.493	2.724	4.937	3.805	5.633	**5.093**

Note: **G** was estimated from the sire covariance matrix in a five-dimensional factor analytic model.

Table 4.2. Genetic covariance matrix (**G**) for the eight logcontrast CHCs in males raised in yeast. Genetic variances are displayed along the diagonal (bold) with covariances below and genetic correlations above (italics).

Logcontrast CHC	(Z,Z)-5,9-$C_{25:2}$	(Z)-9-$C_{25:1}$	(Z)-9-$C_{26:1}$	2-Me-C_{26}	(Z,Z)-5,9-$C_{27:2}$	2-Me-C_{28}	(Z,Z)-5,9-$C_{29:2}$	2-Me-C_{30}
(Z,Z)-5,9-$C_{25:2}$	**2.685**	*0.569*	*0.970*	*0.947*	*0.921*	*0.961*	*0.918*	*0.935*
(Z)-9-$C_{25:1}$	0.357	**0.146**	*0.351*	*0.412*	*0.295*	*0.481*	*0.305*	*0.409*
(Z)-9-$C_{26:1}$	3.016	0.255	**3.603**	*0.956*	*0.961*	*0.952*	*0.955*	*0.943*
2-Me-C_{26}	2.487	0.252	2.909	**2.571**	*0.882*	*0.958*	*0.979*	*0.923*
(Z,Z)-5,9-$C_{27:2}$	3.451	0.258	4.171	3.232	**5.228**	*0.949*	*0.931*	*0.975*
2-Me-C_{28}	2.928	0.342	3.358	2.856	4.036	**3.456**	*0.973*	*0.991*
(Z,Z)-5,9-$C_{29:2}$	3.964	0.307	4.777	4.137	5.612	4.768	**6.947**	*0.963*
2-Me-C_{30}	3.608	0.368	4.217	3.485	5.248	4.337	5.980	**5.545**

Note: **G** was estimated from the sire covariance matrix in a four-dimensional factor analytic model.

Table 4.3 First principal components (i.e. eigenvectors) of the covariance matrices at the sire-level (i.e. **G**) in corn and yeast and at the sire × environment level. Also shown is the vector of directional sexual selection gradients on the eight logcontrast CHCs in males (***β***).

Logcontrast CHC	g_{max} (corn)	g_{max} (yeast)	sire × environment	$\boldsymbol{\beta}$[a]
(Z,Z)-5,9-$C_{25:2}$	0.296	0.294	0	0.061
(Z)-9-$C_{25:1}$	-0.016	0.028	-0.108	-0.175
(Z)-9-$C_{26:1}$	0.308	0.347	0.269	-0.109
2-Me-C_{26}	0.252	0.288	0.550	-0.196
(Z,Z)-5,9-$C_{27:2}$	0.441	0.414	-0.354	-0.141
2-Me-C_{28}	0.340	0.343	0.296	0.442
(Z,Z)-5,9-$C_{29:2}$	0.495	0.483	-0.633	0.742
2-Me-C_{30}	0.446	0.433	-0.024	-0.386

Note: In all three cases the eigenvectors were calculated from a model with four or more dimensions at all levels to avoid potential biases that can result from the underfitting of the number of genetic principal components (Meyer and Kirkpatrick 2008).

[a] this vector represents the linear combination of male CHCs that females prefer most (equivalent to male attractiveness) and is standardized to unit length.

Table 4.4. Model fit statistics of the number of effective dimensions of the additive genetic covariance matrix (**G**) for male CHCs in each environment as determined from a factor-analytic model of the sire-level covariance matrix.

	Corn				Yeast			
dimension	% variance[a]	-2LL[b]	*d.f.*	*P*[b]	% variance[a]	-2LL[b]	*d.f.*	*P*
8	0.0	17083.17	1	1.000	0.0	18044.96	1	1.000
7	0.1	17083.17	2	0.529	0.0	18044.96	2	1.000
6	0.5	17084.44	3	0.245	0.3	18044.96	3	0.119
5	0.7	17088.60	4	0.001	0.6	18050.82	4	0.105
4	0.9	17107.38	5	<0.001	1.3	18058.49	5	0.000
3	2.2	17139.42	6	<0.001	1.8	18090.88	6	0.000
2	3.5	17182.34	7	<0.001	2.2	18138.29	7	0.000
1	92.0	17262.93	8	<0.001	93.9	18219.33	8	0.000
0	-	17679.63	-	-	-	18651.42	-	

[a] Percent of the total genetic variance in male CHCs as calculated from the full (i.e. eight-dimensional) factor analytic model.

[b] -2 log-likelihood value from a likelihood ratio test as to whether eliminating the particular dimension in question significantly worsened the fit of the model, with degrees-of-freedom (*d.f.*) and significance (*P*) as indicated.

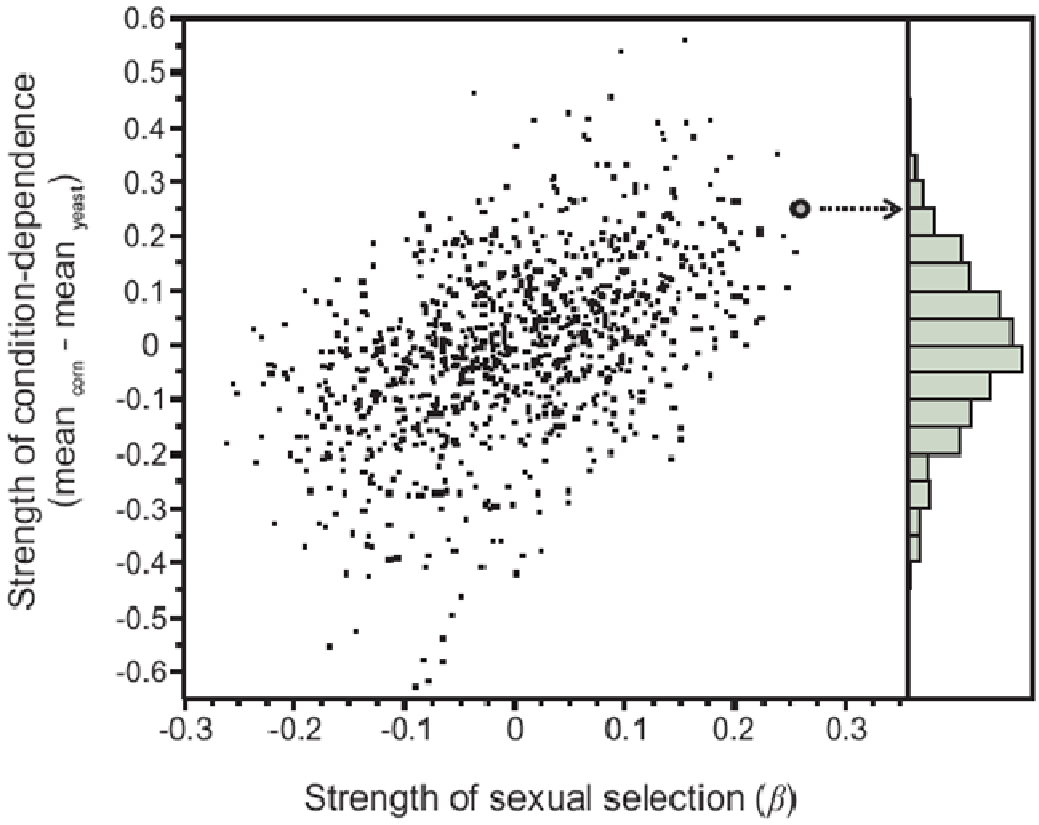

Fig. 4.1. Relationship between the strength of condition dependence, as measured by the difference in mean trait value between corn and yeast-raised males, and the strength of sexual selection, as given by the selection gradient (β) for 1,000 different traits representing different linear combinations of the eight logcontrast CHCs. The correlation (r = 0.482) is significant, as tested via a randomization procedure ($P < 0.001$; see Methods). The large circle indicates the particular combination of CHCs most preferred by females (i.e. male attractiveness); this blend is significantly more condition dependent that would be expected by chance, as shown on the histogram on the right (P = 0.049).

Chapter 5: Evidence for mutation-selection balance attained for sexually selected traits

The work in this chapter is a slightly modified version of a manuscript, coauthored by Mark Blows (School of Biological Sciences, University of Queensland, Australia) and Howard Rundle (Department of Biology, University of Ottawa), that is currently in preparation for submission to a peer-reviewed journal.

Directional selection on quantitative traits is both common in nature and frequently strong (Endler 1986; Hoekstra et al. 2001; Kingsolver et al. 2001; Hereford et al. 2004), yet does not appear to generate sustained evolutionary responses (Svensson and Gosden 2007; Kingsolver and Diamond 2011). Instead, phenotypes in natural populations tend to remain relatively stationary in value over various time-scales (Estes and Arnold 2007), suggesting a limit to trait evolution. A simple lack of genetic variance in target traits is unlikely to provide a general explanation: genetic variance is found for almost all traits (Lynch and Walsh 1998; Barton and Partridge 2000) and, although genetic covariances among traits may constrain their response to multivariate selection (Lande 1979; Walsh and Blows 2009), this is often insufficient to prevent it entirely (Beldade et al. 2002; Conner et al. 2003; Hine et al. 2011). The potential importance of opposing natural selection in limiting trait evolution has long been recognized during artificial selection experiments (Lerner and Dempster 1951; Falconer 1955; Roberts 1966; Enfield 1980; Hine et al. 2011). However, the genetic basis of evolutionary limits in unmanipulated populations has been more difficult to ascertain (Merila et al. 2001; Merila 2009).

Sexual selection provides a particularly striking example of how strong directional selection fails to change trait means in contemporary populations (Svensson and Gosden 2007). An evolutionary response in male sexual displays is not generally observed despite the presence of strong selection (Kingsolver et al. 2001) acting on heritable variation in male traits (Pomiankowski and Moller 1995). Opposing natural selection, arising from pleiotropic effects on non-sexual fitness, has long been recognized as a potential limit to male trait exaggeration (Fisher 1930), although direct evidence is rare (Jennions et al. 2001; Kotiaho et

al. 2001). A recent evolutionary manipulation in *Drosophila serrata* confirmed the potential of this mechanism to halt the evolution of male pheromone displays consisting of cuticular hydrocarbons (CHCs). Artificial selection on CHCs in the direction of female preferences conferred higher male mating success over control populations. However, the response to selection was stopped after seven generations by opposing natural selection, despite a substantial increase in genetic variance in CHCs as this new evolutionary limit was approached (Hine et al. 2011). This suggests that the alleles responding to artificial selection were initially at low frequency in the base population due to their antagonistic effects on male sexual and non-sexual fitness, or in other words that unconditionally beneficial genetic variance was lacking.

If male sexually-selected traits are held at an evolutionary limit by opposing selection, their genetic basis in an unmanipulated population should be at a mutation-selection balance. Here, I show that a genetic prediction unique to the Hill-Keightley model of mutation-selection balance (Keightley and Hill 1988; Barton 1990; Kondrashov and Turelli 1992) is upheld for CHCs in *D. serrata* males. The central feature of this model is that for any allele that affects the phenotype of a measured quantitative trait, its pleiotropic effects on all other aspects of fitness can be summarised into a net deleterious effect on total fitness. At mutation-selection balance, while a given mutation may increase or decrease the value of the measured trait with equal probability, it will always decrease fitness. Consequently, because individuals with more extreme values of the trait will tend to carry more deleterious alleles, this generates the appearance of stabilizing selection on the trait even if it is not under direct phenotypic selection. Using a new analytical approach for the identification of such fitness

optima (McGuigan et al. 2011), I demonstrate that in an unmanipulated population of *D. serrata*, the major axis of genetic variance in male CHCs is under stabilizing selection, explaining why strong directional sexual selection on these traits fails to produce an evolutionary response.

Within a large breeding design, I measured the fitness of 2,118 sons and daughters (Delcourt et al. 2009) and subjected these traits to a multivariate genetic analysis together with the suite of CHCs measured on 1,114 other sons (Delcourt and Rundle 2011). As has been shown in previous studies (Blows et al. 2004; Chenoweth and Blows 2005; Hine et al. 2011), male CHCs were under strong directional sexual selection in a phenotypic analysis (r^2 = 21.2%, P < 0.0001; Table 5.1). Despite significant genetic variance in all traits, however, fitness of neither sex covaried genetically with the phenotypic trait representing the major axis of genetic variance in male CHCs ($CHCg_{max}$, accounting for 93.9% of the total genetic variance in male CHCs; Delcourt and Rundle 2011) (Table 5.2), indicating the absence of directional selection on CHCs at the genetic level.

In contrast to the lack of genetic covariance between male CHCs and fitness, there was a strong covariance between male fitness and the squared deviations of the $CHCg_{max}$ phenotype ($CHCg_{max}^2$), indicating the presence of stabilizing selection on the major axis of genetic variance in CHCs (Table 5.3). This selection on $CHCg_{max}$ genotypes was apparent since the $CHCg_{max}$ phenotype was not itself under stabilizing sexual selection (γ = 0.002; r^2 = 2.3%, P = 0.487), although weak directional sexual selection was observed (β = 0.077, r^2_{adj} = 2.3%, P < 0.0001).

Stabilizing selection on $CHCg_{max}$ genotypes appears to arise from the pleiotropic effect of the underlying alleles primarily on male as opposed to female fitness (i.e. a significant covariance between $CHCg_{max}^2$ and male but not female fitness; Table 5.3). However, the $CHCg_{max}^2$ – fitness covariance matrix has two significant genetic principal components (i.e. eigenvectors: g_1 and g_2) which account for all of the genetic variance in these traits (λ_1 = 57.8%, $P < 0.001$; λ_2 = 42.2%, $P = 0.006$). These eigenvectors differ primarily in the genetic association of male and female fitness, representing sexually antagonistic and sexually concordant fitness variation respectively (Table 5.3). The existence of these two dominant axes of fitness variation is confirmed in a separate genetic analysis of male and female fitness on their own (Fig. 5.1). Re-expressing fitness variation along these two axes provides additional insight into the genetic covariance of $CHCg_{max}$ deviations and fitness, revealing stabilizing selection through sexually-concordant, but not sexually-antagonistic, fitness variation (Fig. 5.2). This suggests that individuals deviating in either direction from the $CHCg_{max}$ optimum tend to carry unconditionally deleterious alleles as opposed to those harmful to males alone.

My results demonstrate apparent stabilizing selection on the major axis of genetic variance in male CHCs in an unmanipulated laboratory population, providing strong evidence that these traits are at mutation-selection balance. Apparent stabilizing selection indicates that this genetic limit arises ultimately as a consequence of pleiotropy, or in other words, that changes in allele frequency in response to directional selection on CHCs has altered other traits, thereby generating opposing selection. That such an evolutionary limit may be

common is suggested by two lines of evidence implying widespread pleiotropy. First, although data are limited, estimates indicate that the per-trait mutation rate is about one-tenth of the genome-wide mutation rate, suggesting only a small number of genetically independent traits within an organism (Johnson and Barton 2005; Walsh and Blows 2009). Second, genetic covariances among suites of traits are often found to restrict genetic variance to a small number of independent or 'effective' dimensions (Kirkpatrick 2009), again suggesting few genetically independent traits. Extensive pleiotropy has also been observed with respect to specific alleles (Brown et al. 1999; Hekerman et al. 2005; Ostrowski et al. 2005), although gene knockout studies have implied greater modularity (Wang et al. 2010). Consistent with an important role for pleiotropy in limiting trait evolution, results of long term artificial selection experiments reveal that genetic variance in the target traits is often present at an apparent selective limit (e.g., Reeve and Robertson 1953; Enfield 1980; Hine et al. 2011). Opposing selection in particular has also been inferred when the trait gain decays when artificial selection is halted (e.g., Lerner and Dempster 1951; Falconer 1955; Roberts 1966; Enfield 1980; Hine et al. 2011).

Sexual display traits in particular are subject to persistent directional selection yet appear to be at an evolutionary limit in natural population (Svensson and Gosden 2007). The classic expectation for the evolution of these traits involves a runaway phase that is eventually halted by opposing selection. Although this process of exaggeration is likely rapid in relation to any subsequent equilibrium (Fisher 1930), attention has focussed on the expectation of a positive covariance between sexual displays and fitness (e.g., Kirkpatrick and Barton 1997; Moller and Alatalo 1999; Houle and Kondrashov 2002). Such an approach

may not be particular insightful, however, if as a consequence of widespread pleiotropy sexual displays in contemporary populations are often at a selective limit. Under such conditions, characterizing the genetic association between sexual displays and fitness requires an alternative approach such as that applied herein (McGuigan et al. 2011).

Mating success is likely the major component of male fitness in species like *D. serrata* in which fathers contribute only genes to their offspring. At mutation-selection balance, a good genes process for the maintenance of costly female mate preferences may depend on the indirect benefits females gain from discriminating against males carrying a greater number of deleterious mutations (Tomkins et al. 2004; McGuigan and Blows 2009). Consistent with this, results from a few studies suggest that mating success is sensitive to the presence of deleterious mutations in manipulated populations (Radwan et al. 2004; Sharp and Agrawal 2008; Hollis et al. 2009; MacLellan et al. in press). My results provide further support for this hypothesis because stabilizing selection appears to have arisen from alleles with sexually concordant effects, decreasing both male and female fitness (Fig. 5.2).

More generally, the genetic covariance of squared trait deviations and fitness may provide a powerful approach to inferring fitness optima without resorting to artificial selection. This may be particularly useful in characterizing evolutionary limits in natural populations for which pedigrees are available. It may also fundamentally alter our view of how costly female mate preferences are maintained at equilibrium under a good genes process.

Supplementary Material

Breeding design

Using a previously described outbred laboratory population of *D. serrata* (Rundle et al. 2006), I conducted a breeding design involving 91 sires mated to each of four dams. Fitness of 1,111 female and 1,007 male offspring were assayed in the standard laboratory environment (i.e. yeast medium) to which the population was long adapted, as previously described (Delcourt et al. 2009). In brief, female fitness was measured as the total number of adults offspring produced over 48 h by a single female when held together with a random stock male. This measure therefore includes her fecundity and the subsequent survival to emergence of her offspring. Male fitness was measured as the ratio of adult offspring sired by a single genetic male relative to two random stock males when in competition for 72 h to fertilize a single random stock female. This measure of male fitness includes his competitive mating success, the subsequent productivity of the female he mated, and the survival to emergence of his male and female offspring. CHCs were assayed on 1,114 separate sons within the context of a sexual encounter by presenting the male with two random stock females (Delcourt and Rundle 2011). Once mating occurred, CHCs were extracted using standard protocols (Blows 2002), analyzed via gas chromatography, and the relative concentrations of each of nine previously identified CHCs (Howard et al. 2003) was determined. Data were transformed as previously described (Delcourt et al. 2009; Delcourt and Rundle 2011) and standardized $\{N \sim(0,1)\}$ prior to analysis.

Genetic analyses

To determine the major axis of genetic variance in CHCs (i.e. $\mathbf{g}_{max}$), the sire-level additive-genetic covariance matrix (**G**) for these eight traits was estimated via restricted maximum likelihood (REML) using a standard mixed model in which dam was nested within sire (Delcourt et al. 2011). Diagonalization of **G** yielded its eigenvectors (i.e. genetic principal components) and associated eigenvalues, the first of which represents the linear combination of the original traits with the greatest genetic variance ($\mathbf{g}_{max}$; Schluter 1996; Blows 2007). As with any eigenvector, $\mathbf{g}_{max}$ of set of metric traits is itself a metric trait and phenotypic scores of individual sons from the breeding design ($CHCg_{max}$) were calculated using: $CHCg_{max} = \mathbf{g}_{max}{}^{T}\mathbf{Z}$, where **Z** is a row vector of the eight observed CHC values for an individual (McGuigan et al. 2011). These phenotypic scores for $CHCg_{max}$ were then used in a genetic analysis, along with male and female fitness, to calculate the sire-level additive genetic covariance matrix (**G**) for these three traits via REML. Significance of the individual (co)variances was determined using a Bayesian approach with a non-informative flat prior and a Markov Chain Monte Carlo algorithm to generate 10,000 posterior samples, as implemented in the MIXED procedure of SAS 9.2. Similar results were obtained using a non-informative version of the Jefferey's prior (SAS 9.2 User Manual). Directional selection on the major axis of genetic variance in CHCs ($CHC\mathbf{g}_{max}$) would be indicated by a significant additive genetic covariance between $CHC\mathbf{g}_{max}$ and male and/or female fitness (Lande and Arnold 1983).

If selection on $CHCg_{max}$ is stabilizing, this would not be detected as a covariance with fitness because individuals deviating from the $CHCg_{max}$ optimum in either direction would

have similarly low fitness (McGuigan et al. 2011). However, in an approach analogous to phenotypic tests for nonlinear selection, stabilizing selection can be detected through a genetic analysis of the squared deviations of $CHCg_{max}$ phenotypes from the population mean (Wright 1935; McGuigan et al. 2011). These deviations were therefore calculated as the square of the individual $CHCg_{max}$ scores when standardized to a mean of zero ($CHCg_{max}^2$). These deviations were then used in place of the original $CHCg_{max}$ scores in a genetic analysis along with male and female fitness. Because the $CHCg_{max}^2$ values are non-normally distributed, significance of the genetic covariances between $CHCg_{max}^2$ and fitness were again evaluated using a Bayesian approach. The genetic principal components of the sire-level covariance matrix were determined via diagonalization and their significance was evaluated via factor analytic modeling (Hine and Blows 2006).

To characterize the covariance structure of male and female fitness, a genetic analysis was conducted on these traits alone and breeding values were estimated as best linear unbiased predictors (BLUPS) from this model. The two genetic principal components were extracted via diagonalization of the sire-level covariance matrix, representing sexually antagonistic and sexually concordant fitness variation respectively. Fitness variation was re-expressed along these two axes by scoring the breeding values of male and female fitness separately for the two eigenvectors. Directional and stabilizing selection on $CHC\mathbf{g}_{max}$ arising from its association with these two new fitness axes was then estimated using first and second-order polynomial regression on the $CHC\mathbf{g}_{max}$ breeding values (calculated as BLUPS from a univariate model).

Phenotypic selection analyses

As part of a separate analysis of the genetic basis of female mate preferences for male CHCs, other daughters from this breeding design were used in mate choice trials in which a single female was presented with five randomly chosen stock males (Delcourt et al. 2010). CHCs were extracted and quantified for the chosen male and one of the four rejected males (randomly chosen) from each trial, yielding 3,315 males in total. Directional sexual selection gradients on the eight logcontrast CHCs were calculated in a phenotypic analysis of these males using a standard first-order polynomial regression model (Lande and Arnold 1983). Significance was determined via logistic multiple regression because mating success is binomially distributed (Fairbairn and Preziosi 1996). Phenotypic sexual selection on CHC$\mathbf{g}_{max}$ was estimated by scoring these males for the first eigenvector of the sire-level covariance matrix for the eight logcontrast CHCs ($\mathbf{g}_{max}$; Table 5.1) and then employing a first and second-order standard polynomial regression (directional and quadratic selection respectively; Lande and Arnold 1983) of mating success on these scored phenotypes, with significance determined via logistic regression.

Table 5.1. Phenotypic sexual selection gradients on the eight logcontrast CHCs (***β***), along with the first genetic principal component ($\mathbf{g}_{max}$) of these traits, accounting for 93.9% of the total genetic variance, calculated as the first eigenvector of the sire-level covariance matrix.

	β	$\mathbf{g}_{max}$ (CHCs)
(Z,Z)-5,9-$C_{25:2}$	0.021*	0.294
(Z)-9-$C_{25:1}$	-0.059**	0.028
(Z)-9-$C_{26:1}$	-0.037**	0.347
2-Me-C_{26}	-0.066**	0.288
(Z,Z)-5,9-$C_{27:2}$	-0.048**	0.414
2-Me-C_{28}	0.150**	0.343
(Z,Z)-5,9-$C_{29:2}$	0.251**	0.483
2-Me-C_{30}	-0.131**	0.433

*P < 0.05; **P < 0.001.

Table 5.2. Genetic (co)variance matrix (**G**) of CHC$\mathbf{g}_{max}$ phenotypes and male and female fitness. Genetic variances are displayed along the diagonal (bold) with covariances below and genetic correlations above (italics).

	CHC$\mathbf{g}_{max}$	female fitness	male fitness
CHC$\mathbf{g}_{max}$	**1.041****	*-0.138*	*-0.095*
female fitness	-0.054	**0.145***	*-0.480*
male fitness	-0.034	-0.065	**0.126***

*P < 0.05; **P < 0.0001.

Table 5.3. Genetic (co)variance matrix from an analysis of the squared deviations of CHC$\mathbf{g}_{max}$ (CHC$\mathbf{g}_{max}^2$) phenotypes and male and female fitness. Variances are displayed along the diagonal (bold) with covariances below and genetic correlations above (italics). $\mathbf{g}_1$ and $\mathbf{g}_2$ are the first and second genetic principal components (i.e. eigenvectors) of the covariance matrix, accounting for of the total genetic variance respectively ($\mathbf{g}_3$ did not account for any detectable genetic variance and was not significant; P = 0.738).

	CHC$\mathbf{g}_{max}^2$	female fitness	male fitness	$\mathbf{g}_1$**	$\mathbf{g}_2$*
CHC$\mathbf{g}_{max}^2$	**0.096**	*-0.162*	*-0.854**	-0.429	-0.563
female fitness	-0.021	**0.172****	*-0.376*	-0.542	0.786
male fitness	-0.096*	-0.056	**0.131***	0.722	0.256

*P < 0.01; **P < 0.001.

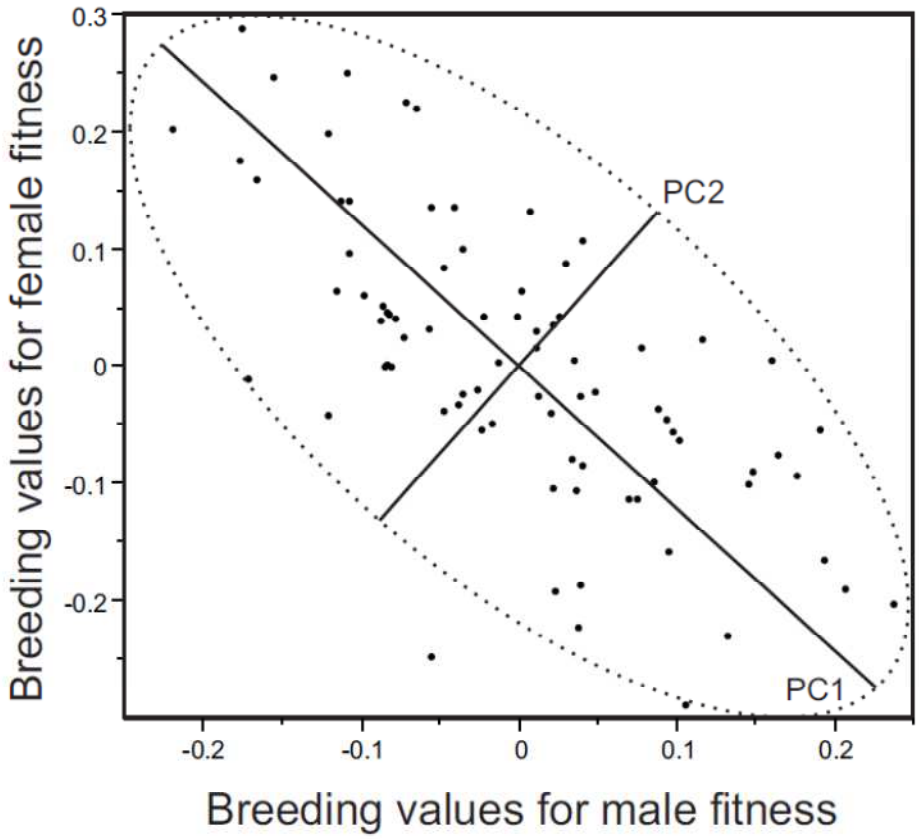

Figure 5.1. Breeding values for male and female fitness, estimated as best linear unbiased predictors from a genetic analysis of these traits. The first and second genetic principal components, depicted within a 95% confidence ellipse, represent genetic variance in fitness with sexually antagonistic (PC1) vs. sexually concordant (PC2) effects respectively.

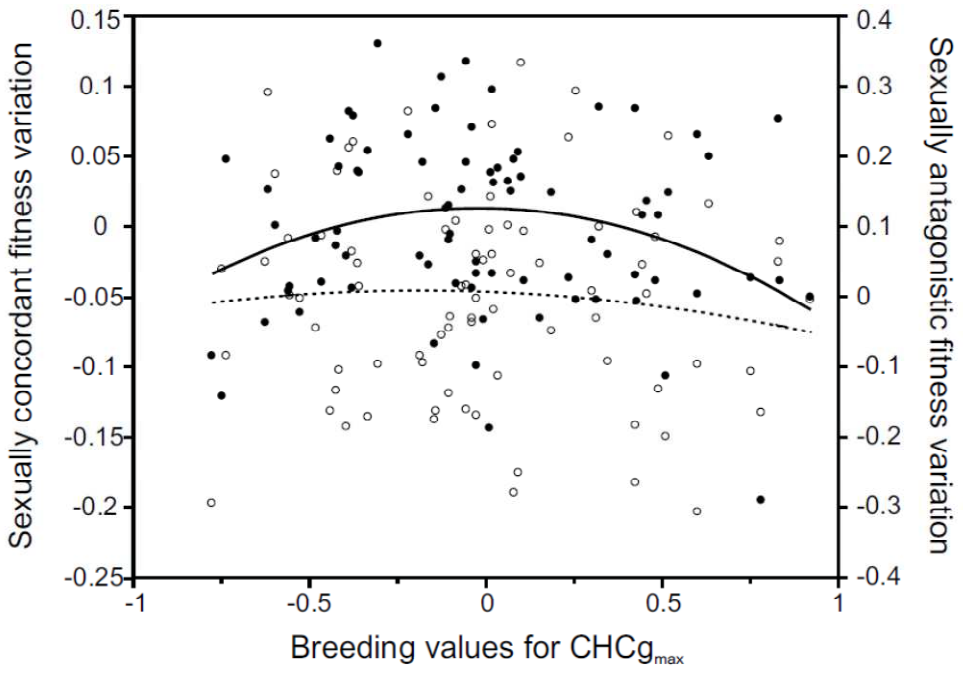

Figure 5.2. Scatterplot depicting the association between genetic variance in fitness and the major axis of genetic variance in CHCs ($CHCg_{max}$). Genetic variance in fitness is expressed along its two dominant axes, representing sexually antagonistic (SA) and sexually concordant (SC) variation (PC1 and PC2 from Fig. 5.1 respectively). There is no evidence of directional selection in either case (linear regression, SA fitness variation: β = -0.023, t_{79} = -0.576, P = 0.577; SC fitness variation: β = -0.011, t_{79}= -0.645, P = 0.521). Stabilizing selection is present with respect to sexually concordant but not sexually antagonistic fitness variation (non-linear regression, SA fitness variation: γ = -0.049, t_{78} = -0.583, P = 0.562; SC fitness variation: γ = -0.081, t_{78} = -2.34, P = 0.022).

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Chapter 6: General conclusions

In this chapter I summarize the results of my research, describing what we have learned from the quantitative genetics analyses of male and female fitness, female mate preferences, and male sexual display traits. In doing so, I highlight what I see as important and unresolved issues with respect to these topics, giving special attention to the implications of my results for the study of good-genes indirect benefits, the original goal of my research. I also take a broader perspective on the importance of pleiotropy and the implications of mutation-selection balance to the study of sexual selection.

In chapter two, I investigated the nature of standing genetic variance in male and female fitness in a laboratory population of *Drosophila serrata*. In line with recent studies in both laboratory and natural populations (e.g., Chippindale et al. 2001; Bonduriansky and Chenoweth 2009; Poissant et al. 2009), a large component of standing genetic variance in this population was sexually antagonistic, as revealed by a negative intersex genetic correlation for fitness (r_W^{mf}). A negative intersex genetic correlation provides strong evidence of ongoing intralocus sexual conflict in this population (Bonduriansky and Chenoweth 2009) which can have important consequences, for example constraining adaptation (Lande 1982) and maintaining genetic variance in fitness (Turelli and Barton 2004).

While the existence of a negative intersex genetic correlation for fitness establishes that intralocus sexual conflict is occurring, it provides no information concerning the shared phenotypes underlying the conflict. Such conflict can arise with respect to a diverse set of shared traits including, for example, mating rate (Arnqvist and Rowe 2005), locomotory

activity (Long and Rice 2007), and diet (Maklakov et al. 2008). The latter is a strong candidate in my case given the sex-specific effects I observed from my diet manipulation and because larval development was likely a major component of fitness in my assays. An important goal of future research is to determine which traits underlie persistent sexual conflict and understand what constrains their sex-specific divergence and thereby prevents the resolution of the conflict (Bedhomme and Chippindale 2007; Cox and Calsbeek 2009).

A negative intersex genetic correlation for fitness will also impact a good-genes process by reducing the benefits of mate choice (Brommer et al. 2007). This is because fitness benefits associated with the expression of advantageous alleles in one sex may be counter-balanced by fitness costs associated with the expression of these same alleles in the opposite sex. This can be seen in equation 1 (*Introduction*) in which the sign of the intersex correlation for fitness, r_W^{mf}, can either enhance (if positive) or diminish (if negative) the indirect benefits gained by females from their choice of mates. More generally, evidence is accumulating that sexually antagonistic selection and intralocus sexual conflict are common (Bonduriansky and Chenoweth 2009; Cox and Calsbeek 2009) and recent theory suggests that this may impose substantial fitness costs when unresolved (Connallon et al. 2010). My results suggest that alleles with antagonistic effects on male and female fitness may be prevalent in a population that is well adapted to its environments (here the yeast environment). This is probably because alleles that are unconditionally beneficial to both sexes are rapidly fixed, and alleles that are left segregating in the population are the ones that are under conflicting/antagonistic selection across sexes. While this may be characteristic of laboratory populations in which the constant environment may allow selection to be

particularly effective at purging unconditionally deleterious mutations (Chapman et al. 2003), negative intersex correlations for fitness have also been found in natural populations including red deer (Foerster et al. 2007) and collared flycatchers (Brommer et al. 2007). That the intersex genetic correlation for fitness appeared to become more negative in the novel corn environment was surprising, although makes sense in light of evidence for sexual conflict over diet choice (Chapter 4). How intersex genetic correlations for fitness change across environments remains largely unexplored, in particular as populations are displaced further from a fitness peak. Such genotype-by-environment interactions may have important consequences for adaptation to novel environments.

In chapter three, I focused on the genetic basis of female mate preferences, taking advantage of recently developed methods for the multivariate genetic analysis of function-valued traits (Meyer and Kirkpatrick 2005; Chenoweth and Blows 2006; McGuigan et al. 2008). I detected significant genetic variance in preferences for CHCs, indicating that they are heritable and could therefore evolve through either direct or indirect selection. One of the major finding of this chapter is that individual preferences for each male CHC were genetically correlated, meaning that segregating alleles affecting female preference for one pheromone also tended to affect their preference for other pheromones. This is in line with another study that found genetic constraint in female mate preference when modeled as a function-valued trait (McGuigan et al. 2008; but see Brooks and Couldridge 1999). Such genetic correlations among preferences for different traits may arise from either linkage disequilibrium or pleiotropic effects of the underlying alleles, and their presence may influence the evolution of both female preference as well as the male traits they target by

restricting genetic variance in these traits within the genotypic space. In particular, the genetic covariance of female preferences for different traits may help explain the observation that females often pay attention to multiple male display traits at the same time (Candolin 2003). Whether the existence of these multiple genetically correlated preferences favour or hamper a good-genes process will depend on whether mate preferences evolve to target specific combinations of male traits (because these combinations are more condition dependent; see chapter 4) and if so, on the effects of these correlations on preference evolution. In my results, the second genetic dimension (i.e. eigenfunction) of female preference approached significance, suggesting the presence of genetic variance for changes in preference direction. While this second dimension could reflect an alternative mating rule that females may use to choose among their mates (Kirkpatrick et al. 2006), it could alternatively reflect among population variation in preference (Higgie et al. 2000; Higgie and Blows 2008) given the origin of the stock population I used in my study. Dissecting the genetic basis of female mate preferences will be key to our understanding of evolution of mating choice and preference divergence.

Finally, the fact that female mate preferences did not change upon colonization of the novel corn-food environment suggests that they might not be overly sensitive to female condition. This apparent absence of condition-dependence is contrary to expectation if preferences are costly and the marginal fitness gain from preference expression is greater for high than for low-condition females (Cotton et al. 2006; Getty 2006). It also contrasts with previous findings from other systems (reviewed in Cotton et al. 2006). Although a direct test is lacking, preferences appear to be costly in *D. serrata* (Rundle et al. 2009) and the absence of

condition-dependence therefore suggests that their expression is independent of female quality. How the costs of mate preferences arise is poorly understood in general and understanding such costs is an important topic for future research. It will also be important to determine the generality of the current results, including whether it holds when changing other aspects of the environment and when manipulating condition genetically.

In chapter four, I investigated the condition-dependence of CHCs in males using a classic diet manipulation. CHCs were found to be condition-dependent with respect to a change in larval diet, and females tended to prefer a combination of CHCs that was unusually condition-dependent relative to other CHC combinations they could have targeted. The positive relationship observed between the strength of selection and the degree of condition-dependence is, as far as I am aware, the first direct test of this prediction of condition-dependent theory. Via an environmental manipulation of condition, I was also able to show that CHC condition-dependence itself had a genetic basis. This suggests that the degree of condition-dependence of male displays can itself evolve over time and potentially strengthen under the action of female preference. Genetic variance in condition-dependence has received limited attention to date (David et al. 2000; Cotton et al. 2004) and more work is needed to determine the generality of this result.

The findings of chapter four have important implications for a good-genes process, suggesting that females are targeting a combination of CHCs through which they are best able to assess male genetic quality. The multivariate approach, taken within the context of a breeding design, also sheds some light onto the effects of persistent mate preferences on

genetic variance in male display traits. In particular, I found that there was little genetic variation in CHCs in the multivariate direction of female mate preference, confirming a previous result from an independent study of a different laboratory population of the same species (Blows et al. 2004). This suggests that condition-dependence is insufficient to maintain much standing genetic variation in the combination of CHCs that females prefer, and therefore raises the fundamental question of how females benefit from choosing among males using this trait. One possibility is that females may benefit from discriminating against males carrying novel deleterious mutations every generation, and that the resulting selection may be sufficiently strong to prevent much standing genetic variance from accumulating (Whitlock 2000; Tomkins et al. 2004; McGuigan and Blows 2009). This suggests that demonstrating good genes indirect benefits may require investigation of the ability of females to detect and avoid mating with males carrying novel mutations (e.g. Radwan et al. 2004), as opposed to studying the covariance between sexual displays and fitness, as is commonly done (Moller & Alatalo 1999; see chapter 5).

My results demonstrate that female mate preferences target an unusually condition dependent combination of male CHCs. The evolutionary origin of this association is a key question. On one hand, CHCs may have evolved condition-dependence because of persistent directional selection arising from female mate choice, with female preferences originally targeting these CHCs for some other reason (e.g., species recognition, see Higgie et al. 2000; Higgie and Blows 2007). On the other hand, female preferences may have evolved to target this particular combination of male CHCs because it was among those most sensitive to condition, thereby providing an honest indicator of male quality. The presence of genetic

variance in female mate preferences in chapter three indicates that the latter is at least possible. A direct test of these alternative scenarios is an important topic for future research. Previous studies have shown the presence of a pre-existing bias in female preference (Endler and Basolo 1998; Ryan 1998; Boughman 2002) in some systems, suggesting the former. However, that the latter is also feasible is suggested by a recent theoretical model (Johnstone 2009), and empirically in *D. serrata* by an evolution experiment in which preferences in this population appeared to have evolved to bring them into closer alignment with the vector of directional natural selection (Rundle et al. 2009).

Chapter five integrates data from chapters two, three and four to investigate the genetic relationship between CHCs and fitness. Despite significant directional sexual selection on these traits at the phenotypic level, I found no significant genetic covariance between the major axis of genetic variance in CHCs ($CHCg_{max}$) and male or female fitness, indicating the absence of directional selection at the genetic level. However, I did detect significant stabilizing selection on the major axis of genetic variance in CHCs ($CHCg_{max}$) via its genetic association with total male fitness. Much attention in sexual selection research has focused on the expectation of a positive genetic covariance between male sexual display traits and fitness (e.g., r_{TW} from equation (1) in the *Introduction*; see Kirkpatrick and Barton 1997; Houle and Kondrashov 2002; Moller & Alatalo 1999). However, while such correlation may be expected during the early exaggeration (or runaway) phase in which the display and the preference coevolve (Fisher 1930), once exaggeration of the display is halted due to opposing selection, the trait is at its fitness optimum and its genetic architecture should be characteristic of mutation-selection balance. In theory, opposing selection could arise from

the effects of the display trait itself on other fitness components (e.g., a long tail is costly because it reduces survival), or it could arise because the alleles underlying the trait have pleiotropic effects on other traits which are themselves under selection. While the former would be detectable in a phenotypic selection analysis of the trait itself, the latter would only be seen in a genetic analysis (i.e. apparent selection). That pleiotropy is extensive and may therefore be key to generating opposing selection is suggested by comparisons of the per-trait and genome-wide mutation rates (the former being only approximately one-tenth of the latter; Walsh & Blows 2009; Johnson & Barton 2005) and the observation that genetic variance in suites of traits is often restricted to a small number of genetic dimensions (Kirkpatrick 2009).

In sexual selection research, comparatively little attention has been given to understanding the maintenance of costly female mate preferences when sexual display traits are at an equilibrium between opposing selection, despite the classic expectation that the runway phase will be rapid (Fisher 1930). The population used in my experiment was well adapted to its laboratory environment and male CHCs appear to be at a fitness optimum. Consistent with such conditions in nature, continued exaggeration and divergence of sexual displays is not generally observed (Svensson and Gosden 2007), although direct tests for the presence and nature of selective limits in the field are generally lacking. In one study on forehead patch size in male collared flycatchers, Qvarnström et al (2006) found only a weak and non-significant genetic correlation with male fitness in a wild population. Quantifying the strength of indirect selection on female mate preference under such condition may therefore

require an alternative approach to that taken in equation (1) and this remains a key topic in good genes research.

Concluding remarks

The original goal of my thesis was to quantify the indirect genetic benefits that females gain through their choice of mates. Estimating parameters of equation (1) is a challenging task when mate choice targets a multivariate suite of sexual displays such as in *D. serrata*. For example, the phenotypic covariance between the preference and CHCs, as well as the heritability of female preference (ρ_{PT} and h_P^2 respectively in equation 1; *Introduction*), cannot be directly estimated when preferences are estimated only at the sire-level and not for individuals daughters. While it may be possible to re-parameterize equation (1) to address this, the non-linear association between CHCs and fitness is a more substantial issue, questioning the assumptions underlying this theory. The evolution and maintenance of costly female preferences via a good-genes process is clearly a topic that will continue to occupy both theoreticians and empiricists for some time to come, and the extent to which "sexual selection will have given its aid to ordinary selection" (Darwin1859) remains one of the most challenging topic in evolutionary biology.

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Figure 6.1: Mating pair of the Australian fruit fly *Drosophila serrata*