GENOTYPE-BY-ENVIRONMENT INTERACTIONS AND SEXUAL SELECTION

Submitted by:

Fiona Caroline Ingleby

to the University of Exeter as a thesis for the degree of Doctor of Philosophy in Biological Sciences

August 2012

I certify that all materials in this thesis that are not my own work have been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

ABSTRACT

Genotype-by-environment interactions (G x Es) describe genetic variation for phenotypic plasticity, such that the relative performance of genotypes varies across environments. These interactions have been studied in the context of natural selection for decades, but research interest in the evolutionary consequences of G x Es in sexual traits is more recent. Theory suggests that G x Es in sexual traits could be of fundamental importance to the operation of sexual selection across heterogeneous environments, but empirical research lags behind the theory. In this thesis, I review the current literature on the role of G x Es in sexual selection and identify areas for further research. Using cuticular hydrocarbons (CHCs) in the fruit fly *Drosophila simulans* as a model system for sexual selection, I examine G x Es in trait expression and quantify the effect of these G x Es in terms of sexual signal reliability and the coevolution of male and female sexual traits.

To do so, I use a combination of quantitative genetics and laboratory environmental manipulations. First, I demonstrate that male CHC profile is subject to sexual selection through female mate choice and find some variation in patterns of mate choice across diets and temperatures (Chapter 3). Next, I identify G x Es in male and female CHC expression across diets and temperatures, although G x Es in male CHC profile across temperatures are weak (Chapter 4). I find that G x Es in male CHC expression can cause sexual signal unreliability, as predicted by theory, since male CHCs do not reliably signal heritable aspects of male attractiveness across diets and temperatures (Chapter 5). I also find G x Es in some aspects of female mate choice across temperatures (Chapter 6). In spite of the evidence for signal unreliability and variation in female mate choice across environments, I show that the overall outcome of mate choice is unaffected by G x Es, such that the same male genotypes are attractive across diets and temperatures (Chapters 5 and 6). From my results, it seems likely that females assess male attractiveness based on multiple male sexual signals, so that whilst male CHCs influence mate choice, CHC profile does not necessarily correlate well with overall male attractiveness. I discuss the implications of these results for the evolution of sexual traits and the genetic covariance between male and female sexual traits across environments. The research in this thesis highlights the importance of multivariate studies of sexual selection across environments for a more complete understanding of the evolution of sexual traits.

ACKNOWLEDGEMENTS

My thanks begin with my parents and my older brother, who have now spent 26 years supporting and encouraging me, no matter where in the world I've been. I can never thank them enough for always being there to listen and to help me.

Thanks to Will Pitchers for cheering me up when I needed it, and for being an invaluable source of confidence when my self-confidence failed. Also, he made sure I had a steady supply of ice cream, which is very important. Thanks to my AMAZING snorkelling and wine-drinking partners, Megan Head (I honestly could not have done my final year without you, so I can almost forgive you for beating me at Wii dance all the time) and Ruth Archer (thanks for smiling even when we were grumpy). I expect them to set up a lab and give me a job so that we can do ALL the science together (and also more snorkelling and wine-drinking). Thankyou Zen Lewis (for words of wisdom and lots of good evenings in the pub), Kaat Brulez (for sea-swimming and a shared love of Scotland), Devi Newcombe (for hugs and an amazing time in Switzerland), Caro Moussy (for surfing and dark chocolate biscuits), Fran Tyler, Kim Stokes, Jenni Sanderson, Damo Smith, Josie Orledge, Xav Harrison, Katy Gavrilchuk and all the many, many others who made the CEC such a fun and friendly place to spend the last few years. Thanks also to the excellent Team Fly - Kristy 'The Rock' Flowers, Sarah 'The Fox' Lane, Mikey 'The Hawk' Hawkes, James 'The Doctor' Rapkin and Corrina 'The Scrubber' Lowry. Some of the data in this thesis wouldn't have been possible without their hard work, and I also want to thank them for making the lab a fun place to be. It couldn't have happened at a better time for me and I was proud to be 'The Boss'. There have also been a number of other people in fly lab who have helped me out and made my PhD more enjoyable along the way - thanks to Ali Skeats, Wayne Rostant, Eoín Duffy, Richa Joag, Sheridan Willis (honorary fly lab member), Clarissa House, Chris Mitchell, Tom Price, Michelle Taylor and MD Sharma. I should probably also thank the 14,349 flies which gave up their hydrocarbons or had their mating behaviour recorded (yes, I really did bother working out the total), and thanks to Natasa Fytrou in Greece, for collecting the wild drossies which I used to set up the lab populations and isolines way back at the start of my PhD.

I'd also like to thank Nina Wedell, who guided me through my MSc and continued to support me throughout my PhD. I can't really put into words how grateful I am for her kindness, moral support and honest advice. Thanks to Ian Dworkin, who patiently tutored

me on Bayesian statistics and is therefore single-handedly responsible for my conversion (although I'm still not totally sure if that's a good thing). Thanks to Dave Shuker, who gave me the encouragement I needed at the end of my Honours degree to believe I could do any of this. Thanks to Scott Pitnick for reminding me about why I love science when I was in the middle of the write-up blues.

Last, but definitely not least, massive thanks to Dave Hosken and John Hunt. Their reputations precede them - when I first started, I lost count of the number of people who said I was 'brave' for doing a PhD with both of them - but in fact, they have been brilliant, and I feel lucky to have had such intelligent and enthusiastic supervisors. I might even miss them a bit because no doubt all future science meetings are going to seem boring compared to my PhD meetings with them. Thanks to John for motivating me and helping me to learn some seriously cool quant gen; and thanks to Dave for being straight-talking and supportive when I needed it most, and of course, for encouraging me to kill pigs.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Chapter 2

Chapter 3

Chapter 4

Chapter 6

AUTHOR'S DECLARATIONS

During the research contributing to this thesis Fiona Caroline Ingleby (FCI) was supported by a studentship from the ESF. All of the chapters presented in this thesis were written by FCI with comments and editing from David J. Hosken (DJH) and John Hunt (JH). The laboratory populations and inbred lines used in Chapters 3-6 were set up by FCI using animals collected by Natasa Fytrou in Greece in April 2010. Further contributors for each chapter are detailed below.

Chapter 2

A version of this chapter was published as a review article in the *Journal of Evolutionary Biology* 23: 2031-2045. FCI, DJH and JH are grateful for the comments of two anonymous reviewers.

Chapter 3

The data in this chapter was collected by FCI, Kristy Flowers (KF), Michael F. Hawkes (MFH), Sarah M. Lane (SML) and James Rapkin (JR). Chris Mitchell provided training for gas chromatography techniques. FCI and JH conducted the statistical analyses.

Chapter 4

The data in this chapter was collected by FCI, KF, MFH, SML and JR. Ian Dworkin (ID), FCI and JH conducted the statistical analyses. FCI wrote the manuscript with comments and editing from DJH, JH and ID. A version of this chapter has been accepted for publication in the *Journal of Evolutionary Biology*.

Chapter 5

The data in this chapter was collected by FCI with assistance from Eoín Duffy and Richa Joag during mating assays. FCI and JH conducted the statistical analyses. A version of this chapter has been accepted for publication in the *Journal of Evolutionary Biology*.

Chapter 6

The data in this chapter was collected by FCI with assistance from Jack Boyle, Nicole Goodey and Claire Young during mating assays. Statistical analyses were carried out by FCI. A version of this chapter is currently in review with *Heredity*.

Appendix 3

This chapter was co-authored by FCI, DJH and JH. A version of this chapter appears in: *The role of genotype-by-environment interactions in sexual selection* (Ed: DJ Hosken and J Hunt). In press with Wiley-Blackwell.

CHAPTER 1: General introduction

1.1 Sexual selection and genotype-by-environment interactions

Since the revival of interest in Darwin's original theory of sexual selection (Darwin 1871), research has documented the causes and mechanisms of the evolution of sexual traits across an incredible range of organisms (Emlen and Oring 1977; Lande 1981; Majerus 1986; Andersson 1994; Andersson and Simmons 2006; Hosken and House 2011). It is only recently, however, that the potential importance of environmental variation has been highlighted, and research has begun to consider sexual selection in more complex scenarios (Cornwallis and Uller 2009). Sexual selection across environmental variation has been looked at in several contexts, including ecological speciation and local adaptation (Ritchie 2007; van Doorn et al. 2009), the role of the environment in determining individual condition and the expression of condition dependent sexual traits (Rowe and Houle 1996; Cotton et al. 2006), and most recently, the effect of genotype-by-environment interactions (G x Es) and their evolutionary consequences for sexual traits (Greenfield and Rodríguez 2004; Bussière et al. 2008; Ingleby et al. 2010).

G x Es in trait expression describe differences between genotypes in the direction and extent of phenotypic plasticity across heterogeneous environments (Lynch and Walsh 1998). In essence, G x Es describe genetic variation for phenotypic plasticity. These interactions have clear implications for agriculture, where crop yield will depend on environmental variation and G x Es, and selective breeding, where the response to selection can be affected by the environment and G x Es (Falconer 1952; Kang and Gauch 1996).

The broad evolutionary consequences of G x Es in natural selection have been explored theoretically (Via and Lande 1985; Via and Lande 1987; Gillespie and Turelli 1989) and empirically (e.g. Mousseau et al. 1987; Wade 1990; Vieira et al. 2000). Recent attempts at modelling G x Es in sexual traits have built on these original evolutionary genetic studies (Kokko and Heubel 2008; Higginson and Reader 2009). As well as confirming the potential for G x Es to maintain genetic variation in traits (which has particular relevance within the field of sexual selection as a resolution to the lek paradox; Kirkpatrick and Ryan 1991), these specific sexual selection models have also shown that G x Es could disrupt sexual signal reliability and the genetic covariance between female preference and male attractiveness

across environments. In Chapter 2, I discuss these model predictions in more detail. Chapter 2 also provides a review of the empirical literature on G x Es in sexual selection.

1.2 Study system

Research on the role of G x Es in sexual selection is complex because of the need to quantify and understand not only the G x E effects themselves, but also the interaction between the male and female which is intrinsic to sexual selection. The research presented in this thesis examines sexual selection on cuticular hydrocarbons (CHCs) in *Drosophila simulans* as a model system to examine G x E in male and female sexual traits, and the consequences of these interactions for sexual selection across heterogeneous environments.

Drosophila CHCs are an ideal trait to consider with respect to G x Es in sexual selection. There is clearly substantial genetic variation for CHC profile, as studies of both *D. serrata* and *D. simulans* have found that CHCs are highly heritable (Hine et al. 2004; Sharma et al. 2012a), and also that CHC profile can evolve through natural and sexual selection (Blows 2002; Chenoweth and Blows 2005; Chenoweth et al. 2008; Rundle et al. 2009; Sharma et al. 2012b). However, natural and sexual selection will not necessarily favour the same combinations of CHCs (Ferveur 2005). Natural selection is likely to favour production of CHCs which offer the insect protection, such as long-chained CHCs which create a stable, protective layer on insect cuticle and prevent desiccation or infection. Sexual selection, on the other hand, will favour more volatile short-chained CHCs which might be transferred or detected more easily and therefore allow communication between individuals. Opposing selection will create environment-dependent optima and trade-offs for CHC expression. Indeed, studies of *D. serrata* have found trade-offs (Blows 2002), opposing selection (Chenoweth et al. 2008) and condition dependence (Gosden and Chenoweth 2011) in CHC expression.

Sexual selection in *D. simulans* is likely to be driven by indirect benefits of female mate choice through heritability of male attractiveness (Taylor et al. 2007; Taylor et al. 2008; Hosken et al. 2008). Since CHC profile in this species is heritable and subject to sexual selection (Sharma et al. 2012a,b), CHCs are an ideal candidate trait to signal heritable aspects of attractiveness and to examine the operation of sexual selection across environments. However, nothing is known about how environmental variation might affect

heritability of CHCs, or how selection on CHCs, through mate choice, might vary across environments.

1.3 Breeding design

Almost all of the research in this thesis uses quantitative genetics. Broadly speaking, the question of what effect G x Es might have on the process of sexual selection is one of how sexually-selected phenotypic variation relates to underlying genetic variation, and how this genotype-phenotype relationship might change across environments. By use of a breeding design and environmental manipulations, I have been able to partition genetic variation in trait expression between genetic and environmental components; as well as calculate heritability and genetic correlations which allow insight into the evolutionary potential of a trait.

A variety of quantitative genetic breeding designs exist, and the choice of breeding design dictates to what level variance can be partitioned. The research in this thesis is based on a large set of inbred lines (iso-female lines, referred to as 'isolines') which I derived from wild-caught female *D. simulans* at the start of 2010. By using isolines, I was able to identify genetic variation in trait expression, V_G, from environmental variation caused by the environmental manipulations. However, the use of isolines (as opposed to a more complex paternal half-sibling breeding design) sacrifices the ability to partition the additive genetic variation, V_A, within estimates of V_G. Since a lot of evolutionary genetic theory is modelled with V_A, this is not ideal, but on the other hand, using a simpler breeding design has made the complex experiments and analyses in this thesis possible.

1.4 Thesis outline

I used a combination of quantitative genetics, environmental manipulations, behavioural assays and phenotypic measurements in order to address the broad question of the role of G x Es in sexual selection. Chapter 2 is a version of a review article which was published in the *Journal of Evolutionary Biology* in 2010. In this article, I discuss G x Es in sexual selection and outline areas for further work in the field, some of which the empirical research in Chapters 3-6 contributes to.

Explicitly, I used inbred lines of *D. simulans* to examine genetic, environmental (diet and temperature) and G x E effects on the expression of CHCs, and examined the

consequences of these G x Es in terms of sexual selection and mate choice in this species. Throughout all my research, I used flies from isolines or an outbred population which were derived from the same wild-caught females, therefore representing the same genetic background, and I used the same axes of environmental variation, such that the results from each experiment are relevant to one another and contribute to a larger picture of G x Es in sexual selection in *D. simulans*.

In Chapter 3, I use a standard selection analysis to examine patterns of sexual selection through female mate choice on male *D. simulans* CHC profile, with males reared across different environments. By combining the results of this selection analysis with the genetic variance-covariance matrices underlying CHC expression identified in Chapter 4, I was also able to calculate the genetic constraints on the evolution of CHC profile within each environment. Chapter 4 identifies G x Es in both male and female CHC expression and by estimating the variance components, heritability and genetic correlation of CHC expression across environments, I was able to assess the potential consequences of these interactions on CHC evolution. Chapter 5 provides a direct test of one of these potential consequences – that, as predicted by theory, G x Es in sexual signal expression will compromise signal reliability. In Chapter 6, I tested for G x E in female mate choice, and specifically examined G, E and G x E variation for female mate choice for male CHCs. I was also able to test the genetic covariance between female preference and male attractiveness across environments. Finally, in Chapter 7 I discuss the thesis as a whole and highlight directions for future research on the role of G x Es in sexual selection.

CHAPTER 2: The role of genotype-by-environment interactions in sexual selection

2.1 Abstract

Genotype-by-environment interactions (G x Es) in naturally selected traits have been extensively studied, but the impact of G x Es on sexual selection has only recently begun to receive attention. Here we review recent models and consider how G x Es might affect the evolution of sexual traits through influencing sexual signal reliability, and also how G x Es may influence variation in sexually selected traits and the process of reproductive isolation. We then assess the current empirical literature on G x Es in sexual selection and conclude by highlighting areas that need additional work. Research on G x Es and sexual selection is an important new area of study for the discipline, which has largely focused on relatively simple mate choice/competition scenarios to date. Investigators now need to apply this knowledge to more complex, but realistic situations, in order to more fully explore the evolution of sexual traits, and in this review we suggest potentially useful directions for future research.

2.2 Introduction

Genotype-by-environment interactions (G x Es) occur whenever the relative performance of different genotypes is dependent on the biotic and/or abiotic environment in which they are expressed (Lynch and Walsh 1998). G x Es have been extensively studied for over half a century in an agricultural context to improve crop yields and the efficacy of selective breeding programmes (see Falconer 1952; Kang and Gauch 1996). However, despite recent modelling attempts (e.g. Kokko and Heubel 2008; Higginson and Reader 2009) and increasing attention in empirical research over the last decade, relatively little is known about the role of G x Es in sexually selected traits and sexual trait coevolution (but see Greenfield and Rodríguez 2004; Bussière et al. 2008).

In this review, we summarise predictions from recent models which have investigated how G x Es might influence sexual selection, and also consider current empirical research on G x Es in male sexual traits and female mating preferences. Our review highlights the paucity of empirical studies of G x Es in sexual traits, and how the theoretical work which has been done would benefit from further empirical testing. We therefore

finish by outlining possible directions that future research may take to improve our understanding of the role that G x Es play in sexual selection.

2.3 G x Es and the expression of phenotypic traits

G x Es influence trait expression so that individuals with identical genotypes can have different phenotypes when exposed to different environments. This can be clearly illustrated as a reaction norm, where the phenotypic expression of a trait is plotted separately for each genotype in alternate environments (Figure 2.1; Lynch and Walsh 1998). In some recent theoretical papers, G x Es have been classified as either "strong" interactions which cause ecological crossover between reaction norms (i.e. the ranked performance of genotypes changes between environment), or as "weak" interactions which do not cause ecological crossover (see Figure 2.1) (Kokko and Heubel 2008; Higginson and Reader 2009). These so-called weak G x Es change the scale of genetic variation across environments, but the rank order of genotypes remains the same in each environment and only the relative strength of the selective advantage varies. That is, the variation in genotype performance is reduced in one environment relative to the other, under the assumption that selection on other traits remains constant between environments. However, this classification of G x Es as either "strong" or "weak" is somewhat idealised and depends strongly on the scale and extent of environmental variation which is considered. In other words, if G x Es are visualised graphically as non-parallel gradients of reaction norms for different genotypes (as in Figures 1b and 1c), then every G x E will involve ecological crossover at some point along an infinite x-axis. Thus, whether a G x E is identified as "strong" or "weak" is merely a consequence of the scale and boundaries of the x-axis (i.e. the range of environmental variation which is studied). As such, these classifications may be useful theoretical concepts, but empirically they may be misleading. It might be more helpful empirically to compare the strength and influence of an interaction on trait expression by direct comparison of reaction norm gradients. It is also important to remember that the "strength" of a G x E will be influenced by the genetic variation for the characters in question. For instance a "strong" G x E, with ecological crossover of reaction norms, may actually have less impact than a "weak" G x E, with changes in the scale of variation, when additive genetic variation is lower in the former instance and larger in the second.

2.4 Modelling G x Es and their potential role in sexual selection

In the context of sexual selection, G x Es are likely to be very important. They could affect the expression of both male sexual traits and female mating preferences for them, which would ultimately influence how these traits co-evolve. Furthermore, G x Es might account for claims that sexual selection generates limited evolution in some free-living populations (Grant and Grant 2002). However, this is a relatively new field of research and even theoretical studies are yet to consider many of the possible ways in which G x Es could potentially influence the evolution of male sexual traits. So far, models have explored how G x Es could disrupt the reliability of sexual signals (Higginson and Reader 2009) and how they might facilitate the maintenance of variation in sexually selected traits (Kokko and Heubel 2008).

The reliability of sexual traits as signals

Many models of sexual selection and the evolution of female mating preferences require that male sexual traits reliably signal some aspect of male quality that enables females to benefit from costly mate choice (Zahavi 1975; Grafen 1990; Johnstone 1995). These benefits can be either direct to the female through materials and resources which might help her produce and raise offspring, or indirect through heritable genetic gains for offspring. If only high quality males are capable of producing exaggerated sexual signals, then females can assess male quality via the sexual trait, secure fitness benefits, and female mate preferences will be advantageous (Grafen 1990).

However, there are a number of circumstances in which G x Es in male sexual signals could disrupt signal reliability, causing females to effectively make the "wrong" mating decision (Greenfield and Rodríguez 2004). As an example, consider male bushcrickets which call to attract females using specialised structures on the wings that are fixed at eclosion to adulthood. During mate choice, females use calls to assess male quality, choosing to mate with high quality males that are able to produce large, nutrient-rich spermatophores. However, if the environment changes between when males develop their wings and when they become sexually mature and start calling, or similarly if migration occurs between these times, then wing morphology, and the resulting quality of song a male produces, represent his condition and quality in the initial environment which is no longer relevant. Consequently, females might choose a male based on an attractive call, but receive a poor

quality spermatophore in return. In this way, G x Es in heterogeneous environments could cause the signal received by the female to be an unreliable indicator of the quality of the male and of the benefits he can provide (Higginson and Reader 2009), and this will have implications in the evolution of mating preferences and could potentially eliminate any selective advantage to mate choice in the first place.

Equally, females can use male sexual signals to assess genetic quality. Indirect genetic benefits are generally mediated through genes that either confer sexual attractiveness or viability to offspring, and studies have found that attractive males do sire attractive sons, for example (e.g. crickets, Wedell and Tregenza 1999; flies, Taylor et al. 2007). However, the reliability of indicators of male attractiveness could be disrupted by G x Es and environmental fluctuations in the same manner as the direct benefits discussed above (Kokko and Heubel 2008; Higginson and Reader 2009), as could the reliability of viability indicators. For instance, male sticklebacks (*Gasterosteus aculeatus*) in good condition can produce brightly pigmented patterns that are attractive to females. In populations with parasites, condition is correlated with resistance to infection, and so females can use these sexual signals as indicators of viability genes which confer parasite resistance to her offspring (Barber et al. 2001). However, parasite populations will vary both spatially and temporally, creating situations where a male might develop in the absence of parasites, and then produce an attractive signal despite not being resistant to infection.

The issue of signal reliability is likely to be even more complex when females assess multiple sexual traits during mate choice, as appears common in many species (Candolin 2003). For example, in the field cricket, *Gryllus campestris*, males produce an advertisement call to attract a mate and females prefer males that produce calls with an increased chirp rate (Holzer et al. 2003) and a lower carrier frequency (Simmons and Ritchie 1996). Carrier frequency and chirp rate are uncorrelated components of the call (Holzer et al. 2003; Scheuber et al. 2003a) and carrier frequency, but not chirp rate, is negative correlated with adult body size. Carrier frequency reliably signals juvenile, but not adult, condition with juveniles experiencing good nutrition during development growing larger and producing a call with a lower carrier frequency (Scheuber et al. 2003b). Conversely, chirp rate is not influenced by juvenile condition but reliably signals adult condition, with adults fed a more nutritious diet calling at an increased chirp rate (Scheuber et al. 2003a).

Consequently, if individuals occupy heterogeneous environments and there are G x Es for these traits, then the signal content of them can become uncoupled, making it difficult for a female to fulfil both preference criteria reliably. It is even possible that females will receive conflicting information from the traits they are assessing (i.e. a male producing a high carrier frequency but producing a high chirp rate).

The reliability of sexual signals is a key assumption in most models of sexual selection, because if not, selection for costly mate choice should be significantly weakened. Some models even predict that mate choice should not evolve in populations where this positive correlation does not exist (Kokko et al. 2006). Two recent models that have considered how G x Es can influence signal reliability use different modelling approaches, but largely reach the same conclusion (see Box 2.1). That is, interactions modelled both with and without ecological crossover can disrupt the reliability of sexual signals (Kokko and Heubel 2008; Higginson and Reader 2009) and, under certain conditions, can result in a negative correlation between female preference and male quality (Higginson and Reader 2009). This situation is not predicted by classical models of sexual selection, but clearly indicates how important G x Es could be in sexual selection.

Kokko and Heubel (2008) explored sexual signal reliability by modelling the costs of mating preferences tolerated by females, which is used as a proxy for the strength of female mating preferences (see Box 2.1). Where G x Es exist in sexual trait expression, a major cost could be the potentially low information content of male signals of quality, and the resulting increased chance that a female will make a mistake when expressing mate choice. The model looks at how gene flow between environments affects the costs of female mating preferences and the results clearly indicate that selection for female mating preferences disappears under high levels of gene flow (with environmental structure) (see Box 2.1). This could be attributed to the high costs of female mate choice, which result from the low reliability of male sexual signals, which are in turn caused by G x Es and environmental variation (change).

Higginson and Reader (2009) test the potential effect of G x Es on sexual signal reliability by modelling the information content of sexual signals. Interestingly, the model highlights the importance of both genetic variation and environmental variation: signal reliability can potentially be compromised both by reduced genetic variation and by increased environmental variation (see Box 2.1). The model also emphasises the influence

of harsh, or stressful, environmental conditions which can severely reduce the information content of sexual signals.

The next obvious theoretical step would be to consider the consequences of unreliable sexual signals on the evolution of female mate preferences. It follows that selection for female choice will be weakened if male sexual traits do not reliably signal some female benefit. This potentially has knock-on effects for trait and preference evolution. Indeed, Greenfield and Rodríguez (2004) suggested that signal reliability in traits affected by G x Es can only be fully maintained when the reaction norms for the size of the male trait and the corresponding female preference are parallel across environments.

Alternatively it is possible that some information is better than none at all, meaning that even when G x Es exist for male sexual traits, females that utilize the little information in these signals have less variance in fitness than females not using "unreliable" signals. Again, this needs explicit testing, by, for example, comparing female choice benefits in constant environments and fluctuating environments, with females not given a choice of mates. Either way, empirical research needs to look at both male trait expression and female mating preferences in order to account for the coevolution of sexual traits.

G x Es and the maintenance of genetic variation in sexual traits

The maintenance of genetic variation in sexual traits is important. If genetic variance is depleted, females may not be able to reliably gain indirect benefits of mate choice. This is the essence of the "lek paradox", which asks how genetic variation (on which mate choice depends when males provide only indirect benefits) can be maintained in the face of sustained directional sexual selection from female choice (Kirkpatrick and Ryan 1991). Many studies have examined how environmental variation might maintain genetic variation, particularly with respect to the effects of stressful or unfavourable conditions, although the focus is typically not on sexually selected traits (reviewed by Hoffmann and Merilä 1999). A recent meta-analysis of the effect of environmental stress on genetic variation in wild populations concluded that stressful conditions cause an overall reduction in genetic variation, although the effect was smaller in traits more closely correlated with fitness than in morphological traits (Charmantier and Garant 2005).

Depletion of the genetic variation in male sexual traits in harsh environments could contribute to signal unreliability. Depending on the mechanism of female mate choice, a

threshold for male attractiveness might not be met in harsh environments, or alternatively mating decisions based on relative male attractiveness might be difficult with decreased variation between males. Higginson and Reader's (2009) model of sexual signal reliability with G x Es suggests as much, with the explicit prediction that signal reliability was greatly reduced in harsh environments, and also that signal reliability was generally lowest with low genetic variation in male quality.

Genetic variation might not only be affected by stressful environmental conditions, but also simply by temporal and spatial environmental fluctuations which are a characteristic of most natural environments. It has long been recognised that environmental heterogeneity could potentially facilitate the maintenance of genetic variation in naturally selected traits (Hedrick et al. 1976), and this idea was modelled explicitly for traits with G x Es by Via and Lande (1985). For a given trait expressed in different environments, evolution is not independent in each environment. With no G x E, there will be a positive correlation between trait expression in one environment and trait expression in another environment. However, where G x Es exist for trait expression, this across environment correlation can be weakened or become negative. Assuming there is some level of gene flow between environments (either spatially between populations or temporally through overlapping generations), genetic variation can then be maintained as a result of disruptive selection across environments. Gillespie and Turelli (1989) focussed on naturally selected traits with a model which demonstrated that significant trait variation could be maintained by the presence of G x Es in heterogeneous environments, and furthermore, that this effect could often be missed when only a narrow range of environmental variation is studied. A number of empirical studies support these predictions. For example, in laboratory populations of *Tribolium castaneum* kept on a variety of food substrates, body size evolution was not independent across environments (Via and Connor 1995), and field-based experiments with *Drosophila melanogaster* have shown that genetic polymorphism in naturally selected traits can be maintained in heterogeneous environments (Mackay 1980; Santos et al. 1999).

More recently, there have been attempts to apply this general theory explicitly to G x Es in sexually selected traits (e.g. Kokko and Heubel 2008; Box 2.1). The idea that G x Es might contribute to the maintenance of genetic variation in sexually selected traits is frequently proposed as a possible solution to the lek paradox, with genetic variance

depleted through female mate choice but also maintained through the effect of G x Es. Kokko and Heubel (2008) modelled the potential of G x Es in sexually selected traits to maintain enough additive genetic variation to sustain indirect benefits of female mate choice when choice was costly, and hence select for the evolution of female mate choice (Box 2.1). Their results demonstrate that G x Es can help to maintain enough genetic variation for the persistence of indirect benefits of female mating preferences, but that this is heavily dependent on the extent to which reproductive individuals from different environments mix (gene flow). With mixing of reproductive individuals from different developmental environments there was increased genetic diversity within populations, maintaining the variation on which females could base mating decisions.

However, as described above, migration or environmental change before mating can disrupt sexual signal reliability (see Box 2.1; Figures 2.2a and 2.2b). As a result, low levels of gene flow between environments selected for female mate choice, but with high levels, costly mating preferences were selected against despite the genetic variation in male traits in the population. Kokko and Heubel's (2008) model highlighted the fact that it can be uninformative to consider how G x Es might maintain variation in sexually selected traits without also testing how they might disrupt sexual signal reliability. Indeed, the influence of genetic variation on the information content of sexual signals was also considered by Higginson and Reader (2009). Signal reliability increased with increasing variation in genetic quality, but this effect was weakened in heterogeneous environments where the correlation between male trait size and genetic quality was disrupted by G x Es (see Figure 2.3a). In heterogeneous environments, G x Es can disrupt male sexual signal reliability and so weaken selection for female mating preferences, but conversely, environmental fluctuations can help maintain genetic variation and so maintain the advantages of mating preferences. A single model that includes both of these aspects would be enlightening.

Many aspects of sexual selection have been examined in quantitative genetics models, and many of these are built on Lande's (1981) original polygenic model. However, quantitative genetic modelling has yet to be applied directly to the role of G x Es in sexual selection. Arguably, the existing quantitative genetics models of sexual selection, combined with methods which have been developed for modelling G x Es (e.g. Nussey et al. 2007), contain all the relevant detail that needs to be brought together to examine G x Es in sexual selection. Alternatively, new models which integrate tests of signal reliability and

maintenance of genetic variation together would be useful, as would polygenic models which can account for male and female sexual trait coevolution in the context of G x Es. The nature of these traits means that quantitative genetics coupled with computer simulations could be another useful direction for theoretical research.

Currently however, existing theoretical models have explored the role of G x Es in sexual selection far more comprehensively than empirical research in this field. While the existing models provide us with a number of predictions on how G x Es might influence genetic variation in sexual traits and the reliability of sexual signalling, the field would benefit greatly from empirical tests of these predictions, as well as tests of the underlying assumptions of these models.

G x Es and population divergence

Given that speciation can be driven by local adaptation to different environmental conditions (Bush 1975), it follows that G x Es in naturally selected traits could affect this process. If a population is subdivided and then subject to different environmental conditions, local selection will drive the evolution of genetic differences between populations, potentially leading to speciation (Wade 2000). The potential role of G x Es in this process appears to depend strongly on whether or not there is gene flow between isolated populations. Where gene flow does occur, G x Es could act as a constraint on local adaptation (Via and Lande 1985) and slow the rate of population divergence, particularly with disruptive selection acting between environments. This has been demonstrated empirically in experimental meta-populations of *T. castaneum* (Wade 1990). However, the potential for G x Es to act as a constraint will be determined by the immigration of genes from other environments, as well as the relative strength of selection and the strength of the G x E. In the absence of gene flow between populations this constraint is removed and genetic divergence should proceed (Wade 2000).

Sexual selection has also been implicated in population divergence due to the strong potential for sexual selection on male sexual traits or female mating preferences to cause reproductive isolation (West-Eberhard 1983). It is therefore likely that G x Es in sexual traits or mating preferences might also affect speciation but, in spite of this, this possibility has not been studied in any depth. Again, as the traits in question are polygenic, quantitative genetic models are ideally needed to account for the co-evolution of male and female

sexual traits. Furthermore, like models of speciation with G x Es in naturally selected traits, gene flow between separated populations is likely to be important. For instance, G x Es in sexual traits combined with high levels of gene flow between populations will compromise the reliability of sexual signals, weakening selection for female mating preferences, as described above. As a consequence, the presence of G x Es in sexual traits could act as a constraint on the evolution of reproductive isolation. Indeed, Etges et al. (2007) found that G x Es in the male courtship song of *D. mojavensis* might slow population divergence due to the disruption of sexual signal reliability. However, without further attention, it is impossible to fully understand the complex ways that G x Es might affect reproductive isolation.

2.5 Empirical studies of G x Es in sexual selection

As a result of recent increased interest in the role of G x Es in sexually selected traits, there is a slowly growing body of empirical work examining the effect of genetic and environmental factors on sexual trait expression. We summarise some of these studies in Table 2.1. This is unlikely to represent an exhaustive list of studies which have had the potential to test for G x Es in sexual traits, as negative results may not have been published. Most studies appear to have focussed on the identification of G x Es in the expression of male sexual traits (e.g. David et al. 2000; Etges et al. 2007; Engqvist 2008; Morrow et al. 2008). Table 2.1 illustrates that G x Es in male sexual traits are apparently common and found across a wide range of species, although notably not ubiquitous (see Miller and Brooks 2005; Kemp and Rutowski 2007). Furthermore, it is clear that G x Es frequently cause ecological crossover of reaction norms across environments, although as discussed previously, this does not necessarily indicate a strong influence of the G x E interaction on sexual trait evolution. The breadth of studies identifying G x Es in male sexual traits is in contrast to the mere two studies to date which have directly tested and found G x Es in female mate preference, both of which have involved laboratory model insect species: the lesser waxmoth, *Achroia grisella* (Rodríguez and Greenfield 2003), and the fruit fly, *D. melanogaster* (Narraway et al. 2010). The latter study further demonstrated that the genetic variance underlying female preferences differed between environments.

It is also clear from Table 2.1 that research so far has primarily focussed on testing how trait expression is affected by abiotic environmental factors. For example, Olvido and

Mousseau (1995) found significant G x Es for calling rate and call duration in male crickets (*Allonemobius fasciatus*) dependent on rearing temperature and photoperiod, and Jia et al. (2000) showed that the pulse rate of the acoustic signal in male *A. grisella* exhibited significant G x Es depending on both rearing temperature and food quality.

A few studies have, however, begun to consider the effect of biotic environmental factors (Table 2.1), which is reassuring since the biotic environment is probably subject to greater and more rapid change than the abiotic environment (Wolf et al. 1999) and therefore likely to have a stronger influence on sexual selection. However, manipulation of the biotic environment has often involved altering density (e.g. Morrow et al. 2008) or brood size (e.g. Mills et al. 2007), and whilst these studies demonstrate G x Es in the male sexually selected traits examined, it is difficult to determine precisely what is causing the variation in the male trait: it could either be a direct consequence of social interactions or an indirect result of reduced food availability. This distinction between social environment and other environments is likely to be important, as different evolutionary dynamics might be caused by social environmental factors, which have a genotype and are selected on themselves. However, a recent study by Kent et al. (2008) explicitly tested the effect of both abiotic and biotic environmental variation using *D. melanogaster* isofemale lines under different light cycles and different social environments. They found that the composition of cuticular hydrocarbons (CHCs), which act as male sexual signals in this species, exhibits significant G x Es with both the abiotic (i.e. light:dark cycle) and social (i.e. social competitors) environmental factors examined.

Another interesting pattern that emerges from the empirical studies on G x Es presented in Table 2.1 is the diversity of breeding designs used to account for the genetic component of sexual trait expression. Although a single given breeding design is not always amenable to all study species, it is important to recognise that these designs differ markedly in the quality of information they provide on G x Es. For example, some studies have quantified G x Es by regressing the sexual trait of the father against that of the son when expressed in alternate environments (i.e. parent-offspring regression) (e.g. Qvarnström 1999). Other studies have measured genetic divergence between isolated populations under different environmental conditions to test for G x Es (e.g. Olvido and Mousseau 1995), or have used either a full-sibling breeding design (e.g. Etges et al. 2007) or isofemale (inbred) lines (e.g. Danielson-François et al. 2006) (Table 2.1). The limitation of these

approaches is that while they show that genes differ in their expression across environments, they are unable to differentiate between genes with an additive effect from those that have a non-additive effect (i.e. dominance and/or epistasis) (Lynch and Walsh 1998). Most quantitative genetic models that examine the evolutionary implications of G x Es are based on additive genetic variance (e.g. Via and Lande 1985), and as such, if empirical results are to be directly linked to existing theory, they should make this distinction also. Of the variety of breeding designs shown in Table 2.1, only the paternal half-sibling design is able to partition the effects of additive and non-additive genetic variance on the expression of sexual traits in alternate environments (Lynch and Walsh 1998).

As well as the breeding design, another consideration when designing experiments should be the substantial statistical power which will be needed in order to detect a G x E interaction. In Table 2.2, we have extracted standardised effect sizes, where possible, from the studies cited in Table 2.1 which identify G x E interactions. G x E effect sizes are clearly very small. In fact, the effect sizes we found are generally slightly lower than those found for "good genes" effect sizes by Møller and Alatalo (1999), who used similar methods to calculate the effect sizes from sexual selection studies. Since testing for G x Es generally involves measurement of how much phenotypic variation is due to the interaction between environmental and genetic factors, they will be subject to a lot of noise, and so in order to detect a significant G x E of such a small effect size will require large studies with high statistical power.

Table 2.1 also highlights the extensive empirical research conducted on G x Es in the waxmoth, *A. grisella*. Not only have G x Es been identified in male sexual traits (e.g. male acoustic sexual signals; Danielson-François et al. 2006), but the first positive identification of a G x E in female mate choice was demonstrated in this system (Rodríguez and Greenfield 2003), and remains to date one of only two studies demonstrating that G x Es in female mating preferences exist (see also Narraway et al. 2010). Furthermore, although a few studies have considered the fitness consequences of potential G x Es, this is the only system in which G x Es in sexual traits have been studied in any depth. Having identified G x Es in both male sexual traits and female mating preferences (Rodríguez and Greenfield 2003; Danielson-François et al. 2006), research then began to consider the role of G x Es in sexual selection. The potential of G x Es to facilitate the maintenance of variation in male sexually-selected traits has been demonstrated (Jia et al. 2000), and it has also been shown that G x

Es can alter the fitness consequences of mate choice (Jia and Greenfield 1997; Danielson-François et al. 2009). This is an important finding and highlights that whilst demonstrating that G x Es exist for sexual traits is an essential starting point for determining the role of G x Es in sexual selection, the next step is to test whether these G x Es alter the fitness consequences of female mate choice. Few of the studies in Table 2.1 have considered the effects G x Es in male sexual traits have on female mate choice and the possible benefits gained by the female. Furthermore, those which have assessed the fitness consequences have only focussed on indirect benefits (e.g. Qvarnström 1999; Welch 2003), and as discussed previously, it is possible that G x Es could also affect the relationship between a male sexual signal and the direct benefits he can offer a female.

These studies show that G x Es can often cause the indirect benefits of mate choice to be dependent on environmental variation, and as a result, G x Es are likely to be highly influential in the evolution of female mating preferences. With knowledge of the frequency of G x Es in male sexual traits and how they might influence fitness, more complex evolutionary questions can then be addressed, concerning the identification of G x Es for female mating preferences, and the effect of G x Es on the co-evolutionary dynamics between female preference and male sexual traits.

2.6 Future directions

Further research on G x Es in sexual selection needs to begin by focussing on female mating preferences, as these have largely been neglected in studies so far. The lack of research on this subject could be in part due to the poor understanding of the evolution and genetics of female mate choice in general (Bakker 1999; Mead and Arnold 2004). Empirical data on G x Es in female mating preferences will firstly allow us to determine whether G x Es are as strong and as widespread as the interactions already documented for many male sexually-selected traits. There are only two studies that have identified G x Es in female mating preferences, one in the lesser waxmoth (Rodríguez and Greenfield 2003) and one in *D. melanogaster* (Narraway et al. 2010). The potential for G x Es in female mate choice has largely been ignored to date, and could represent another way (in addition to unreliable male sexual signals) in which females could make the "wrong" mating decision and fail to gain benefits from mate choice. The fitness consequences of a mating decision should drive the evolution of mate choice, and as such, it is likely that G x Es in sexual traits could have a

strong impact on the evolution of mate preferences. It is also possible that female preferences demonstrate adaptive plasticity (Shuster and Wade 2003), and vary between environments such that preferences track variation in male signals across environments. This possibility requires additional research as so far the influence of G x Es on the adaptive plasticity of mating preferences has only been assessed in female waxmoths (Rodríguez and Greenfield 2003).

Once the occurrence and strength of G x Es has been identified in both male sexual traits and female mating preferences, research should focus on testing the potential roles of G x Es in sexual selection and in the evolution of sexual traits and mating preferences. With this aim in mind, we have outlined the following possible avenues for future research. We discuss: (1) why it is important that research integrates male and female sexual traits in G x E studies in order to consider sexual trait coevolution, (2) gaps in our understanding of how abiotic environmental variation might affect sexual trait expression, (3) the influence of biotic environmental factors and social environment on sexual trait expression, (4) the relevance of the "strength" of G x E interactions and (5) the potential for future research into the genetic mechanisms which underlie G x Es. We hope to emphasise how these research directions could support the existing theory and further develop our understanding of the role of G x Es in sexual selection.

1. Integration of male and female traits

Mating involves an interaction between a male and female through sexual signalling and a mate-choice response, and since these male and female traits are expected to co-evolve, incorporating both into one study is essential to understand evolutionary dynamics. So far, neither modelling nor empirical studies of G x Es have fully attempted this. From a modelling perspective, the existing single-locus models of G x Es in sexual selection represent a good start, but these models remain inadequate because the traits in question are likely to be polygenic. The utility of quantitative genetic models in the study of sexual selection is illustrated by a direct comparison between the one or two loci models of O'Donald (1980), which demonstrated linkage building up between male sexual signal and female preference traits, with Lande's (1981) polygenic model, which not only showed this, but additionally was able to fully demonstrate Fisher's runaway process. Quantitative genetics are necessary to incorporate patterns of both inheritance and selection on

continuously varying traits into models, which is vital when modelling the coevolution of sexual signal and preference traits (Mead and Arnold 2004). More realistic multi-locus models should also be used to examine how G x Es affect the expression of both male and female sexual traits and how this, in turn, affects the interaction between the individuals during mate choice. If G x Es exist in the expression of either the male sexual trait or the female mating preference, then the co-evolution of the two traits could be strongly disrupted by environmental heterogeneity, particularly in light of the results of the recent models which have demonstrated how G x Es can affect sexual signal reliability and levels of genetic variation in sexual traits.

Empirical studies which integrate G x Es in male sexual traits and female mating preferences could specifically test how G x Es influence the coevolution of male and female sexual traits by assessing genetic associations between the two. Empiricists should also evaluate the extent to which sexual signal reliability is disrupted or otherwise altered by G x Es, and the role this might have in the evolution of female mating preferences and on the fitness consequences of mate choice.

2. Abiotic environmental variation

Our review of the empirical literature clearly illustrates that most studies of G x Es in sexual selection have looked for interactions between abiotic environmental factors and the expression of male sexual traits, and there is compelling evidence from a number of species that such G x Es exist. However, these studies should now be developed to directly address the assumptions and predictions of theory. For example, Gillespie and Turelli (1989) pointed out that in order to thoroughly test for maintenance of genetic variation in traits with G x Es, a broad range of environmental variables should be investigated, as there is a risk of failing to detect an effect when studying limited environmental heterogeneity. Identifying G x Es based on multivariate environments might be useful for a number of other reasons, including making studies more realistic, and enhancing our ability to identify interactions between environmental variables that affect trait expression.

Additionally, an explicit prediction made by Higginson and Reader (2009) is that sexual signals should become less reliable with increasingly harsh developmental environments. Indeed, Charmantier and Garant's (2005) meta-analysis suggested that genetic variation is depleted in wild populations under harsh environmental conditions,

which could contribute to unreliable sexual signals. However, it is also thought that in particularly harsh environments, only high quality males are able to afford the costs of exaggerated sexual signals, illustrating Zahavi's handicap principle and how the honesty of sexual signals can be enforced (Hoffmann and Merilä 1999). It is clear that whilst G x Es have been identified, we are still unsure of how trait expression is affected by relative degrees of environmental heterogeneity along one axis of environmental variation, and what the consequences of this are for signal reliability. By widening the range of environments employed in an empirical study, it should be possible to quantify the effects and strengths of G x Es and relate these results to existing models.

3. Biotic environmental variation and social environments

Previous research has also largely been limited to identifying G x Es in sexual traits that result from variation in abiotic environmental factors. These abiotic factors are probably the simplest to manipulate experimentally, but the paucity of studies examining biotic environmental factors, and especially social environmental factors, is surprising since sexual selection typically involves male-male competition, female choice of mates and/or sexual conflict, all of which involve social interactions. It therefore follows that the outcome of sexual selection will be influenced by the surrounding biotic and social context, and that this effect could be strong given the potential for these biotic environmental factors to vary widely over relatively short timescales (Wolf et al. 1999).

In fact, there are many empirical studies which demonstrate that variation in social environment can indeed affect female mating preferences, which illustrates adaptive plasticity of preference, although not explicitly testing for G x Es. These studies generally focus on the effect of a female's previous social experiences; for example, copying the mating preferences of other females (e.g. White and Galef 2000), or expressing preference for males with "familiar" phenotypes to those which they have experienced or interacted with previously (e.g. Hebets 2003; Dukas 2008). These studies illustrate examples from a diverse range of species and suggest that an effect of social environment on mating preferences could be common. Furthermore, work on the field cricket *Teleogryllus oceanicus* has not only demonstrated that previous social environment can affect mating preferences, but has also showed how this affects the outcome of sexual selection through changing the female's preference function and mate choice strategy (Bailey and Zuk 2008;

2009). These studies clearly demonstrate that female mating preferences can be strengthened or weakened dependent on the social environment experienced by the focal individual.

The importance of social environment is further highlighted by recent work on indirect genetic effects (IGEs), where the phenotypic expression of a focal individual is affected by interactions with conspecifics, be these parents, siblings, or unrelated conspecifics (Wolf et al. 1998). There is a considerable body of evidence showing that IGEs are important in sexual selection and the evolution of mating preferences (reviewed by Miller and Moore 2007). Furthermore modelling has shown that evolutionary dynamics can be dramatically altered when IGEs are taken into consideration (e.g. Wolf et al. 2008), and that this seems to be due to two effects that arise when environmental variation is heritable. Firstly, the environment itself will be subject to selection as well as causing selection on the focal individual, and secondly, IGEs can alter the covariance between genotype and phenotype. This covariance is important as it defines how phenotypic selection is translated into changes in gene frequency and thus evolution (Wolf et al. 1998). The distinction between G x Es for social environment and IGEs will depend partly on the question being studied (see Wolf et al. 2004 and Wolf and Moore 2010 for more in-depth discussion). Arguably, the theory developed for IGEs could generally be applied to genotype-by-social environment interactions for sexual traits. However, G x Es describe particular cases where trait expression in the focal individual is not only dependent on the genotypes of surrounding conspecifics (i.e. the social environment), but also on the genotype of the focal individual itself, since a G x E for social environment describes variation between focal genotypes in their response to variation in the social environment. This added layer of complexity may mean that predictions about the effects of genotype by social environment interactions on sexual traits are slightly different than those concerning the effect of IGEs.

G x Es for biotic environmental variation are also somewhat more general than the G x Es for social environment and IGEs. An important point is that whilst social environment covers the influence of interacting individuals of the same species as the focal individual, G x Es can involve interactions with other species in the environment. Interactions with other species could affect the phenotypic expression of a sexual trait, as illustrated in the earlier example of male stickleback pigmentation as a signal of male resistance to parasites, which

females use during mate choice. There are no studies of G x Es in sexual traits which have directly addressed the effect of biotic interactions with other species, such as parasite prevalence or non-conspecific competitors for resources. However, a few studies have recognised the importance of biotic environment and have attempted to test for G x Es in male sexual traits through manipulation of biotic environmental variables. For example, Welch (2003) manipulated population density, which might alter the intensity of competition or levels of mate availability. However, there is the potential to confound biotic factors, such as competition, with abiotic factors, such as food availability, and this may mean that more direct tests of the effect of social interactions through manipulation of the social environment (*sensu* Kent et al. 2008) may be more revealing. Since the outcome of a female's mating decision is based mainly on behavioural responses and the signalling interaction between the male and female, more studies are required if we are to understand how social environments might affect the evolution of sexual traits where G x Es exist.

4. The "strength" of a G x E interaction

As discussed previously, sexual selection models of G x Es have made a distinction between "strong" G x Es which have ecological crossover of reaction norms, and "weak" G x Es which do not (Kokko and Heubel 2008; Higginson and Reader 2009). This has enabled theoretical predictions to be based on an assumption of the strength of the influence a G x E has on trait expression, however, the distinction between these "types" of G x E does not translate easily into an empirical program. A "strong" G x E under this definition might have very little effect on sexual selection if there is low genetic variation for the traits in question, whereas a "weak" G x E could be hugely important where there is a lot of genetic variation.

Empirically, it might be more useful to estimate the "strength" of a G x E interaction as some measure of the genetic effect size of the interaction, or as some measure of the genetic variation between environments. In order to more easily apply some measure of interaction "strength" to empirical studies, we could consider trait heritability between environments, the relative gradients of reactions norms, or even the genetic effect size of the interaction directly. Alternatively, quantification of the strength of an interaction might involve measuring genetic correlation of sexual traits between environments, or measuring the covariance between male and female sexual traits, but this again is something that

requires further assessment. The "strength" of a G x E should be measured in terms which are comparable and can easily relate empiricism to theory. In doing so, there is the potential to test whether or not the "strength" of the G x E interaction is important in the outcome of sexual selection.

5. The mechanistic basis of G x Es

The research directions suggested above largely aim to improve understanding of the evolutionary consequences of G x Es in sexual traits. However, in order to fully explore the role of G x Es in sexual selection, insight into the genetic mechanisms which underlie G x Es will also be necessary. To this end, research into G x Es in sexual selection can begin to make use of what is already known about the genetics of sexual traits, although research in this field has so far largely focussed only on male sexual traits (reviewed by Emmons and Lipton 2003). This information could be used in studies which look at the genetic control of differential gene expression between environments, which is indeed beginning to be investigated in naturally selected traits in yeast, *Saccharomyces cerevisiae* (Landry et al. 2006), and the nematode worm, *Caenorhabditis elegans* (Shook and Johnson 1999; Li et al. 2006). DNA microarrays have been used to identify quantitative trait loci for plasticity of expression in traits which are known to have G x Es. These genomic techniques could similarly be applied to test for pleiotropy and epistatic gene interactions in the expression of sexual traits with G x Es, both of which have the potential to control the genetic mechanism behind G x Es in phenotypic trait expression.

2.7 Conclusions

In conclusion, research into the effect of G x Es on sexual selection has to date primarily concentrated on identifying G x Es in male sexual traits and making predictions based on models of the effects these G x Es might have on sexual selection. Research now needs to test more thoroughly for G x Es in female mating preferences, and then move on to evaluating the theoretical implications of G x Es, such as how G x Es affect sexual signalling and genetic variance, and ultimately the influence they might have on the co-evolution of male and female sexual traits. We have suggested that advances in this field of research will involve theoretical progress, through quantitative genetics models, and empirical progress, through dedicated research programmes similar to that applied to the lesser waxmoth,

where G x Es in both male and female traits are identified and the specific effects of these G x Es on sexual trait co-evolution can quantified. With this aim in mind, we have detailed five potential research directions which we feel are important to increase understanding of the role of G x Es in sexual selection. The last 30 years of sexual selection research has largely been about documenting sexual selection and its mechanisms. It is now time to move beyond this and to consider more complex scenarios and how they influence sexual selection and sexual trait evolution.

Box 2.1 Models of G x Es in sexual traits

Kokko and Heubel (2008) use a population genetics model to explore the effect of G x Es on the maintenance of female mating preferences, which are predicted to be selected for only when there is sufficient variation in sexual traits and when sexual signals reliably signal some kind of benefit for the female. By using the costs of mating preferences tolerated by females as a proxy for the strength of female mating preferences, Kokko and Heubel (2008) test how various scenarios with G x Es might affect the evolution of female mating preferences. The model compares the effect of no G x E against the effect of a G x E with ecological crossover.

The work highlights the importance of the timing of movement between environments (or, similarly, environmental change). Figure 2.2a describes the effect of migration, defined here as movement of males and females into a different environment immediately after birth, such that rearing conditions (the conditions in which viability selection takes place) are different from the conditions an individual is born in. Figure 2.2b shows the effect of male mixing, used in this model to quantify male-specific movement between environments which occurs after development (viability selection), but before mating (sexual selection). Male mixing therefore essentially describes the rate at which a given female encounters males from an alternative developmental environment than her own, as opposed to meeting males who developed in the same environment as herself. As levels of male mixing increase, the probability of a given female encountering a male from her own environment decreases. In both scenarios, the results suggest that generally selection for female mating preferences is low under high levels of gene flow (with environmental structure), although the exact effect of male mixing also depends also on the migration rate: low levels of migration coupled with low levels of male mixing somewhat alleviates the costs of female preferences, although these costs increase as male mixing increases (Figure 2.2b).

Higginson and Reader (2009) use stochastic simulations to explicitly test the effect of sexual trait G x Es without ecological crossover on sexual signal reliability. These simulations consider the information content of a male signal trait under G x Es, where each male is assumed to have a given value of genetic quality which indicates both his ability to survive environmental stress during development and his ability to produce an attractive sexual signal. Firstly, signal reliability is considered under varying degrees of environmental

heterogeneity (Figure 2.3a), and then signal reliability is modelled in environmental conditions of varying “harshness” (Figure 2.3b). Harshness is used here to create a negative relationship between an environmental variable and a male’s ability to both survive development and produce an attractive sexual signal, therefore simulating stressful or unfavourable conditions when environmental “harshness” is high.

The model makes four explicit predictions about the conditions in which male sexual signals are likely to be unreliable indicators of male quality. These are: (1) in highly heterogeneous environments (see Figure 2.3a), (2) when variation in genetic quality is low (Figure 2.3a), (3) in harsh environments where juvenile mortality is common (Figure 3b) and (4) when environmental factors have a strong influence on sexual trait expression relative to the influence of genetic quality (Figure 2.3b). This model is based on G x Es without ecological crossover, and yet it is clear that these so-called “weak” interactions can severely compromise the reliability of sexual signals, even causing a negative correlation between male attractiveness and genetic quality in particularly harsh environments (see Figure 2.3b).

Table 2.1 Summary of studies from the last 15 years which have tested genetic and environmental effects on sexual trait expression and/or the fitness consequences of potential G x Es in sexual traits.

Species	Environmental variable(s) manipulated	Breeding design	G x E in sexual trait	Ecological crossover	Fitness consequences	Reference
Bank vole (*Clethrionomys glareolus*)	Litter size	Full-sibling design	Yes- male testosterone levels (determine dominance)	No	Yes- indirect benefits to male offspring only in constant environments	Mills et al. 2007
Coal tit (*Parus ater*)	Early/late in breeding season	Maternal half-sibling design	Not tested	–	Yes- indirect benefits to offspring only late in the season	Schmoll et al. 2005
Collared flycatcher (*Ficedula albicollis*)	Year of study; brood size	Parent-offspring regression	Not tested	–	Yes- indirect benefits to sons (inheritance of attractive traits from father) only in favourable conditions	Qvarnström 1999
Collared flycatcher (*Ficedula albicollis*)	Year of study	Parent-offspring regression	Not tested	–	Yes- indirect benefits of mating attractive males varied between years	Hegyi et al. 2006
Fruit fly (*Drosophila mojavensis*)	Host plant	Divergent populations	Yes- male courtship song traits	Yes	Not tested	Etges et al. 2007
Fruit fly (*Drosophila melanogaster*)	Photoperiod; social environment	Inbred lines	Yes- composition of cuticular hydrocarbons (male	Yes	Not tested	Kent et al. 2008

			sexual signals)			
Fruit fly (*Drosophila melanogaster*)	Larval density	Inbred lines	Yes- sperm length	Yes	Not tested	Morrow et al. 2008
Fruit fly (*Drosophila melanogaster*)	Larval temperature (cold-shock)	Inbred lines	Yes- female mating preference intensity	Yes	Not tested	Narraway et al. 2010
Gray tree frog (*Hyla versicolor*)	Tadpole density	Maternal half-sibling design	Not tested	–	Yes- female mate choice only conferred benefits in certain environmental conditions	Welch 2003
Guppy (*Poecilia reticulata*)	Food quality	Full-sibling design	Yes- male carotenoid pigmentation	Yes	Not tested	Grether 2000
Guppy (*Poecilia reticulata*)	Social environment	Full-sibling design	No- tested male pigmentation and display behaviour	No	Not tested	Miller and Brooks 2005
Lesser waxmoth (*Achroia grisella*)	Larval density, food quality, temperature and photoperiod	Full-sibling design	Yes- pulse rate of male acoustic sexual signal	Yes	Not tested	Jia et al. 2000
Lesser waxmoth (*Achroia grisella*)	Rearing temperature	Full-sibling design	Yes- female mating preferences	Yes	Yes- threshold for female mating preference differed between environments	Rodríguez and Greenfield 2003

Lesser waxmoth (*Achroia grisella*)	Larval density	Inbred lines	Yes- male acoustic sexual signal	Yes	Not tested	Danielson-François et al. 2006
Lesser waxmoth (*Achroia grisella*)	Larval competitive environment	Inbred lines	Not tested	–	Yes- indirect benefits of attractive males were dependent on environment	Danielson-François et al. 2009
Orange sulphur butterfly (*Colias eurytheme*)	Larval food quality; temperature	Full-sibling design	No- tested male wing pigmentation	No	Not tested	Kemp and Rutowski 2007
Scorpionfly (*Panorpa cognata*)	Larval food availability	Full-sibling design	Yes- male sperm transfer rate	Yes	Not tested	Engqvist 2008
Stalk-eyed fly (*Cyrtodiopsis dalmanni*)	Larval food quality	Full-sibling design	Yes- male eye span	No	Not tested	David et al. 2000
Sticklebacks (*Gasterosteus Aculeatus*)	Light environment	Paternal half-sibling design	Yes- male pigmentation	Yes	Not tested	Lewandowski and Boughman 2008
Striped ground crickets (*Allonemobius fasciatus*)	Rearing temperature; photoperiod	Full-sibling design	Yes- male calling rate and duration	Yes	Not tested	Olvido and Mousseau 1995

Table 2.2 Effect sizes of G x E interactions identified in some of the studies shown in Table 2.1.

Species	Sexual trait	*P* value	Effect size**	Reference
Bank vole (*Clethrionomys glareolus*)	Male dominance	0.006	0.017	Mills et al. 2007
Fruit fly (*Drosophila mojavensis*)	Male acoustic signal	0.006*	0.009	Etges et al. 2007
Fruit fly (*Drosophila melanogaster*)	Male cuticular hydrocarbons	0.2138*	0.046	Kent et al. 2008
Fruit fly (*Drosophila melanogaster*)	Female mate choice	0.0001	0.006	Narraway et al. 2010
Guppy (*Poecilia reticulata*)	Male pigmentation	0.077*	0.015	Grether 2000
Lesser waxmoth (*Achroia grisella*)	Female mate choice	0.013	0.008	Rodríguez and Greenfield 2003
Lesser waxmoth (*Achroia grisella*)	Male acoustic signal	0.035	0.003	Danielson-François et al. 2006
Scorpionfly (*Panorpa cognata*)	Male sperm transfer rate	0.016	0.013	Engqvist 2008
Stalk-eyed fly (*Cyrtodiopsis dalmanni*)	Male eyespan	0.0001	0.013	David et al. 2000
Sticklebacks (*Gasterosteus aculeatus*)	Male pigmentation	0.059*	0.007	Lewandowski and Boughman 2008

* Average *P* value of multiple sexual traits measured in the study
** Effect size, *r*, calculated from standardised *z* values (see method described in Rosenthal (1991))

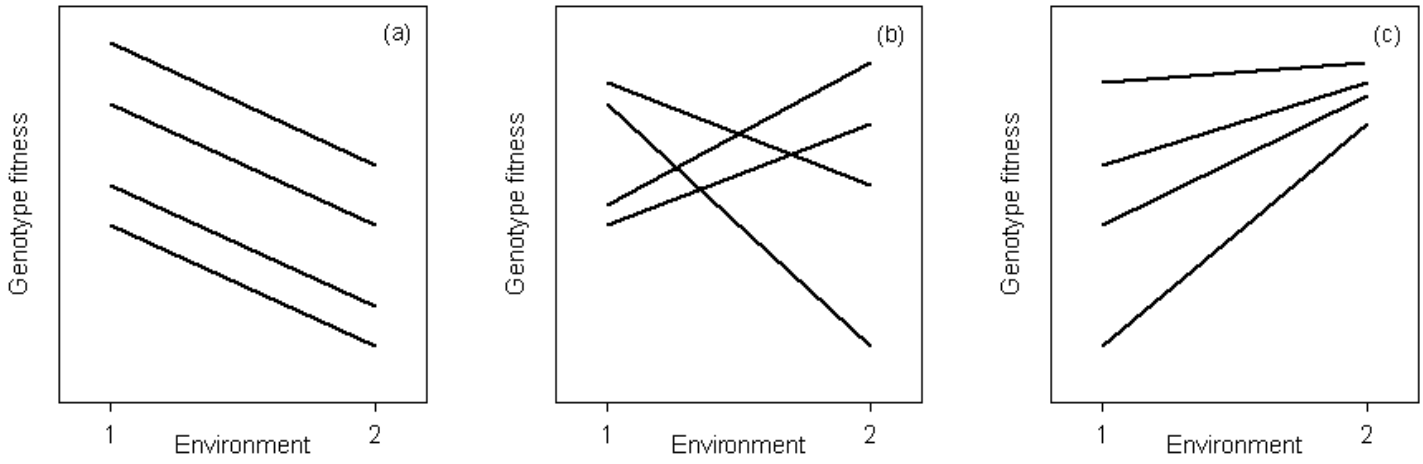

Figure 2.1 Reaction norms for relative fitness of four genotypes each measured in two different environments. (a) No G x E. Genetic variation is indicated by the differences in trait expression within each environment, and the non-zero gradient between environments indicates an effect of environmental variation on trait expression, but there is no interaction between the two and the effect is the same for all genotypes, as shown by the parallel gradients. (b) G x E with ecological crossover of reaction norms. The rank order of genotypes changes between environments, potentially affecting both intensity and direction of selection, and the constancy of relative genotype fitness depends on the environmental constancy. The scale of variation is also likely to be affected under ecological crossover, as shown. (c) G x E where the scale of variation but not the rank order of genotypes differs between environments, which might affect the intensity of selection. A reaction norm for a G x E tested empirically may look like (b) or (c) depending on the range of environmental variation studied (see main text).

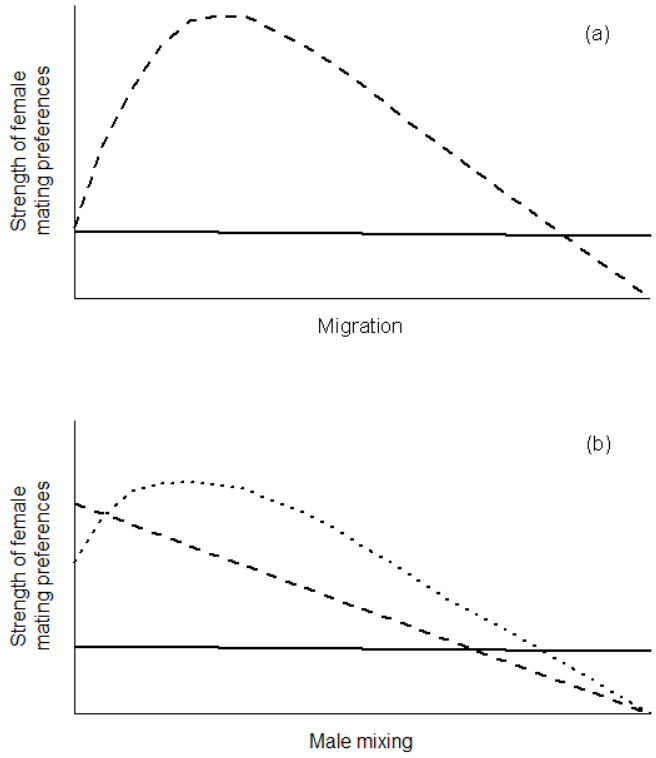

Figure 2.2 The effect of gene flow between different environments on the strength of female preferences, measured as the costs tolerated by females before preference is selected against and disappears from a population. (a) The effect of migration (or environmental change) immediately after birth (e.g. offspring dispersal). Migration is used here to describe an environmental change after birth such that offspring development and mating occur in a different environment from the one an individual is born in. In the absence of a G x E in sexual trait expression (solid line), the effect of migration is negligible. However, when there is a G x E with ecological crossover (dashed line) low levels of migration promote selection for female mating preferences, possibly through increased maintenance of variation in sexual traits. Selection on female mate choice decreases as migration increases, until at high levels of gene flow when individuals are equally mixed between environments, the advantages of female preferences disappear completely, likely to be an effect of unreliability of sexual signals. (b) The effect of male mixing, which describes male-specific movement between environments after trait development but before mating opportunities. As levels of male mixing increase, the probability of a given

female encountering a male from her own environment decreases. With no G x E in sexual trait expression (solid line), the effect of male mixing is negligible. When there is a G x E with ecological crossover, the effect of male mixing depends on levels of migration (or environmental change). With high levels of migration (dashed line), the advantages of female mating preferences are high with no male mixing, then decrease steadily with increasing male mixing. With very low levels of migration (dotted line), the strength of female mating preferences are high with low male mixing, then decrease with as male mixing increases further and the reliability of sexual signals is increasingly disrupted. Figures adapted from Kokko and Heubel (2008).

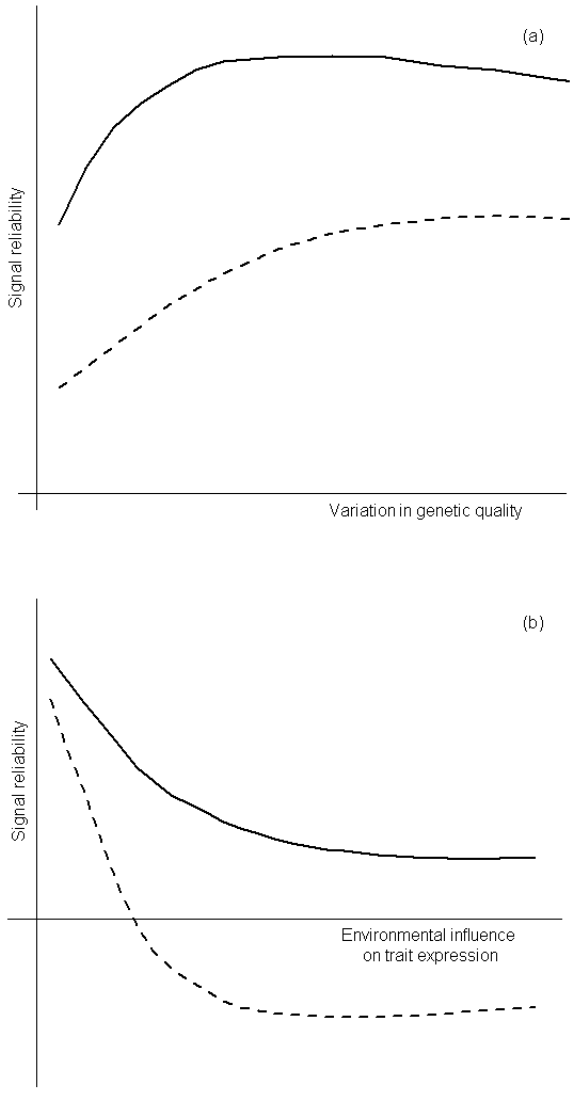

Figure 2.3 Reliability of sexual traits where G x Es affect the scale of variation between trait expression in different environments but do not cause ecological crossover as modelled in different environmental conditions by Higginson and Reader (2009). (a) The effect of genetic variation in quality on the reliability of sexual signals, in environments with low heterogeneity (solid line) and in highly heterogeneous environments (dashed line). Signal reliability, measured here as the correlation between signal trait size and genetic quality, is lower in highly variable environments. However, in both types of environment, signal reliability increases with increasing variation in genetic quality, as the information content of the signal trait will be greater. (b) The effect on sexual signal reliability of the strength of environmental influence on trait expression in favourable environmental conditions (solid

line) and harsh environmental conditions (dashed line). Signal reliability decreases with increasing strength of the environmental influence on trait expression. This effect could be magnified when environmental conditions are harsh (dashed line), even to the extent that the correlation between signal trait size and genetic quality becomes negative in some circumstances. Note that the line falls below zero, indicating this negative correlation. In these conditions, the most exaggerated sexual signals are produced by low quality males, whereas high quality males produce unattractive sexual signals, meaning that the reliability of sexual signals is so disrupted that the correlation between trait size and genetic quality is reversed. Figures adapted from Higginson and Reader (2009).

CHAPTER 3: Environmental heterogeneity, multivariate sexual selection and genetic constraints on cuticular hydrocarbons in *Drosophila simulans*

3.1 Abstract

The role of the environment in sexual selection has been the subject of increasing research interest in recent years. If sexual selection differs between environments then sexual trait evolution will depend on environmental variation. Across a range of diets and temperatures, we studied patterns of sexual selection through female mate choice on male cuticular hydrocarbon (CHC) profiles in *Drosophila simulans*. We find evidence for directional, quadratic and correlational sexual selection on CHCs. We show that the strength and form of sexual selection differs between some of the laboratory environments we examine, particularly across diets rather than temperatures. These results suggest that the trajectory of CHC profile evolution could differ between diets to a greater extent than across temperatures. However, we also identify environment-dependent genetic constraints which are likely to affect these trajectories. Our results highlight the importance of multivariate and cross-environment studies in gaining a more comprehensive understanding of how sexual traits evolve.

3.2 Introduction

Our understanding of sexual selection has improved dramatically over the past few decades (see Majerus 1986; Andersson and Simmons 2006; Hosken and House 2011), but only recently has research really focussed on the role of the environment in sexual selection (Cornwallis and Uller 2010). The potential for sexual selection to vary between environments has been highlighted in a number of different contexts, including the possibility of species divergence through ecological speciation and local adaptation (Ritchie 2007; van Doorn et al. 2009), the role of the environment in determining condition and the condition dependence of sexual traits (Rowe & Houle 1996; Cotton et al. 2006), and most recently, the effect of genotype-by-environment interactions and their evolutionary consequences for sexual traits (Kokko & Heubel 2008; Higginson & Reader 2009; Ingleby et al. 2010). Environmental variation can also be important in the maintenance of genetic variation in sexual traits (Kirkpatrick and Ryan 1991; Kokko and Heubel 2008; Jia et al. 2000), and can affect the expression of sexual signals and mating preferences. This can in turn

affect the coevolution of signal and preference (Greenfield and Rodríguez 2004), which is of key importance to some models of sexual selection (Lande 1981).

In insects, cuticular hydrocarbons (CHC) have been shown to have a strong environmental component and they are also subject to sexual selection. CHCs are particularly well-studied in *Drosophila* species, which produce a variety of different CHCs. Long-chained or heavily-branched hydrocarbons are waxy and largely non-volatile, creating a stable and protective barrier which is thought to help prevent water loss through the cuticle (Ferveur 2005). Studies have shown that *D. mojavensis* (Gibbs et al. 1998), *D. melanogaster* (Savarit and Ferveur 2002), *D. serrata* (Frentiu and Chenoweth 2010) and *D. simulans* (Sharma et al. 2012b; Ingleby et al. in review) produce more long-chained CHCs at higher temperatures, and that desiccation stress exerts selection on CHC profile in *D. melanogaster* (Kwan and Rundle 2009; Foley and Telonis-Scott 2010).

However, short-chained, more volatile CHCs will be useful for chemical communication, functioning as short-range or contact pheromones. Specifically, studies have implicated 7-tricosene and various types of diene in different components of *Drosophila* courtship and mating behaviour (reviewed by Ferveur and Cobb 2010), while some studies of *D. serrata* implicate methyl-branched alkanes (Chenoweth and Blows 2005; Petfield et al. 2005; Delcourt et al. 2010). Consistent with this, experimental evolution of populations of *D. serrata* (Chenoweth and Blows 2005; Blows 2002; Chenoweth et al. 2008; Rundle et al. 2009) and *D. simulans* (Sharma et al. 2012b) found that CHC profile can evolve in response to sexual selection. There is also evidence that *Drosophila* CHCs are costly to produce (Blows 2002; Ferveur 2005), and, accordingly, there is evidence of condition dependence of CHC profiles (Gosden and Chenoweth 2011). As such, it is likely that trade-offs exist between different types of CHCs and their diverse functions.

Drosophila CHCs are therefore an ideal system for studying how environmental variation affects sexual trait evolution. Indeed, experimental evolution with both *D. serrata* and *D. simulans* found an interaction between the effects of natural and sexual selection on CHC profile (Blows 2002; Sharma et al. 2012b), suggesting that sexual selection on CHC profile could differ between environments. However, variation in patterns of selection across environments does not necessarily give an accurate representation of how CHCs will evolve. The response to selection will also depend on genetic variation for CHC expression, and especially how much genetic variation there is in the direction of selection. For this

reason, studies which characterise cross-environment patterns of sexual selection and genetic variation for multiple traits will provide detailed insight into how selection and genetic constraints contribute to trait evolution (Blows 2007; Blows and Walsh 2009).

Here, we examine how sexual selection (through female mate choice) acts on the CHC profiles of male *D. simulans* reared across a range of different laboratory environments, by measuring both male attractiveness and CHC expression and carrying out a standard selection analysis following Lande and Arnold (1983). We identify complex patterns of directional, quadratic and correlational sexual selection, and find some evidence of variation between environments and between different components of the CHC profile. Furthermore, by using estimates of the **G** matrix underlying male CHC expression (from Ingleby et al. in review), we are able to calculate the genetic constraint on CHC evolution within each environment. Together, these analyses describe selection and constraints on male CHCs across environments and allow us to predict how CHC evolution might vary across heterogeneous environments.

3.3 Methods

Stock populations

Female *D. simulans* were collected from Greece in April 2010 and their offspring were used to set up a laboratory population (as well as a set of inbred lines (isolines), see section on genetic constraints below). This population was maintained at an approximate size of 500 individuals, with overlapping generations, for 8 months prior to this study. Flies were kept on a cornmeal-based diet (supplied by Applied Scientific, UK; consisting of sugar, cornmeal, deionised water, yeast, agar, benzoic acid, methyl paraben and propionic acid) at 25^{o}C.

Environmental manipulations

We carried out the experiment in 7 blocks, with each environmental treatment replicated in each block. We reared male flies from the laboratory population in each of four different experimental environments. We used two different diets; the cornmeal-based diet (diet 1), and a novel diet (diet 2) made from oatbran, sugar, deionised water, yeast agar and methyl paraben. These diets were chosen purely to create variation in dietary environment rather than to test diet quality. 100 small vials of each of these two diets (40ml vials with 8ml of medium) were put into the population cage for 24 hours to allow egg laying on both diets.

Over the same 24-hour period, large vials (150ml vials with 30ml of medium) of a potato-based diet (diet 3; supplied by Blades Biological, UK) were also added to the cage, to rear females. All vials were removed from the cage after 24 hours and incubated at 25^{o}C on a 10:14 hour light:dark cycle during offspring development.

Peak eclosions from these vials occurred after 11 days. Virgin females were collected from the large vials of diet 3 and transferred to individual 40ml vials with 8ml of diet 3 and incubated at 25^{o}C. All females were treated identically in order to create a stock of virgin females from a common environment, which was distinct from the experimental treatments, for use in mating trials.

Virgin males were collected from the 100 vials of diet 1 and 100 vials of diet 2. One male was collected from each of the laying vials, so that we could eliminate effects of common rearing environment within each laying vial. Each male was transferred to an individual glass vial of the same diet as development. Males from each diet were then split equally between two post-eclosion temperatures, 23^{o}C and 25^{o}C, creating four treatments: diet 1 at 23^{o}C = treatment A; diet 2 at 23^{o}C = treatment B; diet 1 at 25^{o}C = treatment C; and diet 2 at 25^{o}C = treatment D. Treatment C closely replicated the environment in which these flies had become lab-adapted.

Male attractiveness assays and hydrocarbon extraction

Male attractiveness was assessed in mating assays carried out at 3 days post-eclosion between a standard female and a male from one of the treatments. Each assay lasted 3 hours during which courtship and mating behaviour were recorded. We measured attractiveness as a binary response - the male either mated or did not mate during the 3 hour period. Males which were not observed courting a female at any point in the 3-hour assay were excluded from the dataset. *Drosophila* females have control over acceptance or rejection of courting males (Speith 1974; Markow 1996), and so males which courted and achieved a mating are likely to be more attractive than males which courted but did not mate. Indeed, many previous *Drosophila* studies have used no-choice mating assays to assess overall male attractiveness and female preference (e.g. Speith 1974; Kyriacou and Hall 1986; Barth et al. 1997; Ritchie et al. 1999; Acebes et al. 2003; Shackleton et al. 2005; Taylor et al. 2007; Hosken et al. 2008; Narraway et al. 2010).

After mating had occurred, or after 3 hours in the case of males which did not mate, the male and female were separated using an aspirator. Females were discarded and males were frozen at -80^{o}C in individual glass auto-sampler vials (supplied by Chromacol, UK) prior to CHC extraction. Hydrocarbon extractions were carried out in sets of 100 samples per day, and randomised throughout by treatment. Hydrocarbon extractions and analysis followed a protocol optimised previously for *D. simulans* (see details in Sharma et al. 2012b and Ingleby et al. in review).

Statistical analyses

Principal components analysis

We quantified expression of 22 CHCs for each male. Prior to analysis, we calculated relative peak size by dividing peaks within each individual by the size of the internal standard peak (pentadecane) present in each sample. Relative peak sizes were then log-transformed to fit into a normal distribution. We ran principal components analysis (PCA) to reduce the dimensionality of the data. PCs were extracted using the correlational matrix. We identified multivariate outliers based on Mahalanobis distances and removed these from the data, leaving 645 individuals in subsequent analyses which were spread fairly equally across treatments (148-167 individuals in each treatment). We extracted three orthogonal vectors with eigenvalues greater than 1 which cumulatively explained ca. 75% of the total variation in male CHC expression (Table 3.1). We used factor loadings > 0.25 to interpret the biological significance of these vectors (Tabachnick and Fidell 1989).

Environmental components of CHC expression

We tested for an environmental component of male CHC expression with a multivariate analysis of covariance (MANCOVA) in SAS, with the 3 PCs of CHC expression as response variables; diet, temperature and diet x temperature interaction as fixed effects; and experimental block as a covariate.

Selection analysis of sexual selection on male CHCs

From the mating assays, males were scored mating success of either 1 (mated) or 0 (unmated) during the 3-hour assay. Preliminary analysis with a GLM tested for differences between male rearing environments in the proportion of males which mated, with block as

a covariate, diet, temperature and diet x temperature interaction as fixed effects, and specifying a binomial distribution.

We calculated individual relative fitness within each environment by dividing individual fitness score by the mean fitness score for each treatment. PCs 1-3 were also standardised to the mean score within each treatment. In order to test whether the strength and form of sexual selection differed between treatments, we used the sequential model building approach, as outlined in Appendix A of Chenoweth and Blows (2005). Pairwise comparisons of treatments were used to determine where these differences lay. Since we identified significant differences between most treatments, selection analyses were done separately for each treatment.

Relative fitness was used as the response in a standard selection analysis (following Lande and Arnold 1983) examining sexual selection through female preference on the 3 standardised PCs of CHC expression. For each treatment, we fitted a linear regression to estimate $\boldsymbol{\beta}$, the vector of linear (directional) selection on each PC. The absolute values of these linear selection gradients give an indication of the strength of directional sexual selection. Next, we estimated the matrix of nonlinear selection, $\boldsymbol{\gamma}$, using a quadratic regression model which incorporated linear, quadratic and correlational selection terms for each PC in each treatment. The quadratic selection terms give an estimate of the curvature around the mean trait value, such that higher gradients have a steeper curvature, and negative (positive) gradients suggest stabilising (disruptive) selection. Using this matrix, we performed a canonical analysis to produce the **M** matrix, which gives the shape of the fitness surface described by the major axes on nonlinear selection. We interpret the eigenvalues as a measure of the strength of nonlinear ($\boldsymbol{\lambda_i}$) selection along the eigenvectors (m_1-m_3) and the strength of linear selection along each eigenvectors is given by $\boldsymbol{\theta_i}$. The significant vectors of nonlinear selection for each treatment were plotted using thin plate splines in R (v.2.13.0). For each of these analyses, we used randomisation tests to assign significance to selection gradients.

Genetic constraints

In a previous study (Ingleby et al. in review), we reared *D. simulans* from isolines which were derived from the same genetic background as the population in this study across the same range of laboratory environments, and extracted the same 3 PCs of CHC expression as we do

here. From this data, we are therefore able to estimate the **G** matrix for male CHC expression within each environment, although note that these genetic estimates will be based on V_G as opposed to V_A. Using these **G** matrices along with the $\boldsymbol{\beta}$ vectors identified here, we calculated the predicted response of male CHC profile to sexual selection (using the multivariate breeder's equation; Lande and Arnold 1983), and then tested for genetic constraints by measuring the alignment of the $\boldsymbol{\beta}$ vector and the vector of predicted responses to selection within each environment (Blows and Walsh 2009). Genetic constraint can be estimated as the angle between these two vectors, with a 95% credible interval (CI) used to determine the significance of this angle. These calculations were carried out with a novel Bayesian approach, implemented in R (v. 2.13.0). For details and R code, see Appendix 1.

3.4 Results

Principal components analysis

From the results of PCA, we extracted 3 PCs of CHC expression (Table 3.1). PC1 clearly represents overall investment in CHC production, as each peak is positively and highly loaded. For PC2, short-chained CHCs are generally negatively loaded whereas long-chained CHCs positively loaded, and so we interpret this vector as describing the trade-off between short- and long-chained CHCs, with individuals with high PC2 scores biasing production of long-chained CHCs over short-chained CHCs. The interpretation of PC3 is less clear as only 9 of the 22 peaks have loadings over 0.25, but there appears to be a similar pattern to PC2, since 4 short-chained CHCs are negatively loaded and 4 long-chained CHCs are positively loaded. Seven of the 9 peaks significantly loaded on PC3 are ones which do not contribute significantly to PC2. We therefore interpret PC3 as another vector of variation in the trade-off between short- and long-chained CHCs, although involving fewer CHCs.

Environmental components of CHC expression

Temperature has a significant effect on overall male CHC profile, but there is no multivariate effect of either diet or temperature x diet interaction (Table 3.2). Individual analysis of each of the 3 PCs shows that environmental components of CHC expression are concentrated on PC3, where temperature has a strong effect and there also is an effect of diet (Table 3.2; Figure 3.1). Individuals on diet 2 generally have higher PC3 scores than individuals on diet 1,

and on both diets, individuals from the lower temperature have consistently lower PC3 scores than individuals from the higher temperature (Figure 3.1).

Selection analysis of sexual selection on male CHCs

We found a significant difference between diets in the proportion of males mated from each treatment ($F_{1,644}$ = 7.598; P = 0.006), but no difference between temperatures ($F_{1,644}$ = 0.874; P = 0.350), and no significant block effect ($F_{1,644}$ = 0.206; P = 0.650) or interaction between diet and temperature ($F_{1,644}$ = 0.129; P = 0.720). Males reared in Treatment C (diet 1 at 25^{o}C; which is closest to the environment in which these populations were lab-adapted) appear to be the most attractive as the highest proportion of them mated during the 3-hour assay (Table 3.3).

Patterns of linear ($F_{3,619}$ = 4.453, P = 0.004), quadratic ($F_{3,616}$ = 7.209, P < 0.001) and correlational ($F_{3,604}$ = 4.312, P = 0.005) sexual selection were all significantly different across treatments. From the individual interaction terms in the sequential model, we find that differences in linear sexual selection across treatments are driven by PC2 and PC3 (PC1: $F_{1,619}$ = 0.321, P = 0.571; PC2: $F_{1,619}$ = 5.888, P = 0.016; PC3: $F_{1,619}$ = 6.551, P = 0.011); differences in quadratic sexual selection between treatments were attributable to PC2 (PC1: $F_{1,616}$ = 0.894, P = 0.444; PC2: $F_{1,616}$ = 5.398, P = 0.001; PC3: $F_{1,616}$ = 1.248, P = 0.292); and differences in correlational selection were due to differences in selection on PC2 and PC3 across treatments (PC1: $F_{1,604}$ = 0.410, P = 0.746; PC2: $F_{1,604}$ = 2.615, P = 0.041; PC3: $F_{1,604}$ = 2.989, P = 0.031).

The results of pairwise comparisons of sexual selection between treatments are shown in Table 3.4. Clearly, patterns of sexual selection are quite different between most of the environments we studied, particularly in terms of nonlinear selection. Each form of selection (linear, quadratic and correlational) differed between Treatments A and B (23^{o}C across diets) and Treatments A and D (change in both diet and temperature). No form of sexual selection differed significantly between treatments B and D (ie. diet 2 across temperatures).

In Treatment A (diet 1 at 23^{o}C), we found significant negative directional selection and disruptive selection on PC2 (Table 3.5), which probably contributes to similar patterns of selection on vector m_1 which is heavily loaded for PC2 (Table 3.6). There is also negative correlational selection on PC1 and PC3, and stabilising selection on PC1 (Table 3.5). PC1 is

highly loaded on vector m_3, which is also under significant stabilising selection (Table 3.6). The fitness surface in Figure 3.2a illustrates these significant vectors of sexual selection, and shows the highest fitness peak at low m_1 scores and intermediate m_3 scores. Based on the vector loadings in Table 6, the most attractive males from this environment produce an intermediate overall amount of CHCs (PC1), but invest heavily in short-chained CHCs (PC2).

The fitness surface in Figure 3.2c (for Treatment C; diet 1 at 25^{o}C) shows some similar patterns of sexual selection. PC1 is under stabilising selection, such that intermediate scores are most attractive (Table 3.5) and this is reflected in vector m_3, which is under stabilising selection and is heavily-loaded for PC1 (Table 3.6). Vector m_1 is under negative directional and disruptive sexual selection and is heavily-loaded for PC2 and PC3, indicating that extreme combinations of these vectors, and especially extreme negative values, are preferred (Table 3.6). The fitness peak in Figure 3.2c shows that attractive males are likely to have strongly negative PC2 and PC3 scores and intermediate PC1 scores (see loadings in Table 3.6). Sexual selection on males reared on diet 1 does not vary much across different temperatures.

In Treatment B (diet 2 at 23^{o}C), only PC2 is subject to significant sexual selection, shown by stabilising selection on PC2 in Table 5. There are two significant vectors of nonlinear selection in Treatment B: m_1 is under weakly disruptive selection, and m_3 is under stabilising selection (Table 3.6). Figure 3.2b shows fitness peaks at extremes of m_1 and intermediate scores on m_3. Based on the loadings for each PC for these vectors, the most attractive males in this environment have intermediate PC2 scores, indicating a balance of short- and long-chained CHCs.

On the same diet at 25^{o}C (Treatment D), there is also stabilising selection on PC2 (Table 3.5) and on vector m_3, which is heavily-loaded for PC2 (Table 3.6). There is also significant sexual selection on vector m_1, which shows positive directional and disruptive selection, indicating highly positive m_1 scores are most attractive, which suggests high overall investment in CHCs (i.e. high PC1 scores). Vectors m_1 and m_3 are plotted in Figure 3.2d, where the fitness peak shows that males which invest heavily in CHCs, with a balance of short- and long-chained CHCs, are most attractive (from the loadings in Table 3.6). Thus, there is little difference between sexual selection in Treatments B and D (diet 2 across temperatures), but patterns of selection are very different from those in Treatments A and C (diet 1 across temperatures).

Genetic constraints

The genetic constraints on male CHC profile within each environment are shown in Table 3.7. In each environment, the constraint is significantly different from zero, such that there is some degree of constraint in all environments. The interval estimates are quite wide, but clearly the constraint is weak in Treatment C, which most closely matches the environment in which these populations were lab-adapted. The greatest constraints were found for males reared on diet 2 (Treatments B and D).

3.5 Discussion

We find that although the strength and form of sexual selection imposed through female mate choice on male *D. simulans* CHC profiles can differ between environments, these effects vary for specific CHC components and with the form of environmental variation. For instance, differences in female choice across environments seem to be driven by selection on PC2 and PC3, but not on PC1. Further, the clearest differences in selection are generally found across diets rather than across temperatures. The microevolutionary response of male sexual traits to sexual selection will also depend on genetic constraints, and these also appear to vary between environments. Clearly, patterns of cross-environment sexual selection are very complex even in the simple experimental paradigm we employed, and it is likely that studies which do not examine multiple traits or multiple environments will give an over-simplified view of sexual selection and the evolution of sexual traits.

Sexual selection across environments

Differences in sexual selection are clearer across diets than across temperatures. Interestingly, males reared on diet 1 are significantly more attractive than males reared on diet 2 (a higher proportion of males from diet 1 were mated), suggesting the overall intensity of sexual selection differs across diets. The selection analyses also revealed more detailed differences in the strength and form of sexual selection across diets.

For example, PC1, which represents variation in overall investment in CHC production, is under stabilising selection on diet 1. This suggests that attractive males invest moderately in CHC production, whereas on diet 2 (although only in Treatment D) high overall investment in CHCs is most attractive. Without information on diet quality, these

patterns of sexual selection are difficult to interpret. However, in light of the extensive literature on the condition dependence of sexual traits (Rowe and Houle 1996), these results may warrant further research, especially since diet-mediated condition dependence of sexually selected aspects of male CHC profile has been identified in *D. serrata* (Gosden and Chenoweth 2011).

There are also clear differences across diets in sexual selection on the trade-off between long- and short-chained CHCs (represented by PC2 and to a lesser extent PC3). On diet 1, attractive males invest heavily in short-chained CHCs, whilst on diet 2, attractive males have more balanced investment in long- and short-chained CHCs. Previous research with *Drosophila* suggests that short-chained CHCs could be most favoured by sexual selection, as they are volatile and act as effective short-range and contact pheromones (reviewed by Ferveur and Cobb 2010). Our results show that biasing CHC production towards investment in short-chained CHCs is only attractive in certain environments.

There is no clear evidence that sexual selection on the trade-off between long- and short-chained CHCs is affected by temperature. This is unexpected given the large body of evidence which suggests that whilst sexual selection should favour production of short-chained CHCs (reviewed by Ferveur and Cobb 2010), natural selection through temperature variation should favour production of long-chained CHCs which are more effective at preventing dessication (Gibbs et al. 1998; Savarit and Ferveur 2002; Frentiu and Chenoweth 2010; Foley and Telonis-Scott 2010). Indeed, we find here that males consistently produce more long-chained CHCs at the higher temperature, but this is not coupled with any clear differences in sexual selection across temperatures.

Environmental components of male CHC expression

There is little evidence for an effect of diet on CHC expression, despite dietary effects previously identified in both *D. serrata* (Gosden and Chenoweth 2011) and *D. simulans* (Ingleby et al. in review). However, in *D. simulans*, there was evidence for strong G x E across the same diets used here, whereas the effect of diet itself was weak (Ingleby et al. in review), and so perhaps without being able to partition G, E and G x E effects, we were unable to detect an overall dietary effect on CHC profile here. We did find that males have higher PC3 scores, regardless of temperature, on diet 2 compared to diet 1. This shows that

the resources available through diet have some influence on male resource allocation to CHC production.

Changes in PC3 expression over temperatures drive the overall multivariate effect of temperature. High PC3 score indicates that males invest strongly in some specific long-chained CHCs at the cost of investing in particular short-chained CHCs. As might be expected, therefore, males consistently have higher PC3 scores at 25^{o}C than at 23^{o}C, suggesting increased investment in protective long-chained CHCs when the risk of desiccation is higher (consistent with Gibbs et al. 1998; Savarit and Ferveur 2002; Frentiu and Chenoweth 2010; Foley and Telonis-Scott 2010; Ingleby et al. in review).

The response to selection across environments

Whether or not male CHC profile evolves in response to the selection we measure here will depend on genetic variation underlying CHC expression, and if it aligns with the direction of selection. Previous studies have used experimental evolution to demonstrate that CHC profile can evolve through sexual selection in both *D. simulans* (Sharma et al. 2012b) and *D. serrata* (Blows 2002; Chenoweth and Blows 2005; Chenoweth et al. 2008; Rundle et al. 2009). Furthermore, some of these studies have found an interaction between natural and sexual selection on CHCs, implying that variation in the physical environment might cause differences in patterns of sexual selection and the evolution of CHC profile.

In a previous study, we measured genetic variation in CHC expression in the same population of *D. simulans* across the same environments studied here (Ingleby et al. in review). We found strong G x E effects across diets, and these effects weakened the cross-environment genetic correlation in CHC expression and gave the potential for the response to selection to differ across diets. Here, we show that sexual selection does differ across these diets, and also estimated the genetic constraint on CHC evolution within each environment to test how well aligned genetic variation in CHCs is with the direction of selection. The strength of these constraints differed between diets, suggesting that the evolutionary trajectory of male CHCs could be dramatically different between diets and this is not purely because of differences in selection.

The consequences of temperature variation on CHC evolution are less clear. Whilst there is a strong overall temperature effect on CHC expression (Ingleby et al. in review; this study), there is little evidence of G x E across temperatures (Ingleby et al. in review), nor are

there any clear differences in sexual selection across temperatures. If there are no significant differences in sexual selection across temperatures, and natural selection across temperatures consistently favours certain CHC profiles, then we might expect genetic variation in CHC plasticity across temperatures to be depleted by persistent selection. The lack of evidence for G x E across temperatures in *D. simulans* CHC expression supports this idea (Ingleby et al. in review), and so there may be limited potential for CHCs to evolve independently across temperatures.

We did not consider variation in female mate choice across female rearing environments, and attempted to minimise such variation by using females from a standard environment. Given the evidence for plasticity and context-dependency of mating preferences in many species (reviewed by Jennions and Petrie 1997; Cotton et al. 2006), it is likely that manipulation of female rearing environment might also strongly affect sexual selection across environments, but this will require further research. Further examination of variation in female preference across female environments will also be necessary to make any inference on whether the covariance between male signal and female preference varies between environments.

This study provides evidence for strong sexual selection through female mate choice on male *D. simulans* CHC profile. Furthermore, we find variation in both sexual selection and genetic constraints over some male rearing environments, both of which will influence the evolution of male CHC profile. These results emphasise the importance of multivariate studies and cross-environment studies of sexual selection in order to reveal the potential for evolutionary trajectories of sexual traits to differ between environments.

Table 3.1 Principal component analysis for CHC expression in both sexes. Three principal components with eigenvalues > 1 were extracted for further analyses, explaining just over 70% of the total variation in CHC profile. Biological significance of each component was interpreted from factor loadings > 0.25 (in bold). CHCs are named where known; unnamed CHCs (asterisks) are described by basic chemical structure. CHCs are listed in order of increasing chain length.

	PC1	**PC2**	**PC3**
Eigenvalue	9.731	3.933	1.979
% variance	44.232	17.875	8.997
Loadings:			
Octadecadiene	**0.680**	**-0.252**	0.029
Docosene	**0.403**	-0.039	**-0.314**
Docosane	**0.836**	0.102	**-0.328**
Branched alkane*	**0.714**	**-0.427**	-0.179
7-Tricosene	**0.845**	**-0.271**	0.138
Tricosene	**0.685**	**-0.286**	-0.138
Tricosane	**0.723**	-0.110	-0.025
Branched alkane*	**0.729**	**-0.430**	-0.205
Branched alkane*	**0.797**	-0.206	**-0.388**
Branched alkane*	**0.725**	**-0.405**	-0.075
Tetracosane	**0.700**	**0.613**	**-0.284**
Pentacosadiene	**0.651**	**0.459**	**-0.265**
Alkene*	**0.591**	0.029	**0.588**
Pentacosene	**0.537**	**0.258**	**0.400**
Pentacosane	**0.752**	**0.595**	0.017
Branched alkane*	**0.776**	0.071	**-0.258**
Hexacosane	**0.536**	**0.787**	-0.208
Heptacosane	**0.736**	**0.344**	-0.040
Branched alkane*	**0.500**	0.206	**0.589**
Alkane*	**0.540**	**0.800**	0.122
Alkane*	**0.417**	0.178	**0.592**
Alkane*	**0.486**	**0.807**	-0.147

Table 3.2 Results of a MANCOVA showing the effects of diet and temperature, plus their interaction, on the CHC profile of male *D. simulans*. Significance is highlighted in bold.

Overall MANCOVA			
	Pillai's trace	$F_{3,368}$	*P*
Diet	0.007	1.477	0.220
Temperature	**0.059**	**13.053**	**0.0001**
Diet x temperature	0.001	0.312	0.816
Block	0.010	2.203	0.087
Univariate ANCOVAs			
		$F_{1,630}$	*P*
PC1			
	Diet	0.298	0.585
	Temperature	0.025	0.875
	Diet x temperature	0.225	0.635
	Block	1.179	0.278
PC2			
	Diet	0.008	0.928
	Temperature	0.355	0.552
	Diet x temperature	0.214	0.644
	Block	0.610	0.435
PC3			
	Diet	**3.785**	**0.042**
	Temperature	**33.634**	**<0.001**
	Diet x temperature	0.481	0.488
	Block	1.438	0.231

Table 3.3 The proportion of males from each rearing environment which successfully mated within the 3-hour assay. Differences between diets were significant, differences between temperatures, the block effect and the interaction between diet and temperature were all non-significant (see text).

Treatment	**Proportion males mated**
A (diet 1; 23^{o}C)	0.58
B (diet 2; 23^{o}C)	0.48
C (diet 1; 25^{o}C)	0.62
D (diet 2; 25^{o}C)	0.51

Table 3.4 Pair-wise comparison of the strength of linear (β), quadratic (γ_{ii}) and correlational (γ_{ij}) sexual selection operating on CHCs in male *D. simulans*.

	A: diet 1, 23°C	**B: diet 2, 23°C**	**C: diet 1, 25°C**
B: diet 2, 23°C	**β: $F_{3,303}$ = 2.788, P = 0.041** **γ_{ii}: $F_{3,297}$ = 4.781, P = 0.003** **γ_{ij}: $F_{3,291}$ = 2.920, P = 0.034**		
C: diet 1, 25°C	β: $F_{3,299}$ = 1.102, P = 0.348 **γ_{ii}: $F_{3,293}$ = 3.159, P = 0.021** **γ_{ij}: $F_{3,287}$ = 3.337, P = 0.020**	β: $F_{3,318}$ = 0.965, P = 0.409 **γ_{ii}: $F_{3,312}$ = 2.900, P = 0.035** **γ_{ij}:$F_{3,306}$ = 4.015, P = 0.008**	
D: diet 2, 25°C	**β: $F_{3,301}$ = 2.878, P = 0.036** **γ_{ii}: $F_{3,295}$ = 5.055, P = 0.002** **γ_{ij}: $F_{3,289}$ = 3.024, P = 0.030**	β: $F_{3,320}$ = 2.255, P = 0.073 γ_{ii}: $F_{3,314}$ = 0.444, P = 0.722 γ_{ij}: $F_{3,308}$ = 0.180, P = 0.910	β: $F_{3,316}$ = 0.378, P = 0.769 **γ_{ii}: $F_{3,310}$ = 4.466, P = 0.004** **γ_{ij}: $F_{3,304}$ = 3.531, P = 0.015**

Table 3.5 Results of standard selection analysis (Lande and Arnold 1983) for sexual selection (through female preference) on 3 PCs of male *D. simulans* CHC expression, across each combination of dietary and temperature environments. The vector of standardised directional selection gradients is shown by β, and the matrix of standardised quadratic (diagonal) and correlational (below diagonal) selection gradients is shown by γ. Values in bold are significant ($P < 0.05$) after randomisation tests.

		γ		
	β	**PC1**	**PC2**	**PC3**
A: Diet 1, 23°C				
PC1	0.060	**-0.190**		
PC2	**-0.180**	0.099	**0.304**	
PC3	-0.014	**0.199**	-0.150	-0.156
B: Diet 2, 23°C				
PC1	0.001	-0.032		
PC2	0.083	0.047	**-0.266**	
PC3	0.025	-0.001	0.013	0.242
C: Diet 1, 25°C				
PC1	0.047	**-0.206**		
PC2	-0.097	-0.027	0.156	
PC3	**0.222**	**0.222**	**0.253**	0.090
D: Diet 2, 25°C				
PC1	0.040	0.184		
PC2	-0.100	-0.035	**-0.244**	
PC3	**-0.233**	-0.096	0.059	0.140

Table 3.6 The **M** matrix containing the major vectors of linear ($\boldsymbol{\theta}_i$) and nonlinear ($\boldsymbol{\lambda}_i$) selection acting on male *D. simulans* CHCs in each treatment. Values in bold are significant at $P < 0.05$ after randomisation tests.

	M			**Selection**	
	PC1	**PC2**	**PC3**	$\boldsymbol{\theta}_i$	$\boldsymbol{\lambda}_i$
A: Diet 1, 23°C					
m_1	0.084	0.964	-0.252	**-0.165**	**0.176**
m_2	0.710	0.120	0.694	0.011	0.010
m_3	0.700	-0.237	-0.674	0.094	**-0.208**
B: Diet 2, 23°C					
m_1	0.001	0.026	0.999	0.027	**0.121**
m_2	0.982	0.190	-0.006	0.016	-0.012
m_3	-0.190	0.982	-0.025	0.081	**-0.138**
C: Diet 1, 25°C					
m_1	0.223	0.680	0.699	**-0.145**	**0.203**
m_2	-0.580	0.669	-0.465	-0.033	0.002
m_3	0.783	0.301	-0.543	0.077	**-0.186**
D: Diet 2, 25°C					
m_1	0.767	-0.125	-0.630	**0.190**	**0.135**
m_2	0.640	0.073	0.765	**-0.160**	0.033
m_3	0.049	0.989	-0.136	-0.065	**-0.127**

Table 3.7 Genetic constraint on male CHC profile, estimated as θ, the angle between the vector of the predicted responses to sexual selection of each PC ($\boldsymbol{\Delta z}$) and the vector of linear selection gradients on each PC ($\boldsymbol{\beta}$). $\theta = 0^{\circ}$ would indicate the vectors are perfectly aligned and there is no constraint; $\theta = 90^{\circ}$ would indicate the vectors are orthogonal and the constraint is absolute.

Treatment	**Constraint, θ [95% CI]**
A (diet 1; 23°C)	38.41 [25.50 – 51.08]
B (diet 2; 23°C)	47.48 [35.80 – 60.57]
C (diet 1; 25°C)	28.48 [17.24 – 37.71]
D (diet 2; 25°C)	46.49 [36.54 – 57.43]

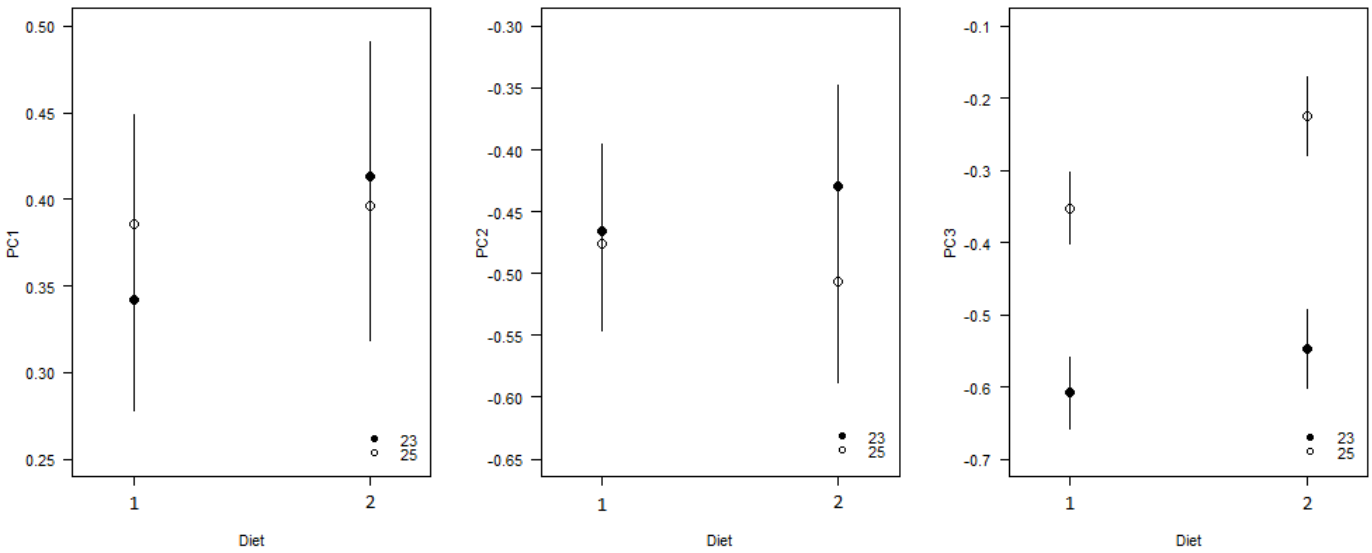

Figure 3.1 Mean PC score (± standard error) for PCs 1-3 (left-right) across diets 1 and 2, with separate points for 23°C (filled points) and 25°C (open points) post-eclosion temperatures.

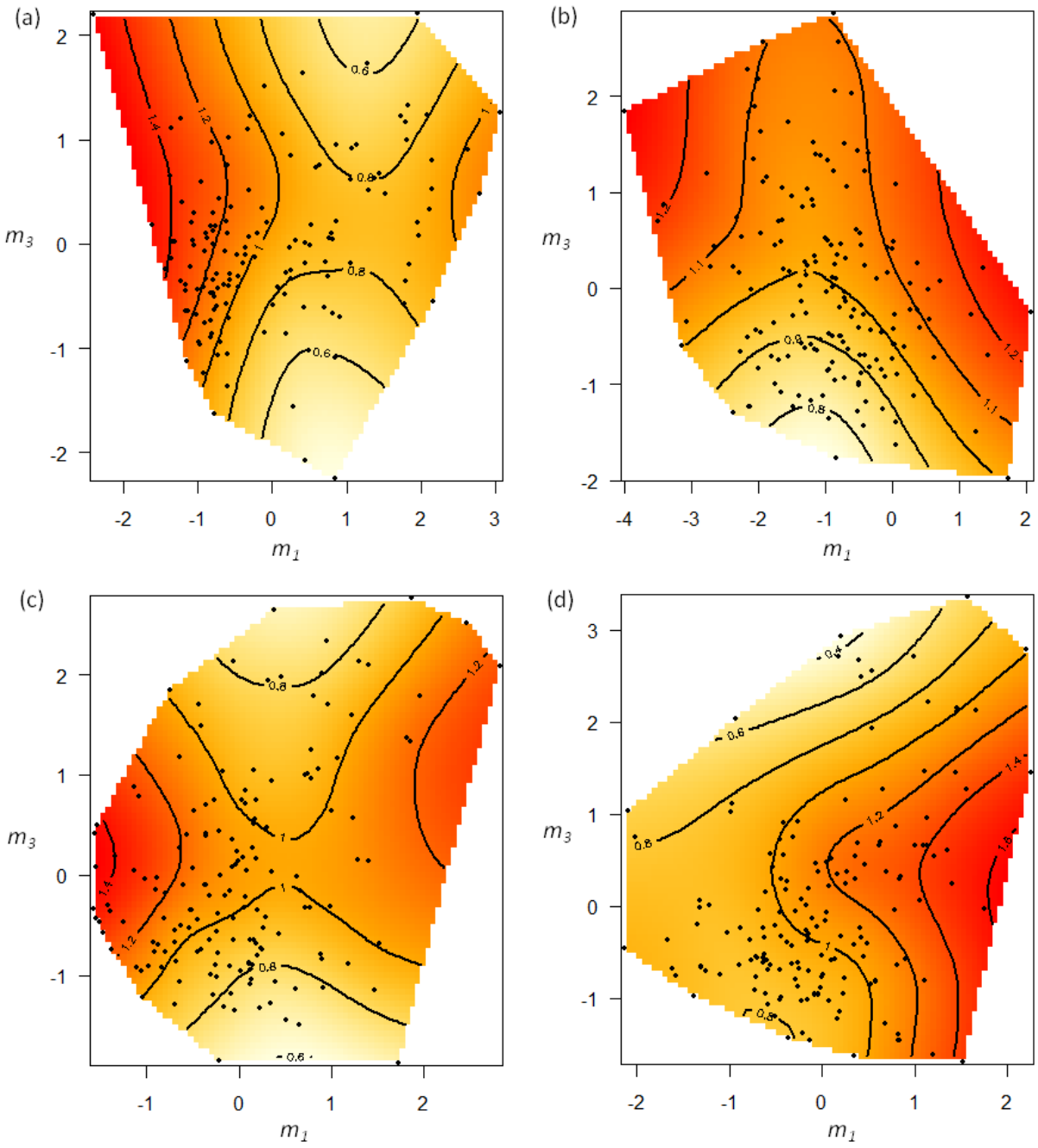

Figure 3.2 Fitness surfaces plotted on the two significant axes (m_1 and m_3) of nonlinear sexual selection on male CHCs for (a) diet 1 at 23°C (Treatment A); (b) diet 2 at 23°C (Treatment B); (c) diet 1 at 25°C (Treatment C); and (d) diet 2 at 25°C (Treatment D). Points represent individual males. Contours describe relative fitness within each environment. Red colouration indicates a peak in the fitness surface and pale yellow indicates a trough.

CHAPTER 4: Genotype-by-environment interactions for cuticular hydrocarbon expression in *Drosophila simulans*

4.1 Abstract

Genotype-by-environment interactions (G x Es) describe genetic variation for phenotypic plasticity. Recent interest in the role of these interactions in sexual selection has identified G x Es across a diverse range of species and sexual traits. Additionally, theoretical work predicts that G x Es in sexual traits could help to maintain genetic variation, but could also disrupt the reliability of these traits as signals of mate quality. However, empirical tests of these theoretical predictions are scarce. We reared iso-female lines of *Drosophila simulans* across two axes of environmental variation (diet and temperature) in a fully factorial design and tested for G x Es in the expression of cuticular hydrocarbons (CHCs), a multivariate sexual trait in this species. We find sex-specific environmental, genetic and G x E effects on CHC expression, with G x Es for diet in both male and female CHC profile and a G x E for temperature in females. We also find some evidence for ecological crossover in these G x Es, and by quantifying variance components, genetic correlations and heritabilities, we show the potential for these G x Es to help maintain genetic variation and cause sexual signal unreliability in *D. simulans* CHC profiles.

4.2 Introduction

Genotype-by-environment interactions (G x Es) represent changes in the relative performance of different genotypes in alternate environments (Lynch and Walsh 1998). G x Es are often interpreted as genetic variation for phenotypic plasticity, such that the direction and extent of plasticity in trait expression across environments differs between genotypes. These interactions have been extensively studied in agricultural research (Falconer 1952; Kang and Gauch 1996) and in evolutionary genetics (Via and Lande 1985; Via and Lande 1987), but research on G x Es in the specific context of sexual selection has only more recently received research attention (see Greenfield and Rodríguez 2004; Bussière et al. 2008; Ingleby et al. 2010).

Recent mathematical models have begun to explore the potential consequences of G x Es in sexual selection (Kokko and Heubel 2008; Higginson and Reader 2009). Broadly, the results of these models suggest that G x Es could help to maintain genetic variation in sexual

traits (Kokko and Heubel 2008; providing a solution to the lek paradox, which states that strong directional selection from female choice should deplete the genetic variance in male sexual traits which is necessary for female choice to act on (Kirkpatrick and Ryan 1991)). This result is consistent with previous theory developed on G x Es in evolutionary genetics more generally (Via and Lande 1987). In addition, a second prediction made from the models suggests that G x Es could disrupt the information content, and hence reliability, of sexual traits as signals or displays of mate quality (Higginson and Reader 2009). The concept of signal reliability hinges upon a predictable relationship between phenotype and underlying genotype, and theory has demonstrated that G x Es for trait expression could weaken or completely remove any such relationship (Higginson and Reader 2009). These theoretical outcomes highlight the influence that G x E effects could have on the evolutionary dynamics of sexually selected traits. For example, mate choice can evolve through indirect benefits of choice (viability or attractiveness) associated with signal traits. This depends on there being genetic variation in sexual traits, as well as sexual signals being reliable indicators of the underlying genotype of a potential mate (Zahavi 1975; Grafen 1990). If G x Es in sexual trait expression cause signals to become unreliable, mating preferences will be costly and should be selected against. Understanding how sexual selection operates in nature therefore depends on understanding the role of G x Es in sexual selection and the empirical evaluation of existing theory.

So far, the focus of empirical work on G x Es in sexual selection has been on identifying whether or not there are G x Es for sexual trait expression. It is clear from this body of research that G x Es are widespread across a variety of sexual traits for a range of species (e.g. male eyespan in stalk-eyed flies (David et al. 2000); male acoustic signalling (Danielson-François et al. 2006) and female preference (Rodríguez and Greenfield 2003) in waxmoths; dominance in male bank voles (Mills et al. 2007); sperm length in flies (Morrow et al. 2008); male pigmentation in sticklebacks (Lewandowski and Boughman 2008) and guppies (Grether 2000); and genital morphology in treehoppers (Rodríguez and Al-Wathiqui 2011)). However, there are some inherent difficulties in relating such empirical work to current theory on G x Es in sexual selection. In particular, theory has defined ‘strong’ G x Es as ones which have ecological crossover of reaction norms and therefore involve a change in the ranked order of genotypes across environments, while ‘weak’ G x Es are those which do not have ecological crossover and represent only a change in the scale of genetic variation

across environments (see Figure 4.1). This distinction is explicit in both Kokko and Heubel's (2008) model, which assumes ecological crossover of G x E reaction norms, and Higginson and Reader's (2009) model, which tests interactions with no crossover. Although the classification of 'strong' and 'weak' G x Es is interesting and potentially useful, defining the strength of G x Es in this way makes interpretation in an empirical context difficult, since it is likely that the presence or absence of crossover in an empirical G x E will not be absolute. Rather, G x E variance will result from a combination of changes in the scale of variation across environments, as well as crossover. Therefore whilst it is possible to test for significant ecological crossover of reaction norms in a particular experiment, interpreting this as the sole indicator of interaction strength could potentially be misleading (Ingleby et al. 2010). Furthermore, with or without crossover, if a G x E only explains a small proportion of the overall phenotypic variation, it is unlikely to be very significant in evolutionary terms. Therefore, whilst the quantification of the degree of reaction norm cross-over will be useful, estimation of variance components, cross-environment genetic correlations and trait heritabilities are also likely to be revealing. Both Falconer (1952) and Via and Lande (1985) noted that one trait expressed in multiple environments can be thought of as multiple genetically correlated traits, and in this way, the genetic correlation describes the extent to which the phenotypic expression of the trait in different environments has the same genetic basis. In other words, the stronger the genetic correlation of trait expression across environments, the weaker the G x E effect on the expression of that trait (Falconer 1952; Via and Lande 1985).

In this study, we test for G x Es in the expression of cuticular hydrocarbons (CHCs) in *Drosophila simulans*. CHCs are thought to function as chemical signals between insects, and shorter-chained, more volatile CHCs are especially implicated in this (Ferveur 2005). This role of CHCs in chemical signalling has been studied in several contexts. This includes signalling between conspecific males in order to assess levels of male-male competition in crickets (Thomas and Simmons 2009), species recognition (Singer 1998; Blows 2002; Ferveur 2005), and intraspecific mating preferences in a wide range of insect species, including bees (Vereecken et al. 2007) and crickets (Ivy et al. 2005), as well as a number of *Drosophila* species (Cobb and Ferveur 1995; Blows 2002; Wicker-Thomas 2007). These studies suggest that CHC profiles are likely to be subject to sexual selection.

It has also been argued that insect CHCs originally evolved as a chemical barrier to help prevent water loss (Ferveur 2005), and as such it is likely that CHC profiles are subject to natural selection as well as sexual selection. Indeed, evidence from experimental evolution in *Drosophila* has demonstrated that CHC profile evolves through both natural and sexual selection (*D. serrata*, Rundle et al. 2009; and *D. simulans*, Sharma et al. 2012b), and as expected from these evolutionary responses, CHCs are heritable (*D. serrata*, Hine et al. 2004; and *D. simulans*, Sharma et al. 2012a). However, natural and sexual selection might favour different CHC profiles. It appears that long-chained CHCs form a more stable and protective layer than short-chained CHCs, and studies have shown that long-chained CHCs provide desiccation resistance in *D. melanogaster* (Savarit and Ferveur 2002; Foley and Telonis-Scott 2011), *D. mojavensis* (Gibbs et al. 1998), and *D. serrata* (Frentiu and Chenoweth 2010). The role of sexual selection on CHC profile has been most thoroughly studied in *D. serrata*, where female preferences for male CHC profiles have been examined in detail (Chenoweth and Blows 2005; Chenoweth et al. 2008; Rundle et al. 2008) and these preferences appear to exert directional selection on male CHC profile (Chenoweth and Blows 2005). Given that production of CHCs is thought to be costly (Blows 2002; Ferveur 2005), it is also likely that the different CHC functions will be traded against each another, making these hydrocarbons a particularly interesting multivariate sexual trait on which to focus.

In *D. simulans*, previous work has demonstrated that there are no direct benefits or direct costs to mate choice (Taylor et al. 2008; Taylor et al. 2010) but that male attractiveness is heritable (Taylor et al. 2007). Research has also shown that individual CHCs and overall CHC profiles are heritable in *D. simulans* (Sharma et al. 2012a). Specific CHCs and overall CHC profiles also influence male mating success (Ferveur and Cobb 2010; Berry et al. in prep; Chapter 3). *D. simulans* CHC profiles are therefore subject to sexual selection (Sharma et al. 2012a). Furthermore, both dietary (Berry et al. in prep; Chapter 3) and temperature (Sharma et al. 2011b; Chapter 3) effects on *D. simulans* CHCs have been identified. Given this evidence for both genetic and environmental variation in *D. simulans* CHC profile, it is likely that G x Es in CHC expression will be important.

Here, we used a quantitative genetic design to estimate the importance of G x Es for male and female CHC expression in *D. simulans.* Flies from a total of 60 iso-female lines were reared on different diets as larvae and then exposed to two temperature regimes

post-eclosion in a fully-factorial design, enabling the examination of any possible synergy between these environmental variables, as well as their individual effects. We estimated cross-environment genetic correlations and trait heritabilities for CHC expression, variance components for each of the G x E interactions, and the degree of ecological crossover between environments in order to interpret the biological significance of any G x Es and their potential consequences for the operation of sexual selection in this species.

4.3 Methods

Isolines and maintenance

Approximately 100 female *D. simulans* were collected from Greece in April 2010 and used to found iso-female lines (henceforth referred to as isolines) in the laboratory (*N* = 65). Within each isoline, approximately 25 male and 25 female offspring were used to found each generation. This process of inbreeding was repeated for 19 generations prior to this experiment, such that each isoline had been heavily inbred and can be considered a distinct genotype (David et al. 2005). Isolines were maintained on a cornmeal-based diet (supplied by Applied Scientific, UK; made from 1L deionised water boiled with 90g cornmeal, 80g brown sugar, 25g yeast, 12g agar and 2g methyl paraben) at 25^{o}C on a 10:14 hour light:dark cycle.

Environmental manipulations

We used male and female flies from a total of 60 isolines. The experimental setup used for each individual isoline in our quantitative genetic design is shown in Figure 4.2. At 3-4 days post-eclosion, adult flies from each isoline were established in small (40ml) vials with either 8ml standard cornmeal diet (diet A) or 8ml of a novel diet (diet B; made from 1L of deionised water boiled with 102g brown sugar, 72g oatbran, 24g yeast, 12g agar and 2g methyl paraben). We set up two replicate vials per isoline x environment combination, with two males and two females in each vial. These flies were given 3 days in which to lay in these vials before adults were removed, and the vials were then incubated at 25^{o}C on a 10:14 hour light:dark cycle during offspring development. Peak offspring eclosions occurred 10 days after laying, at which point male and female virgin offspring were collected from each replicate vial for each isoline x environment combination. Each virgin was transferred into a small (5ml) individual glass vial containing 1ml of the same diet on which they had

developed, and flies were then split equally between two post-eclosion temperatures (23^{o}C and 25^{o}C) in which they were incubated for 3 days. This created four environments, one from each combination of diet and post-eclosion temperatures in a 2x2 factorial design. In total, we reared 6 males and 6 females in each of these four environments from each isoline (*N* = 2880; Figure 4.2). After 3 days in the post-eclosion temperature treatments, each fly was transferred into a glass auto-sampler vial (Chromacol, UK) using an aspirator and stored at -80^{o}C prior to CHC analysis.

Cuticular hydrocarbon extractions

CHC extractions were carried out in sets of 100 samples per day, and randomised throughout by diet, post-eclosion temperature, sex and isoline. CHC extraction was carried out by soaking each individual fly in 50µl of a solution of 10ppm pentadecane in HPLC-grade hexane for 5 minutes, using a vortex for the duration of the final minute to agitate the solution and maximise CHC extraction. The fly was then removed from the vial using metal forceps which had been cleaned in hexane between each sample.

From each hydrocarbon sample, 2µl was injected into a GC-FID (Agilent 7890) fitted with two injectors, and two DB-1 columns of 30m x 0.25mm internal diameter x 0.25µm film thickness. We used hydrogen as a carrier gas. The inlet was set at 250^{o}C, and the injection was in pulsed splitless mode. Separation of the extract was optimized using a column profile which operated at 70^{o}C for one minute, and then increased at 20^{o}C/minute to 180^{o}C, then 4^{o}C/minute to 220^{o}C, and finally 15^{o}C/minute to 320^{o}C, where it was held for two minutes. Column flow was set at 1.2ml/minute. The FID detector heaters were set at 300^{o}C. The H_2 flow was 20ml/minute, and the air flow was 200ml/minute. Nitrogen was used to make up the column flow to 30ml/minute. This protocol has been optimised previously for *D. simulans* (Sharma et al. 2012b). Peak integration of hydrocarbon data was carried out using GC ChemStation software (version B.04.02.SP1).

Statistical analysis

Data reduction using principal components analysis

We quantified expression of 22 different hydrocarbons and used principal components analysis (PCA) to reduce the dimensionality of the data. CHC expression in *D. simulans* is quantitatively sexually dimorphic (Sharma et al. 2012b; present study), but the same CHCs

are produced by males and females. We were therefore able to identify and quantify expression of the same CHCs for males and females. We carried out PCA on the complete data set with male and female data combined, in order to obtain the same principal components (PCs) for males and females and allow examination of sex-specific patterns of CHC expression. PCs were extracted using the correlation matrix. We identified multivariate outliers based on Mahalanobis distances and removed these from the data, leaving 2429 individuals in subsequent analyses. Three orthogonal vectors with eigenvalues greater than 1 were extracted using PCA which together explained just over 70% of the total variation in CHC expression (Table 4.1). We interpret factor loadings for each CHC peak to these eigenvectors of more than 0.25 as biologically significant (Tabachnick and Fidell 1989).

Model fit and evaluation

We used Bayesian inferences implemented by the MCMCglmm package (v.2.12; Hadfield 2010) in R (v.2.13.0) to test multivariate generalised linear mixed effects models for the three PCs describing the variation in male and female CHCs in *D. simulans.* We included environmental terms (i.e. diet and post-eclosion temperature) as fixed effects, and genetic and G x E terms as random effects. We ran chains for 200,000 iterations with a burn-in of 10,000 and a thinning interval of 30.

In each model, we used a relatively uninformative prior (v = 0.02 for both fixed and random effects), which means that models were fitted with very little *a priori* information about the expected parameter estimates. We tested all models with a more informative prior (v = 2) and found that our results were robust to changes in prior specification. However, the results presented here used the relatively uninformative prior distribution for all models.

For the random effects terms, we used the 'idh' variance structure, which fits a unique variance for each PC whilst assuming the random effect covariance between PCs is zero. We also ran models using unstructured variances (with the 'us' variance structure in MCMCglmm), where all variances and covariances between PCs are estimated, and found that the results were similar. We used the 'idh' variance structure in the model results presented here since we were estimating environment-specific genetic variances, and so the use of 'idh' meant fewer estimated parameters and therefore reduced the need for highly informative priors.

We used preliminary analyses to test for the presence of sexual dimorphism in CHC expression. Firstly, we formed a full model with sex, diet, post-eclosion temperature and the interaction between environmental variables as fixed effects, and isoline and the interactions between isoline, diet and post-eclosion temperature as random effects. We then used the deviance information criterion (DIC) (see Spiegelhalter et al. 2002) to compare this full model with and without sex as a fixed effect. A lower DIC estimate indicates a better-approximating model, and the DIC of the model with sex as a fixed effect was far lower than that of the model without (ΔDIC = 1769.87). Comparison of these preliminary models therefore gave very high support for sexual dimorphism in CHC expression. We also used between-sex genetic correlations for each PC (calculated from a simplified model with no G x Es) to examine overall differences between male and female CHC expression. As these sex differences were large, we ran separate models for each sex. For each sex, we tested a set of six plausible models (see Table 4.2). These models are multivariate models which include all 3 PCs of CHC expression, but the inclusion of a 'trait' term in each model allows us to examine effects on individual PCs (details below). We estimated the support for each of these models using the model DIC, and also by calculating an approximate posterior probability for each model. This calculation takes into account the DIC of each model tested, and for each provides a probability that can be used to identify the best approximating model out of the set being tested.

Model interpretation

From the posterior distribution of the best model for each sex, we calculated the effect of the fixed environmental factors on CHC expression. From these models, we also partitioned variance into genetic and G x E components (following Lynch and Walsh 1998), and predicted mean trait value for each isoline in each environment from the posterior distribution (as an approximate equivalent to BLUPs). We used these scores to plot reaction norms for each G x E term in the best model for each sex.

In all calculations, we scaled the PC scores by the standard deviation of each PC in order to measure each PC on a comparable scale. We used variance standardisation since PCs are already mean-centred. Then, we ran a separate model for each possible G x E (isoline x diet and isoline x temperature separately) in each sex (leaving fixed effects unchanged). We used these simpler models instead of the best model for each sex for ease

of interpretation of the variance-covariance matrix. Following Lynch and Walsh (1998), we calculated cross-environment genetic correlations and heritabilities (with ±95% credible intervals around each estimate) both between and within environments for each PC, using estimates extracted from the posterior distribution variance-covariance matrix of each model. For the between-environment genetic correlations, we interpreted an estimate that deviated significantly from 1 (i.e. the credible interval did not overlap 1) as evidence for ecological crossover, since a correlation of 1 would have indicated a perfect correlation with no crossover. In addition, we calculated the proportion of crossover within each G x E we identified, in order to quantify the crossover which can be seen in the reaction norms. This was calculated (following Danielson-François et al. 2006) as the number of pairwise comparisons of isolines which had intersecting reaction norms, divided by the total number of possible pairwise comparisons. The heritabilities represent broad-sense rather than narrow-sense estimates due to our use of isolines (David et al. 2005). For our diet and temperature manipulations, we compared between-environment heritability estimates with the mean within-environment heritability estimates using a paired t-test, to show if heritability differed significantly between and within environments.

4.4 Results

Principal components analysis

From the PCA, we extracted three PCs with eigenvalues exceeding 1 (Table 4.1). PC1 describes the absolute quantity of CHCs produced, as each of the 22 CHCs measured exhibited a positive loading greater than 0.25. For PC2, 14 of the 22 hydrocarbons have a loading greater than 0.25, with short-chained hydrocarbons negatively-loaded and long-chained hydrocarbons positively-loaded. We interpret PC2 as a trade-off between production of long and short-chained CHCs. For PC3, only 9 of the 22 CHCs have loadings over 0.25, with a mixture of negative (mostly short-chained CHCs like docosene and docosane) and positive loadings (mostly long-chained CHCs such as pentacosene and a heavy alkene and branched alkane). This eigenvector therefore appears to describe a similar trade-off between these specific CHCs.

Model selection

The set of 6 models tested with male and female data are summarised in Table 4.2, along with the DIC estimate and approximate posterior probability associated with each model as a measure of statistical support. We have identified model (d) as the best model for the male data, which includes an isoline x diet interaction; and model (e) as the best model for the female data, which includes both isoline x diet and isoline x post-eclosion temperature interactions. Neither of these models includes the three-way interaction (isoline x diet x temperature). We present the results of each of these models (model (d) for males and model (e) for females) here in more detail. However, it is worth noting that whilst these models have the best support from the DIC and posterior probability, other models for each sex also have modest statistical support; namely, models (e) and (f) for males, and model (f) for females. Most importantly though, it is clear from all these models that G x E components of CHC expression are important in both sexes.

We modelled male and female CHC expression separately since preliminary analyses suggested high levels of sexual dimorphism (see Methods). These sex differences were highlighted throughout the process of model selection for each sex, and resulted in a different best model for male and female CHC expression (Table 4.1). Furthermore, the between-sex genetic correlation for each PC demonstrated that the genetic correlation of PC1 between sexes was quite high (r = 0.917; 95% credible interval: 0.723-0.993), was slightly lower for PC3 (r = 0.619; 95% CI: 0.372-0.807) and very weak for PC2 (r = 0.302; 95% CI: -0.433-0.855), indicating an advanced stage of sexual dimorphism for PC2 and PC3 (Lande 1980).

Environmental effects

The fixed effects of post-eclosion temperature, diet and the interaction between these two environmental variables were found in the best model for both male and female CHC expression (Table 4.2). There was a trend for both males (Figure 4.3) and females (Figure 4.4) to produce more CHCs overall (PC1) on the standard diet (diet A), although this effect was stronger in females as there was no overlap of credible intervals between diets.

Expression of PC2 and PC3 are dramatically different in males and females: males consistently have negative scores for PC2 and PC3 across all environments, whereas females consistently have positive scores. For males in the higher post-eclosion temperature, PC2

and PC3 have higher scores than at the lower temperature (Figure 4.3), indicating decreased investment in short-chained CHCs when the temperature is raised. This effect is clearest for PC3, and the difference in PC3 score between temperatures appears slightly larger on diet B (Figure 3). In females, credible intervals for PC2 and PC3 scores are large and overlap widely across all environments (Figure 4.4), and as such environmental effects appear to be much weaker for PC2 and PC3 than for PC1.

Genetic and G x E effects

As shown in Table 4.2, the best models for males and females both included a genetic (isoline) component of CHC expression, although the genetic variance in PC2 was very low for both sexes (Table 4.3). However, male and female CHC expression differed in terms of which G x E effects were important.

In males, only the isoline x diet interaction appears in the best model. The variance in this interaction was quite high for male PC1 compared to the other male PCs (Table 4.3). The isoline x diet interaction for each male PC is shown in the reaction norms in Figure 4.5 (a-c), where a large reduction in the extent of genetic variation in PC2 from the standard diet (diet A) to the novel diet (diet B) can be seen. Genetic correlation of each male PC across diets (Table 4.4) gives some evidence for ecological crossover, as the credible interval in each case does not overlap 1, showing a weakened genetic correlation across environments. However, the genetic correlation for PC3 was high, showing that male PC3 scores were still strongly correlated across diets and crossover is therefore unlikely to be of high importance. The proportion of crossover calculated within each interaction was 25% in PC1, 22% in PC2 and 19% in PC3.

The best approximating model for female CHC expression included both isoline x diet and isoline x temperature terms. The isoline x diet interaction explained substantial variance in PC1 (Table 4.3), and an increase in genetic variation for PC1 from the standard diet (diet A) to the novel diet (diet B) is evident from the reaction norm (Figure 4.5 (d-f)). Table 4.4 shows that there is evidence for some ecological crossover of female reaction norms across diets. The genetic correlation across diets for female PC1 and PC2 was low, whilst genetic correlation across diets for female PC3 was high, and so crossover is likely to be more important in PC1 and PC2 than in PC3. The proportion of crossover calculated for each PC

suggests a higher extent of crossover in PC2, and this can be visualised in the reaction norms presented in Figure 5 (d-f) (PC1: 12%; PC2: 35%; PC3: 12%).

The female isoline x temperature interaction accounted for a low level of variance in each PC (Table 4.3). Reaction norms for this interaction are shown in Figure 4.6. Genetic correlation of female PCs across temperatures again gives some evidence for crossover, as each interval estimate is lower than 1, although the correlation of PC3 across temperatures is high, indicating less crossover within this interaction (Table 4.4). The proportion of crossover calculated in the isoline x temperature interaction was 23% in PC1, 25% in PC2 and 16% in PC3.

Across males and females, the interval estimated for all cross-environment genetic correlations does not overlap 1 (Table 4.4), indicating a weakened correlation of trait expression between environments and providing evidence for significant ecological crossover. However, this effect is generally very small in both male and female PC3 across diets and temperatures. We also calculated heritabilities within and between environments for each PC for males (Table 4.5) and females (Table 4.6). Overall, PC2 exhibited low heritability estimates in both sexes ($0 < H^2 < 0.152$). There was more heritable genetic variation for PC1 ($0.077 < H^2 < 0.344$) and the heritability of PC3 was quite high ($0.391 < H^2 < 0.715$). For both sexes, heritability estimates are consistently lower between different environments than within the same environment. Paired *t*-tests showed that within-diet heritability was significantly higher than between-diet heritability ($t = 7.56$; $n = 6$; $P = 0.0006$). The same pattern, although slightly weaker, was found across the temperature manipulation, as heritability ($t = 6.05$; $n = 6$; $P = 0.002$) was higher within the same temperature than between different temperatures.

4.5 Discussion

Genotype-by-environment interactions (G x Es) for sexually selected traits have received increasing attention in recent years (Greenfield and Rodríguez 2004; Bussière et al. 2008; Ingleby et al. 2010) and theory has predicted they could help to maintain genetic variation in sexual traits subject to strong sexual selection (Kokko and Heubel 2008), whilst also having the potential to disrupt the reliability of sexual traits as signals of underlying mate quality (Higginson and Reader 2009). Here, we measured CHC expression of male and female *D. simulans* from isolines reared across two axes of abiotic environmental variation

(diet and post-eclosion temperature). Our results show that there are G x Es for diet in both male and female CHC expression and a G x E for temperature in female CHC expression. We also find some evidence for ecological crossover in each of the G x Es identified. We quantify each interaction using variance components, genetic correlations and heritabilities, and examine the potential implications for the operation of sexual selection in this species.

Not all of the variation in CHC expression described within the principal component vectors we analysed will necessarily be subject to sexual selection. However, the G x Es identified here are very likely to have an effect on the operation of sexual selection on *D. simulans* CHC profile for the following reasons: (1) in another study, we have examined sexual selection through female choice on exactly the same PCs of CHC expression across exactly the same environments, and we find evidence of sexual selection on each of the principal component vectors examined here (Chapter 3). Furthermore, these PC vectors are significantly aligned with PCs 1-3 in Sharma et al. (2012b), where experimental evolution caused these CHC combinations to evolve through sexual selection. (2) Analysis of individual CHCs shows significant G x Es in male and female expression of some specific CHCs which have previously been strongly implicated in *D. simulans* courtship and mating behaviour (7-tricosene, octadecadiene and pentacosadiene (see Ferveur and Cobb (2010); data not shown). Considered alongside previous research which has found strong genetic correlations between the expression of different CHCs in *D. simulans* (Sharma et al. 2012a), it is therefore extremely likely that sexual selection on particular combinations of CHCs drives the evolution of overall CHC profile through direct selection as well as correlated responses.

Our experimental design also enabled us to test for a G x E x E interaction (between isoline, diet and temperature), which would have indicated synergy between the different G x E effects studied here. However, this three-way interaction was not important in either sex. Studies of synergy between environmental variables have previously given mixed results, and so further research on the role of interactions between environmental factors has been encouraged (Sih et al. 2004). The lack of G x E x E interaction in our study indicates a very low level of genetic variation for the interaction between diet and temperature, and it is therefore unlikely that this interaction will have a significant effect on the evolution of CHC profile in *D. simulans*.

Isoline x diet and dietary effects on CHC expression

In both males and females, we found an isoline x diet interaction which shows genetic variation for diet-dependent aspects of CHC expression. In particular, there is high variance in this interaction for PC1, which describes the overall production of CHCs. Given the extensive research documenting the condition dependence of sexual traits (Rowe and Houle 1996), it is not surprising that the resources accumulated through diet affect overall investment in CHC production, although our data does not explicitly provide evidence for condition dependence. Gosden and Chenoweth (2011) found that dietary manipulation revealed condition dependence of male CHC expression in *D. serrata*, but they found no evidence for genetic variation underlying this diet-mediated plasticity. We find that male and female *D. simulans* in our study generally produce more CHCs on diet A than diet B, but the isoline x diet interaction in both sexes reveals that there is genetic variation underlying patterns of resource allocation across diets. The capture of genetic variance in condition dependent traits is an idea which has been discussed previously as a mechanism to maintain genetic variation in sexual traits (Rowe and Houle 1996; Tomkins et al. 2004; Kokko and Heubel 2008). We find weakened cross-environment genetic correlations for heritable aspects of male and female CHC profile between diets, and so our results are at least consistent with the concept that the response to selection can differ between dietary environments and genetic variation could be maintained.

The potential for G x E to maintain genetic variation in sexually-selected aspects of male CHC expression is of course dependent on whether there is G x E for female preference for male CHCs, and if the reaction norms for preference and signal perfectly match across environments (Greenfield and Rodríguez 2004). Without data on female preference G x E, we are unable to provide a definitive test for this. However, the G x E for diet in female CHC expression could also have evolutionary significance, as there is some evidence for male mate choice in *D. melanogaster* (Byrne and Rice 2006) and *D. serrata*, where males prefer specific female CHC profiles (Chenoweth and Blows 2005), such that G x Es in female CHC profile could influence the evolution of these female signals and the associated male mating preferences. However, experimental evolution in *D. simulans* suggests that the influence of sexual selection on female CHC profile might be weak compared to that of natural selection (Sharma et al. 2012b) hence the evolutionary significance of this G x E in female CHC expression is unclear. We do, however, find quite a

high between-sex genetic correlation for PC1, and so evolution along PC1 in females might correlate quite strongly with that of PC1 in males.

Post-eclosion temperature and isoline x temperature effects on CHC expression

Whilst there is a clear overall temperature component to male CHC expression, we only find an isoline x temperature interaction in female CHCs. This G x E indicates that the effect of temperature on female CHC expression differs between genotypes, however, the overall effect of temperature on females is not as strong as in males. The low variance in the female isoline x temperature interaction probably reflects, in part, the low overall variance in female CHC expression between temperatures. Female *D. simulans* generally have larger body size than males, and so the surface area to volume ratio is lower in females, and this might therefore explain the weaker response to temperature variation in females. Furthermore, if the main function of short-chained CHCs is likely to be as sexual signals to allow males to attract females, then selection for female investment in short-chained CHCs might be weak, and females might invest more in long-chained, protective CHCs (Foley and Telonis-Scott 2011) .In agreement with this, we find that female PC2 and PC3 scores are consistently positive across both temperatures, indicating a bias towards production of long-chained CHCs regardless of temperature.

The lack of G x E for post-eclosion temperature in male CHC expression indicates that differences between temperatures are relatively consistent across genotypes. The effect of temperature on male CHCs is strong and can be seen clearly in the trade-off described by PC2 and PC3. Long-chained CHCs are likely to be naturally selected for desiccation resistance, and risk of desiccation will be elevated at higher temperatures (Savarit and Ferveur 2002; Foley and Telonis-Scott 2011). Consistent with this, we find that at lower temperatures, the decreased risk of desiccation appears to allow males of all genotypes to invest less in long-chained CHCs, and therefore allocate more resources towards producing smaller, more volatile CHCs which could improve male attractiveness. Male attractiveness might therefore be affected by post-eclosion temperature and we might expect female preferences to differ across a temperature gradient, although this remains to be established.

However, while there was no isoline x temperature interaction for male CHC expression in the best model, this interaction was important in two other male models with

some statistical support, albeit more limited. This perhaps explains why we find a similar pattern in heritability and genetic correlation of male CHC expression across both temperature and diet manipulations, although there is some evidence from the comparison of within- and between-environment heritability estimates that this pattern is weaker across temperatures than across diets. Given the large body of evidence for strong temperature-dependent selection on the trade-off between long- and short-chained CHCs in *Drosophila* (e.g. Gibbs et al. 1998; Ferveur 2005; Frentiu and Chenoweth 2010), it is possible that the optimal male response in PC2 and PC3 to temperature variation has become canalised between genotypes. Alternatively, selection on this trade-off might simply have eroded genetic variation for plasticity in male CHC profile across temperatures (Roff and Fairbairn 2006). This explanation is unconvincing for PC3, where estimates of genetic variance are high, but may be the case for PC2, and therefore evolution along PC2 could be constrained by the low heritable genetic variation in this vector.

Quantifying G x E effects

Theory has distinguished between G x E interactions with or without ecological crossover as strong or weak interactions, respectively (Kokko and Heubel 2008; Higginson and Reader 2009). However in empirical studies, this definition of interaction strength is difficult to apply. In part, this is because the influence an interaction has on trait expression and evolution will not only depend on changes in the rank order of genotypes between environments, but also on the extent of genetic variation present. Whilst we show here that there is evidence for ecological crossover of reaction norms in each of the G x Es we identify, we also find that the extent of this crossover varies, such that each G x E appears to result from a combination of ecological crossover and a change in the scale of variation across environments. This is likely to be the case in most empirical studies of G x Es, and so relating these results to theoretical work which is based on an absolute presence or absence of crossover is difficult.

Here, we calculate cross-environment trait heritability and genetic correlations in an attempt to quantify the effect of each G x E and predict its evolutionary significance. The application of cross-environment genetic correlations to the study of G x Es in evolutionary genetics was demonstrated over 25 years ago by Via and Lande (1985), but has not been used to a great extent in the recent spate of studies on G x Es in sexual traits (but see Jia et

al. 2000 and Rodríguez and Al-Wathiqui 2011). With G x Es, heritability and genetic correlation will be weakened across different environments, and this is the overall pattern we find here. Furthermore, if crossover represents a strong interaction, we would expect the extent to which these estimates are weakened between different environments to be larger where there appears to be more crossover. However, this trend is not consistent within the genetic correlations and proportion of crossover we calculate from our data. Reaction norms and crossover are useful to examine phenotypic effects of G x Es, but as we show here, trait heritability and genetic correlation are also important when interpreting results in terms of sexual selection and trait evolution.

There is evidence from a range of insect species (Ivy et al. 2005; Vereecken et al. 2007; Wicker-Thomas 2007), including *Drosophila* (Hine et al. 2002; Sharma et al., 2012b), that CHCs have evolved a role as sexual pheromones which females might use to assess male attractiveness. In *D. simulans*, we have found heritable genetic variation and isoline x diet interactions for aspects of male CHC expression which are likely to be sexually selected. For CHC profiles to be reliable signals of the underlying genetic quality of a male there must be a predictable relationship between phenotype and the benefits of mating with a particular individual. Theoretical work has made the prediction that when there are G x Es in the expression of a sexual signal, the genotype-phenotype relationship can be disrupted by environmental change and environmental stress (Greenfield and Rodríguez 2004; Higginson and Reader 2009). This prediction is supported here by very low trait heritability between environments. Previously, heritability estimates have been used in this way to infer potential signal unreliability through environmental stress in the bank vole, *Clethrionomys glareolus* (Mills et al. 2007). We studied what we believe to be an unstressful range of environmental variation, and also find potential for CHC sexual signal reliability to be disrupted in *D. simulans*.

A large body of research has addressed the potential problem of the maintenance of genetic variation in sexual traits which are subject to directional selection through mating preferences (Kirkpatrick and Ryan 1991; Rowe and Houle 1996; Radwan 2008). Recent theoretical work has proposed that G x Es could help to maintain genetic variation in sexual traits in heterogeneous environments (Greenfield and Rodríguez 2004; Kokko and Heubel 2008). Indeed, there is already evidence from the waxmoth, *Achroia grisella*, which suggests that G x Es in male acoustic sexual signals contribute towards the maintenance of genetic

variation (Jia et al. 2000). We show here that G x Es have the potential to maintain genetic variation in sexually selected components of male CHC profile. Theoretical work highlights the importance of G x Es with crossover in the maintenance of genetic variation (Kokko and Heubel 2008), but in our data we find that G x Es which vary in the extent of crossover have the potential to maintain genetic variation in sexually selected components of male CHC profile. Our results illustrate the usefulness in an evolutionary context of considering estimates of genetic variation alongside phenotypic measurement of G x Es.

In conclusion, across the range of environmental variation studied here, we find G x Es in the expression of components of *D. simulans* CHC profile which are likely to function as sexual traits. Our data demonstrates G x Es for diet for males and females, and a G x E for temperature for females. We find some evidence for ecological crossover in these G x Es, and show that G x Es in this system cause weakened cross-environment genetic correlations and heritabilities. Therefore, as predicted by theory, G x Es in *D. simulans* CHC profile have the potential to contribute to the maintenance of genetic variation, as well as the potential to disrupt the reliability of sexual signals. These are fundamental concepts in sexual selection research, and so further work will therefore be necessary to test the consequences of these G x Es on sexual selection, and particularly on the evolution of mating preferences.

Table 4.1 Principal component analysis for CHC expression in both sexes. Three principal components with eigenvalues > 1 were extracted for further analyses, explaining just over 70% of the total variation in CHC profile. Biological significance of each component was interpreted from factor loadings > 0.25 (in bold). CHCs are named where known; unnamed CHCs (asterisks) are described by basic chemical structure. CHCs are listed in order of increasing chain length.

	PC1	**PC2**	**PC3**
Eigenvalue	9.731	3.933	1.979
% variance	44.232	17.875	8.997
Loadings:			
Octadecadiene	**0.680**	**-0.252**	0.029
Docosene	**0.403**	-0.039	**-0.314**
Docosane	**0.836**	0.102	**-0.328**
Branched alkane*	**0.714**	**-0.427**	-0.179
7-Tricosene	**0.845**	**-0.271**	0.138
Tricosene	**0.685**	**-0.286**	-0.138
Tricosane	**0.723**	-0.110	-0.025
Branched alkane*	**0.729**	**-0.430**	-0.205
Branched alkane*	**0.797**	-0.206	**-0.388**
Branched alkane*	**0.725**	**-0.405**	-0.075
Tetracosane	**0.700**	**0.613**	**-0.284**
Pentacosadiene	**0.651**	**0.459**	-0.265
Alkene*	**0.591**	0.029	**0.588**
Pentacosene	**0.537**	**0.258**	**0.400**
Pentacosane	**0.752**	**0.595**	0.017
Branched alkane*	**0.776**	0.071	**-0.258**
Hexacosane	**0.536**	**0.787**	-0.208
Heptacosane	**0.736**	**0.344**	-0.040
Branched alkane*	**0.500**	0.206	**0.589**
Alkane*	**0.540**	**0.800**	0.122
Alkane*	**0.417**	0.178	**0.592**
Alkane*	**0.486**	**0.807**	-0.147

Table 4.2 Summary of the set of six models tested. Male and female data was modelled separately. The model with the highest support for each sex is in bold, chosen using the DIC and supported by the approximate posterior probability.

	Model rationale	**Model formula *** *[fixed effects (random effects)]*	**Males** *[DIC (posterior model probability)]*	**Females** *[DIC (posterior model probability)]*
(a)	No genetic component	E*d* + E*t* + E*d*:E*t*	15254.58 (<0.0001)	14105.49 (<0.0001)
(b)	Genetic component but no G x E interactions	E*d* + E*t* + E*d*:E*t* + (G)	14977.33 (0.003)	13913.21 (0.005)
(c)	G x E for post-eclosion temperature manipulation only	E*d* + E*t* + E*d*:E*t* + (G + G:E*t*)	14978.71 (0.001)	13906.52 (0.13)
(d)	**G x E for dietary manipulation only**	**E*d* + E*t* + E*d*:E*t* + (G + G:E*d*)**	**14966.68 (0.53)**	13910.55 (0.02)
(e)	**G x Es for both environmental manipulations**	**E*d* + E*t* + E*d*:E*t* + (G + G:E*t* + G:E*d*)**	14968.10 (0.25)	**13903.59 (0.58)**
(f)	Both G x Es plus G x ExE	E*d* + E*t* + E*d*:E*t* + (G + G:E*t* + G:E*d* + G:E*t*:E*d*)	14968.50 (0.21)	13905.16 (0.26)

* E*d* = diet; E*t* = post-eclosion temperature; G = isoline

Table 4.3 Posterior mean of genetic (Isoline) and G x E (Isoline x diet for males; Isoline x diet and Isoline x temperature for females) variance components (with 95% credible interval) for PC1, PC2 and PC3 for each sex. Components are calculated from the best model for each sex.

	Males		**Females**		
	Isoline	*Isoline x diet*	*Isoline*	*Isoline x diet*	*Isoline x temperature*
PC1	0.947	0.256	0.466	0.106	0.055
	(0.491-1.604)	(0.071-0.549)	(0.183-0.878)	(0.014-0.276)	(0.005-0.173)
PC2	0.078	0.025	0.054	0.028	0.024
	(0.022-0.171)	(0.007-0.106)	(0.014-0.119)	(0.005-0.068)	(0.003-0.069)
PC3	0.266	0.036	0.236	0.019	0.027
	(0.165-0.407)	(0.012-0.071)	(0.144-0.369)	(0.003-0.047)	(0.006-0.062)

Table 4.4 Cross-environment genetic correlations (with 95% credible interval) for each male and female PC calculated from the posterior distribution of separate models for each possible G x E (fixed effects unchanged), using variance-scaled PC scores.

	Males		**Females**	
	Diet	*Temperature*	*Diet*	*Temperature*
PC1	0.562 (0.133-0.872)	0.662 (0.256-0.921)	0.446 (-0.065-0.794)	0.489 (-0.008-0.818)
PC2	0.307 (-0.512-0.877)	-0.262 (-0.767-0.383)	0.228 (-0.296-0.662)	0.209 (-0.317-0.660)
PC3	0.778 (0.551-0.928)	0.937 (0.835-0.988)	0.765 (0.533-0.912)	0.741 (0.505-0.896)

Table 4.5 Heritability (with 95% credible interval) of each male PC, as calculated from the posterior distribution of separate models for each possible G x E (fixed effects unchanged), using PC scores scaled by standard deviation. Within-environment heritability is unshaded and between-environment heritability is shaded.

Environment		**PC1**	**PC2**	**PC3**
Temperature	*within 23^{o}C*	0.254 (0.110-0.462)	0.152 (0.060-0.299)	0.535 (0.309-0.871)
	between temperatures	0.158 (0.048-0.300)	-0.029 (-0.103-0.039)*	0.527 (0.321-0.827)
	within 25^{o}C	0.233 (0.099-0.420)	0.081 (0.022-0.181)	0.598 (0.353-0.961)
Diet	*within diet A*	0.344 (0.165-0.594)	0.095 (0.032-0.198)	0.584 (0.335-0.950)
	between diets	0.145 (0.028-0.294)	0.017 (-0.027-0.068)	0.500 (0.275-0.816)
	within diet B	0.198 (0.081-0.374)	0.035 (0.006-0.092)	0.715 (0.424-1.144)

* Interpreted as H^2 = 0 since the credible interval overlaps 0

Table 4.6 Heritability (with 95% credible interval) of each female PC, as calculated from the posterior distribution of separate models for each possible G x E (fixed effects unchanged), using PC scores scaled by standard deviation. Within-environment heritability is unshaded and between-environment heritability is shaded.

Environment		PC1	PC2	PC3
Temperature	*within 23^{o}C*	0.176 (0.070-0.344)	0.117 (0.051-0.223)	0.449 (0.252-0.732)
	between temperatures	0.081 (-0.001-0.190)	0.027 (-0.038-0.102)	0.391 (0.204-0.648)
	within 25^{o}C	0.155 (0.059-0.310)	0.141 (0.054-0.285)	0.624 (0.355-1.016)
Diet	*within diet A*	0.115 (0.043-0.234)	0.138 (0.056-0.268)	0.506 (0.284-0.840)
	between diets	0.077 (-0.010-0.185)	0.027 (-0.032-0.099)	0.392 (0.205-0.657)
	within diet B	0.256 (0.112-0.461)	0.101 (0.042-0.198)	0.524 (0.296-0.850)

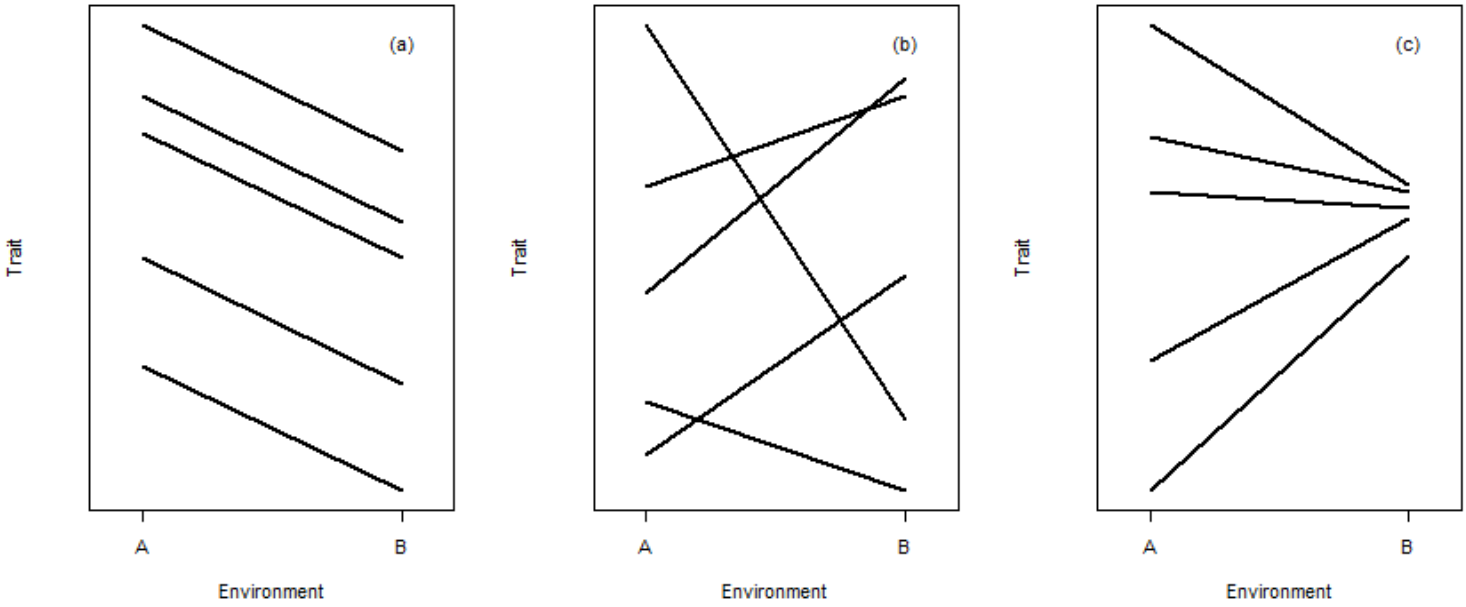

Figure 4.1 Reaction norms showing some measure of trait expression (y-axis) across two hypothetical environments (x-axis). Each line represents a different genotype. (a) Phenotypic plasticity between environments but no G x E interaction, as each genotype responds to the environmental variation in the same way. (b) G x E interaction with ecological crossover of reaction norms. The direction and extent of phenotypic plasticity varies between genotypes and results in crossover in this example, where the ranked order of genotypes changes between environments. (c) G x E interaction without ecological crossover. Again, the direction and extent of phenotypic plasticity differs between genotypes, but here, reactions norms do not cross, and the main effect of the interaction is that the scale of genetic variation between genotypes changes between environments.

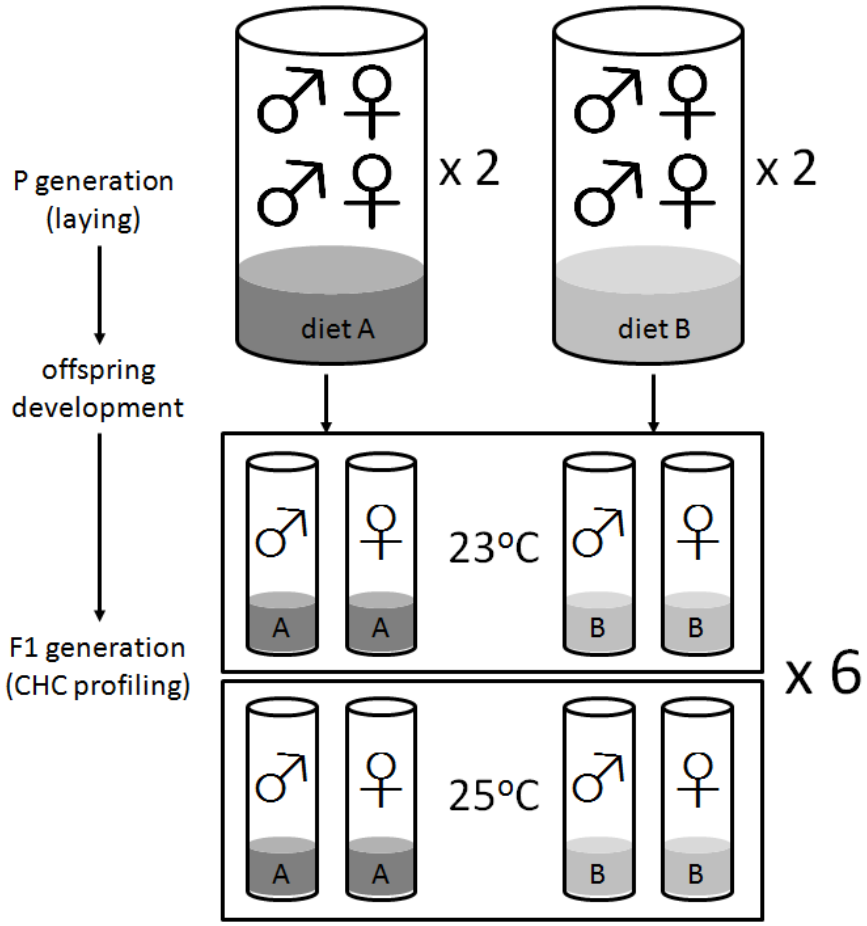

Figure 4.2 Experimental setup for each individual isoline (*N* = 60). Isoline adults (P generation) were set up in laying vials of either diet A or diet B. There were two replicate vials of each diet for each isoline, and each vial had two males and two females. These adults were given a laying period of 3 days before being removed, and the vials were incubated at 25°C on a 10:14 light:dark cycle during offspring development. 10 days after laying, peak offspring eclosions occurred and male and female virgin offspring were collected from each diet (F1 generation). Offspring were housed individually on the same diet as development and assigned to either 23°C or 25°C post-eclosion temperature, creating a 2x2 factorial design of four environmental treatments. From each isoline, CHC expression was analysed for 6 males and 6 females from each of the four treatments.

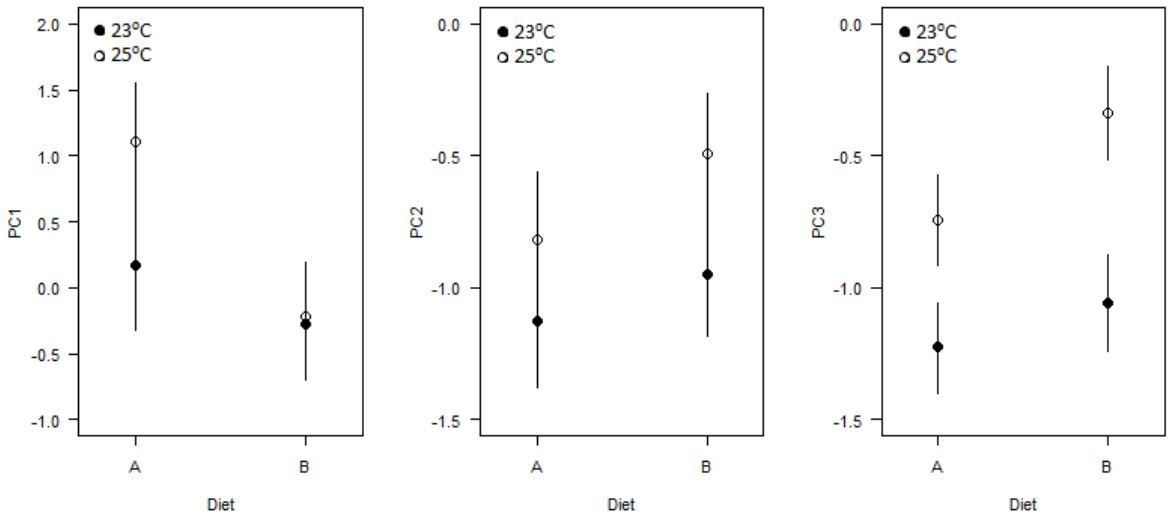

Figure 4.3 Overall posterior mean ± 95% credible interval from the best model for male CHC expression, showing the effect of diet (A or B; x-axis) and post-eclosion temperature (23°C or 25°C; see legend) on male PC1, PC2 and PC3 scores (left to right).

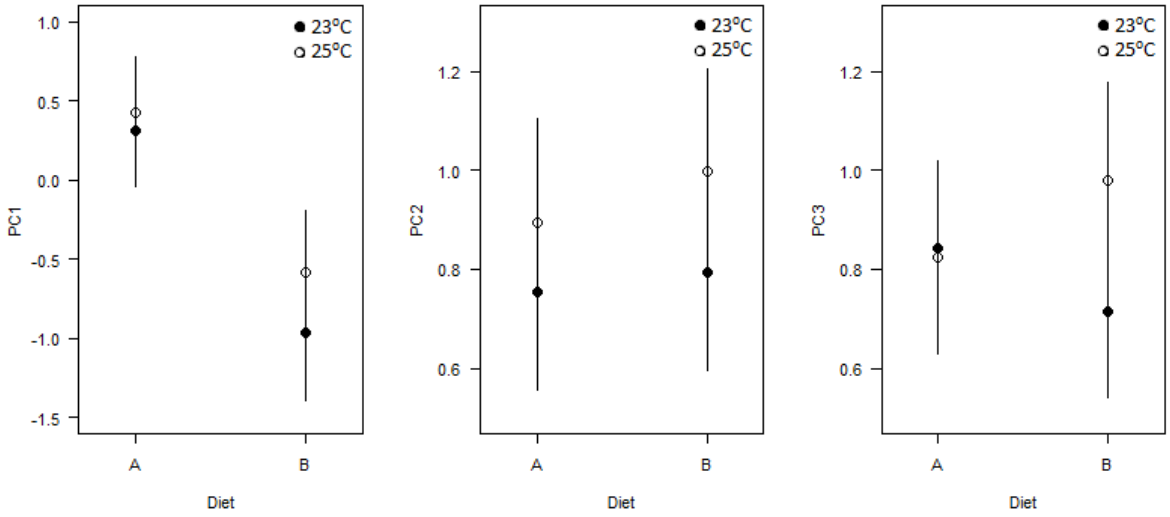

Figure 4.4 Overall posterior mean ± 95% credible interval from the best model for female CHC expression, showing the effect of diet (A or B; x-axis) and post-eclosion temperature (23°C or 25°C; see legend) on female PC1, PC2 and PC3 scores (left to right).

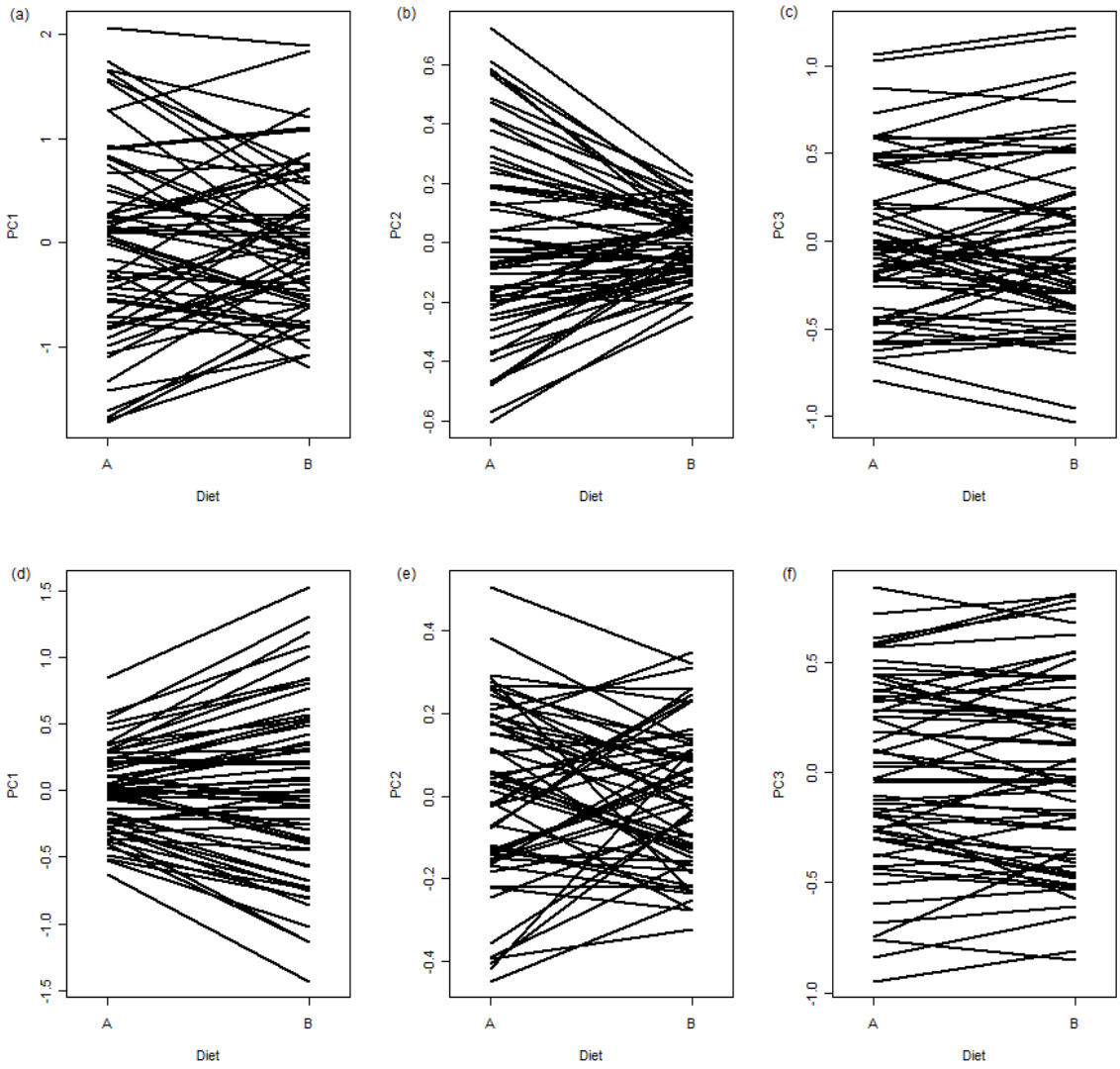

Figure 4.5 Reaction norms for the isoline x diet interaction in male CHC expression for (a) PC1, (b) PC2 and (c) PC3; and for female CHC expression for (d) PC1, (e) PC2 and (f) PC3.. Each point represents the posterior mean for a given isoline in each environment, as an approximate equivalent to BLUPS, calculated from the posterior distributions of the best models for male and female CHC expression. Calculation of the proportion of crossover in male reaction norms showed 25% in PC1, 22% in PC2 and 19% in PC3; and in female reaction norms showed 12% in PC1, 35% in PC2 and 12% in PC3.

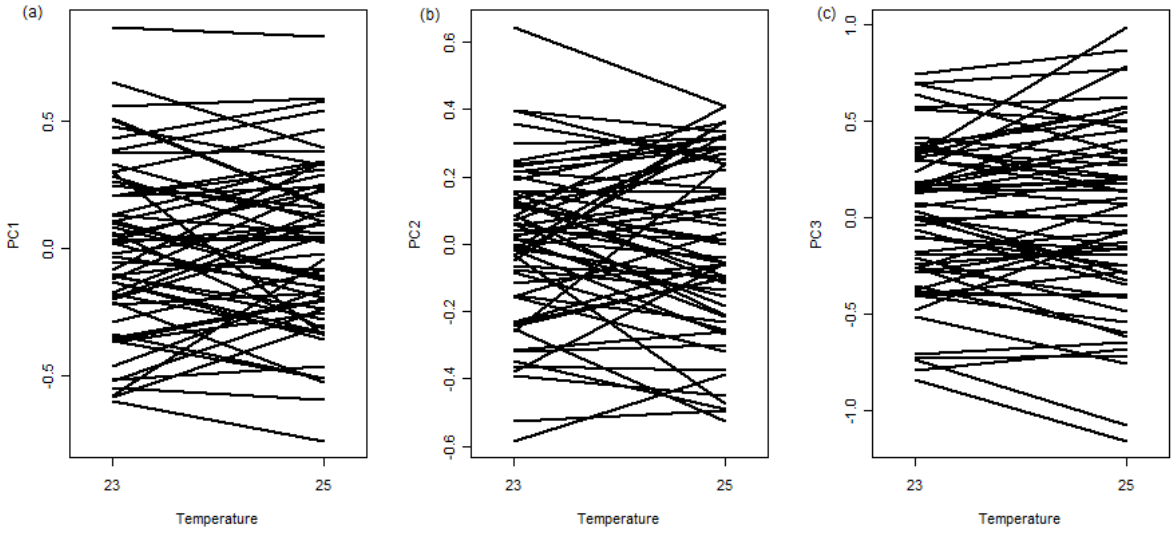

Figure 4.6 Reaction norms for the isoline x post-eclosion temperature interaction in female CHC expression for (a) PC1, (b) PC2 and (c) PC3. Each point represents the posterior mean for a given isoline in each environment, as a Bayesian equivalent to BLUPS, calculated from the posterior distribution of the best model for female CHC expression. Calculation of the proportion of crossover showed 23% in PC1, 25% in PC2 and 16% in PC3. There was no isoline x temperature interaction in the best model for male CHC expression.

CHAPTER 5: Heritability of male attractiveness persists despite evidence for unreliable sexual signals in *Drosophila simulans*

5.1 Abstract

Sexual signals can be used to attract mates, but to be honest indicators of signaller quality they need to convey information reliably. However, environmental variation and genotype-by-environment (G x E) interactions have the potential to compromise the reliability of sexual signals. Here we test the reliability of cuticular hydrocarbons (CHCs) as signals of heritable aspects of male attractiveness in *Drosophila simulans*. We examined the heritability of male attractiveness and a measure of the difference between fathers and sons' CHC profiles across dietary and temperature environments. Our results show that environmental heterogeneity disrupts the similarity of some components of father and son CHC profile. However, male attractiveness is heritable within and across environments, so that sire attractiveness is a good predictor of son attractiveness even with environmental heterogeneity. This suggests that although some components of a male's CHC profile are disrupted by environmental variation, on average, attractive genotypes retain their attractiveness across environments.

5.2 Introduction

Mating choice (usually by females) can be based on sexual signals expressed in the opposite sex (usually males), and sexual signals may reflect the possible benefits of mating with that individual. Benefits of choice can be direct through resources provided to the female (Andersson 1994; Møller and Jennions 2001), or indirect through attractiveness or viability benefits inherited by the offspring (Lande 1981; Kirkpatrick 1982; Wedell and Tregenza 1999; Head et al. 2005; Taylor et al. 2007). The evolution of mating preferences for sexual displays depends to some extent on signal reliability, since expressing preferences for signals which do not contain reliable information about benefits will be costly and should be selected against (Maynard Smith and Harper 2003).

For sexual signals to reliably indicate benefits to females, we expect a positive correlation between benefits and signal attractiveness. This positive correlation has been found in several species with direct benefits of mate choice (see Andersson 1994), although clear evidence from species with indirect benefits of mate choice is less common (e.g.

Qvarnström et al. 2006). It is also thought that information contained in sexual signals can vary between environments (Greenfield 1994; Candolin 2003). If male signal expression is dependent to some extent on the environment, then this environmental component could mean that the signal is a less reliable indicator of benefits across heterogeneous environments. In this way, sexual signals may become unreliable if the environmental conditions change or if males move between different environments (Greenfield and Rodríguez 2004; Higginson and Reader 2009; reviewed by Ingleby et al. 2010). This potential for environmental heterogeneity to compromise the reliability of sexual signals could affect the evolution of mating preferences and the operation of sexual selection by female choice in heterogeneous environments.

In terms of direct benefits of mate choice, if a male advertises for mates in different environmental conditions from those experienced during development, then female assessment of the male could be based on a signal phenotype which does not reflect the ability of the male to provide direct benefits in the current environment. On the other hand, a reliable signal of indirect benefits of mate choice needs to contain information about the underlying genetic quality of a potential mate. If genotype performance differs between environments, then a male could develop an attractive signal in one environment, but the genes inherited by the offspring may not yield benefits if offspring experience different environmental conditions (Ingleby et al. 2010).

Consequently, sexual signals of genetic quality could be unreliable when the genetic variance underlying sexual trait expression (V_G; the variance explained by genetic effects) changes between environments. Changes in genetic variation across heterogeneous environments can be driven by environmental stressors or differential selection between environments (reviewed by Hoffmann and Merilä 1999). In addition to these overall environmental effects, genotype-by-environment interactions (G x Es) can be associated with changes in genetic variation across environments. A G x E in trait expression will mean that individuals with identical genotypes can differ phenotypically when exposed to different environmental conditions, and that the extent and direction of this plasticity differs between genotypes (Lynch and Walsh 1998). G x Es can be interpreted as genetic variation for phenotypic plasticity and might therefore involve changes in genetic variation across environments, although this is not necessarily always the case. Recent theoretical work has demonstrated that G x Es can potentially disrupt sexual signal reliability in

heterogeneous environments (Greenfield and Rodríguez 2004; Kokko and Heubel 2008; Higginson and Reader 2009; reviewed by Ingleby et al. 2010), and a few empirical studies also find this (Jia et al. 2000; Mills et al. 2007). Consequently, it appears that both environmental heterogeneity with and without G x Es in sexual signal expression can potentially decrease the reliability of a sexual signal for indirect benefits by disrupting the relationship between trait expression and underlying genetic quality. Indeed, some studies have also shown G x E for a variety of fitness components (Mills et al. 2007; Danielson-François et al. 2009; Lewis et al. 2012). Ultimately, the breakdown of the relationship between signal phenotype and underlying genotype has the potential to degrade the correlation between signal and preference which is essential to many models of sexual selection for indirect benefits (Kokko et al. 2006), although empirical work examining this idea is scarce.

In *Drosophila*, body size (Partridge et al. 1987), courtship behaviour (Hall 1994) and sex comb structure (Markow 1996) have all been shown to contribute to male attractiveness. More recently, research on a number of species has found that cuticular hydrocarbons (CHCs) are an important determinant of male attractiveness, and that short-chained, volatile CHCs can act as sexual pheromones to attract mates (Cobb and Ferveur 1995; Blows 2002; Wicker-Thomas 2007). Experimental evolution has shown that CHC profiles evolve in response to sexual selection in *D. serrata* and *D. simulans* (Blows 2002; Rundle et al. 2009; Sharma et al. 2012b). In *D. serrata,* female mate preference exerts strong directional selection on male CHC profiles (Chenoweth and Blows 2005). There is also evidence for a strong environmental component of CHC expression in *Drosophila* species. In particular, temperature variation is thought to favour longer-chained CHCs which form a stable and protective chemical barrier to water loss, and in *D. melanogaster* (Savarit and Ferveur 2002; Foley and Telonis-Scott 2011), *D. mojavensis* (Gibbs et al. 1998), and *D. serrata* (Frentiu and Chenoweth 2010) studies have shown that long-chained CHCs can provide desiccation resistance.

Here, we present a quantitative genetic study of male attractiveness and CHC profile in *D. simulans* across a range of environmental variation. Research on our study population has indicated not only that there are temperature and diet effects on CHC expression, but also that there are G x Es with the potential to disrupt signal reliability (Ingleby et al. in review). Previous work on *D. simulans* has demonstrated that there are no direct benefits or

costs to mate choice (Taylor et al. 2008; Taylor et al. 2010; Sharma et al. 2012c), but that male attractiveness is heritable (Taylor et al. 2007; Hosken et al. 2008). Thus, genetic benefits could drive the evolution of female mating preferences in this species. As noted above, *D. simulans* CHCs are heritable and evolve through sexual selection (Sharma et al. 2012b), and we have also found that male CHC profile influences female mating preferences (Berry et al. in prep; Chapter 3). Therefore, CHCs in *D. simulans* are an ideal trait on which to focus to examine how environmental variation and G x Es might affect sexual signalling. We reared male *D. simulans* from forty-seven iso-female lines across a range of abiotic environmental variation (post-eclosion temperature and diet) for two generations. For both sires and sons, we quantified CHC expression and attractiveness. We test heritability of male attractiveness across environments, in order to assess cross-environment indirect benefits of female preference in this species. We then relate this to the expression of male CHC profiles across these environments, as a sexual signal in *D. simulans*. We also test variation across environments in the absolute difference between sire and son CHC profile, and attempt to relate this to environmental and G x E components of CHC expression. Based on theoretical work, we make two predictions: (1) Environmental heterogeneity and G x Es for male CHC expression will make CHCs an unreliable sexual signal of heritable aspects of male attractiveness; and (2) Where sexual signals are unreliable, benefits of mating preferences will be compromised through reduced heritability of male attractiveness across environments.

5.3 Methods

Isolines and maintenance

Approximately 100 female *D. simulans* were collected from Greece in April 2010 and used to found iso-female lines (isolines) in the laboratory (N = 65 as some isolines were lost during the inbreeding regime). A random subset of 47 of these remaining isolines were used in this experiment. Within each isoline, 25 male and 25 female offspring were used to produce each new generation, and this process of inbreeding was repeated for 21 generations prior to this experiment, such that each isoline had been heavily inbred and can be considered as a distinct genotype (David et al. 2005). The wild-collected flies were also used to set up an outbred population containing the same genetic variation as the isolines. The outbred population was maintained with overlapping generations at an approximate population size

of 500 flies. All flies were maintained on a standard cornmeal-based diet (Applied Scientific, UK) at 25^{o}C on a 10:14 hour light:dark cycle, unless stated otherwise.

Environmental manipulations

We carried out post-eclosion temperature and dietary manipulations as separate experiments but with equivalent experimental design (see Figure 5.1). We set up the same set of 47 isolines for the post-eclosion temperature and dietary manipulations, but only 44 of these isolines survived on the novel diets used for the diet manipulation. Flies from the outbred population were reared on the standard cornmeal diet, to generate a stock of outbred flies from a standard environment for use in attractiveness assays (described below).

For each experiment, adult flies were taken from each isoline and used to set up two replicate laying vials (40ml vials with 8ml of medium) for each isoline/environment combination, with two males and two females in each. For the post-eclosion temperature manipulation, all flies laid on the cornmeal-based diet. For the dietary manipulation, we used two novel diets: a homemade oat-based medium (consisting of oatbran, sugar, and yeast set in water and agar; diet A) and a soy-based medium (Genesee Scientific, USA; diet B). These diets were chosen purely to create dietary environmental variation rather than to manipulate diet quality. Flies were given 72 hours in which to lay eggs in these vials before being removed, and the vials were then incubated at 25^{o}C during offspring development. During this 72-hour laying period, flies from the outbred population laid in large vials (150ml) with 30ml of the standard cornmeal diet, and these vials were then kept in the same incubator during offspring development.

Peak offspring eclosion occurred after 11 days, and virgin flies were collected from both isoline and outbred population vials. Vials were cleared out between 7am and 8am. Newly-eclosed virgin adults were collected between approximately 11am and 1pm, and again between 5pm and 7pm. Virgin females were collected from the outbred population vials and subsequently housed individually in small vials of the standard cornmeal diet (40ml vial with 8ml of medium). This created a stock of outbred females reared in a standard environment for use in attractiveness assays and avoided biasing assays through any co-evolved genetic effects within isolines. In this first experimental generation, both virgin males and females were collected from the isoline vials and transferred into small individual

glass vials (5ml with 1ml of medium). For the dietary manipulation, flies were kept on the same food type as development at 25^{o}C. For the post-eclosion temperature manipulation, all flies were kept on the standard cornmeal diet but split evenly between two post-eclosion temperatures: 23^{o}C or 25^{o}C. Flies were given 72 hours to mature in their allocated environment.

After 72 hours, approximately six replicate males from each isoline/environment combination were used to measure male attractiveness. We assessed male attractiveness in assays between a focal (isoline) male and a standard (outbred population) female, where we made courtship observations and measured copulation latency (the time elapsed between placing a male and female together and the start of copulation) within a 3-hour period. In *Drosophila*, females have control over acceptance or rejection of courting males (Speith 1974; Markow 1996), and so preferred, attractive males are able to achieve copulation more rapidly. Indeed, many previous studies have used copulation latency as a metric to assess overall female preference and male attractiveness (e.g. Speith 1974; Kyriacou and Hall 1986; Barth et al. 1997; Ritchie et al. 1999; Acebes et al. 2003; Shackleton et al. 2005; Taylor et al. 2007; Hosken et al. 2008; Narraway et al. 2010), and copulation latency in *D. simulans* is determined by both male (attractiveness) and female (preference) effects (Sharma et al. 2010). Copulation latency is highly positively correlated with latency between first courtship and copulation (Taylor et al. 2007), but is easier to accurately observe and record.

The results of these assays provided measures of “sire” attractiveness. Five further replicate males from each isoline/environment combination were transferred into glass auto-sampler vials (supplied by Chromacol, UK) and frozen at -80^{o}C for storage prior to CHC profiling. This provided the “sire” CHC data.

The remaining isoline males were combined with the isoline females (as above) in order to produce subsequent “offspring”, with two vials for each isoline/environment combination with two males and two females in each. Males and females from the same environments were housed together. As before, adults were given 72 hours in which to mate and lay eggs before being removed, and again, large vials of standard cornmeal diet were set up in the outbred population during this 72-hour period. All vials were then incubated at 25^{o}C during offspring development.

Peak eclosions for the "offspring" generation occurred after 11 days and virgin collection was carried out as described above. This time females were collected only from the vials from the outbred population. These females were housed exactly as before and again used in attractiveness assays with isoline males. Males were collected from the isoline vials set up in the previous generation. Male collection, housing and CHC and attractiveness measurements were carried out exactly as described for the sire generation. The CHC and attractiveness measurements in this second experimental generation provided "son" data.

Cuticular hydrocarbon extractions

We used gas chromatography to analyse CHC profile of sires and sons (*N* = 1820). Hydrocarbon extractions were carried out in sets of 100 samples per day, and randomised throughout by experiment, environment, isoline and generation. Hydrocarbon extraction involved soaking the fly in 50µl of a solution of 10ppm pentadecane in HPLC-grade hexane for 5 minutes, using a vortex for the duration of the final minute to agitate the solution and maximise extraction. The fly was then removed from the vial using forceps sterilised in hexane.

2µl of each hydrocarbon sample was injected into a GC-FID (Agilent 7890) fitted with two injectors, and two DB-1 columns of 30m x 0.25mm internal diameter x 0.25µm film thickness. We used hydrogen as a carrier gas. The inlet was set at 250^{o}C, and the injection was in pulsed splitless mode. Separation of the extract was optimized using a column profile which began at 70^{o}C for one minute, and then increased at 20^{o}C per minute to 180^{o}C, then 4^{o}C per minute to 220^{o}C, and finally 15^{o}C per minute to 320^{o}C, where it was held for two minutes. Column flow was set at 1.2ml per minute. The FID detector heaters were set at 300^{o}C. The H_2 flow was 20ml per minute, and the air flow was 200ml per minute. Nitrogen was used to make up the column flow to 30ml per minute. This protocol has been optimised previously for *D. simulans* (Sharma et al. 2012b). Peak integration of hydrocarbon data was carried out using GC ChemStation software (version B.04.02.SP1).

Statistical analysis

Data handling

We used copulation latency to assess male attractiveness (see above). Only males which were observed courting were included in analyses. Of these males, individuals which did not

mate during the 3-hour assay (*N* = 140 in post-eclosion temperature experiment; *N* = 133 in diet experiment) were allocated a time of 3 hours as a conservative estimate of copulation latency in terms of our experiment. Random variation between mating assays on different days was controlled for by standardising copulation latency to the daily mean.

We measured the expression of 22 CHCs for each male. Prior to analysis, we calculated relative peak area by dividing each peak by the area of the internal standard peak (pentadecane), and then used a log transformation to normalise the data. We used principal components analysis (PCA) in SPSS (v.19) to reduce the dimensionality of this dataset. We used the correlation matrix of male CHC expression data from both the post-eclosion temperature and dietary manipulations together, so that we could extract the same principal components (PCs) of CHC expression across both manipulations. Multivariate outliers were identified by Mahalanobis distances and removed from the dataset, leaving a slightly unbalanced experimental design, with a total of 1686 individuals across all isolines and environments used in the analysis. Five PCs with eigenvalues greater than 1 were extracted, which together explained approximately 82% of the total variation in CHC expression, and we interpret biological significance of these PCs from factor loadings exceeding 0.25 (Tabachnick and Fidell 1989).

In another study and different dataset, we had extracted 3 PCs from PCA and used these PCs in a selection analysis which demonstrated that all 3 PCs were subject to sexual selection (Chapter 3). We tested the correlation between these 3 sexually selected PC vectors from the unpublished dataset with PCs 1-3 extracted from the current study, and found significant and strong correlations between each (PC1: $r = 0.521$, $df = 20$, $P = 0.013$; PC2: $r = 0.636$, $df = 20$, $P = 0.001$; and PC3: $r = 0.581$, $df = 20$, $P = 0.004$). PCs 1-3 for both datasets therefore align well and as a result PCs 1-3 in the current study describe vectors of CHC expression which are sexually selected (although it is unclear if PCs 4 and 5 are subject to sexual selection). To further verify this, we projected the CHC dataset from the current study into the 3-dimensional multivariate space described by the 3 PCs from the unpublished dataset, thereby obtaining PC scores for the individuals used in the current experiment along the exact same PC vectors which were shown to be sexually selected (the unpublished dataset). We repeated all subsequent analyses with both sets of PCs (the 5 PCs extracted from PCA in this dataset and the 3 PCs calculated by projecting the current data into the unpublished PCA space) and found that the results are nearly identical for both,

further supporting our view that PCs 1-3 in both manuscripts are closely aligned. See Appendix 2 for the results of the analyses with the projected PCs. Here, we present the analyses with the 5 PCs extracted from PCA with the current dataset, as these PCs will more fully represent the variation in CHC expression measured in the current experiment.

Environmental and G x E effects

Further analyses were carried out separately for data from the dietary and post-eclosion temperature manipulations, although analysis for each experiment was equivalent. For each experiment, we tested for G x Es in sire and son attractiveness using a GLMM, and tested for G x Es in sire and son CHC expression using a mixed model MANOVA (in SAS version 9.2, SAS Institute Inc., Cary, NC, USA) with the five PCs of CHC expression as response variables. In each model, we specified generation, environment (either temperature or diet) and the generation x environment interaction as fixed effects, and isoline and all interactions including isoline (isoline x generation, isoline x environment and isoline x generation x environment) as random effects. We used the error structure for mixed models following Zar (1999). The generation term effectively acts as a blocking term since it was impossible to assay sires and sons simultaneously. We interpreted both isoline x environment and isoline x environment x generation interactions as indicative of a G x E, although this effect might be inconsistent between generations in the case of an isoline x environment x generation interaction.

Heritability of attractiveness

For these analyses, we calculated the mean standardised sire and son copulation latency for each isoline in each environment and used these values as estimates of male attractiveness.

Since heritability of male attractiveness has previously been identified as a benefit of female preference in *D. simulans* (Taylor et al. 2007), we tested heritability of male attractiveness across environments. We estimated heritability by linear regression (in R v.2.13.0) of sire and son isoline means. We did this for each of the four possible sire/son environmental combinations (henceforth referred to as “treatments”) in each manipulation. The four treatments were as follows for the post-eclosion temperature manipulation: (1) sire and son both in 23^{o}C; (2) sire and son both in 25^{o}C; (3) sire in 23^{o}C and son in 25^{o}C; and (4) sire in 25^{o}C and son in 23^{o}C; and for the dietary manipulation: (1) sire and son both on

diet A; (2) sire and son both on diet B; (3) sire on diet A and son on diet B; and (4) sire on diet B and son on diet A. In this way, we tested two constant environment treatments ((1) and (2)) and two changing environment treatments ((3) and (4)) for each manipulation. Regression of sire and son between different environments meant re-analysis of the same data used in the regression of sire and son within the same environment, and so all *P*-values for heritability are Bonferroni-adjusted to correct for two tests. These heritability estimates represent broad-sense heritability (H^2, the proportion of total phenotypic variance, V_P, explained by the genetic variance, V_G) because we were using isolines (David et al. 2005).

Absolute difference between sire and son attractiveness and CHC expression

These analyses used the mean attractiveness scores as calculated above. Similarly, we calculated mean sire and son PC score (for each of the five PCs of CHC expression) for each isoline in each environment.

For each isoline, we estimated the absolute difference between sires and sons attractiveness and PC scores by calculating the difference between mean sire and son scores within each of the four treatments, and representing this as an absolute value (= |x|). We used this measure to show how similar sire and son CHC expression was both when the environment remained constant between generations and when the environment changed. This measure of absolute difference could be more informative than heritability estimates when the phenotypic mean is different between generations. We tested for variation in these absolute differences between treatments using a separate GLMM (in R v.2.13.0) for attractiveness and for each PC, with treatment as a fixed effect and isoline as a random effect. Again, this involved using the dataset once for the constant environment treatments and again for the changing environment treatments, and so the *P* values associated with these tests are also Bonferroni-adjusted to correct for two tests.

5.4 Results

Principal components analysis

We extracted five PCs with eigenvalues greater than 1 from the results of PCA (Table 5.1). All 22 peaks had positive loadings for PC1 (although the loading for octadecadiene was less than 0.25), and so this PC appears to represent the overall quantity of CHCs produced. We interpret both PC2 and PC4 as a trade-off between the production of long- and short-

chained CHCs. PC2 is weighted in favour of long-chained CHCs, with positive loadings greater than 0.25 for several long-chained CHCs (pentacosane, hexacosane, heptacosane and some longer alkanes and branched alkanes), and negative loadings less than 0.25 for short-chained CHCs such as docosene and tricosene. PC4 is weighted in favour of shorter CHCs, although only 5 of the 22 peaks have loadings over 0.25 for PC4. Short-chained CHCs (octadecadiene and docosene) are highly positively loaded, and long-chained CHCs (pentacosadiene and two branched alkanes) are negatively loaded. PC3 and PC5 are positively loaded for different long-chained CHCs (including pentacosene and a branched alkane in PC3, and heptacosane and some long-chained and branched alkanes in PC5), whereas loadings for short-chained hydrocarbons are mixed (PC3 is positively loaded for octadecadiene and heavily negatively loaded for docosane, and PC5 is positively loaded for octadecadiene). These vectors describe investment in long-chained CHCs with some trade-offs between other specific CHCs.

Attractiveness and CHC expression across post-eclosion temperatures

There were no environmental or G x E effects on overall male attractiveness across temperature (Table 5.2), but there was a strong genetic (isoline) component (Table 5.2). Consistent with this, the heritability of overall attractiveness was high and repeatable across all temperature treatments (Table 5.3). This was also reflected in the absolute difference between sire and son attractiveness scores, which did not vary significantly across treatments (Figure 5.2). Sire attractiveness is clearly a reliable predictor of son attractiveness across these temperatures.

Across post-eclosion temperatures, significant isoline and temperature effects revealed genetic and environmental components of male CHC expression (Table 5.3). We also found evidence for G x E across temperatures from isoline x temperature and isoline x temperature x generation interactions in overall CHC expression (Table 5.3). Examination of these effects for individual PCs shows that the isoline x temperature interaction is not significant for any PC individually (Table 5.3). The significant isoline x temperature x generation interaction for PC3 (Table 5.3), however, shows that there is a G x E, but that this effect is not consistent between generations. There is a strong effect of temperature on PC2, and to a slightly lesser extent on PC3 and PC4 (Table 5.3). The effect of temperature

and isoline x temperature interaction on each PC is shown in the reactions norms in Figure 5.3.

The absolute difference between sire and son PC2 scores varies significantly between treatments, with a much larger difference between sires and sons in the changing environment treatments than in the constant environment treatments ($F_{3,47} = 37.818$; $P < 0.001$; Figure 5.2). There is also significant variation between temperature treatments in the absolute difference between sire and son PC3 scores ($F_{3,47} = 11.072$; $P = 0.022$; Figure 5.2), although there is no evidence that this difference is larger when sires and sons are reared in changing environments than when they are reared in constant environments.

CHC expression and attractiveness across diets

Neither diet nor isoline x diet interaction had any significant effect on overall male attractiveness (Table 5.2), and attractiveness was largely determined by isoline (Table 5.2). Again, heritability of overall attractiveness was high and consistent across diets (Table 5.3), and the absolute difference between sire and son attractiveness scores did not vary significantly across diet treatments (Figure 5.4). Sire attractiveness therefore reliably predicts son attractiveness across the diets studied here.

Male CHC expression across diets had a significant genetic (isoline) and dietary component (Table 5.5). Evidence for G x Es across diets is shown in the significant isoline x diet and isoline x diet x generation interactions in Table 5.5. There are G x Es for each PC individually: the isoline x diet interaction is significant for PC2 (Table 5.5), and the isoline x diet x generation interaction is significant for each PC except PC2, where it is marginally non-significant (Table 5.5). Overall dietary components of CHC expression are significant for PC1, PC3 and PC5 (Table 5.5). Reaction norms of CHC expression across diets are shown for each PC in Figure 5.5.

The absolute difference between sire and son PC scores varies significantly between diet treatments for PC1 ($F_{3,44} = 20.458$; $P < 0.001$), PC3 ($F_{3,44} = 12.588$; $P = 0.012$) and PC5 ($F_{3,44} = 11.712$; $P = 0.016$), and whilst there is some evidence that the difference between sire and son is greater in changing environment than in constant environment treatments, this trend is not consistent (Figure 5.4).

5.5 Discussion

Theory predicts that sexual signal reliability can be compromised by environmental heterogeneity and G x Es in sexual trait expression (Higginson and Reader 2009). In *D. simulans*, female mating preferences are likely to be driven by genetic benefits through heritable male attractiveness (Taylor et al. 2007; Hosken et al. 2008), and CHCs are an important heritable component of male attractiveness in this species (Sharma et al. 2012b; Ingleby et al. in review). Here, we have found G x Es and strong environmental components of male CHC expression. PCs 1-3 are sexually selected vectors of CHC expression, and the evidence that differences in sire and son scores for PCs 1 and 3 vary across diets and PCs 2 and 3 vary across temperatures suggests that these aspects of CHC profile are unreliable indicators of male genetic quality across some of the environments studied here. In light of this, we would also expect heritability of male attractiveness to vary across environments, but our results clearly demonstrate that heritability of male attractiveness is maintained consistently across all environments we examined.

These results might seem surprising given the evidence from a number of studies that CHCs contribute significantly to male attractiveness in many *Drosophila* species (e.g. Cobb and Ferveur 1995; Blows 2002; Wicker-Thomas 2007; Sharma et al. 2012b). However, our measure of male attractiveness represents total attractiveness, and whilst CHCs influence mate choice, CHC profile is not the only determinant of total attractiveness. Indeed, we only find evidence for signal unreliability in certain sexually-selected aspects of CHC profile here and not others, and so total attractiveness might be maintained across environments as a result of reliable aspects of CHC signals and by other sexual traits. In addition to CHCs, a number of traits are known to influence mating decisions in *Drosophila*, including body size (Partridge et al. 1987), courtship behaviour (Hall 1994) and sex comb structure (Markow 1996). Multiple signals are thought to contain more information than a single trait, and could compensate for any signal unreliability of particular sexual cues and environmental variation in signal expression (Candolin 2003). For instance, if females assess males based on multiple sexual traits then we might expect that even if one particular signal is an unreliable indicator of mate quality across some environments, other signals could be reliable. In this way, females will still be able to gauge overall male quality and the overall outcome of female mate choice will be unaffected by unreliability of any particular sexual signal, as we find here. In fact, it is likely that selection will favour female preferences which

focus on reliable aspects of male sexual signals. Preferences for signals which have G x E or strong environmental components may be selected against due to signal unreliability across heterogeneous environments, and these signals might evolve to be less important in mating decisions.

Male CHC expression across diets

In our dietary manipulation, we found differences in the absolute difference between sire and son PC1, PC3 and PC5 scores between diets. Overall, it appears that PC1 and PC3 represent CHC vectors that are unreliable sexual signals across diets, since we have reason to believe these PCs are under sexual selection. The other sexually-selected vector, PC2, appears to be a more reliable aspect of CHC profile, since the absolute difference between sire and son PC2 scores was relatively consistent across treatments. Each sexually-selected PC had a significant G x E component (isoline x diet in the case of PC2; isoline x diet x generation for PC1 and PC3), yet only PC1 and PC3 show evidence of signal unreliability across diets. It is possible that the G x E for PC2 does not involve changes in V_G across environments, and so it is less likely that the similarity between sire and son along this vector will be disrupted across environments. Alternatively, the consistency of sire and son similarity along PC2 across environments could reflect the lack of significant overall effect of diet on this vector. PC1 and PC3 each had significant diet and G x E components, suggesting that signal unreliability might be due to a combination of environmental and G x E effects.

PC1 described a vector representing variation in the overall quantity of CHCs produced, and PC3 appeared to describe investment in long-chained CHCs, with some specific trade-offs between other CHCs. The effect of diet on these vectors is consistent with the large body of research on condition dependence of sexual traits (Rowe and Houle 1996), and the evidence that CHCs are costly to produce (Blows 2002; Ferveur 2005). Indeed, there is evidence from *D. serrata* of condition dependent expression of male CHCs, and this was revealed by a diet manipulation (Gosden and Chenoweth 2011). There was no evidence of genetic variation for plasticity of diet-mediated CHC expression in *D. serrata* (Gosden and Chenoweth 2011), but we find genetic variation underlying variation in resource allocation patterns in *D. simulans*. It appears that this genetic variation contributes to the unreliability of some sexually selected CHC components across diet manipulation in the present study.

Male CHC expression across post-eclosion temperatures

In the post-eclosion temperature manipulation, the absolute difference between sire and son PC2 and PC3 scores varied between temperatures. If sire and son scores for these vectors are very different from one another, it suggests that these vectors of sire CHC profile are not good predictors of the corresponding son PC score, and so the CHC components described by these vectors are likely to be unreliable as sexual signals. There were both significant G x E and environmental components to PC3, and both are likely to contribute to signal unreliability. However, there was no evidence of any G x E effects for PC2, and so the extremely strong environmental component of expression could account for signal unreliability in this instance.

We interpreted the vector described by PC2 as a trade-off between long- and short-chained CHCs, and PC3 as investment in long-chained CHCs with some trade-offs between other specific CHCs. Short-chained CHCs are thought to function mainly as sexual pheromones, whereas long-chained CHCs are likely to have a protective role in preventing water loss through the cuticle (Ferveur 2005; Wicker-Thomas 2007). The strong influence of temperature on relative investment in long- and short-chained CHCs has been shown before, not only in our study system (Ingleby et al. in review), but also in *D. melanogaster* (Savarit and Ferveur 2002; Foley and Telonis-Scott 2011), *D. mojavensis* (Gibbs et al. 1998), and *D. serrata* (Frentiu and Chenoweth 2010). As such, it is likely that the trade-off described by PC2 will be under strong temperature-dependent selection, and furthermore that a raised temperature could be stressful to the flies in terms of this trade-off in CHC investment. This would provide a mechanism through which environmental variation, even without G x Es, could cause disrupt the similarity of sire and son PC2 scores between temperatures (Hoffmann and Merilä 1999).

Sexual signal reliability

We have shown that in *D. simulans*, a combination of G x E and environmental effects cause elements of CHC profiles to be unreliable across diets and temperatures, providing valuable support for model predictions which have suggested that G x Es in sexual signals can cause signal unreliability across environments (Higginson and Reader 2009). The role of G x Es in this process was less obvious across temperatures than across diets, however, and overall our results suggest that whilst G x Es may be important, environmental heterogeneity by

itself could also lead to signal unreliability through changes in the genetic variance between environments.

Either way, it is clear from our results that some aspects of CHC profile cannot act as reliable indicators of male quality across different diets and temperatures. In spite of this, we show that the heritability of attractiveness across the same range of environmental variation is maintained. A recent study in the field cricket, *Gryllus lineaticeps*, found a similar result (Tolle and Wagner 2011). In this species, males provide direct benefits to the females through seminal fluid substances which increase female fecundity. It was found that in spite of G x Es rendering male acoustic signals unreliable across different diets, the quality of the direct benefits provided to females varied only with male genotype, with no G x E. Our results describe a similar phenomenon, but in a system where females benefit indirectly from mate choice. In addition, we demonstrate that whilst G x Es can disrupt signal reliability, it is not always necessary to invoke G x Es to explain unreliable signals, as environmental heterogeneity can cause disrupt signal reliability even without G x Es in signal expression.

Nonetheless, in both the cricket study and our own, sexual signal unreliability did not alter the overall outcome of female choice. It is therefore likely that multiple sexual signals, and possibly selection for females to pay less attention to more unreliable aspects of male attractiveness, could attribute for these results, as discussed above. However, it is also possible that genetic, environmental and G x E effects on female preference enable female mating decisions to track the environmental differences in male sexual signal expression and reliability. In this way, adaptive plasticity in female preferences might account for the heritability of male attractiveness across different environments. Environmental variation in female preferences has been found across a number of species, and some studies have shown genetic variation underlying female preferences (reviewed by Jennions and Petrie 1997), but very little is known about G x E effects on mate preference (although see Rodríguez and Greenfield 2003; Narraway et al. 2010). Our study randomised these effects across treatments by using females from a standard environmental and genetic background. However, further investigation of genetic variation in female preference across environments will be useful. This is because the genetic covariance between male signal and female preference is central to many models of sexual selection (Kirkpatrick and Ryan 1991;

Kokko et al. 2006), but little is known about how environmental variation could influence this.

Across the diet and post-eclosion temperature variation studied here, we show that G x Es and environmental heterogeneity can disrupt the reliability of male CHC profiles to function as a signal of heritable aspects of male quality in *D. simulans*. However, we show that the heritability of total male attractiveness is maintained in spite of unreliable sexual signal components. Thus, we can see how mate choice for genetic benefits could operate in an ecological context with environmental variation. Further work should consider how female preferences might vary across environments and with G x Es, and also the consequences of unreliable sexual signals and environmental heterogeneity on the genetic covariance between male sexual traits and female mating preferences.

Table 5.1 Principal component analysis for CHC expression in both post-eclosion temperature and dietary manipulations. Five principal components with eigenvalues greater than 1 were extracted, explaining 82% of the total variation in CHC expression. Biological significance of each component was interpreted from factor loadings exceeding 0.25 (in bold). CHCs are named where known; unnamed CHCs (asterisks) are described by basic chemical structure. CHCs are listed in order of increasing chain length.

	PC1	*PC2*	*PC3*	*PC4*	*PC5*
Eigenvalue	11.100	3.431	1.463	1.180	1.041
% variance	50.453	15.597	6.650	5.365	4.733
Loadings:					
Octadecadiene	.211	-.180	**.255**	**.696**	**.252**
Docosene	**.611**	**-.294**	.233	**.441**	.031
Docosane	**.762**	-.218	**-.527**	.158	-.076
Branched alkane*	**.825**	**-.329**	.089	-.077	-.177
7-Tricosene	**.886**	**-.294**	.134	.084	-.092
Tricosene	**.715**	-.225	**.351**	.061	.034
Tricosane	**.844**	-.189	-.195	.231	.025
Branched alkane*	**.851**	-.121	**-.256**	-.014	-.208
Branched alkane*	**.859**	-.038	**.352**	.002	-.155
Branched alkane*	**.809**	-.170	-.240	-.060	-.192
Tetracosane	**.834**	.158	**.437**	.054	-.107
Pentacosadiene	**.575**	**-.561**	-.195	**-.322**	.172
Alkene*	**.801**	.231	**.268**	-.238	-.176
Pentacosene	**.849**	.126	**.363**	-.120	-.178
Pentacosane	**.809**	**.345**	-.012	.059	.097
Branched alkane*	**.646**	**-.469**	.037	**-.264**	**.403**
Hexacosane	**.637**	**.646**	-.249	.064	-.057
Heptacosane	**.664**	**.330**	-.008	-.207	**.481**
Branched alkane*	**.534**	**.471**	**.369**	**-.252**	-.105
Alkane*	**.544**	**.763**	-.074	.187	.006
Alkane*	**.467**	**.581**	-.025	-.020	**.470**
Alkane*	**.435**	**.716**	-.028	-.075	**.289**

Table 5.2 Results from separate GLMMs for temperature and diet manipulations with male attractiveness as the response variable; environment (temp or diet), generation (gen) and environment x generation interaction as fixed effects; and isoline, isoline x generation, isoline x environment and isoline x environment x generation as random effects. Significance is highlighted in bold.

	F	*df*	*P*
Temperature:			
Isoline	**3.709**	**46,18**	**<0.001**
Temperature	0.003	1,46	0.955
Generation	0.052	1,47	0.821
Isoline x Gen	1.194	46,46	0.183
Isoline x Temp	0.946	46,46	0.576
Temp x Gen	0.950	1,47	0.330
Isoline x Temp x Gen	1.051	46,809	0.385
Diet:			
Isoline	**2.810**	**43,19**	**<0.001**
Diet	1.111	1,43	0.292
Generation	0.227	1,44	0.634
Isoline x Gen	0.534	43,43	0.994
Isoline x Diet	0.811	43,43	0.802
Diet x Gen	0.108	1,44	0.743
Isoline x Diet x Gen	0.785	43,867	0.838

Table 5.3 Broad-sense heritability estimates (H^2 [SE]) of male attractiveness for each sire/son environment combination. All heritabilities are significant ($P < 0.05$) after Bonferroni correction.

Sire/son environment	H^2 (SE)
Temperature	
23/23	0.774 (0.128)
23/25	0.652 (0.147)
25/23	0.744 (0.115)
25/25	0.608 (0.135)
Diet	
A/A	0.944 (0.103)
A/B	0.832 (0.108)
B/A	0.686 (0.131)
B/B	0.602 (0.133)

Table 5.4 Results from a mixed model MANOVA with PCs 1-5 of CHC expression as response variables; temperature, generation and temperature x generation interaction as fixed effects; and isoline, isoline x temperature, isoline x generation and isoline x temperature x generation as random effects. Results of individual univariate GLMMs for each PC are given in the second section of the table. Significance is highlighted in bold. Error structure was specified following Zar (1999).

Overall MANOVA				
	Wilks' λ	*F*	*df*	*P*
Generation	**0.678**	**3.99**	**5,42**	**0.005**
Temperature	**0.188**	**36.38**	**5,42**	**<0.001**
Isoline	**0.228**	**4.65**	**230,3096**	**<0.001**
Gen x Temp	**0.740**	**2.95**	**5,42**	**0.023**
Gen x Isoline	**0.583**	**1.54**	**230,3096**	**<0.001**
Temp x Isoline	**0.646**	**1.23**	**230,3096**	**0.011**
Gen x Temp x Isoline	**0.635**	**1.29**	**230,3096**	**0.003**
Univariate GLMMs				
		F	*df*	*P*
PC1				
Generation		0.57	1,47	0.454
Temperature		0.19	1,48	0.664
Isoline		**3.60**	**46,10**	**0.017**
Gen x Temp		0.32	1,48	0.572
Gen x Isoline		1.09	46,46	0.389
Temp x Isoline		0.67	46,46	0.912
Gen x Temp x Isoline		1.11	46,625	0.290
PC2				
Generation		0.000	1,48	0.964
Temperature		**132.78**	**1,47**	**<0.001**
Isoline		**3.42**	**46,22**	**0.001**
Gen x Temp		**9.40**	**1,48**	**0.004**
Gen x Isoline		0.97	46,46	0.535
Temp x Isoline		1.40	46,46	0.131
Gen x Temp x Isoline		1.12	46,625	0.279
PC3				
Generation		**11.52**	**1,47**	**0.001**
Temperature		**7.03**	**1,48**	**0.011**
Isoline		**6.39**	**46,10**	**0.002**
Gen x Temp		0.78	1,47	0.381
Gen x Isoline		1.10	46,46	0.373
Temp x Isoline		0.67	46,46	0.913
Gen x Temp x Isoline		**1.74**	**46,625**	**0.002**
PC4				
Generation		0.43	1,47	0.517
Temperature		**48.47**	**1,47**	**<0.001**

Isoline	1.77	46,21	0.079
Gen x Temp	1.71	1,47	0.197
Gen x Isoline	1.42	46,46	0.121
Temp x Isoline	0.91	46,46	0.626
Gen x Temp x Isoline	1.30	46,625	0.094
PC5			
Generation	**5.03**	**1,47**	**0.030**
Temperature	0.02	1,47	0.891
Isoline	1.51	46,34	0.107
Gen x Temp	0.99	1,48	0.325
Gen x Isoline	**1.66**	**46,46**	**0.045**
Temp x Isoline	1.37	46,46	0.145
Gen x Temp x Isoline	1.14	46,625	0.252

Table 5.5 Results from a mixed model MANOVA with PCs 1-5 of CHC expression as response variables; diet, generation and diet x generation interaction as fixed effects; and isoline, isoline x diet, isoline x generation and isoline x diet x generation as random effects. Results of individual univariate GLMMs for each PC are given in the second section of the table. Significance is highlighted in bold. Error structure was specified following Zar (1999).

Overall MANOVA				
	Wilks' λ	*F*	*df*	*P*
Generation	**0.351**	**14.45**	**5,39**	**<0.001**
Diet	**0.096**	**73.25**	**5,39**	**<0.001**
Isoline	**0.166**	**6.97**	**215,3452**	**<0.001**
Gen x Diet	**0.433**	**10.21**	**5,39**	**<0.001**
Gen x Isoline	**0.572**	**1.91**	**215,3452**	**<0.001**
Diet x Isoline	**0.484**	**2.52**	**215,3452**	**<0.001**
Gen x Diet x Isoline	**0.548**	**2.07**	**215,3452**	**<0.001**
Univariate GLMMs				
		F	*df*	*P*
PC1				
Generation		**15.40**	**1,44**	**<0.001**
Diet		**21.10**	**1,43**	**<0.001**
Isoline		**3.55**	**43,13**	**0.008**
Gen x Diet		1.58	1,44	0.215
Gen x Isoline		0.56	43,43	0.969
Diet x Isoline		1.45	43,43	0.112
Gen x Diet x Isoline		**1.70**	**43,697**	**0.004**
PC2				
Generation		**6.82**	**1,44**	**0.012**
Diet		0.44	1,43	0.509
Isoline		**2.77**	**43,34**	**0.001**
Gen x Diet		**28.80**	**43,34**	**<0.001**
Gen x Isoline		1.55	43,43	0.078
Diet x Isoline		**1.66**	**43,43**	**0.049**
Gen x Diet x Isoline		1.39	43,697	0.055
PC3				
Generation		1.57	1,44	0.218
Diet		**90.81**	**1,44**	**<0.001**
Isoline		**6.10**	**43,4**	**0.049**
Gen x Diet		0.00	1,43	0.982
Gen x Isoline		0.74	43,43	0.838
Diet x Isoline		0.68	43,43	0.892
Gen x Diet x Isoline		**2.82**	**43,697**	**<0.001**
PC4				
Generation		**28.43**	**1,44**	**<0.001**
Diet		1.93	1,43	0.172
Isoline		**2.08**	**43,18**	**0.046**

	Gen x Diet	3.79	1,43	0.058
	Gen x Isoline	0.69	43,43	0.886
	Diet x Isoline	1.64	43,43	0.055
	Gen x Diet x Isoline	**2.31**	**43,697**	**<0.001**
PC5				
	Generation	0.67	1,43	0.419
	Diet	**105.39**	**1,44**	**<0.001**
	Isoline	**1.98**	**43,25**	**0.036**
	Gen x Diet	**18.16**	**1,44**	**<0.001**
	Gen x Isoline	**1.69**	**43,43**	**0.045**
	Diet x Isoline	1.00	43,43	0.506
	Gen x Diet x Isoline	**2.04**	**43,697**	**<0.001**

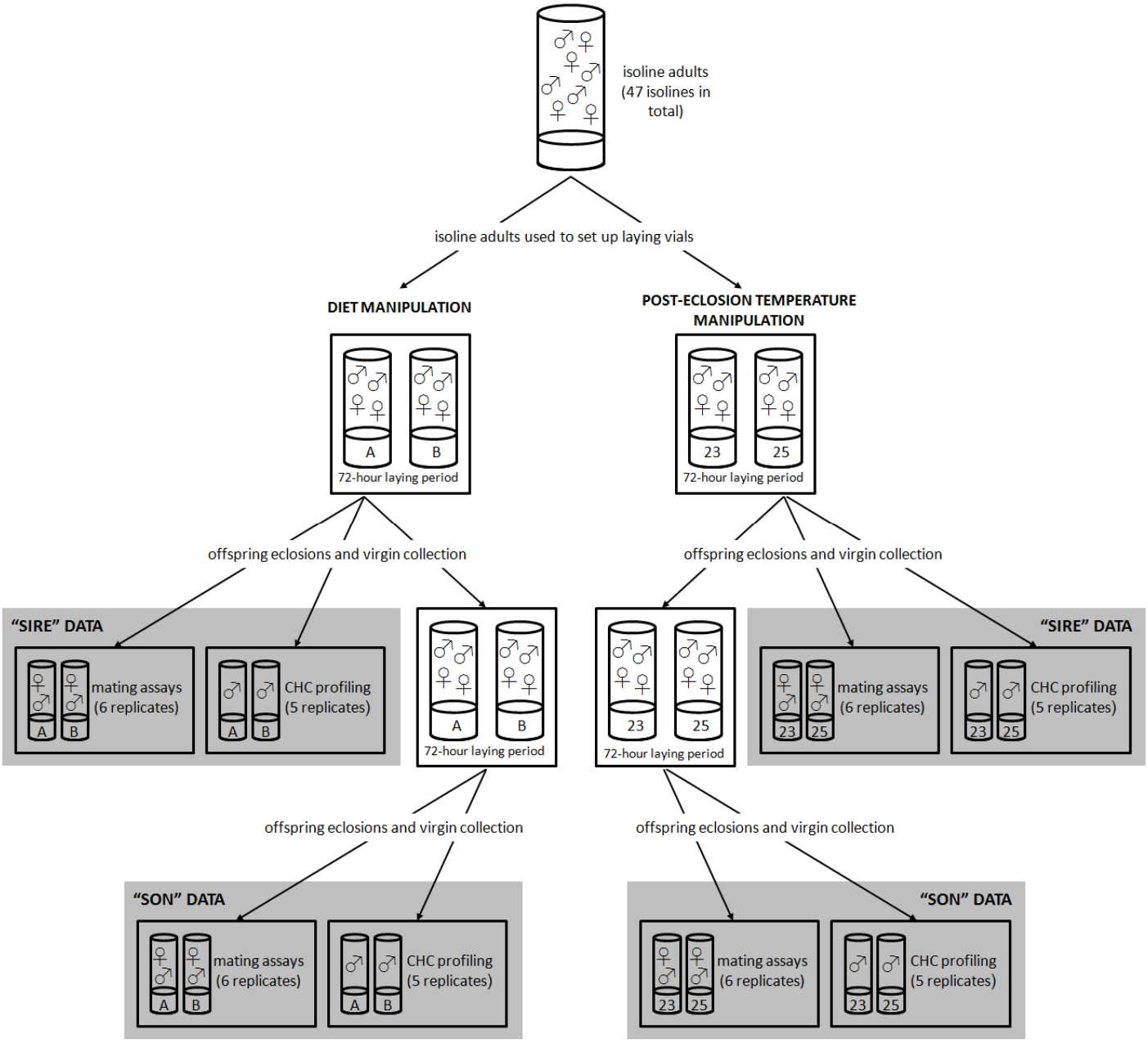

Figure 5.1 Experimental design, showing the setup for a single isoline. Vials marked "A" and "B" denote flies which were reared on oat-based and soy-based diets, respectively. Vials marked "23" and "25" denote flies which were kept in 23°C and 25°C post-eclosion temperatures, respectively. Adult flies from each isoline were set up in laying vials as shown (with 2 replicate vials of each isoline/environment combination) and allowed a 72-hour laying period. During this period, vials of the standard diet were also set up for laying in the outbred population. Offspring development took 11 days (until peak eclosions) at 25°C. Virgin collection was carried out as described in text. Offspring were used in 3 ways. (1) 6 replicate males from each isoline/environment combination were used in mating assays

with outbred females, to assess male attractiveness (detail in text). (2) 5 replicate males from each isoline/environment combination were used for CHC profiling. Mating assays and CHC analysis in this generation provided “sire” data. (3) Isoline males and females from each environment were combined in fresh laying vials for 72 hours (with 2 replicate vials of each isoline/environment combination as before). Again, laying vials were placed in the stock population during this period. Offspring development and virgin collection were carried out as in the previous generation. Mating assays and CHC profiling were carried out as before, providing “son” data from this generation.

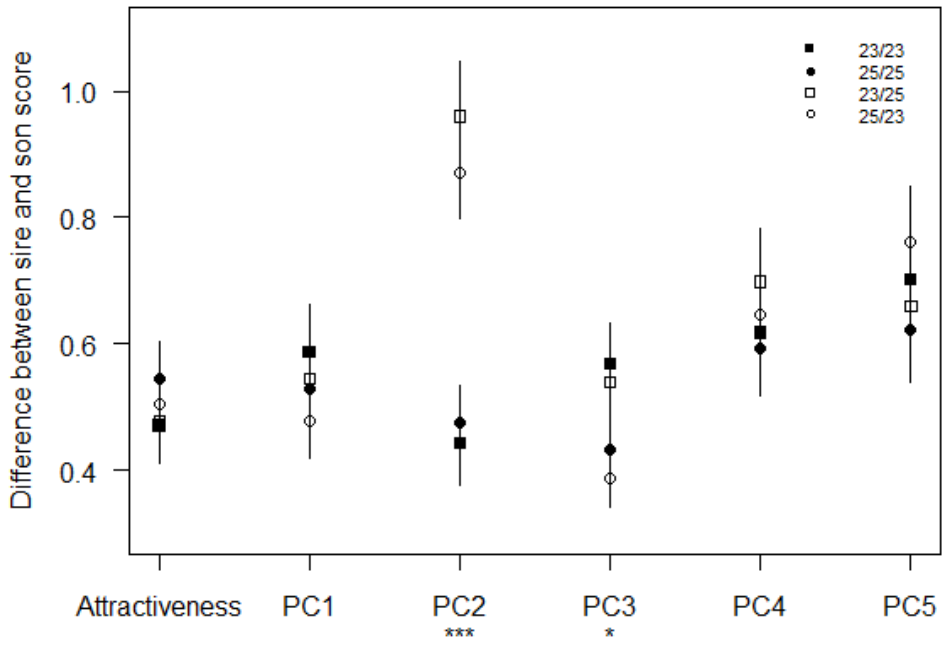

Figure 5.2 Mean absolute difference (±SE) between isoline sire and son attractiveness (as standardised copulation latency) and PC scores (PC1-5) in each post-eclosion temperature treatment (see key for sire/son environment). Filled points represent constant environment treatments and open points represent changing environment treatments. Variation between treatments is significant in PC2 and PC3 (asterisks).

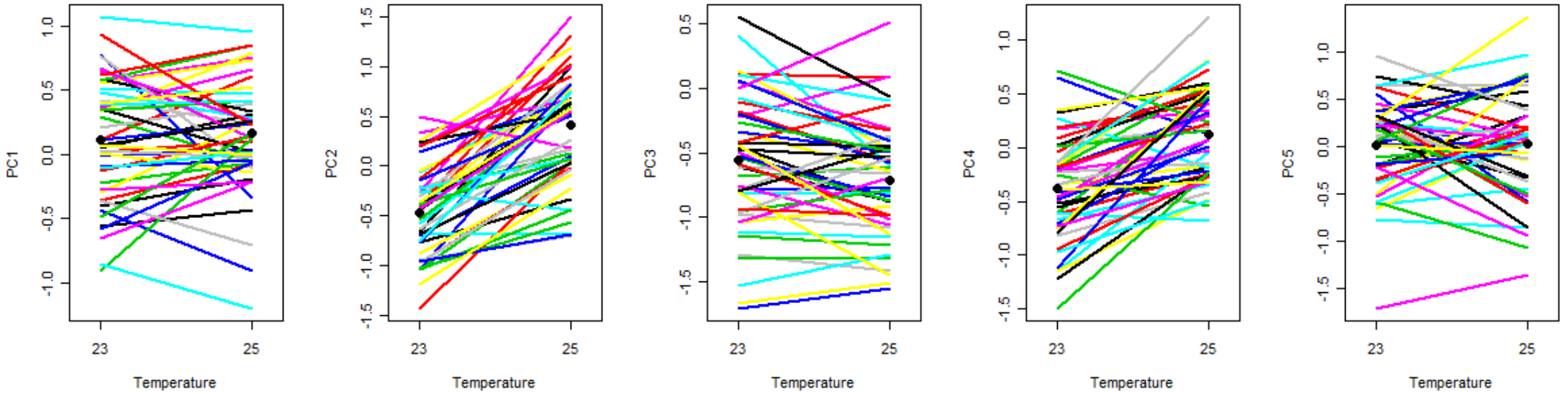

Figure 5.3 Reaction norms for PCs 1-5 (left-right) of male CHC expression across post-eclosion temperatures. Each line represents an isoline. Points represent overall mean PC score across all isolines within each temperature.

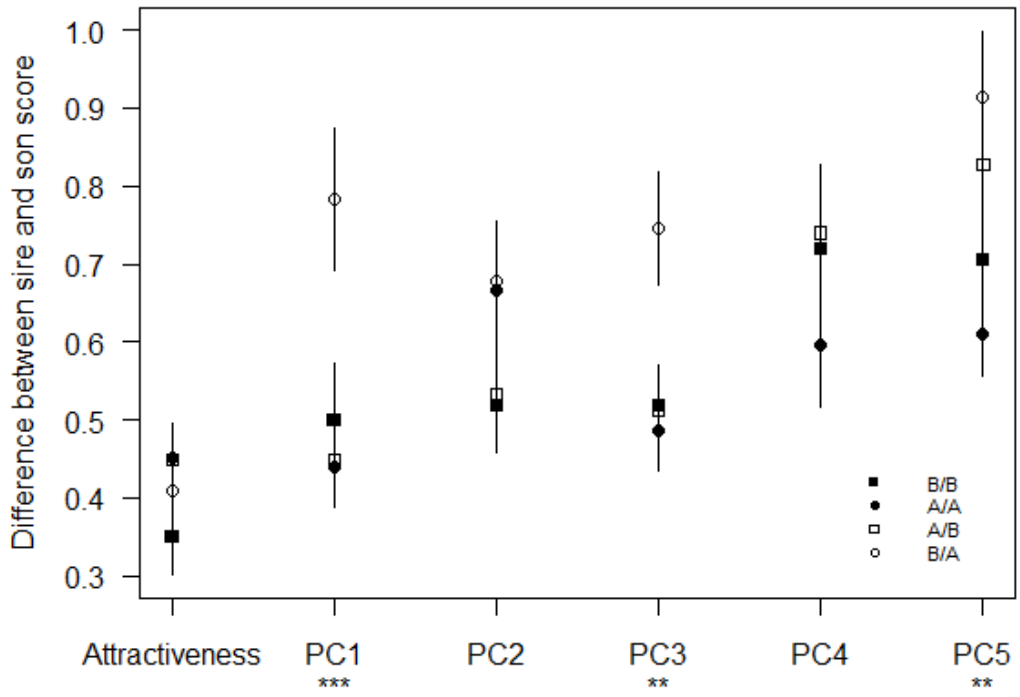

Figure 5.4 Mean absolute difference (±SE) between isoline sire and son attractiveness (as standardised copulation latency) and PC scores (PC1-5) across dietary treatments (see key for sire/son environment). Filled points represent constant environment treatments and open points represent changing environment treatments. Variation between treatments is significant in PC1, PC3 and PC5 (asterisks).

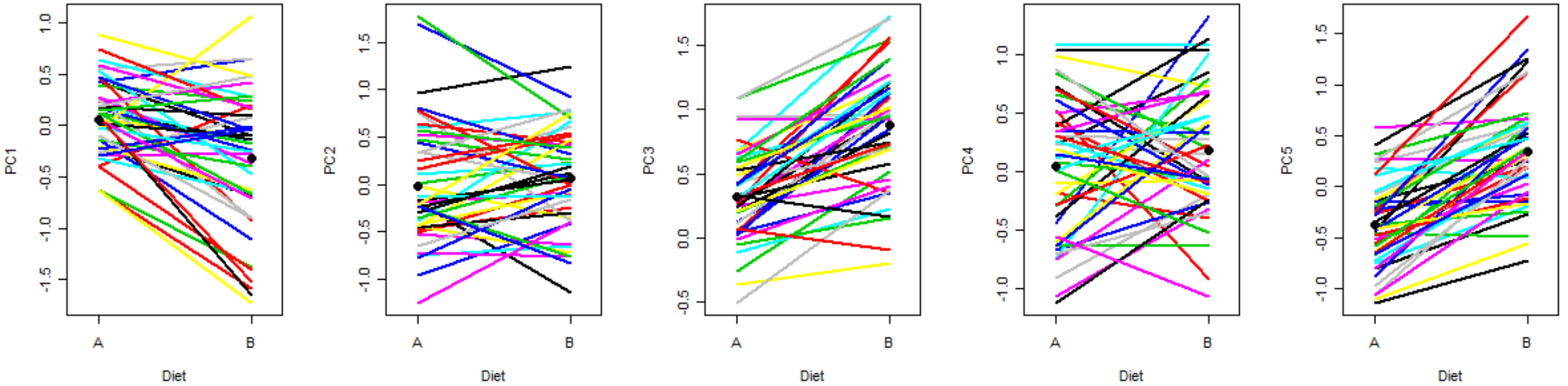

Figure 5.5 Reaction norms for PCs 1-5 (left-right) of male CHC expression across diets. Each line represents an isoline. Points represent overall mean PC score across all isolines within each diet.

CHAPTER 6: Genotype-by-environment interactions for female mate choice of male cuticular hydrocarbons in *Drosophila simulans*

6.1 Abstract

Recent research has highlighted the potential importance of environmental and genotype-by-environment (G x E) variation in sexual selection, but most studies have focussed on the expression of male sexual traits. Consequently, our understanding of genetic variation for plasticity in female mate choice is extremely poor. In this study we examine the genetics of female mate choice in *Drosophila simulans* using isolines reared across two post-eclosion temperatures. There was evidence for G x Es in female choosiness and preference, which suggests that the evolution of female mate choice behaviour could differ across environments. However, the ranked order of preferred males was consistent across females and environments, so the same males are favoured by mate choice in spite of G x Es for choosiness and preference. Our study highlights the importance of taking cross-environment perspectives in order to gain a more comprehensive understanding of the operation of sexual selection.

6.2 Introduction

Female mate choice exerts strong sexual selection on males and is thought to drive the evolution of many elaborate sexual traits and displays (Jennions and Petrie 1997). Despite an initial reluctance to recognise the importance of mate choice in sexual selection (O'Donald 1979; Hosken and House 2011), research in this area has advanced and female mate choice has been documented in many species and is understood in considerable detail (Jennions and Petrie 1997; Andersson and Simmons 2006). Studies have demonstrated that females can benefit from mate choice directly through resources provided by the male (Møller and Jennions 2001), or indirectly via offspring gaining viability or attractiveness genes (Lande 1981; Kirkpatrick 1982; Head et al. 2005; Taylor et al. 2007). However, given the evidence for plasticity and context-dependency of mate choice in a wide range of species (reviewed by Jennions and Petrie 1997; Cotton et al. 2006), it seems unlikely that mate preferences will be static and that all females will prefer the same males in every environment.

Unfortunately little is known about the genetics underlying plasticity in mate choice, and we therefore have a very limited understanding of the operation and evolution of mate choice across heterogeneous environments. The potential significance of this plasticity in mate choice has been highlighted by recent interest in genotype-by-environment interactions (G x Es) in sexual selection (Greenfield and Rodríguez 2004; Ingleby et al. 2010). G x Es describe changes in the relative performance of genotypes across environments (Lynch and Walsh 1998), and have been studied within the field of evolutionary genetics for well over twenty years (Via and Lande 1985). Interest in G x Es in a sexual selection context is more recent, but theoretical and empirical work suggests that G x Es could be of fundamental importance to the operation of sexual selection (reviewed in Ingleby et al. 2010).

Theory suggests that G x Es in the expression of male sexual signals and displays can make sexual signals unreliable (Higginson and Reader 2009), but can also contribute towards the maintenance of genetic variation in sexual traits (Kokko and Heubel 2008), and there is some empirical support for these predictions (Jia et al. 2000; Tolle and Wagner 2011; Ingleby et al. in review). G x Es in mate choice could also have important implications for sexual selection. Where there are G x Es, genetic variation underlying mate choice varies across environments and the evolution of mate choice will be dependent on the environment. In this way, G x Es could allow for adaptive plasticity in mating decisions (Kokko and Heubel 2008). For example, the prediction that male sexual signals can be unreliable indictors of male quality across heterogeneous environments may not hold if the reaction norms for female mate choice and male signals match one another. When this occurs, changes in the direction and extent of signal plasticity will be mirrored by changes in mate choice across environments, and benefits of mate choice could therefore be maintained in spite of G x Es for trait and preference (Greenfield and Rodríguez 2004). If reaction norms for female mate choice and male signals do not match, then the genetic covariance between signal and preference could vary in strength and sign between environments. Sexual selection by a Fisherian runaway process is to a large extent determined by the strength of genetic correlation between female preference and male sexual trait (Lande 1981; Hosken and House 2011), and so environmental heterogeneity and G x Es in preferences and signals could affect the operation of sexual selection.

Empirical studies suggest that G x Es in male sexual traits are widespread (e.g. David et al. 2000; Danielson-François et al 2006; Lewandowski and Boughman 2008; Rodríguez and Al-Wathiqui 2011), although not ubiquitous (e.g. Miller and Brooks 2005; Gosden and Chenoweth 2011). However, very few studies have examined G x Es in female mate choice. Those which have find evidence for G x Es in aspects of mate choice behaviour in the waxmoth, *Achroia grisella* (Rodríguez and Greenfield 2003), and in *Drosophila melanogaster* (Narraway et al 2010), but there is no evidence for G x E in *D. serrata* mate preferences (Delcourt et al 2010). Further study is therefore needed to determine how common G x Es for female mate choice are, and explore their consequences for sexual selection.

Here, we test for G x Es and examine the genetics of female mate choice in *D. simulans*. Previous work has shown that there are no direct costs or benefits of mate choice in this species (Taylor et al 2008), but that females benefit indirectly through heritable male attractiveness (Taylor et al 2007; Hosken et al 2008). Furthermore, we have evidence that male attractiveness is heritable across environments, although aspects of male sexual signalling with cuticular hydrocarbons (CHCs) are unreliable (Ingleby et al in review) because of G x Es in male CHC expression (Ingleby et al in review). Using females from isolines reared across two temperature environments, we measure two important aspects of female mate choice: choosiness and preference. As defined by Jennions and Petrie (1997), preference describes the willingness of a female to mate, which we measure here as a binary response of whether or not a female mates within a given period of time. We are also able to examine female preference functions, as we assay females from each isoline with the same set of male isolines. Choosiness describes the time and effort a female spends assessing potential mates (Jennions and Petrie 1997), and here we measure this as copulation latency, the time between introduction of a male and female and the start of mating, which is a common metric used in *Drosophila* studies (e.g. Speith 1974; Barth et al 1997; Ritchie et al 1999; Taylor et al 2007; Hosken et al 2008; Narraway et al 2010). We assay mating behaviour in trials with single males and females (i.e. the choice is whether or not to mate with a given male; *sensu* Shackleton et al 2005) since this allows us to uncouple mate choice from male-male competition, which would be confounded in trials using multiple males. Note also that in *Drosophila*, studies using single and multi-male assays produce identical results (e.g. Avent et al 2008; Taylor et al 2008). We also quantify male CHC expression, which allows us to analyse female mate choice as a function of a male sexual signal, as well as test the

genetic covariance between female preference and male attractiveness across environments.

6.3 Methods

Isolines and maintenance

Female *D. simulans* were collected from Greece in April 2010 and used to found 60 iso-female lines (isolines) in the laboratory. Within each isoline, 25 male and 25 female offspring were used to found each new generation. Isolines had been maintained in this way for 34 generations prior to this experiment and so each isoline can be considered a distinct genotype (David et al 2005). Isolines were maintained on a standard cornmeal-based diet (supplied by Applied Scientific, UK) at 25^{o}C on a 12:12 hour light:dark cycle throughout the experiment (unless stated otherwise).

We used a subset of 28 isolines in this study, 8 of which were used to derive experimental males (henceforth referred to as 'male isolines'). These male isolines were chosen based on results from previous experiments (Ingleby et al. in review), in order to provide male genotypes with broad variation in attractiveness. The other 20 isolines were used to derive experimental females ('female isolines') and had been chosen haphazardly from the remaining isoline stock, such that males and females were derived from different isolines.

Environmental manipulation and mating assays

The experiment was carried out in 7 blocks. For each block, adult flies were taken from each of the male and female isolines and used to set up two replicate laying vials per isoline, each with five males and five females in 150ml vials with 30ml of food. After a 48-hour laying period, the adult flies were removed and the vials were incubated at 25^{o}C during offspring development. Development took 11 days until peak eclosion, at which point virgin flies were collected. Any eclosed adults were cleared from vials at 7am. Newly-eclosed virgin adults were collected between 11am and 1pm, and again between 5pm and 7pm. Virgin males were collected from each of the 8 male isolines and housed by isoline (10 males per 40ml vial with 8ml of food) at 24^{o}C. From each of the 20 female isolines, virgin females were collected and housed individually in a 40ml vial with 8ml of food. Females were split approximately equally between two post-eclosion temperatures, 23^{o}C and 25^{o}C.

These males and females were used in mating assays which were carried out at 3 days post-eclosion. Over the entire experiment, 6-8 females from each female isoline x environment combination were assayed with a male from each of the male isolines (6-8 replicate females from 20 female isolines x 2 post-eclosion temperatures x 8 male isolines = 2239 assays, carried out in 7 approximately equal blocks). Each assay was carried out at 24^{o}C and lasted 3 hours, during which courtship and mating behaviour was observed. We recorded the time when each pair started to mate, providing mate acceptance data (as a binary measure of whether or not a pair successfully mated during the 3-hour assay) and copulation latency data (the time between introduction and the start of copulation). Copulation latency measured this way is highly positively correlated with latency between first courtship and copulation (Taylor et al. 2007), but is easier to accurately observe and record. In *Drosophila*, females have control over acceptance or rejection of courting males (Speith, 1974), and so preferred males should copulate more rapidly. From our data, we therefore had two measures of overall mate choice for females from each female isoline x male isoline x environment combination.

Assessing male CHC profile

Two sets of virgin males for CHC profiling were also collected during virgin collection (see above). Firstly, males were collected from each of the 8 male isolines (56-63 males from each isoline, *N* = 485) to provide CHC data for the male genotypes used in the mating assays. This allowed us to examine female preference for these genotypes as a function of average male CHC profile. We did not sample CHCs from the same individuals used in the mating assays since CHC profiles can change with mating (Ferveur and Cobb 2010). However, the CHCs sampled from virgin males from the same isolines will closely represent the CHC profiles of virgin males used in the assays since the isolines are effectively genetically identical, and we reared males for mating assays and CHC profiling in identical environmental conditions (10 males per 40ml vial with 8ml of food kept at 24^{o}C), and both mating behaviour and CHCs were assayed at 3 days post-eclosion. The second set of males were collected from each of the 20 female isolines (12-14 males per isoline, *N* = 270). These males were split between the same two post-eclosion temperatures as the females from these isolines (23^{o}C and 25^{o}C). Males were housed together according to isoline and temperature in 40ml vials with 8ml of food. CHC profiling of these males gave us male CHC

data from each female isoline x environment combination, and, in combination with the data on female mate choice from the same female isoline x environment combinations, allowed us to calculate the genetic covariance between male sexual signal and female mate choice for these 20 isolines across both temperatures.

Males for CHC profiling were transferred to individual glass auto-sampler vials (supplied by Chromacol, UK) at 3 days post-eclosion, and stored at -80^{o}C prior to hydrocarbon extraction. Hydrocarbon extractions were carried out in sets of 100 samples per day, and randomised throughout by isoline and environment. Hydrocarbon extractions and analysis followed a protocol optimised previously for *D. simulans* (see details in Sharma et al. 2012b and Ingleby et al. in review).

Statistical analyses

All analyses were carried out using R (v.2.13.0) and copulation latency (female choosiness) and mate acceptance (female preference) were examined separately. Mate acceptance was scored as 0 (unmated) or 1 (mated) (N = 2239), and copulation latency (seconds elapsed between introducing the male to the vial and the start of copulation) was log-transformed prior to analysis to fit a normal distribution. Copulation latency was analysed using only the pairs which successfully mated during the assay (N = 1674).

Model fit and evaluation

We used generalised linear mixed models and Bayesian inference as implemented by the MCMCglmm package (v.2.12; Hadfield 2010). Temperature was specified as a fixed effect, and female and male isoline as random effects. We used a Gaussian distribution for the copulation latency data and a 'categorical' distribution (in MCMCglmm notation) to handle the binary mate acceptance data. For each model, we ran Markov chains for 400,000 iterations with a burn-in of 20,000 and a thinning interval of 25. Each model used unstructured variances ('us' in MCMCglmm notation), therefore estimating all variance and covariance parameters. We tested models both with an informative (v = 2) and a relatively uninformative prior (v = 0.02) and found that results were robust to changes in prior specification. We present results from models with relatively uninformative priors (v = 0.02), which means that models were fitted with very little *a priori* information about the expected parameter estimates.

A set of 7 plausible models were tested for each response, which examined combinations of male isoline, female isoline, environmental and G x E components of mate choice (see Table 6.1 for the biological rationale of each model). Statistical support for each model was estimated using the deviance information criteria (DIC), and also by calculating the approximate posterior probability. This calculation takes into account the DIC of each model tested, and for each, provides a probability that can be used to identify the best approximating model out of the set being tested. Models were tested with and without experimental block as a covariate, but inclusion of a block term did not alter our results and model fit was consistently better without a block term (Table 6.1), and so further analyses do not include block.

Model interpretation

Reaction norms were plotted to illustrate female isoline x temperature interactions for both copulation latency (female choosiness G x E) and mate acceptance (female preference G x E). For latency and acceptance individually, we estimated the cross-environment genetic correlation, heritability between and within environments, and variance components for female isoline, male isoline and female isoline x temperature. These estimates were made from the simplest model to include all the relevant parameters (i.e. female isoline x temperature + male isoline; see Table 6.1). Genetic correlation, heritability and variance components were calculated following Lynch and Walsh (1998).

The male isoline term in the models in Table 6.1 was interpreted as genetic variation in male attractiveness. Female isoline x male isoline, male isoline x temperature and female isoline x male isoline x temperature interaction terms were interpreted as female G, E and G x E variance (respectively) in choosiness function (for latency data) or preference function (for acceptance data), as these interactions describe variation in female choosiness or preference as a function of male genotype.

The CHC data for the male isolines was used to examine mate choice in terms of male CHC phenotype (as opposed to male genotype as above). Expression of 22 hydrocarbon peaks was quantified for each male. We calculated relative peak size by dividing each peak by an internal standard (pentadecane) within each sample, and then normalised the CHC data using log transformation prior to analysis. The pooled male CHC data from both male and female isolines was used in a principal components analysis (PCA)

to reduce the dimensionality of the data and extract the same vectors of CHC variation for males from male and female isolines. PCs were extracted from the correlational matrix and vectors with eigenvalues greater than 1 were used in subsequent analyses. This gave four PCs which together explained 83% of the total variation in CHC expression. We plotted copulation latency (choosiness) and mate acceptance (preference) for each female isoline as a function of male CHC profile (using the ranked PC scores for each male isoline) with the 'smooth.spline' function in R ('stats' package).

The set of models in Table 6.1 was re-analysed without the male isoline term, instead using the four PCs of male CHC profile as covariates to account for male effects on female mate choice in terms of male phenotype. Since our measure of CHC expression is an average CHC profile for each male isoline, the best models for both copulation latency and mate acceptance using male CHC data are analogous to the best models identified using the male isoline term. From the posterior distribution of the best model for each response, we were able to estimate overall β, the linear selection gradient, to quantify sexual selection through mate choice on each PC. In addition, an estimate of β (for each PC) for each female isoline x temperature combination was also extracted from the posterior distribution of the model including the female isoline x temperature x male isoline interaction, in order to examine genetic and environmental variation in β.

Genetic covariance between female preference and male attractiveness

The cross-environment genetic covariance between female preference and male attractiveness (calculated from CHC profile) was analysed using the male and female data from the 20 female isolines in each post-eclosion temperature. For males from each female isoline x environment combination, a mean attractiveness score was assigned based on CHC profile. These attractiveness scores were calculated from the results of a discriminant function analysis of PCs 1-4 of CHC expression for the males from the male isolines that were used in the mating assays, using mate acceptance (0 or 1) as the response (using the 'lda' function in the 'MASS' package in R). The discriminant function identified the vector of male CHC variation that best distinguished between mated and unmated males and could therefore be used as a surrogate of the attractiveness of a male's CHC profile. Since both sets of male CHC data were pooled for PCA (see above), we had characterised the same 4 PCs for males from the female isolines as we did for the males from the male isolines. The

data for the males from the female isolines could therefore be directly projected onto the vector identified by the discriminant function analysis, providing a univariate attractiveness score for males from each female isoline x environment combination.

Using the MCMCglmm package as before, we tested for G x E in male attractiveness scores across temperatures. Models were specified as described above. We used a Gaussian distribution, with temperature as a fixed effect and female isoline as a random effect. We tested two models: one including a G x E (female isoline x temperature) for male attractiveness score, and the other with only G and E effects. Using the same methods described above, we assessed model fit, and calculated the cross-environment genetic correlation of male attractiveness score from the model which included the G x E term.

The genetic correlation between female preference and male CHC attractiveness both within and across temperatures was calculated following Lynch and Walsh (1998), using mean female mate acceptance and mean male CHC attractiveness scores for each female isoline x temperature combination. Genetic correlations were calculated with bootstrapped 95% confidence intervals and significance assigned by randomisation test (with 10,000 iterations).

6.4 Results

Female choosiness

The model with the strongest support for the copulation latency data (Table 6.1) shows that there is significant genetic (female isoline) variation in female choosiness and male attractiveness (male isoline), although genetic variance in female choosiness is low (Table 6.2). There is a clear overall temperature effect, with females reared at 25^{o}C mating more quickly than females reared at 23^{o}C (Figure 6.1a).

There were no significant interaction effects. The lack of G x E (female isoline x temperature) in the best model suggests that the effect of temperature on female choosiness does not vary significantly between female genotypes. This is reflected in the low variance explained by the G x E interaction (Table 6.2). However, despite the lack of significant G x E, crossover can be seen in the reaction norms in Figure 6.1a, and there is evidence for substantial changes in genetic variation in choosiness between temperatures (Table 6.3). In fact, both the cross-environment genetic correlation and the between-environment heritability are significantly lower than 1 but not significantly different from 0,

showing a very weak genetic correlation across environments and very low heritability between temperatures, although the intervals around these estimates are wide. Note also that there is some (weak) statistical support for inclusion of a G x E effect in the copulation latency models (Table 6.1).

Female preference

The model with the highest support for the mate acceptance data indicates significant genetic variation for female preference (female isoline) and male attractiveness (male isoline) (Table 6.1). The overall temperature effect on mate acceptance is very slight (Figure 6.1b). Both female and male isoline terms contribute strongly to variance in mate acceptance, indicating that genetic variation in preference and attractiveness is high (Table 6.2). This inference is supported by the high heritability of female preference within each temperature (Table 6.3). However, there is significant genetic variation in plasticity of female preference across temperatures (i.e. a G x E component; Table 6.1 and Figure 6.1b). The variance explained by this G x E effect is fairly high (Table 6.2), consistent with the strong interaction shown in Figure 6.1b, and the weakened cross-environment genetic correlation and between-temperature heritability, which are both significantly lower than 1 (Table 6.3).

From these models, we were also able to test for G, E and G x E variation in female preference as a function of male isoline (i.e. was there variation in female preference functions). However, none of the models with these terms (female x male, male x temperature and female x male x temperature interactions) were a good fit for the data (Table 6.1), suggesting that female isolines tend to 'agree' on which male isolines are preferred.

Female mate choice for male CHCs

The results of PCA on male CHC data gave us 4 PCs of CHC expression which together explain ca. 83% of the total variation in CHC profile. We used these vectors to reduce the dimensionality of the CHC data, whilst capturing a large proportion of the overall variation in CHC profile in order to describe female mate choice in terms of male phenotype. We do not examine CHC expression in detail, since we quantify cross-environment patterns of genetic variation in CHC profile in the same population of *D. simulans* isolines elsewhere

(Ingleby et al. in review), and very similar results are found from this data (analysis not shown).

Re-analysis of the set of models in Table 6.1 with the 4 PCs describing male CHC expression as covariates confirmed that using male isoline or male CHC data gives the same best model for copulation latency and mate acceptance (results not shown). This was expected since we used an average CHC profile for each male isoline. However, we do find that accounting for male effects on mate choice using genotype (i.e. male isoline in the models in Table 6.1) gives a much better model fit than is achieved using phenotype (i.e. male CHC PCs substituted into the models in Table 6.1), which suggests that male genotype accounts for more variation in mate choice than CHC phenotype (ΔDIC = 25.559 for the best model for copulation latency data; ΔDIC = 59.235 for the best model for mate acceptance data).

Since the best models with CHC data suggest that choosiness and preference functions for male CHC profiles do not vary across female genotypes, environments or with G x E (i.e. no female x male, male x temperature or female x male x temperature interactions), the overall posterior estimates for β (with 95% credible interval) for each PC (Table 6.4) clearly show strong sexual selection on PC3 and PC4. Additionally, whilst PC2 does not significantly influence female preference, it does significantly influence female choosiness (Table 6.4).

Our use of an average CHC profile per male isoline could have limited our ability to detect female G, E and G x E variation in mate choice for male CHCs, and so we examined mate choice on CHCs in more detail. Genetic variation in choosiness function and preference function (Figures 6.2 and 6.3, respectively) is illustrated as a function of each PC vector of CHC variation. Some genetic variation in mate choice for CHCs is clear by eye, particularly in the vectors of CHC profile under significant sexual selection (PCs 2-4). Estimation of β for each female isoline and environment combination for each PC individually also shows some evidence of genetic variation in β (Figure 6.4), but a lack of variation between temperatures.

Genetic covariance between female preference and male attractiveness

There was some statistical support for the model including a G x E across temperatures in male attractiveness score (DIC [posterior probability] = 1222.708 [0.707]), and the model including only G and E had lower support (DIC [posterior probability] = 1224.466 [0.293]).

This suggests there is G x E in male attractiveness, however, the small change in DIC suggests the G x E effect is weak, and this is reflected in the cross-environment genetic correlation, which is high and only very marginally different from a correlation of 1 (0.977 [0.874-0.999]). Since male attractiveness score was calculated from female preference and male CHC expression data, this interaction could suggest the potential for the genetic correlation between female preference and male CHC attractiveness to vary across environments. However, genetic correlations both within (23^{o}C: r_g = 0.23 (-0.23-0.61), P = 0.165; 25^{o}C: r_g = 0.38 (-0.07-0.71), P = 0.063) and between temperatures (female 23 ^{o}C, male 25 ^{o}C: r_g = 0.24 (-0.23-0.62), P = 0.156; female 25 ^{o}C, male 23 ^{o}C: r_g = 0.10 (-0.36-0.52), P = 0.339), are positive but non-significant (although the correlation within 25^{o}C is only marginally non-significant). We therefore find no evidence for genetic covariance between female preference and male attractiveness (as calculated from CHC profile) across any of the temperatures we studied.

6.5 Discussion

Despite recent interest in the role of the environment and genotype-by-environment interactions in sexual selection, relatively little is known about the genetics of plasticity in female mate choice (Ingleby et al 2010). Here, we examine the genetics of two aspects of female mate choice, choosiness and preference, across two post-eclosion temperatures. We find evidence for genetic, environmental and G x E components of both choosiness and preference, making this one of a small number of studies to investigate the cross-environment genetics of mate choice behaviour (Greenfield and Rodríguez 2003; Delcourt et al. 2010; Narraway et al. 2010). However, the lack of female G, E and G x E variation in the ranked order of preferred male genotypes suggests that females generally agree on which males are most attractive, and so the outcome of mate choice is unlikely to differ across these temperatures.

The definitions used here for female choosiness and preference follow Jennions and Petrie (1997) and are also consistent with Cotton et al. (2006). Whilst there may be slight overlap in the information provided by latency and acceptance data, these metrics represent good proxies for components of female mate choice. The distinction between choosiness and preference can be useful, since female choosiness can vary (e.g. through changes in the costs or benefits of mate assessment) without necessarily altering overall

preference (Jennions and Petrie 1997). Therefore, separate consideration of choosiness and preference provides insight into both the evolution of female mate choice behaviours and the overall outcome of mate choice. Based on the G, E and G x E variation we identify, we consider the implications of our findings below.

Female choosiness

The best model for copulation latency identifies a genetic basis of both female choosiness and male attractiveness, as well as a strong effect of female environment (post-eclosion temperature). Females reared at the higher temperature are less choosy on average than females from the lower temperature, which was expected since it has been found previously that female *D. melanogaster* respond more quickly to males when kept at higher temperatures (Barron 2000).

There was no significant genetic variation for plasticity in choosiness across temperatures (i.e. a female choosiness G x E was not included in the best statistical description of the data). However, the cross-environment genetic correlation and between-environment heritability provide evidence for substantial changes in genetic variation in choosiness between temperatures indicative of a G x E. The wide intervals around these estimates perhaps explain the lack of a significant statistical interaction, but the intervals overlap 0 and are distinct from 1 and so it is likely that there is some genetic variance in plasticity in female choosiness across temperatures. This G x E could mean that the evolution of this aspect of female mate choice will depend on the environment even across the narrow range of environmental variation assessed here, and note that this narrowness could also explain why the G x E term did not fall into the best-fit model. The results are therefore largely consistent with the only other studies we are aware of which test for G x E in female choosiness: Rodríguez and Greenfield (2003) found a G x E for female responsiveness in *A. grisella* reared across different temperatures and Narraway et al. (2010) identified G x E for female choosiness in *D. melanogaster* dependent on temperature stress during development.

Female preference

From the mate acceptance data, we find high genetic variance in female preference and male attractiveness, and very little overall temperature effect. However, there is substantial

genetic variation in the effect of temperature, shown by a strong female preference G x E. The combination of high heritability and G x E variation means that there is considerable opportunity for the evolution of different female preferences, and variation in the strength of sexual selection, across environments. However, our results clearly demonstrate that preference as a function of male genotype does not differ across female genotypes or environments, nor with G x E. Therefore the ultimate outcome of mate choice does not vary across female genotypes or environments, and hence the same male genotypes are always preferred.

Female mate choice for male CHCs

The ability to detect significant G, E and G x E variation in mate choice for male CHCs could have been limited by the experimental design (by using an average male CHC profile per male isoline in the analysis). However, we were still able to quantify female mate choice for male CHC profiles across female genotypes and environments, and this reveals some interesting patterns underlying CHC attractiveness which potentially warrant further research. In particular, there appears to be genetic variation in female mate choice for aspects of male CHC profile, although no indication of variation in choice across environments. This is consistent with a study on *D. serrata* (Delcourt et al. 2010), where a genetic basis for female preference functions for male CHC profiles was identified, but there was no evidence of plasticity or G x E across a dietary manipulation.

Male CHCs function as sexual signals in a number of *Drosophila* species (Ferveur and Cobb 2010; Delcourt et al. 2010) including *D. simulans* (Sharma et al. 2012b; Chapter 3). Consistent with these studies, we find evidence for significant sexual selection acting on 3 of the 4 vectors of CHC expression examined. Interestingly, PCs 3 and 4 are under selection using either component of mate choice (preference or choosiness), and therefore are likely to contribute to overall attractiveness of male CHC profile. On the other hand, PC2 only explains variation in female choosiness, perhaps indicating that CHC variation in this vector influences female responsiveness during courtship.

Despite the clear influence of male CHC profile on overall female mate choice, male CHC profile accounts for less variation in mate choice than male genotype, indicating that there is important sexually selected phenotypic variation in other traits which were not measured in this study. This is consistent with what is known of *Drosophila* courtship and

elements of it that we did not assess, such as song and dance (Speith 1974). Nonetheless, there is evidence that mate choice for aspects of male CHC phenotype varied between female genotypes, and in a previous study on the same population of *D. simulans* isolines, we found complex patterns of G, E and G x E variation in male CHC profile (Ingleby et al. in review). However, overall male attractiveness was strongly genetically determined and consistently heritable across a range of environments (Ingleby et al. in review). The results of the current study are consistent with previous conclusions that females assess male attractiveness using multiple sexual traits (Speith 1974), including aspects of CHC profile, and so although CHCs influence female mate choice, the overall attractiveness of a given male correlates more strongly with male genotype than with a particular phenotypic trait.

Genetic covariance between female preference and male attractiveness

Analysis of the genetic covariance between female preference and male attractiveness lends further support to the idea that multiple sexual traits contribute to the overall attractiveness of a given male. Despite measuring a positive genetic correlation between female preference and male CHC attractiveness across each temperature, none of these correlations were significant. Similar results were found in a study of the cross-environment genetic covariance between female preference and a male sexual signal in *A. grisella* (Zhou et al. 2011). At first glance, this is highly unexpected. In *D. simulans*, there is evidence for strong heritability of overall male attractiveness (Taylor et al. 2007; Ingleby et al. in review) and female preference (this study), and additionally there is no evidence of any direct benefits of mate choice to females (Taylor et al. 2008). We therefore expect sexual selection to operate through a Fisherian runaway process, and a positive genetic correlation is expected to evolve between female preference and male attractiveness (Lande 1981).

However, in the present study, male attractiveness (of males from the female isolines) was scored as a function of male CHC phenotype, and so the lack of covariance between female preference and male attractiveness could be an artefact of the complex multivariate nature of sexual signalling and preference. If females use multiple sexually selected cues to assess overall male attractiveness, then calculating male attractiveness scores from only the CHC data will overlook sexually selected variation in other male signals, thus resulting in the weakly positive genetic correlations we find between male attractiveness score and female preference. A more accurate method for scoring male

attractiveness might therefore involve either measuring additional sexual traits, or the overall attractiveness of male genotypes.

A strong positive genetic correlation between preference and attractiveness is predicted to facilitate the runaway evolution of sexual traits (Lande 1981), and so a weak correlation implies that although Fisherian sexual selection could operate, it is unlikely to result in accelerating trait evolution. In *D. simulans*, it seems likely that the strength of the genetic covariance between female preference and male CHC profile could be mediated by a combination of (1) indirect benefits of mate choice through heritable male attractiveness (Taylor et al. 2007; Ingleby et al. in review), (2) multiple sexual signals contributing to overall variance in male attractiveness (Ingleby et al. in review; this study), and (3) the balance between naturally and sexually selected optima in CHC profile. This balance is particularly relevant with respect to sexual selection across temperatures in *Drosophila*, given evidence that temperature-dependent natural selection will favour the production of different CHCs than sexual selection (Ferveur and Cobb 2010). However, in our data there were no clear differences in the male-female genetic correlation across temperatures, and so it remains uncertain how important this factor is.

In conclusion, we find genetic, environmental and G x E variation in female choosiness and preference, but find no such variation in the ranked order of preferred males, such that the same male genotypes are likely to be favoured by sexual selection even across different environments and females. Therefore whilst the evolution of female mate choice behaviour could differ between environments, the ultimate outcome of mate choice may be relatively consistent. However, there is evidence of genetic variation for female mate choice based on male CHC profile, and shows that CHC profile by itself is not as effective a predictor of total male attractiveness as male genotype. Furthermore, the genetic covariance between female preference and male attractiveness, scored by CHC profile, is weak and consistent with the idea that other male sexual signals contribute to overall attractiveness. This study highlights the importance of multivariate and cross-environment perspectives in order to gain a full understanding of sexual selection.

Table 6.1 Summary of the sets of models tested for (I) copulation latency (female choosiness) and (II) mate acceptance (female preference) data. All models include post-eclosion temperature (*t*) as a fixed effect. Female isoline (F), male isoline (M) and any interactions are added as random effects, as shown. The best model is highlighted in bold and chosen using the DIC (supported by the approximate posterior probability) and models are ranked from best model fit (lowest DIC) to poorest model fit (highest DIC). Results are shown for models with and without block as a covariate. Results are qualitatively identical with and without a block effect, but model fit is improved slightly by removing block.

	Model rationale	Random effects	DIC (posterior probability) without block	DIC (posterior probability) with block
I. Copulation latency				
1.	**Genetic variation for both choosiness and attractiveness**	**F + M**	**4153.165 (0.852)**	**4155.402 (0.839)**
2.	G x E for female choosiness and G for male attractiveness	F x *t* + M	4156.730 (0.143)	4158.780 (0.155)
3.	Genetic and environmental variation for female choosiness function	F x M + M x *t*	4163.460 (<0.001)	4166.307 (<0.001)
4.	Genetic variation for male attractiveness	M	4172.226 (<0.001)	4174.435 (<0.001)
5.	Genetic variation for female choosiness function	F x M	4195.469 (<0.001)	4196.404 (<0.001)
6.	G x E for female choosiness function	F x M x *t*	4198.091 (<0.001)	4200.514 (<0.001)
7.	Genetic variation for female choosiness	F	4257.801 (<0.001)	4260.257 (<0.001)
II. Mate acceptance				
1.	**G x E for female preference and G for male attractiveness**	**F x *t* + M**	**2363.753 (0.986)**	**2365.790 (0.963)**

2.	Genetic variation for both preference and attractiveness	F + M	2372.393 (0.013)	2374.284 (0.037)
3.	Genetic and environmental variation for female preference function	F x M + M x *t*	2395.824 (<0.001)	2398.063 (<0.001)
4.	Genetic variation for female preference function	F x M	2406.907 (<0.001)	2409.905 (<0.001)
5.	G x E for female preference function	F x M x *t*	2408.196 (<0.001)	2409.950 (<0.001)
6.	Genetic variation for female preference	F	2441.910 (<0.001)	2443.997 (<0.001)
7.	Genetic variation for male attractiveness	M	2449.585 (<0.001)	2450.941 (<0.001)

Table 6.2 Variance in copulation latency (female choosiness) and mate acceptance (female preference) accounted for by male isoline, female isoline and female isoline x temperature (G x E). 95% credible intervals around each estimate are in brackets. Components included in the best model for each response are highlighted in bold.

	Copulation latency	*Mate acceptance*
Male isoline	**0.084 (0.024-0.258)**	**0.421 (0.111-1.299)**
Female isoline	**0.017 (0.005-0.040)**	**0.425 (0.176-0.902)**
Female isoline x temperature	0.004 (0.001-0.013)	**0.090 (0.008-0.256)**

Table 6.3 Cross-environment genetic correlation and between- and within-environment heritability of copulation latency (female choosiness) and mate acceptance (female preference). 95% credible intervals around each estimate are in brackets. Interval estimates which are distinct from 1 are highlighted in bold.

	Copulation latency	*Mate acceptance*
Genetic correlation, r_g	**0.606 (-0.115 – 0.934)**	**0.679 (0.130 – 0.982)**
Heritability, H^2:		
within 23°C	0.752 (0.209 – 1.435)	0.850 (0.368 – 1.390)
between temperatures	**0.555 (-0.097 – 0.906)**	**0.646 (0.123 – 0.961)**
within 25°C	1.248 (0.565 – 1.791)	1.150 (0.610 – 1.633)

Table 6.4 Overall estimates for β, the linear selection gradient, on each PC of CHC expression. 95% credible intervals around each estimate are in brackets. Interval estimates which are significantly different from 0 are highlighted in bold. Note that consistent sexual selection will have the opposite sign for latency and acceptance.

	Copulation latency	*Mate acceptance*
PC1	-0.067 (-0.198 – 0.066)	0.083 (-0.284 – 0.449)
PC2	**-0.143 (-0.275 – -0.013)**	0.015 (-0.372 – 0.395)
PC3	**-0.516 (-0.656 – -0.377)**	**0.618 (0.214 – 1.022)**
PC4	**0.401 (0.310 – 0.493)**	**-0.460 (-0.717 – -0.197)**

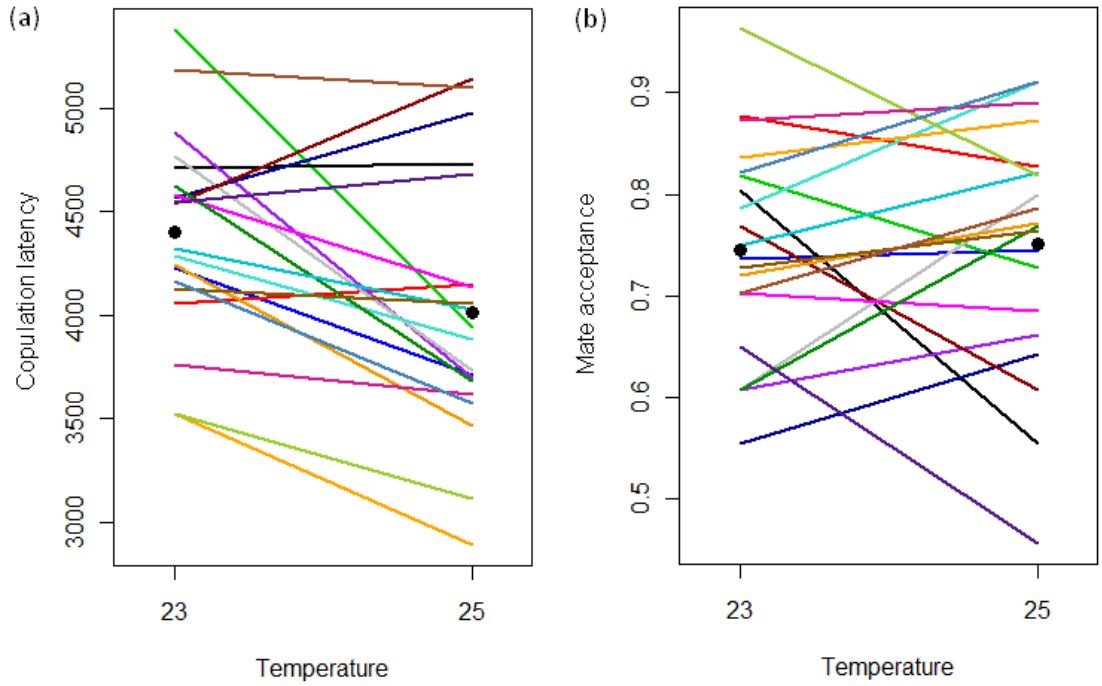

Figure 6.1 Female G x E reaction norms for *(a)* copulation latency (female choosiness); and *(b)* mate acceptance (female preference) across post-eclosion temperatures. Each coloured line represents the mean score for each female isoline (*N* = 20 isolines). Points represent the overall mean score within each temperature across all isolines.

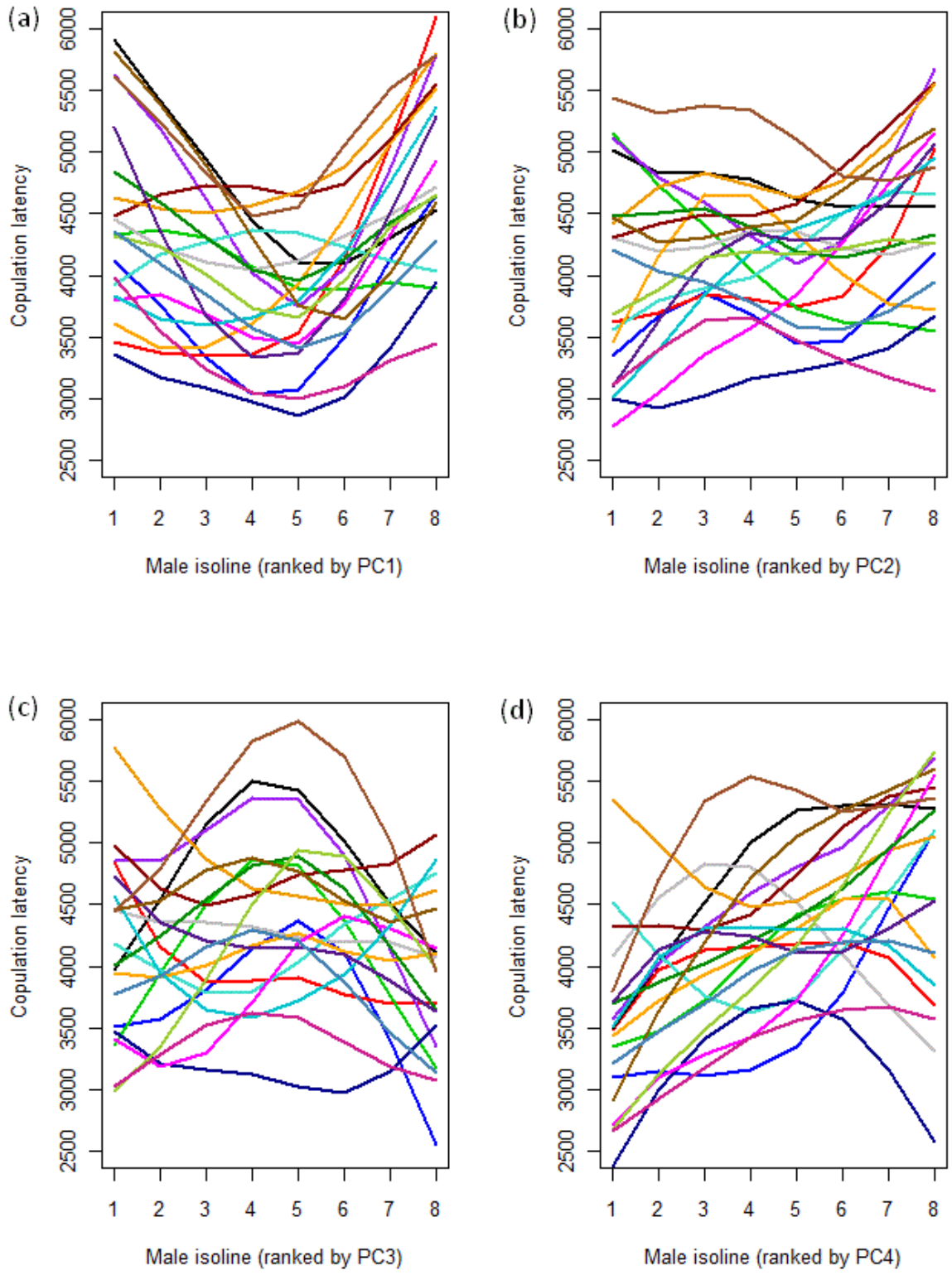

Figure 6.2 Genetic variation in copulation latency (female choosiness) as a function of male CHC profile (PCs 1-4, *(a)-(d)*). Male isolines (*N* = 8 isolines) are ranked on the x-axis according to mean PC score (left (low) to right (high) along axis). Each coloured line represents a female genotype (*N* = 20 isolines) pooled across temperatures. Note that low copulation latency indicates high male attractiveness.

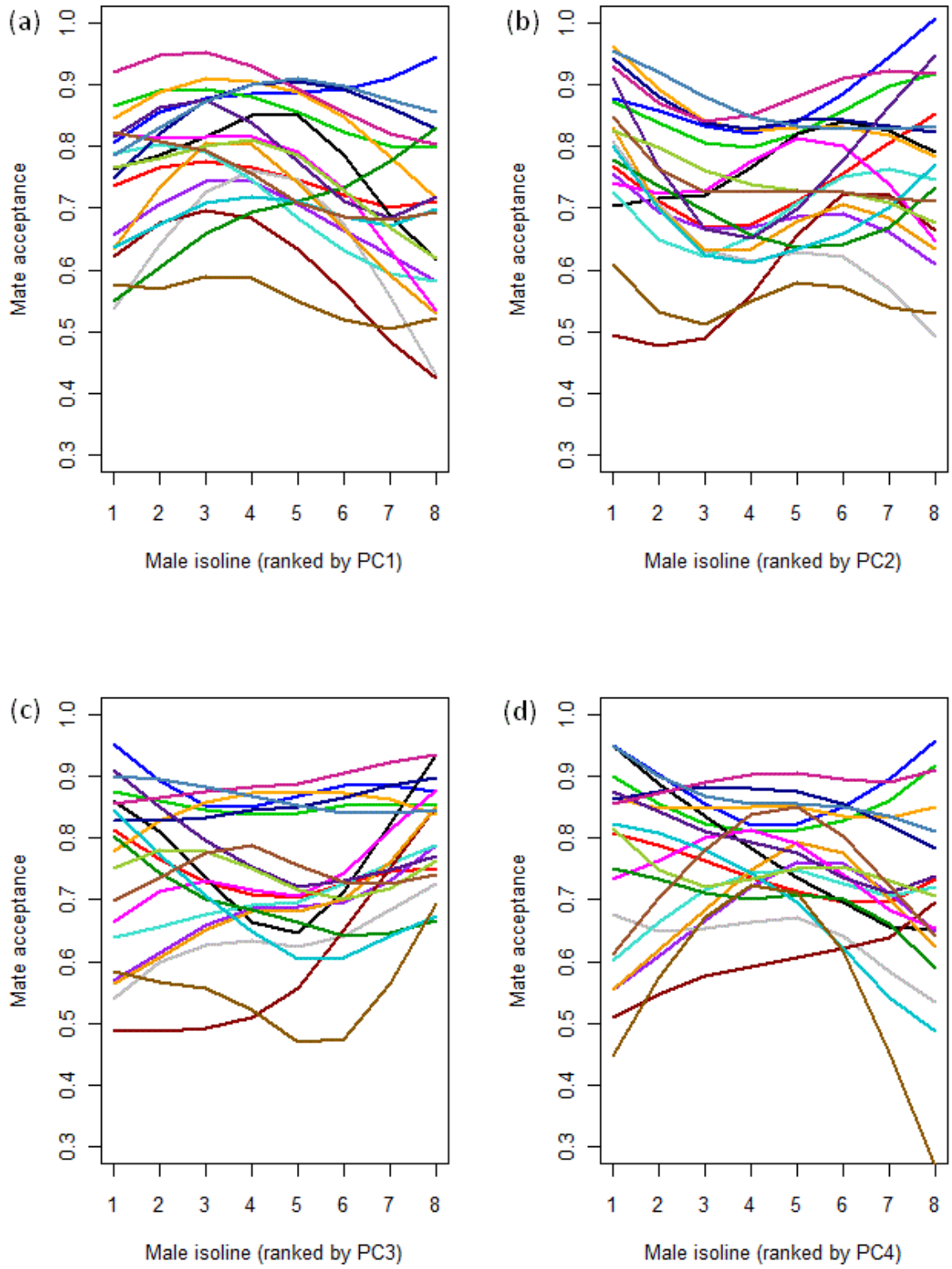

Figure 6.3 Genetic variation in mate acceptance (female preference) as a function of male CHC profile (PCs 1-4, *(a)-(d)*). Male isolines (*N* = 8 isolines) are ranked on the x-axis according to mean PC score (left (low) to right (high) along axis). Each coloured line represents a female genotype (*N* = 20 isolines) pooled across temperatures.

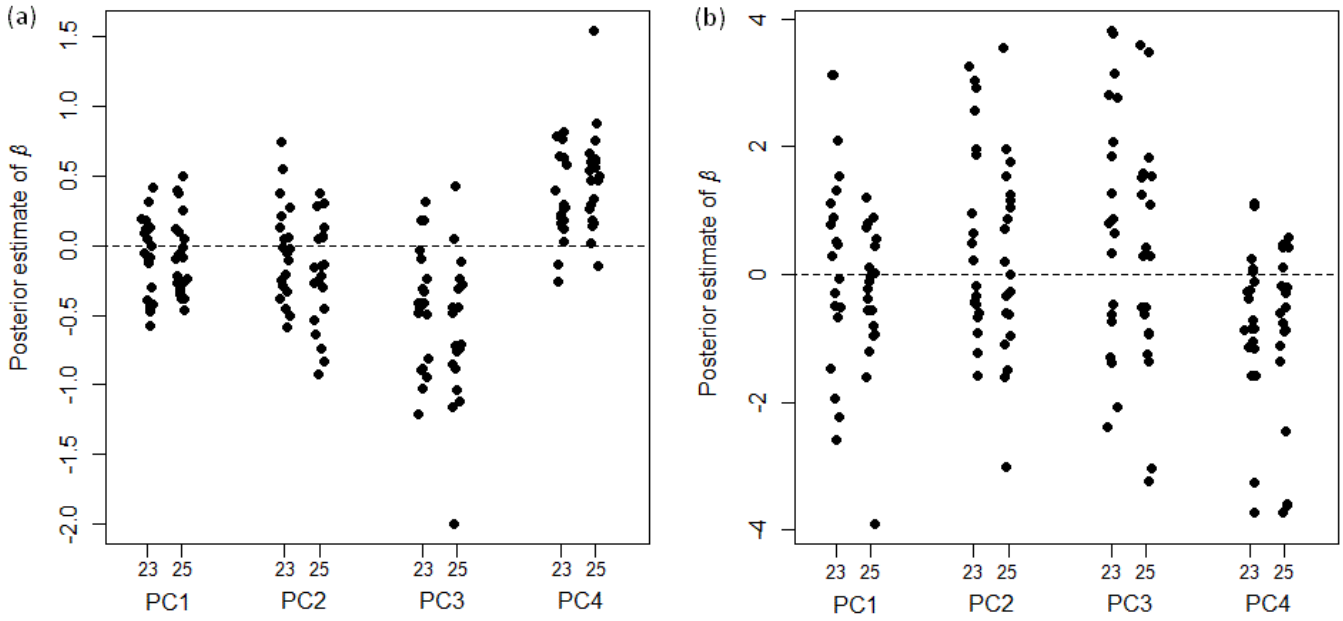

Figure 6.4 Posterior estimates of β, the linear selection gradient, on each PC of male CHC expression for each female isoline x temperature combination using *(a)* copulation latency (female choosiness) and *(b)* mate acceptance (female preference) data. Each point represents a female isoline and the dashed line denotes $\beta = 0$ (i.e. no linear selection). Linear selection was significant overall on PC3 and PC4 for both choosiness and preference, and also on PC2 for choosiness (see text for details).

CHAPTER 7: General discussion

The role of genotype-by-environment (G x E) interactions in sexual selection has been the subject of increasing research interest in recent years. However, as highlighted in Chapter 2 (Ingleby et al. 2010), empirical research has somewhat lagged behind the theory. In particular, Chapter 2 emphasises the importance of further research providing empirical tests of theoretical predictions, integrating research on male and female sexual traits, and exploring different types of environmental variation (abiotic and biotic). Here, I will discuss how the research in this thesis has contributed to these areas and suggest directions for future research.

7.1 Empirical tests of theoretical predictions

Higginson and Reader (2009) demonstrated the potential for G x Es in sexual signals to break down the relationship between genotype and phenotype across environments, causing signal unreliability. If females cannot rely on male sexual signals to honestly advertise potential benefits of mate choice, then female mate choice should be costly and be selected against. The potential for G x Es to cause sexual signal unreliability is clear from studies which have shown that sexual signal heritability varies across environments (e.g. Mills et al. 2007; Chapter 4), and in Chapter 5, I explicitly demonstrated signal unreliability in some sexually-selected aspects of male *D. simulans* CHC profile. However, I found that these unreliable signals did not alter the overall outcome of mate choice, such that females consistently gained genetic benefits of mate choice across environments.

These results show that variation in an individual's overall attractiveness is determined by more than just CHC profile, indicating that females use multiple sexual signals to assess overall male attractiveness. This interpretation is consistent with the results of Chapter 6, and also with studies which have identified multiple sexual traits in *Drosophila* (e.g. Speith 1974; Markow 1996), as well as supporting the idea that multivariate signalling could be a mechanism to compensate for signal unreliability (Candolin 2003). Multiple sexual signals could also account for the results of a similar study on field crickets (Tolle and Wagner 2011), which found that direct benefits of female mate choice were maintained across environments despite G x Es in the expression of a male acoustic signal. If G x Es cause some aspects of sexual signals to be unreliable, then female mate choice could

be under selection to weight more importance on reliable aspects of signalling. Therefore, whether or not unreliable sexual signals affect the overall outcome of female mate choice will depend on how many sexual signals influence a mating decision, and on the relative importance of individual signals (in terms of the amount of variation in female mate choice each signal accounts for). It will also be interesting to see if, in other species, benefits of mate choice can be maintained across environments through use of multiple sexual signals.

A second model showed that G x Es could contribute to the maintenance of genetic variation in sexual traits (Kokko and Heubel 2008). Studies which have shown that G x Es weaken the genetic correlation of trait expression across environments (e.g. Jia et al. 2000; Chapter 4) demonstrate the potential for traits to evolve independently (at least to some extent) in different environments, and for genetic variation to be maintained.

However, the depletion of genetic variation is only a problem assuming persistent selection. I find significant variation in sexual selection (Chapter 3), genetic constraints (Chapter 3) and genetic variation (Chapter 4) for male CHC profile across diets, suggesting that the evolutionary trajectory of male CHC profile could be dramatically different across the diets I studied. Across temperatures, on the other hand, variation in selection through female preference for CHCs is less clear (Chapters 3 and 6). Coupled with a lack of strong G x E in male CHCs across temperatures (Chapters 4 and 5), genetic variation in male CHCs could be depleted. Interestingly, there was low genetic variation in the vector of male CHC expression describing a trade-off between long- and short-chained CHCs (Chapter 4), which is likely to be subject to consistent natural selection (for desiccation resistance across temperatures; Gibbs et al. 1998; Savarit and Ferveur 2002; Frentiu and Chenoweth 2010) as well as sexual selection (Chapter 3). A lack of genetic variation could therefore be a major constraint on the evolution of some aspects of CHC profile across temperatures.

7.2 Integrating studies of male and female sexual traits

As discussed in Chapter 2, integrating studies of male and female sexual traits could greatly improve our understanding of the evolutionary consequences of G x Es. Specifically, only by combining male and female perspectives can we assess the potential for G x Es to breakdown the genetic covariance between male attractiveness and female preference which is necessary for sexual selection to operate through a runaway process (Lande 1981). This has been measured in the waxmoth, *Achroia grisella* (Zhou et al. 2011), and in *D.*

simulans (Chapter 6). Both of these studies failed to find a significant genetic covariance between male attractiveness and female preference, despite evidence for genetic benefits of mate choice in each species (Taylor et al. 2007; Zhou et al. 2011; Chapters 5 and 6).

A positive covariance between male attractiveness and female preference is predicted to drive the evolution of exaggerated sexual traits. Assessing male attractiveness based on a single sexual trait might have limited the ability to detect a strong covariance between preference and signal, due to female use of multiple sexual signals, such that realistically a significant positive genetic covariance might only be found between overall preference and overall attractiveness. However, calculating the genetic covariance based on one sexual trait does provide useful insight into the evolution of that trait. A weak covariance precludes the ability for runaway selection to result in extreme exaggerated sexual phenotypes.

This genetic covariance will be mediated by the magnitude of genetic benefits; the multivariate nature of sexual signalling and the relative importance of the signal in mate choice; and, across environments, the balance between naturally and sexually selected optima in trait expression. Therefore, the genetic covariance between preference and attractiveness will be highly context- and species-specific, and more studies which examine this cross-environment covariance will be needed to provide further insight.

Further research could also consider G x Es and selection on sexual traits which are expressed in both sexes. In Chapter 4, I identified G x Es in female CHC expression across both diets and temperatures. The evolutionary consequences of these G x Es, however, are unclear, since I did not measure selection on female CHCs. Studies have shown that female CHC profile can evolve through both natural and sexual selection (e.g. Blows 2002; Sharma et al. 2012b), and male mate choice based in female CHCs has been shown in *D. serrata* (Chenoweth and Blows 2005). Further, several roles of female CHCs in *Drosophila* mating interactions have been identified, including signals of female receptivity (Marcillac and Ferveur 2004), post-mating changes in female CHCs (Petfield et al. 2005) and species recognition between closely-related species (e.g. Higgie et al. 2000). G x Es in female CHC profile could therefore have similar effects as G x Es for male CHCs, for example, contributing to the maintenance of genetic variation or disrupting the reliability of signals.

Furthermore, Chapter 4 shows that despite male and female *D. simulans* producing qualitatively the same CHCs, there is quantitative sexual dimorphism. There is also sexual

dimorphism in the direction and extent of G, E and G x E effects on CHC expression. Further research could examine the potential for G x sex interactions, which would indicate an escape from genetic constraints on the evolution of sexually dimorphic CHC profiles, and even G x E x sex interactions, which would create complex environment-dependent dynamics in the evolution of sexual dimorphism.

7.3 Abiotic and biotic environmental variation

Previous studies of G x Es in sexual selection have focussed largely on manipulation of a single axis of abiotic environmental variation (Chapter 2). As such, we have a poor understanding of how G x Es might differ between different types and magnitudes of environmental variation. In this thesis I have shown that it is not necessary for environments to be particularly harsh or stressful in order for G x Es to have a significant effect (Hoffmann and Merilä 1999), since the effects I found were all identified across a fairly narrow range of environmental variation which is within the normal bounds that *D. simulans* might encounter. Further, although I found no evidence for synergistic effects of diet and temperature (Chapter 3 and 4), I did find that diet and temperature have very different effects both on CHC expression (Chapters 3-5) and on patterns of sexual selection (Chapter 3). Similarly, although a strong G x E in *D. simulans* male CHC expression across diets was identified (Chapter 4), there was no evidence of G x E in *D. serrata* male CHC expression across diets (Gosden and Chenoweth 2011). Whilst these results could amount to species differences, these are closely-related *Drosophila* species, and the most striking contrast between the two studies is the difference in the environmental manipulation: I used different larval rearing diets but did not manipulate diet quality (Chapter 4); whereas Gosden and Chenoweth (2011) manipulated adult diet quality. Together, these results highlight the importance of studying G x Es across a range of different environments, since it is clear that even studies of the same trait can create a very different picture by looking at different types of environmental variation.

Further work should not only explore different types and extents of environmental variation, but also take into consideration the temporal scale of environmental fluctuations. Theory suggests that 'fine-scaled' environmental fluctuations, where the environment varies a lot within a generation, will generate strong selection which could erode genetic variation in plasticity and minimise G x E effects, but that over more 'coarse-scaled' environmental

variation, where the environment is relatively stable within a generation, this will not be the case and we might expect G x E effects to be stronger (Levins 1968). In Chapter 5 there was no indication that the effect of G x Es differed when the environment changed between generations compared to when it stayed constant, but more thorough empirical tests of this idea could be useful (Rodríguez 2012).

Another aspect of environmental variation to consider will be the social environment. Variation in the social environment has the potential to have strong and complex effects on the evolution of sexual traits, since the social environment can vary widely over short timescales (Wolf et al. 1999), and can alter the intensity of sexual selection, since social interactions are intrinsic to sexual selection through competition for mates, mate choice and mating itself. The importance of social environment is apparent from the few existing studies which directly test for G x E (e.g. Kent et al. 2008), as well as from experimental evolution, which has demonstrated the evolvability of phenotypic plasticity across social environments through sexual selection, indicating that there must be genetic variation for this plasticity (Chenoweth et al. 2010). Given the importance of social environment in studies of sexual selection, and the paucity of research on the subject, this is clearly a vital area for future research.

7.4 Conclusions

Sexual selection research has begun to recognise the importance of examining sexual trait evolution in increasingly complex scenarios, taking into consideration environmental variation and interactions between individuals. My research highlights the importance of such complexity, demonstrating that G x Es can significantly impact on sexual trait evolution across environments. I have shown that examining patterns of genetic variation and multivariate selection across environments, and consideration of both male and female sexual traits, can provide a more comprehensive understanding of sexual selection.

However, there are many potentially useful directions for future research. For example, our understanding of plasticity in female mate choice and sexual selection on females is still poor, despite some progress in recent years. Further work could also attempt more empirical tests of fundamental theoretical predictions - such as the potential for G x Es to breakdown sexual signal reliability and the genetic covariance between males and

females - across a wider range of species and contexts in order to generalise the results of the few existing studies.

Clearly, research into the role of G x Es in sexual selection presents some considerable empirical challenges due to the complex nature of the cross-environment and multivariate approaches which are needed. However, the research in this thesis highlights the importance of these complexities in order to examine the interacting evolutionary dynamics of male and female sexual traits across different environments, and to gain a better understanding of sexual selection.

APPENDIX 1: Calculation for genetic constraint angles with 95% credible intervals

Here, the calculation for one environment is described. This was done separately for each of four environments. Annotated R code (built in R v.2.13.0) for each step is provided in the boxes. We used the 'MCMCglmm' package (Hadfield 2010).

First, a Bayesian generalised linear mixed model was used to model genetic variation for the traits of interest (in this case, isoline variation for PCs 1-3 of CHC expression from the dataset 'G.data'). The posterior distribution of the **G** matrix can be extracted from this model.

```
G.model <-  MCMCglmm (cbind (PC1,PC2,PC3) ~ trait - 1,
            random=~ us(trait):isoline, rcov=~ us(trait):units,

            # PCs 1-3 as a multivariate response and isoline as a
            # random effect, with unstructured (us) variances so all
            # genetic variances and covariances are estimated.

            prior= list (R= list (V= diag(3)/3, nu=0.02),
            G= list (G1= list (V= diag(3)/3, nu=0.02))),

            # Specifies an uninformative prior distribution, where
            # diag(3) reflects the dimensions of the G matrix.

            data= G.data, family= rep ("gaussian",3),

            # Different data distributions can be used. Here, each
            # PC fits a Gaussian distribution.

            nitt= 400000, burnin= 20000, thin= 25, pr= T)

            # Gives a posterior distribution based on 15200 estimates
            # of each parameter.
```

Next, a Bayesian linear regression was used to model $\boldsymbol{\beta}$, the linear selection gradient, on each trait (PCs 1-3 modelled against relative fitness). The posterior distribution of $\boldsymbol{\beta}$ for each PC can be extracted from this model.

```
selection.model <-  MCMCglmm (rel.fitness ~ PC1 + PC2 + PC3,
                    data= selection.data,
                    nitt= 400000, burnin= 20000, thin= 25)

                    # Gives a posterior distribution based on 15200
                    # estimates of each parameter.
```

The posterior distribution of each of these models consists of 15200 rows, each row with an estimate of each component of the **G** matrix and an estimate of $\boldsymbol{\beta}$ on each PC. For each row,

the predicted response to selection is calculated according to the multivariate breeder's equation (Lande 1983) (i.e. the response to selection, $\Delta \mathbf{z} = \mathbf{G}\boldsymbol{\beta}$). The angle between the vector of predicted responses ($\Delta \mathbf{z}$) and the vector of linear selection ($\boldsymbol{\beta}$) gives the genetic constraint (Blows and Walsh 2009). This is calculated as follows:

```
angles <-  numeric (15200)

           # Creates a vector of length 15200 (i.e. same length as
           # there are rows in the posterior distribution), and the
           # angle calculated for each row is stored in this vector
           # as follows:

for (i in 1 : 15200) {

G <-  matrix (G.model $ VCV[i,1:9], 3)

      # Creates a 3 x 3 G matrix from the variance and covariance
      # estimates in each row of the posterior distribution.

b <-  selection.model $ Sol[i,2:4]

      # Creates a vector from the beta estimates in each row of the
      # posterior distribution.

delta.z <-  G %*% b

            # Calculates the predicted response to selection for each
            # row of the posterior distribution.

angles[i]<-  acos ((t(delta.z) %*% b) / ( (sqrt (t(delta.z) %*%
             delta.z)) * (sqrt (t(b) %*% b)))) * (180/pi) }

             # Calculates the genetic constraint for each row of the
             # posterior distribution and stores it in the 'angles'
             # vector.
```

The mean genetic constraint angle estimate (in degrees) and 95% credible intervals can be called directly from 'angles'.

APPENDIX 2: Re-analysis of Chapter 5 using projected PC vectors

Table A Results from a mixed model MANOVA with PCs 1-3 of CHC expression (projected PC scores) as response variables; post-eclosion temperature, generation and temperature x generation interaction as fixed effects; and isoline, isoline x temperature, isoline x generation and isoline x temperature x generation as random effects. Results of individual univariate GLMMs for each PC are given in the second section of the table. Significance is highlighted in bold. Error structure was specified following Zar (1999).

Overall MANOVA				
	Wilks' λ	*F*	*df*	*P*
Generation	**0.823**	**3.150**	**3,44**	**0.034**
Temperature	**0.326**	**30.361**	**3,44**	**0.0001**
Isoline	**0.462**	**4.222**	**138,1992**	**0.0001**
Gen x Temp	**0.850**	**2.842**	**3,44**	**0.049**
Gen x Isoline	**0.772**	**1.304**	**138,1992**	**0.014**
Temp x Isoline	**0.630**	**1.328**	**138,1992**	**0.010**
Gen x Temp x Isoline	**0.621**	**1.684**	**138,1992**	**0.0001**
Univariate GLMMs				
		F	*df*	*P*
PC1				
Generation		0.601	1,47	0.441
Temperature		0.891	1,48	0.351
Isoline		**3.471**	**46,10**	**0.023**
Gen x Temp		1.415	1,48	0.242
Gen x Isoline		1.020	46,46	0.475
Temp x Isoline		0.704	46,46	0.888
Gen x Temp x Isoline		1.070	46,664	0.349
PC2				
Generation		0.062	1,47	0.803
Temperature		**93.103**	**1,47**	**0.0001**
Isoline		**3.734**	**46,19**	**0.0014**
Gen x Temp		**4.966**	**1,47**	**0.031**
Gen x Isoline		1.205	46,46	0.267
Temp x Isoline		0.997	46,46	0.507
Gen x Temp x Isoline		1.271	46,664	0.115
PC3				
Generation		**7.056**	**1,47**	**0.011**
Temperature		**26.778**	**1,47**	**0.0001**
Isoline		**4.213**	**46,22**	**0.0003**
Gen x Temp		**7.953**	**1,47**	**0.007**
Gen x Isoline		1.231	46,46	0.243
Temp x Isoline		1.110	46,46	0.361
Gen x Temp x Isoline		**1.756**	**46,664**	**0.0019**

Table B Results from a mixed model MANOVA with PCs 1-3 of CHC expression (projected PC scores) as response variables; diet, generation and diet x generation interaction as fixed effects; and isoline, isoline x diet, isoline x generation and isoline x diet x generation as random effects. Results of individual univariate GLMMs for each PC are given in the second section of the table. Significance is highlighted in bold. Error structure was specified following Zar (1999).

Overall MANOVA				
	Wilks' λ	*F*	*df*	*P*
Generation	**0.611**	**8.708**	**3,41**	**0.0001**
Diet	**0.484**	**14.564**	**3,41**	**0.0001**
Isoline	**0.334**	**7.132**	**129,2083**	**0.0001**
Gen x Diet	**0.647**	**7.463**	**3,41**	**0.0004**
Gen x Isoline	**0.712**	**1.943**	**129,2083**	**0.0001**
Diet x Isoline	**0.659**	**2.415**	**129,2083**	**0.0001**
Gen x Diet x Isoline	**0.720**	**1.874**	**129,2083**	**0.0001**
Univariate GLMMs				
		F	*df*	*P*
PC1				
Generation		**16.323**	**1,44**	**0.0002**
Diet		**19.761**	**1,43**	**0.0001**
Isoline		**3.363**	**43,12**	**0.012**
Gen x Diet		2.156	1,44	0.150
Gen x Isoline		0.555	43,43	0.973
Diet x Isoline		1.435	43,43	0.122
Gen x Diet x Isoline		**1.787**	**43,697**	**0.0019**
PC2				
Generation		**5.932**	**1,44**	**0.019**
Diet		3.664	1,44	0.062
Isoline		**3.809**	**43,34**	**0.0001**
Gen x Diet		**11.406**	**43,34**	**0.0015**
Gen x Isoline		1.392	43,43	0.142
Diet x Isoline		**1.832**	**43,43**	**0.026**
Gen x Diet x Isoline		1.324	43,697	0.086
PC3				
Generation		0.157	1,44	0.698
Diet		**7.643**	**1,44**	**0.008**
Isoline		**4.001**	**43,19**	**0.0008**
Gen x Diet		**15.126**	**1,44**	**0.0003**
Gen x Isoline		1.067	43,43	0.429
Diet x Isoline		1.223	43,43	0.257
Gen x Diet x Isoline		**2.029**	**43,697**	**0.0002**

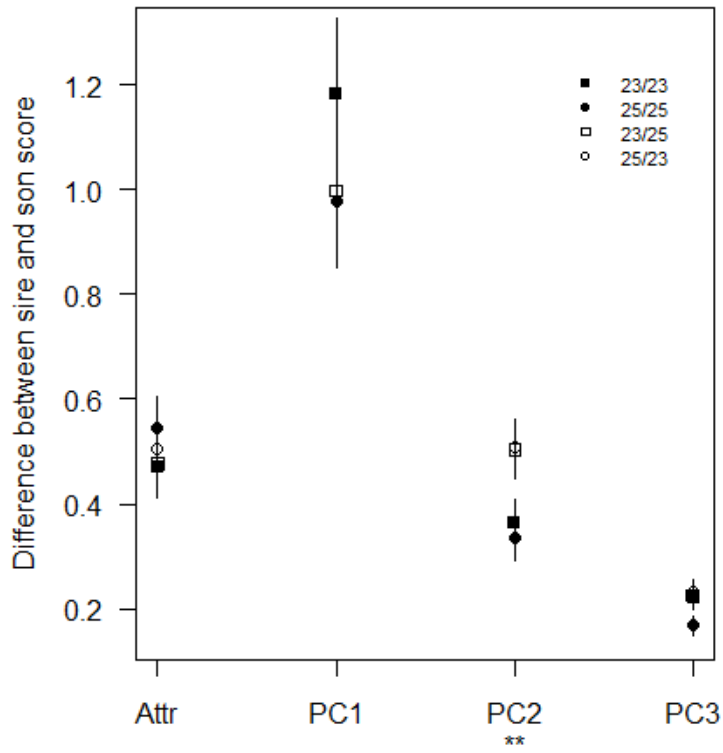

Figure A Mean absolute difference (±SE) between isoline sire and son attractiveness (as standardised copulation latency) and PC scores (PC1-3) across temperatures (see key for sire/son temperature). Filled points represent constant environment treatments and open points represent changing environment treatments. Variation between treatments is significant only in PC2 (asterisks; $F_{3,47}$ = 9.052, P = 0.035), although variation between treatments in PC3 only became non-significant after Bonferroni correction ($F_{3,47}$ = 5.060, P = 0.080).

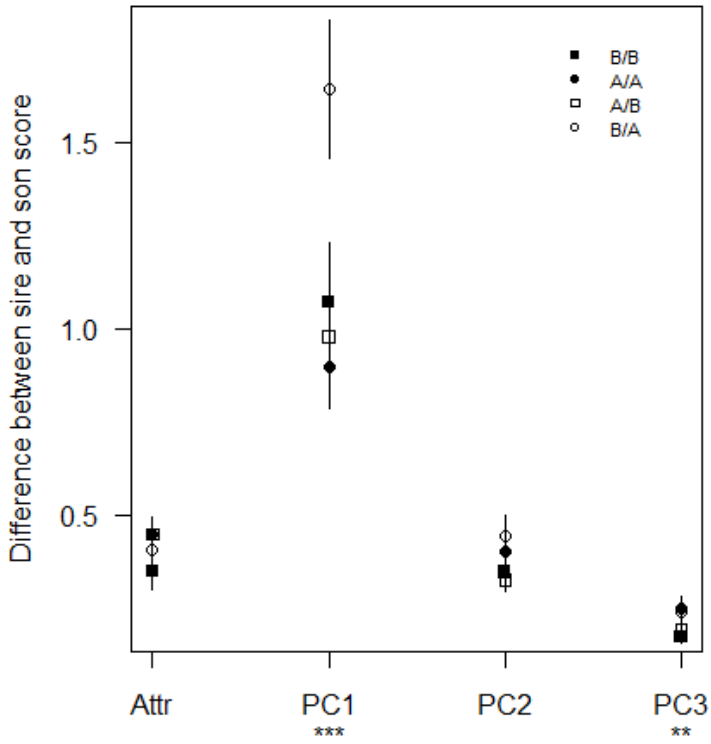

Figure B Mean absolute difference (±SE) between isoline sire and son attractiveness (as standardised copulation latency) and PC scores (PC1-3) across diets (see key for sire/son diet). Filled points represent constant environment treatments and open points represent changing environment treatments. Variation between treatments is significant in PC1 (asterisks; $F_{3,44} = 15.452$, $P < 0.001$) and PC3 (asterisks; $F_{3,44} = 6.634$, $P = 0.040$).

APPENDIX 3: Sexual selection and genotype-by-environment interactions in *Drosophila* cuticular hydrocarbons

A version of this chapter appears in: *The role of genotype-by-environment interactions in sexual selection* (Ed: DJ Hosken and J Hunt). In press with Wiley-Blackwell.

Cuticular hydrocarbons (CHCs) have important functions in insects, from waterproofing to species recognition cues to dominance cues. They can act as pheromones and facilitate chemical mimicry, can be relatively simple or complex (Figure A) and they have been the subject of much research (Blomquist and Bagnères 2010). Similarly, insects have been the subject of much sexual selection research, being used as models to investigate costs of signalling (e.g. Bailey et al. 1993; Hunt et al. 2004; Okada et al. 2011), female mate preference (e.g. Moore 1989; Rodriguez and Greenfield 2003; Tregenza et al. 2006), sperm competition (e.g. Gage 1994; Simmons et al. 1999; Holman and Snook 2008) and benefits of mate choice (e.g. Friberg and Arnqvist 2003; Hosken et al. 2003; Brown et al. 2004). There is considerable commonality in these fields of investigation and *Drosophila* loom large as key study species in the area of overlap.

The function of CHCs has been studied in depth across a wide range of *Drosophila* species. As more generally, non-volatile CHCs create a protective waxy layer on the cuticle, helping the insect resist desiccation. This has been demonstrated by empirical studies manipulating temperature (*D. mojavensis*; Gibbs et al. 1998) and humidity (*D. melanogaster*; Foley and Telonis-Scott 2010), and CHC expression across natural clines in rainfall and humidity are consistent with this as well (*D. serrata*; Frentiu and Chenoweth 2010). CHCs are also involved in chemical communication in *Drosophila*, acting as both contact pheromones and short-range signals, and these CHC signals are integral to fly sexual behaviour. For example, the level of 7-tricosene expressed by male *D. melanogaster* is particularly important in influencing female receptivity (Grillet et al. 2006), and it also influences courtship behaviour in *D. simulans* (Savarit et al. 1999). Methyl-branched alkanes have also been implicated in sexual selection in *D. serrata* (Chenoweth and Blows 2005; Petfield et al. 2005; Delcourt et al. 2010). Female CHCs have also been shown to induce courtship and prolong mating in *D. melanogaster* (Marcillac and Ferveur 2004), and studies of *D. melanogaster* (Yew et al. 2008), *D. simulans* (Ferveur and Jallon 1993) and *D. serrata*

(Petfield et al. 2005) show that CHC profiles are not static, with changes in CHC expression occuring during courtship and mating. These alter male attractiveness and female receptivity, as well as inhibiting courtship behaviour. CHCs are also thought to allow males to assess the level of competition for mates in *D. melanogaster* (Savarit et al. 1999; Bretman et al. 2011), and species-specific CHC profiles are thought to enable species recognition between closely-related *Drosophila* species (e.g. *D. serrata* and *D. birchii*, Higgie et al. 2000; *D. santomea* and *D. yakuba*, Mas and Jallon 2005).

In-depth reviews of the extensive research on CHC functions in *Drosophila* already exist (see Ferveur 2005; Wicker-Thomas 2007; Ferveur and Cobb 2010), and it is clear from this body of research that both natural and sexual selection could drive the evolution of CHC profiles. This inference is supported by experimental evolution in laboratory populations of *D. serrata* and *D. simulans*, which show that CHC profiles in these species are subject to natural and sexual selection (Chenoweth and Blows 2005; Blows 2002; Chenoweth et al. 2008; Rundle et al. 2009; Sharma et al. 2012b). Furthermore, it appears that natural and sexual selection favour different types of CHC profile, as indicated by an interaction between the effects of natural and sexual selection in *D. serrata* (Blows 2002), and by sex-specific patterns of selection on CHCs in both *D. serrata* (Chenoweth and Blows 2005, Chenoweth et al. 2008) and *D. simulans* (Sharma et al. 2012b). Long-chained CHCs are likely to be favoured by natural selection, since non-volatile CHCs will create a more effective waterproof layer on the cuticle, whereas short-chained, more volatile CHCs might contribute more to sexual signalling, although this is undoubtedly a somewhat simplified view of the complex biochemistry of CHCs. Nonetheless, if different CHC profiles are favoured in different selective contexts, then there could well be trade-offs between CHC components and their functions, the more so because CHCs are costly to produce (Blows 2002; Ferveur 2005).

The full gambit of selection acting on *Drosophila* CHCs, because of the range of functions they fulfil, makes these hydrocarbons a particularly interesting sexual trait on which to focus in studies of plasticity across environments. This, coupled with the significant genetic basis to CHC expression that has been found in *D. serrata* (Hine et al. 2004) and *D. simulans* (Sharma et al. 2012a) for example, potentially makes GEIs in CHC expression important for CHC evolution. In this chapter we examine studies of plasticity in CHC profile across abiotic and biotic environments. The importance of GEIs on trait

evolution more generally is discussed at length in other chapters in this volume. Here we restrict our discussion to summarising existing evidence for genetic variation underlying *Drosophila* CHC plasticity (GEIs), and explore the potential effects of plasticity and GEIs on the evolution of CHC profiles in the context of sexual selection and signalling across heterogeneous environments.

Abiotic environments

So far, research has largely focussed on CHC expression across abiotic environments. Research on temperature and dietary effects on *Drosophila* CHC profiles has provided valuable insight into the interaction between natural and sexual selection on CHCs. To a large extent this work has shown that these selective episodes favour different CHC combinations.

Temperature and desiccation stress

It makes intuitive sense that an increase in temperature will select for increased investment in the production of long-chained hydrocarbons as these confer greater desiccation resistance, while lower temperatures, with their decreased risk of desiccation will allow individuals to invest more in the short-chained CHCs important in mate attraction. Indeed, there is evidence supporting this trend, both from clinal variation in Australian populations of *D. melanogaster* and *D. serrata* (Frentiu and Chenoweth 2010), and from experimental manipulation of temperature in *D. affinis* (Jackson 1996), *D. mojavensis* (Gibbs et al. 1998) and *D. melanogaster* (Savarit and Ferveur 2002). In all cases more longer chained CHCs were produced in higher temperature environments. A similar pattern was found in an experimental manipulation of humidity, where long-chained CHCs provided greater desiccation resistance in *D. melanogaster* (Foley and Telonis-Scott 2010). Furthermore, experimental evolution with *D. simulans* at two different temperatures showed that males at the higher temperature evolved to produce more long-chained CHCs (Sharma et al. 2012b), demonstrating that the genetic variation in CHC expression responded to environmental variation in temperature.

Clearly, temperature is an important determinant of *Drosophila* CHC profiles, and plasticity in CHC expression allows individuals to adjust to the thermal environment. However, evidence for GEI across temperatures is scarce, and to our knowledge, has only

been tested in *D. simulans* (Ingleby et al. in review). In this study, there was a strong temperature effect on male CHC expression, which was most clearly manifest in a vector describing the trade-off between long- and short-chained CHCs. Males in the warmer temperature generally invested more in long-chained CHCs, and there was no evidence for significant GEI in male CHC expression across temperatures, indicating that this effect was fairly consistent between genotypes. Given the evidence for strong temperature-dependent selection on *Drosophila* CHCs, the lack of genetic variation underlying plasticity across temperatures could suggest that the optimal male response to temperature variation has become canalised between genotypes (Roff and Fairbairn 2006).

From the same study (Ingleby et al. in review), it appears that the overall effect of temperature on female CHC profile is weaker, and that there is evidence for genetic variation in female CHC plasticity across temperatures (GEI). Temperature-mediated selection on female CHC profile might be weaker than on males, since female *Drosophila* are generally larger than males, and the risk of desiccation will be lower for larger flies with a lower body volume:surface area ratio. Interestingly, Foley and Telonis-Scott (2010) found that the positive correlation between production of long-chained CHCs and desiccation resistance in *D. melanogaster* was stronger in females than in males. This could be explained if females are generally less at risk of desiccation than males. That is, if natural selection on the balance between long- and short-chained CHCs is weaker on females than on males, this would go some way to explaining why genetic variance for plasticity remains for female CHC expression across temperatures, but not for males: weaker selection erodes genetic variation more slowly.

The validity of this explanation also depends on the relative strength of sexual selection acting on the sexes, and it is unclear exactly how sexual selection might affect female CHC profiles. There is considerable evidence to suggest that females will be under selection to produce signalling CHCs. For example, there is evidence for male mate choice in *D. melanogaster* (Byrne and Rice 2006), female receptivity signals (Marcillac and Ferveur 2004; Ferveur and Cobb 2010), and a role for CHCs in species recognition (Higgie et al. 2000; Mas and Jallon 2005). However, this selection is probably not as strong as that caused by female mate choice on male CHCs, and generally sexual selection is stronger on males than females (Shuster and Wade 2003). Indeed, Sharma et al. (2012b) found that the effect of sexual selection was much weaker on female *D. simulans* CHC profiles than its influence on

male CHCs. In another study, Chenoweth and Blows (2005) found that whilst female preference caused strong directional selection on male CHCs in *D. serrata*, male mate-choice produced stabilising selection on female CHC profiles. These kinds of sex-specific patterns of selection could give rise to the sexual dimorphism in GEIs and CHC plasticity found across temperatures.

Dietary effects and condition dependence

While there is substantial evidence for dietary effects on CHC profiles, the nature of these effects are not intuitive, and the picture produced by studies which have examined this in *Drosophila* is less clear than that from the temperature studies discussed above. Given the evidence for condition dependence of CHC expression and that CHCs appear to be costly to produce (Blows 2002; Ferveur 2005), it is perhaps unsurprising that the resources available to an individual will affect CHC production. Additionally, while many CHCs are synthesised by insects, dietary hydrocarbons can nonetheless be directly incorporated into CHCs (Blomquist 2010), suggesting another avenue for diet effects.

Consistent with these general expectations, adult male *D. serrata* reared on a diet containing yeast have vastly different CHC profiles to males reared on low quality, no yeast diets (Gosden and Chenoweth 2011). This study also demonstrated that sexually selected aspects of male CHC profile exhibited higher levels of condition dependence than non-sexually selected aspects. That is, the low quality diet had the greatest impact on the sexual CHC components. If this condition dependence evolved as a result of a trade-off between investment in CHCs which help to attract mates and investment in CHCs which confer protection from desiccation and environmental stress, then we would expect to find genetic variation underlying this condition dependence. In other words, a dietary GEI could be seen. By using a paternal half-sibling breeding design, Gosden and Chenoweth (2011) estimated the additive genetic variation underlying the CHC reaction norms across diets and found very little in sexually selected aspects of CHC profile. The lack of GEI indicates a constraint in the evolution of condition dependence across diets. From this data, it appears more likely that female mate preferences have evolved to focus on traits which are useful indicators of some aspect of male condition (Johnstone et al. 2009).

In contrast to this, manipulation of larval and adult diet in *D. simulans* found evidence of genetic variation in both male and female reaction norms for CHC expression

(Ingleby et al. in review). Whether or not these results can be interpreted as condition dependence is unclear, but it does suggest that there is genetic variation underlying resource allocation patterns across diets in this species, or at least there is in the diets employed in this study. These effects were sex-specific, with clear sex differences in overall CHC expression and in patterns of plasticity across diets. There was particularly high genetic variance in plasticity across diets in a principal component vector of CHC expression which describes overall investment in CHCs, and this vector has previously been identified as being subject to sexual selection in *D. simulans* (Chapter 3). In males, this GEI means that there is the potential for the maintenance of genetic variation in a sexual trait across diets. This could even occur in the face of strong and consistent female preferences for particular CHC profiles and responses to selection could differ between diets.

The discrepancy between these studies on *D. simulans* and *D. serrata* could simply be attributed to species differences. Perhaps more interestingly, the different results could be explained by differences in the dietary manipulations employed, in terms of the types of diet used and the relative diet qualities, or in terms of the difference between manipulating adult diet only or manipulating larval and adult diet. This could of course be dependent on the domestication process and the use of more or less "natural" diets and their constancy in laboratory populations. Any generality to dietary GEI effects across species and across manipulations of the physical environmental is an area for future research.

Biotic environments

Biotic environmental variation has the potential to have a strong impact on sexual selection, since biotic factors can vary much more widely than abiotic factors, particularly across relatively short timescales. Furthermore, when the biotic environment in question is composed of other individuals – parasites, predators, interspecific competitors or conspecifics (see section below on the social environment) – then the environment itself will be subject to selection and is likely to evolve, and even coevolve, with the focal individual. This is likely to be very important in sexual selection, which by definition depends on sexual competitors. This creates a complex picture involving interacting evolutionary dynamics, but this complexity is relevant and important to a full understanding of sexual selection on CHCs.

The importance of biotic environmental variation in sexual selection has been highlighted in a variety of taxa. For example, an ongoing project with Hawaiian populations of the field cricket, *Teleogryllus oceanicus*, reported that parasite prevalence in the environment is responsible for the extremely rapid evolutionary loss of the male acoustic signal which serves to attract females (and parasites) (Zuk et al. 2006). Predation pressure on guppies, *Poecilia reticulata*, affects the colouration of sexual signals (Endler 1991), while in the gray tree frog, *Hyla versicolor*, male acoustic calls are supposed to signal offspring viability benefits to the females, but these benefits are subject to GEI across different tadpole densities (Welch 2003). GEI across larval densities has also been found to affect sperm length in *D. melanogaster* (Morrow et al. 2008).

However, with regards to CHCs in *Drosophila*, relatively little research has been undertaken on the effect of biotic factors. Biotic environmental variation might be more difficult to experimentally manipulate than abiotic variation, but some elements of the biotic environment could potentially have a strong effect on *Drosophila* CHCs. For example, as a costly trait to produce, competition for resources could be significant, as suggested by the evidence for dietary impacts on CHC expression described above. It is also possible that parasite prevalence in the environment might be important, given that CHCs can form a protective barrier on the cuticle, which helps to prevent infections (Golębiowski et al. 2011).

Social environment, however, will almost certainly be an important determinant of *Drosophila* CHC profile, since chemical communication is an integral part of insect social behaviour (Wyatt 2003), and some research already supports this view. We use social environment here to describe specific types of biotic environmental variation which involve interactions between the focal individual and other individuals of the same species. This is a particularly interesting and useful distinction within the context of sexual selection, since sexual selection must involve social interaction to some extent, through female assessment and choice of potential mates, male sexual displays to females, male-male competition for mates, and mating itself. *Drosophila* CHCs are probably involved in each of these sexually-selected social-interactions, as studies have identified a role for CHCs in signalling male attractiveness (e.g. Savarit et al. 1999; Grillet et al. 2006), female receptivity (e.g. Marcillac and Ferveur 2004) and levels of male competition (e.g. Savarit et al. 1999). Furthermore, Billeter et al. (2009) used transgenic *D. melanogaster* which did not produce CHCs to demonstrate that CHCs play are vital in social communication and recognition.

Social environment is therefore bound to have an important influence on the evolution of CHCs through sexual selection. Aspects of these social interactions and their evolutionary significance have been described by the large body of research on indirect genetic effects (IGEs), which have been examined in-depth elsewhere (see Wolf et al. 1998; Wolf et al. 1999; Miller and Moore 2007). IGEs describe interactions between individuals, and the consequences of these social interactions for trait expression and evolution. Studies on *D. serrata* and *D. melanogaster*, in particular, examined IGEs and CHCs and the potential for GEI across social environments.

IGEs on D. serrata CHC expression

In *D. serrata*, there is evidence that males alter CHC expression when females or their cues are in the environment (Petfield et al. 2005). This experiment also demonstrated the extremely plastic nature of *Drosophila* CHC expression, as male CHC profiles were altered within minutes of encountering females. This effect was attributed to males using visual and olfactory cues to detect females in the environment. In fact, the evidence suggests that males adjusted their CHC profile in response to assessment of females during courtship, since the vector showing the male IGE was very similar to a vector describing genetic variance in female CHCs. Approximately 20% of the variation in the male response was explained by the variance in the corresponding female vector. Based on the biological significance of this female vector (overall investment in CHCs), it appeared that, during courtship, males might be assessing female condition or size and respond by changing their CHC profile. This has the potential to increase male fitness returns if altered profiles mean more siring success.

Chenoweth et al. (2010) demonstrated the potential of IGE-based plasticity in male *D. serrata* CHCs to evolve, which suggests that genetic variation for IGE interactions must exist, although they did not explicitly test for GEI across social environments. They used experimental evolution to create populations of *D. serrata* which were under sexual selection, natural selection, or a combination of both, and assayed male CHCs from each population in both solitary and social environments. Sexual selection, but not natural selection, was found to drive the evolution of the IGE interaction coefficient, *psi*, for certain aspects of the male CHC profile, particularly methyl-branched alkanes. These alkanes have

also been implicated in sexual selection and IGE in other studies with *D. serrata* (Chenoweth and Blows 2005; Petfield et al. 2005; Delcourt et al. 2010).

Chenoweth et al. (2010) explain the evolutionary change in *psi* as a response to the evolution of other CHC components. If this CHC 'background' differs between naturally and sexually selected populations, as it probably would (see above), then male flies attempting to produce an attractive CHC profile across social environments will need to evolve different responses to do so. This explanation downplays the potential for changes in female CHC cues to drive the evolution of the male CHC profile, which is justified in this particular case by the fact that female CHCs only evolved through natural selection in the experimental populations. However, in general, female CHC signals of receptivity or condition, as well as female preference for male CHCs, will clearly be important to examine across social environments, and this has so far been neglected.

Circadian clocks and the social environment in D. melanogaster CHC expression

Drosophila species have a daily cycle which concentrates courtship and mating behaviour into specific times of the day, often the morning, or the beginning of the light cycle in laboratory populations. Recent work on *D. melanogaster* has brought together this temporal aspect of mating behaviour with manipulation of the social environment in order to examine effects on male CHC expression.

Through manipulation of the light cycle, Krupp et al. (2008) showed that male CHC production varied diurnally and responded to light cues. They found that this circadian rhythm is controlled by a peripheral molecular clock – distinct from the main clock in the central nervous system of the fly – located in the oenocytes, where CHCs are produced. The light environment appears to act as a stimulus setting this circadian rhythm, which influences the expression of three clock genes (*period*, *timeless* and *Clock*) in the oenocytes, as well as the activity of the enzyme *desaturase1*, which is necessary for CHC production.

In addition, male CHC expression responded to social cues. By comparing the CHC profiles of males from groups consisting solely of wild-type *D. melanogaster* to those of males from groups consisting of a mixture of wild-type and arrhythmic clock mutants (with a non-functional *period* gene), they found that CHC expression varied between social environments (Krupp et al. 2008). In fact, approximately a third of the overall variation in CHC expression was attributed to the social environment, suggesting a remarkably strong

IGE. The change in CHC expression between environments appeared to result from altered expression of both the clock genes and *desaturase1*. Furthermore, there was some evidence that particular CHCs involved in sexual communication were the most strongly influenced by social environment (e.g. 7-tricosene; Savarit et al. 1999; Grillet et al. 2006). Consistent with this, further experiments demonstrated that changes in CHC expression were associated with changes in mating behaviour. Wild-type males which experienced a mixed social environment mated more frequently than those from a purely wild-type social group.

Kent et al. (2008) found complex patterns of genetic variation underlying these environmental effects on the CHC expression of male *D. melanogaster*. Not only was the molecular clock controlling CHC production influenced by the light environment, but the genetic variation underlying the response to light cues revealed GEI for CHCs across light environments. In addition, they found genetic variation for the IGEs identified previously, in that the response to the social environment depended on male genotype. In fact, far from having a genetically deterministic molecular clock controlling CHC production, CHC profile in *D. melanogaster* appears to be highly plastic, and influenced by a complex combination of interacting genetic, temporal and social effects. As suggested above, CHC expression is not simple.

Whilst existing research on GEI in CHC expression across social environments is scarce, these studies in *D. serrata* and *D. melanogaster* have highlighted the potential for social environment to strongly influence the evolution of CHC expression and sexual signalling interactions. It is clear that without considering social environments, our understanding of the evolutionary responses of CHC profile to sexual selection is incomplete.

Part of the difficulty of studying the effects of the social environment on *Drosophila* CHCs will be distinguishing between active and passive changes in CHC profile. That is, are changes in CHC profile caused by altering CHC expression in the focal individual, or by CHCs transferred by contact between individuals? This distinction is likely to be an interesting subject for future research, and will probably require mechanistic studies of gene expression, or the use of *Drosophila* mutants for CHC production. Another future research direction will be to develop our understanding of female CHC expression. Existing studies of CHC expression across social environments have focussed solely on male CHCs, but female

CHCs also have an important function in sexual interactions (Savarit et al. 1999; Marcillac and Ferveur 2004) and female CHC profile has been shown to vary across some abiotic environments (see above). Nonetheless, nothing is known about the role of the social environment on females. Sexual selection across social environments is complex and a full understanding of it will ultimately require integration of male and female phenotypes and the signalling interactions between them.

Consequences of GEI and environmental variation in *Drosophila* CHCs and directions for future research

Clearly, the influence of the physical environment on *Drosophila* CHC expression is better understood than that of the biotic environment. However, whilst GEI in CHCs across aspects of the abiotic environment have been identified in some studies, other studies have failed to find any evidence for GEI. There is obviously substantial variation in the effect of abiotic factors on CHCs, and further research will help to determine species-specific effects, as well as the effects of different types and strengths of environmental variation. Whilst examination of every aspect of the abiotic environment for every species is unrealistic, it will be possible to build on the existing research on CHC function and natural and sexual selection on CHCs in order to focus on interesting questions about GEI across abiotic environments. To date, studies across abiotic environments have mostly been identifying GEIs in *Drosophila* CHC expression, and have therefore laid the groundwork for future research to focus on the consequences of these GEI for the operation of sexual selection across heterogeneous environments. With respect to biotic environments, this groundwork is still very much needed in order to assess how widespread GEI are, and how strong GEI effects are, before research can move on to consider the roles such GEI might play in sexual selection.

For both abiotic and biotic environments, empirical work evaluating the evolutionary consequences of environmental variation and GEI lags far behind the extensive theoretical examinations of the issue (Via and Lande 1985; Via and Lande 1987; Wolf et al. 1998; Hoffmann and Merilä 1999; Wolf et al. 1999; Kokko and Heubel 2008; Higginson and Reader 2009). The evolutionary significance of GEI in sexual traits such as CHCs could have profound implications for sexual selection, and we outline some of these here.

Consistent with research on GEI in evolutionary genetics more generally (Via and Lande 1987), theory which has focussed explicitly on sexual selection has shown the potential for GEIs in sexual trait expression to help to maintain genetic variation (Kokko and Heubel 2008). Mechanisms for the maintenance of genetic variation in sexual traits are of particular interest because of the long standing issue of the lek paradox in sexual selection. The paradox states that strong directional selection from female choice, which has clearly operated on many (exaggerated) sexual traits, should deplete the genetic variance in these characters. In many species females only obtain genes from their mates and therefore genetic variation is needed for costly female choice to be maintained. Hence the paradox, costly female choice for genetic benefits requires genetic variation and yet erodes it (Kirkpatrick and Ryan 1991). The maintenance of genetic variation with GEI has been tested in some species (e.g. *Achroia grisella*, Jia et al. 2000), but no studies have explicitly addressed this for *Drosophila* CHCs. There is some indirect evidence for the maintenance of genetic variation from Ingleby et al. (in review), which identified GEI for CHCs across diet and temperature environments, but largely speaking this is a question that would benefit from more empirical attention.

Another important prediction from theory is that GEI in sexual traits could affect the reliability of these traits to act as honest sexual signals (Higginson and Reader 2009). If sexual signals, such as CHCs, do not reliably indicate mate quality across heterogeneous environments, then the benefits of mating preferences could be undermined. Again, in terms of *Drosophila* CHCs, empirical research mainly touches on this idea by implicitly demonstrating the potential for signal unreliability. In a more direct assessment of the theory, Ingleby et al. (in review) examined the heritability of sexually-selected components of male *D. simulans* CHC profile across diet and temperature environments, and related this to the heritability of male attractiveness across the same environments. This is pertinent as male attractiveness seems to be the only (indirect) benefits of mate choice in this species (Hosken et al. 2008; Taylor et al. 2008; Sharma et al. 2012c). While there was evidence that GEI caused some aspects of male CHC expression to be unreliable indicators of heritable aspects of male attractiveness across environments, as predicted by theory, there was no GEI for overall male attractiveness. So sexual selection operated consistently across environments, with the same genotypes always more attractive to females in spite of the GEI for some aspects of attractiveness, which made them unreliable signals. To our

knowledge, this is the only study that has explicitly tests the reliability of CHCs as sexual signals across environments. Given the evidence for GEIs in CHC expression, and the extensive research documenting the significant role of CHCs in chemical communication between the sexes during courtship and mating, this is clearly an important direction for further research.

A lack of reliability in sexual signals could also affect the genetic covariance between male signal and female preference across environments, and this covariance is central to many models of sexual selection (e.g. Lande 1981; Kirkpatrick 1987; Wade 1987), and so GEIs in sexual signals could fundamentally affect the operation of sexual selection. Equally, GEIs in female preferences could have the same effect, although if reaction norms for the sexual signal match the reaction norms for female preference for the signal, then plasticity in sexual traits across environments could be advantageous (Greenfield and Rodríguez 2004). With so little known about GEI in female preferences, it is impossible to empirically evaluate these ideas.

Delcourt et al. (2010) analysed female *D. serrata* preference functions for male CHCs across diets of different quality. Surprisingly, they found very little evidence for a condition dependent component to the preference functions, although they did not assess other aspects of preference, such as female choosiness. It was clear that the combination of male CHCs preferred by females was independent of the resources available to them, and there was no evidence for GEI. In fact, female preferences appeared to be strongly genetically determined. This genetic basis of female preference for male CHCs has also been found in *D. bunnada* (McGuigan et al. 2008) and *D. simulans* (Ingleby et al. in review), and is consistent with evidence for a genetic component of both preference functions and choosiness in *D. melanogaster* (Narraway et al. 2010), although this study did not explicitly associate female preference with a particular male sexual trait. Narraway et al. (2010) also identifed GEIs across temperature environments for both female mate preference and female choosiness. However, the results from a recent experiment examining female preference functions and choosiness for male CHCs in *D. simulans* across temperature environments found a slightly different picture (Ingleby et al. in review). Here preference and choosiness had strong genetic components, and there was a GEI for female preference for male CHCs. This indicates that female preferences changed across environments and this environmental effect differed between genotypes. Female choosiness, on the other

hand, did not exhibit a GEI, but there was an overall effect of temperature, in that females at higher temperatures were generally quicker to mate than those at lower temperatures.

These studies provide some insight into GEIs and female preference, but the overall neglect of the female perspective hinders a detailed understanding of the evolutionary consequences of GEI in sexual selection. There is considerable evidence for female choice of male CHCs, and for environmental and GEI components of CHC expression, but we know relatively little about how female preference for CHCs changes across environments.

Finally, it is clear from functional studies of *Drosophila* CHCs that females do not only receive these chemical signals, but produce CHC cues that males might use to assess females. This highlights the profound importance of studying female mating behaviour across environments, and, in particular, across social environments. The female perspective has been overlooked with respect to social environmental variation, and this is a clear direction for future research, given that both males and females contribute actively to chemical communication during courtship and mating interactions. However, it also true that to study these complex interacting phenotypes, particularly within the quantitative genetic framework necessary to examine GEI, represents a considerable empirical challenge, but it is nonetheless one that future research in this area will need to overcome.

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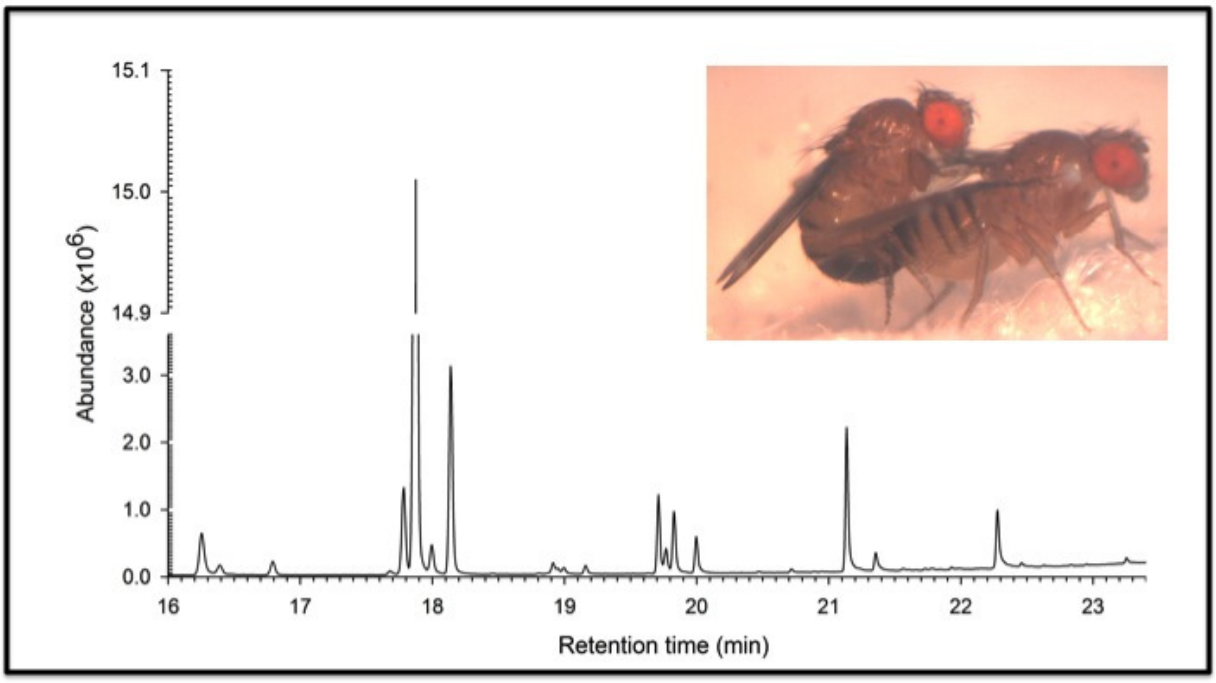

Figure A A gas chromatograph of the typical cuticular hydrocarbon profile of a male *Drosophila simulans* (the insert is a pair of these flies in copula). The x-axis shows the retention time and the y-axis the relative abundance of each CHC component. There are 25 repeatably detectable CHCs in this species, which contrasts with some *Drosophila,* which can have as few as 7 peaks.

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