The genetic limits to trait evolution for a suite of sexually selected male cuticular hydrocarbons in *Drosophila serrata*

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Abstract

Directional selection is prevalent in nature yet phenotypes tend to remain relatively constant, suggesting a limit to trait evolution. The genetic basis of evolutionary limits in unmanipulated populations, however, is generally not known. Given widespread pleiotropy, opposing selection on a focal trait may arise from the effects of the underlying alleles on other fitness components, generating net stabilizing selection on trait genetic variance and thus limiting evolution. Here, I look for the signature of stabilizing selection for a suite of cuticular hydrocarbons (CHCs) in *Drosophila serrata*. Despite strong directional sexual selection on CHCs, genetic variance differed between high and low fitness individuals and was greater among the low fitness males for seven of eight CHCs. Univariate tests of a difference in genetic variance were non-significant but have low power. My results implicate stabilizing selection, arising through pleiotropy, in generating a genetic limit to the evolution of CHCs in this species.

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Chapter 1

1.0 Sexual Selection

Sexual selection is a process that occurs when a particular trait varies among individuals within a population and when the association between an individual's value of the trait and their reproductive fitness is non random. Unlike other forms of selection, it does not arise from a struggle for survival but from a struggle for reproductive success. Sexual selection was first recognized by Darwin (1859) in his attempt to reconcile how conspicuous male traits, which would otherwise reduce survival, can evolve and be maintained within populations, and it was one of his more controversial ideas (Andersson 1994).

The opportunity for sexual selection arises from variance among individuals in their reproductive success, and this variance can arise from both pre- and post-copulatory processes (i.e. those taking place before or after mating). Most often, it is the case that males compete for access to females, whilst females choose amongst competing males. Although these roles are reversed in some species (Bonduriansky 2001), I focus on the classic scenario of male competition and female choice. Pre-copulatory processes may involve searching for or otherwise attracting a

mate to the male's location, potentially generating indirect (e.g., scramble) competition among males in their attempts to do so. Pre-copulatary processes can also include direct competition among males for access to females, and courting or otherwise coercing a female into mating. Post-copulatory processes may include competition among sperm from different males for fertilization of the ova (Parker 1970), differential sperm use by females (cryptic female choice), and mate guarding by males after mating.

Through these pre- and post-copulatory processes, two forms of sexual selection arise. The first, termed intra-sexual selection, occurs when members of one sex compete, either directly or indirectly, for access to the members of the other sex. This form of sexual selection often results in the evolution of traits such male weaponry used in pre-copulatory competitions for access to mates, or accessory gland proteins (Acps) used in post-copulatory sperm competitions. Weaponry can include traits such as tusks seen in the African King cricket, *Libanasidus vittatus*, or antlers of the red deer, *Cervus elaphus* (Bateman 2000; Clutton-Brock et al. 1979), whereas proteins such as Acp29AB, which plays a role in sperm storage, mediate competitive interactions among the sperm of different males in *Drosophila melanogaster* (Wong et al. 2008).

Intersexual selection, the second form of sexual selection, occurs when individuals of one sex (usually females) choose amongst members of the opposite sex (usually males), expressing preferences for particular values of traits in their potential mate. These preferences are often based on sexual displays or ornaments, with intersexual selection directing the evolution of often striking and exaggerated sexual displays. These sexual displays can range from the bright

plumage of the house finch, *Carpodacus mexicanus* (Hill 1990), to elaborate courtship calls of field crickets, *Gryllus bimaculatus* (Hedrick 1986), and pheromonal displays of the fruit-fly, *Drosophila serrata* (Howard et al. 2003). The elaboration of male sexual displays through female preferences has generated much of the historical and current interest in sexual selection research, and their evolution (or lack thereof) is the focus of this thesis.

Though Darwin questioned the importance of sexual selection (Darwin 1859), subsequent research has underscored its key role in generating much of the diversity we see around us. For example, sexual selection has been shown to contribute to phenotypic divergence among populations (Price 1998), it plays a central role in the evolution of sexual dimorphism (Lande 1980), and ultimately may be a key mechanism of speciation (Coyne & Orr 2004). Conceptual models propose that sexual selection may aid natural selection in the purging of deleterious mutations from populations, increasing population mean fitness, promoting adaptation, and preventing mutational meltdown and extinction, although empirical evidence for this is limited (Whitlock & Agrawal 2009). Additionally, a recent review indicates that sexual selection may often be stronger than forms of selection acting on components of non-sexual fitness (Kingsolver et al. 2001).

The evolution of male sexual displays and female preferences

The evolution of male sexual displays is now widely recognized to be driven through female preferences, although a variety of selective processes may underlie preference evolution, with

their relative importance a subject of much debate (Andersson 1996, 1994; Andersson & Simmons 2006). These selective processes that shape male sexual displays can be divided broadly into two categories depending on the cost of female preference.

When females do not incur a cost of preference, male sexual displays may evolve through a statistical co-evolutionary process called Fisherian runaway selection (Fisher 1930). This process can occur when the preference for a display trait arises in a population for some reason (including by chance), and leads to higher reproductive success of males who express this trait. Females carrying the preference mate with males carrying the trait, and their offspring thus carry alleles for both the trait and preference for it. Sexual selection for the male display, therefore, also serves to increase the frequency of the preference due to the build up of linkage disequilibrium between the underlying alleles. The result is a self reinforcing co-evolutionary process that drives the exaggeration of both the male sexual display and the female preference for it. Early models demonstrate that this process can result in the elaboration of costly sexual displays, with preference and displays reaching a stable line of equilibria (Kirkpatrick 1982; Lande 1981). However, if females incur any cost of expressing their preference (i.e. direct selection on preference), the preference is rapidly eliminated from the population and male sexual displays collapse to their naturally selected optima (Arnqvist & Rowe 2005).

When female preferences are costly, the evolution of male sexual displays requires that such costs be overcome through either direct or indirect benefits of the preference. If males provide direct benefits to their mate (e.g., parental care, predator protection, nuptial gifts, or the absence

of sexually-transmitted diseases), preferences may evolve in response, thereby driving the exaggeration of male sexual displays that reflect the quality of the direct benefits provided. In order to gain proportionately more resources, females prefer the most elaborate displays (Møller & Jennions 2001), generating directional selection on male sexual displays and their subsequent exaggeration. Direct benefits to females are not limited to those that are resource based, but can also include benefits gained by minimizing the cost of sexual conflict, which arises through differences in the reproductive interests of the sexes (Arnqvist & Rowe 2005). Direct benefits arising in this way may also generate directional selection on male sexual displays and thus drive their exaggeration, as described above.

Male sexual displays may also indicate a male's ability to provide indirect benefits or 'good genes' to the female they mate with. Under a 'good genes' model, when elaborate displays are costly to produce (for example, if their expression is condition dependent), they act as honest indicators of overall genetic quality because only high quality males are able to bear the costs of the most elaborate displays (Houle & Kondrashov 2002; Iwasa & Pomiankowski 1999; Rowe & Houle 2007). By mating with these males, females procure high quality genes for their offspring (Andersson & Simmons 2006; Mead & Arnold 2004). There is currently much debate surrounding whether the magnitude of indirect benefits of mate preferences are sufficient to overcome their costs, due in part to conflicting results from different theoretical models characterizing this process (Houle & Kondrashov 2002; Kirkpatrick & Barton 1997; Kirkpatrick 1996). Before the importance of 'good genes' indirect benefits can be determined, better

empirical estimates of the direct and indirect costs and benefits of female preferences are needed (Arnqvist & Rowe 2005).

Finally, male sexual displays can also evolve through female sensory biases (Fuller, Houle, & Travis 2005). In this case, the exaggeration of sexual displays occurs as a by-product of female preferences which have been shaped by natural selection for other reasons, such as foraging efficiency (Andersson & Simmons 2006; Endler & Basolo 1998; Fuller et al. 2005). Males evolving traits which exploit this bias become favoured by females, and exaggeration of the sexual display may follow. Female preferences for carotenoid based male sexual displays in guppies, *Poecilia reticulata*, are an example of how a sensory bias has resulted in the genetic association between a male sexual display and female preference, and subsequently guided the evolution of these displays (Grether et al. 2005; Rodd et al. 2002).

As outlined above, a diverse set of mechanisms may underlie the evolution of female mate preferences, and these various scenarios are not mutually exclusive (Arnqvist & Rowe 2005). Nevertheless, whatever their origin, mate preferences are common (Andersson 1994) and have been shown to generate strong and persistent directional sexual selection (Kingsolver et al. 2001), ultimately resulting in the vast diversity of striking sexual displays observed in contemporary populations. While there has been much empirical and theoretical attention given to understanding the origins and maintenance of female mate preferences and their role in the exaggeration of male sexual displays, what eventually limits this exaggeration is poorly understood. It is this topic on which my thesis research is focused.

1.1 Limits to trait evolution

Observations from natural populations indicate that directional selection on quantitative traits is both common and frequently strong (Endler 1986; Hereford, Hansen, & Houle 2004; Hoekstra et al. 2001; Kingsolver et al. 2001), yet in the absence of environmental changes this often does not appear to generate a sustained evolutionary response (Kingsolver & Diamond 2011; Svensson & Gosden 2007). Instead, phenotypes in natural populations tend to remain relatively stationary in value over various time-scales, with the evolution of these traits being best described by a model of stabilizing selection around a slowly moving optimum (Estes & Arnold 2007). The inability of directional selection to generate a sustained evolutionary response suggests a limit to trait evolution.

Sexual selection is an ideal context in which to study the genetic limits to trait evolution. Empirical investigations in nature and in the laboratory tend to find strong and persistent directional sexual selection on male display traits (Delcourt, Blows, & Rundle 2010; Kingsolver et al. 2001; Rundle, Chenoweth, & Blows 2009). Significant heritable variation also appears to exist for most sexual displays (Jennions, Moller, & Petrie 2001; Kruuk et al. 2002), yet these traits rarely show continued exaggeration in contemporary populations (Svensson & Gosden 2007), indicating that they have reached an evolutionary limit. The genetic basis of this limit is a largely unresolved issue and could arise in a number of ways, as outlined below.

Genetic Constraints

A lack of genetic variance could prevent quantitative traits, including sexual displays, from evolving under directional selection. Following Fisher's fundamental theorem, quantitative traits closely linked to fitness should exhibit low levels of additive genetic variance due to persistent directional selection (Fisher 1930). However, substantial research addressing the amount of genetic variance in components of fitness has found that traits that are closely linked to fitness often exhibit the highest levels of genetic variance (Burt 1995; Fowler et al. 1997; Houle 1992), including sexual display traits in particular (Jennions et al. 2001). Various explanations for this observation, including the 'genic capture' hypothesis, have been proposed (Rowe & Houle 1996), although compelling empirical evidence for these hypotheses is limited (Johnson & Barton 2005; McGuigan & Blows 2009).

Multivariate constraints arising from the genetic co-variance structure among a set of traits may restrict or eliminate genetic variance in the direction of selection (Barton & Partridge 2000; Blows & Hoffmann 2005), but these appear insufficient to generate absolute constraints (Beldade, Koops, & Brakefield 2002; Conner, Franks, & Stewart 2003; Hine, McGuigan, & Blows 2011), at least for bivariate trait combinations. Furthermore, genetic co-variances among traits can also facilitate evolution if they increase genetic variance in the direction of selection, and it seems that genetic architecture may facilitate evolution as often as it constrains it (Agrawal & Stinchcombe 2009). In general, there is little evidence to suggest that the genetic limits to trait evolution are commonly generated by a simple lack of genetic variance in either univariate or multivariate trait space.

Overdominance

Overdominance at loci underlying traits that are subject to directional selection may also cause an evolutionary limit. Overdominance, also known as heterozygote superiority, occurs when heterozygous individuals have a higher fitness than either homozygote (Hartl & Clark 2007). When traits are at equilibrium under overdominance, all genetic variance will arise from dominance variance and, consequently, there is no additive component of genetic variance on which selection can act. The presence of additive genetic variance in traits which have reached their evolutionary limit, which is often observed in artificial selection experiments (Enfield, 1980; Hine et al., 2011; Reeve & Robertson, 1953), is inconsistent with overdominance generating these limits. Furthermore, if overdominance is a significant cause of selective limits, when a population at its limit for a particular trait is inbred, the mean trait value for *every* inbred line must rapidly decline to a value below the founding population's outbred mean. This is because inbreeding increases the frequency of the less fit homozygous genotypes until fixation of either homozygote is reached; the trait value of an inbred line, therefore, cannot equal or surpass the mean value of the founding population in which both alleles were segregating. This pattern is generally not observed in selective breeding programs where populations have undergone inbreeding after reaching an evolutionary limit, also inconsistent with overdominance (Eisen 1980; Falconer 1971). Currently there is, therefore, little empirical evidence to suggest that overdominance is a common feature of selection limits, at least for artificially selected populations (Falconer & Mackay 1996; Lynch & Walsh 1998).

Opposing selection

Opposing selection arising through other fitness components has also been proposed as a potential limit to trait evolution. With respect to sexual displays, Fisher's (1930) original model for their runaway exaggeration suggested that the process would eventually be halted by opposing natural selection. Such opposing selection may arise from a direct effect of the trait(s) on non-sexual fitness, for example if an exaggerated sexual display increases the risk of predation or parasitism (Kotiaho 2001). In guppies (*Poecilia reticulata*), the sexually selected bright body patterns of males have been shown to directly increase mortality risk when paired with less brightly coloured conspecific males during encounters with a natural predator (Godin & McDonough 2003), providing evidence for such opposing selection. While it is often observed that predators exploit male sexual displays, such as conspicuous plumage, mate calling, or pheromones, surprisingly few empirical studies have evaluated the direct fitness costs associated with these sexual displays (Godin & McDonough 2003; Hoefler, Persons, & Rypstra 2008; Kotiaho 2001).

Opposing selection may arise not only from the direct effects of sexual displays on non-sexual fitness, but also from the pleiotropic effects of the underlying alleles on other traits that are also under selection. The idea is that, when populations are well adapted to their environment, changes in allele frequencies in response to directional selection on a particular trait will alter other traits due to pleiotropy, displacing these latter traits from their fitness optima and thereby generating opposing selection. For example, in the field cricket, *Teleogryllus commodus*, increased early life mate calling has been shown to have a pleiotropic effect on non-sexual

fitness through a decreased lifespan (Hunt et al. 2004). Additionally, male *Drosophila melanogaster* that have a high sexual competitive ability have been shown to also have decreased immune function (McKean & Nunney 2007).

More generally, two observations suggest that pleiotropy is widespread across the genome, implying that it may be an important mechanism in generating evolutionary limits. First, strong genetic co-variances are often observed among suites of traits, causing the multivariate distribution of genetic variance to be confined to only a small subset of independent genetic dimensions (Kirkpatrick 2009). Second, estimates of the per trait mutation rate indicate that is about one tenth of the genome-wide mutation rate, suggesting that organisms are comprised of only a small number of genetically independent traits (Johnson & Barton 2005; Walsh & Blows 2009). The consequences of such widespread pleiotropy with respect to opposing selection may be difficult to detect in a phenotypic analysis because it is challenging to identify and include all of the relevant traits (McGuigan et al. 2011). As outlined in the following section, detecting the consequences of pleiotropy in a genetic analysis may be more straightforward.

The potential importance of opposing selection in limiting trait evolution is suggested from various artificial selection experiments in which a plateau in trait response is reached despite the presence of additive genetic variance in the traits under selection (Falconer 1955; Hine et al. 2011; Roberts 1966; Yoo 1980). Opposing selection is often directly inferred because, when artificial selection is relaxed, trait values rapidly revert towards their initial values. Classic examples include Yoo's (1980) selection experiment in *Drosophila melanogaster,* where the

response to selection for increased abdominal bristle number eventually plateaued and rapidly returned towards its original value once selection was relaxed. A more recent example is provided by Hine et al. (2011) in which *Drosophila serrata* males were artificially selected for increased attractiveness of their cuticular hydrocarbon pheromones (CHCs). The traits responded, indicating the presence of genetic variance in the ancestral (i.e. unmanipulated) population, yet this response plateaued after about seven generations. This plateau was not the result of a lack of genetic variance: genetic variance in this trait combination actually increased during artificial selection. When selection was relaxed, however, CHCs reverted towards their original trait values, demonstrating that natural selection was opposing their further exaggeration.

While artificial selection experiments suggest the potential importance of opposing selection in generating evolutionary limits, these limits are the result of a manipulation imposed through the selection regime. The importance of opposing selection in generating evolutionary limits in unmanipulated populations is not known, however, and has proven challenging to infer (Merilä 2009; Merilä, Sheldon, & Kruuk 2001). My goal is to ascertain how the evolutionary limits to sexual display trait (CHC) evolution arise in an unmanipulated population of *D. serrata*, and in particular to infer the importance of opposing selection arising through pleiotropy in generating these limits.

1.3 A pleiotropic model of mutation-selection balance

If an evolutionary limit to sexual display trait exaggeration in an unmanipulated population is caused by opposing selection arising from the pleiotropic effects of the underlying alleles of sexual displays on other traits, the genetic architecture of these traits should be characteristic of a pleiotropic model of mutation-selection balance (Barton 1990; Keightley & Hill 1988). Although sexual displays are the target of directional phenotypic sexual selection, the underlying genetic variance will be subject to stabilizing selection through its association with net fitness. Under the Hill-Keightley model of mutation-selection balance, newly arising mutations affecting a trait of interest also affect many other traits, and although these other traits are unmeasured, mutational effects on them are subsumed into a pleiotropic effect on fitness. At mutation-selection balance, although novel mutations may increase or decrease the value of the measured trait, their pleiotropic effect on fitness will almost always be deleterious. Individuals with more extreme values of the measured trait will tend to carry more mutations, each with pleiotropic fitness costs, thereby generating the appearance of stabilizing selection on the measured trait (Keightley & Hill 1990). Stabilizing selection is apparent because it arises through the pleiotropic effects of segregating alleles on unmeasured traits and, as such, is best detected in a genetic as opposed to a phenotypic analysis.

Under stabilizing selection, individuals with intermediate values of a measured trait will have the highest fitness, and fitness will decline as individuals deviate from this optimum in either direction. A group of low fitness males will, therefore, contain individuals with both high and low phenotypic values of the measured trait, and genetic variance of the trait will be greater

among these individuals as compared to among the high fitness individuals (Fig. 1) (McGuigan et al. 2011). This unique prediction of the Hill-Keightley model of mutation-selection balance provides a straightforward and potentially powerful test for the presence of stabilizing selection on the genetic variance underlying a trait or suite of traits, providing direct insight into the importance of pleiotropy in imposing genetic limits to trait evolution in unmanipulated populations.

Testing for this signature of stabilizing selection arising through pleiotropy can be undertaken via a classic breeding design or by applying the animal model (Kruuk 2004; Lynch & Walsh 1998) in a natural populations with a known pedigree (McGuigan et al. 2011). A strength of this method is that it does not require detailed estimates of lifetime fitness, which are often empirically difficult to obtain, but rather only requires a broad classification of individuals into high vs. low fitness groups. One attempt has been made to apply this technique in *Drosophila bunnanda*, demonstrating asymmetries in genetic variance among high and low fitness males consistent with stabilizing selection on a suite of male sexual displays (cuticular hydrocarbons) (McGuigan & Blows 2009), although a direct test of the significance of this difference was not performed.

Using this framework, I examine differences in genetic variance among high and low fitness males in a different species, *Drosophila serrata*, in order to ascertain whether opposing natural selection arising through pleiotropy limits the evolution of male sexual displays in this species, providing additional insight into the generality of these limits in unmanipulated populations. I

also extend the previous approach to provide a more direct statistical test for differences in genetic variance, providing an important methodological contribution to the field.

The subsequent data chapter of this thesis was written as a stand-alone manuscript in the style of an academic journal article. As such, there is some overlap in the background material presented, although the purpose of the current introduction was to provide a more in-depth treatment of the subject than was possible in manuscript form. I have standardized the format of the references, tables, and figures for the purpose of this thesis

Figures

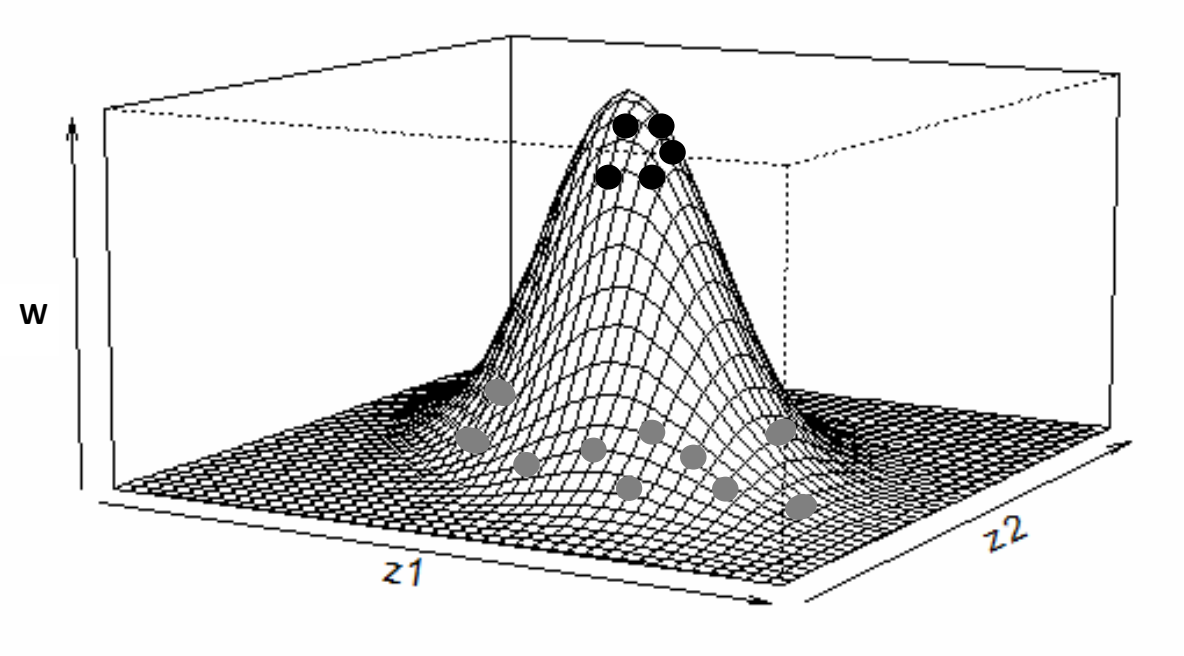

Figure 1.1: A multivariate normal distribution of the sire breeding values for two traits (z1 and z2) and fitness (w), with a simulated distribution of high fitness males (black) and low fitness males (grey). High fitness males have intermediate values of the measured trait, and therefore have decreased genetic variance, as compared to individuals with more extreme phenotypic values (both high and low).

Chapter 2

Reduced genetic variance of high vs. low fitness individuals: evidence of mutation-selection balance for sexually selected traits

Introduction

Studies of the evolution of quantitative traits in nature have yielded two general observations: directional selection on heritable traits is common and often quite strong (Endler 1986; Hereford et al. 2004; Hoekstra et al. 2001; Kingsolver et al. 2001), but in the absence of environmental change, a sustained evolutionary response of these traits is rare (Kingsolver & Diamond 2011; Svensson & Gosden 2007). Rather, investigations over various timescales indicate that phenotypic trait means tend to remain relatively constant, with change best described by a model

of stabilizing selection around a slowly moving optimum (Estes & Arnold 2007). The failure of directional selection to produce a prolonged response suggests that these traits have reached some form of evolutionary limit. The genetic nature of this limit in natural populations, however, remains a largely unresolved issue.

Directional selection erodes genetic variance in target traits, making a simple lack of genetic variance in the direction of selection a possible explanation. Empirical data, however, suggest that this is not a general explanation: variation exists for the majority of traits studied (Barton & Partridge 2000; Lynch & Walsh 1998; but see Hoffmann et al. 2003), with those most closely linked to fitness often exhibiting the highest levels of genetic variance (Houle 1992). Although multivariate genetic constraints arising from the genetic co-variance structure among suites of traits may often decrease the genetic variance available to selection (Barton & Partridge 2000; Blows & Hoffmann 2005), such co-variances appear insufficient to produce absolute constraints, at least for bivariate trait combinations (Beldade et al. 2002; Conner et al. 2003; Hine et al. 2011). Furthermore, the co-variance structure of a set of traits can also increase genetic variance in the direction of selection, and it appears that genetic architecture may facilitate an evolutionary response as often as constraining it (Agrawal & Stinchcombe 2009). Overdominance of loci responding to selection may also impose an evolutionary limit by restricting the additive genetic variance available to selection (Falconer & Mackay 1996). However, there is currently little evidence to suggest that overdominance is a general source of selection limits (Eisen 1980; Falconer & Mackay 1996; Lynch & Walsh 1998).

It has long been recognized that trait responses may eventually be halted by opposing natural selection (Fisher 1930), providing an alternative mechanism for an evolutionary limit. Consistent with this, artificial selection experiments often observe plateaus in trait responses despite the presence of additive genetic variance. Traits also tend to regress towards their former values when artificial selection is relaxed (Enfield 1980; Falconer & Mackay 1996; Hine et al. 2011; Reeve & Robertson 1953), suggesting an important role for opposing natural selection in generating evolutionary limits. Whether the genetic limits arising from artificial selection experiments are representative of those occurring more generally in unmanipulated populations is not known, in part, because ascertaining the nature of these limits in such populations has proven a challenging endeavour.

Sexual display traits provide a striking example of how directional selection often fails to generate sustained evolutionary change (Kruuk et al. 2002) and such traits, therefore, provide an ideal system to study the genetic basis of evolutionary limits. Directional sexual selection is often strong in nature (Kingsolver et al. 2001), is thought to be persistent, and commonly targets traits that are associated with high levels of genetic variance (Pomiankowski & Moller 1995). Sexual selection's role in the exaggeration of sexual displays is also well established, the end result of which is the pervasive sexual dimorphism that constitutes a substantial component of existing phenotypic diversity (Darwin 1871; Andersson 1994). The continual exaggeration of sexual displays, however, is generally not observed in contemporary populations (e.g., Kruuk et al., 2002), and sexual selection appears insufficient to drive the divergence of sexual displays among populations on its own (Svensson & Gosden 2007).

Opposing natural selection, arising from the pleiotropic effects of sexual displays on non-sexual fitness, has historically been recognized as a potential limit to the sustained exaggeration of sexual displays (Fisher 1930). Consistent with this, sexual displays have been observed to evolve more readily in association with a change in natural selection (Svensson & Gosden 2007), and costs of sexual displays to specific components of non-sexual fitness have been demonstrated in some cases (Fernandez & Morris 2008; Moller 1989; Ryan, Tuttle, & Rand 1982). However, direct evidence that natural selection opposes further trait exaggeration in unmanipulated populations is generally lacking (Jennions et al. 2001; Kotiaho 2001).

Opposing selection may arise not only as a direct cost to nonsexual fitness of a sexual display itself, but it may also occur due to the pleiotropic effects of the underlying alleles on other traits affecting fitness. Two general observations implicate widespread pleiotropy throughout the genome, suggesting that pleiotropic costs may often limit sexual display trait evolution (McGuigan et al. 2011). First, data on mutation rates indicates that the per trait rate may be as high as one tenth of the mutation rate for an individual (Johnson & Barton 2005), suggesting that there are few genetically independent traits within an organism. Second, quantitative genetic analyses of suites of traits generally find that a small number of independent trait combinations account for the majority of genetic variance, again suggesting the presence of strong pleiotropic co-variances among traits (Kirkpatrick 2009)

If sexual displays are held at an evolutionary limit due to opposing natural selection, genetic variance underlying these traits will be subject to stabilizing selection with respect to net fitness, and the genetic basis of the traits should be characteristic of a pleiotropic model of mutation-selection balance (Johnson & Barton 2005; Keightley & Hill 1988, 1990; McGuigan et al. 2011). In the Hill-Keightley model of mutation-selection balance, alleles affecting a measured quantitative trait also have pleiotropic effects on many other traits. Although not measured, these effects are captured in the model as net pleiotropic effects on fitness. At mutation-selection balance, while a given mutation may increase or decrease the value of the measured trait, its pleiotropic effect on fitness through unmeasured traits, will almost certainly be deleterious. Individuals with more extreme values of the measured trait are expected to harbour more mutations (with deleterious pleiotropic effects on fitness), thereby generating the appearance of stabilizing selection on the focal traits. Selection is apparent in this model because it arises from the pleiotropic effects of the alleles on unmeasured traits. Because the traits underlying these pleiotropic costs are generally not known, stabilizing selection is more easily uncovered by a genetic as opposed to a phenotypic analysis (McGuigan et al. 2011).

Here we apply a recently suggested empirical approach (McGuigan et al. 2011) to demonstrating the presence of a fitness optimum arising from the pleiotropic costs of a measured trait on other fitness components. The approach tests for the signature of stabilizing selection by comparing the genetic variance in the measured trait among individuals of high and low fitness. In particular, because under stabilizing selection low fitness individuals will tend to have more extreme values of the measured trait, genetic variance of the trait will be greater for these

individuals than for high fitness individuals. This approach has been applied in a recent study of sexual display traits in *Drosophila bunnanda*, uncovering a difference in genetic variance between sexually successful and sexually unsuccessful males consistent with stabilizing selection (McGuigan and Blows, 2009). The genetic basis of these traits differed significantly between these two fitness groups, consistent with a difference in genetic variance, although a direct statistical test of this difference was lacking. The results suggest that opposing selection arising through pleiotropy may be a key evolutionary limit for sexual displays.

Here I apply this approach within the context of a half-sibling breeding design in *Drosophila serrata*. *D. serrata* is an ideal system in which to study the genetic basis of evolutionary limits to sexual display trait exaggeration, because sexual selection on male pheromonal displays, arising through female mate preferences, has been previously investigated using a series of quantitative genetic studies, behavioural assays, and evolution experiments. In particular, female mate choice within populations targets a particular combination of long chain cuticular hydrocarbons (CHCs) that act as contact pheromones, generating consistent and often strong directional sexual selection on these traits (Chenoweth & Blows 2005, 2003; Delcourt et al. 2010; Higgie & Blows 2007; Higgie, Chenoweth, & Blows 2000; Rundle et al. 2009). Results of a recent evolution experiment have implicated opposing natural selection in generating a new evolutionary limit when artificial selection on CHCs was applied in the direction of female mate preferences (Hine et al. 2011). This experiment also demonstrated that alleles conferring increased male attractiveness were segregating in the original base population at low frequency,

presumably due to opposing natural selection on them. Here, I undertake a direct test for the signature of the opposing selection in an unmanipulated *D. serrata* laboratory population.

Methods

Half-Sibling Breeding Design:

A paternal half-sibling breeding design was conducted using a previously described outbred and laboratory adapted stock population of *D. serrata* (Chenoweth, Rundle, & Blows 2008; Rundle, Chenoweth, & Blows 2006). Eighty sires were each mated to three virgin dams in two successive rounds (i.e. dam 1, 2, 3, 1, 2, 3). Dams were allowed to oviposit for 72 h following each mating round, and sons from these half-sib families were collected upon emergence for use in binomial mate choice trials and subsequent extraction of their CHCs. The breeding design was conducted in two blocks, each consisting of 40 sires, spanning two generations of the laboratory stock population, and resulting in 240 full- and half-sib families. Virgin males were collected from both oviposition vials for each dam using light CO_2 anaesthesia within 24 h of eclosion, with a total of ten sons collected per family. All flies used in experimental assays were held as virgins at a density of six flies/vial, and were five to seven days old at the time of the assays.

Mating Success and CHC Assay:

For the binomial mate choice assay, randomly chosen virgin females from an outbred *D. serrata* population fixed for a recessive mutation causing an orange-eye phenotype were presented with a random virgin male from the same population ('competitor male') and a virgin son from the breeding design ('focal male', wild-type eye colour). Trios were observed until a male and female pair had begun copulating, and the mating success (chosen or rejected) of the focal male was recorded. Orange-eye competitor males were chosen in 49% of the mating trials overall, indicating that this phenotype had little effect on mating success.

Female *D. serrata* actively control mating and can prevent males from mounting and achieving intromission (Hoikkala, Crossley, & Castillo-Melendez 2000), implicating a central role of female mate choice in determining male fitness. Although the design of these mating trials does not preclude male-male competition nor the possibility that female choice may target other correlated traits, several lines of evidence indicate that CHCs are a direct target of sexual selection arising from female mate preferences, and that binomial choice trials are a suitable technique for quantifying female choice (Delcourt et al. 2010; Higgie & Blows 2008). The evidence includes results of a manipulative evolution experiment in which artificial selection in the direction of female mate preferences, as determined from binomial choice trials, was shown to increase male mating success over control populations (Hine et al. 2011). Because mating success is the primary determinant of male fitness in species where males contribute only their genes to their offspring, the outcome of binomial mate choice trials is a straightforward way to sort males into high and low fitness categories.

Before copulation between the female and chosen male was complete, flies were anesthetized with light CO_2 and the focal male (chosen or rejected son from the breeding design) was isolated for immediate extraction of its CHCs. Focal males were individually washed in 100μl of hexane for 3 min, followed by 1 min of agitation on a vortex mixer. Flies were then removed from the hexane and the resulting CHC samples were stored at -20°C. Individual CHC samples were analyzed using a dual-channel Agilent 6890N fast gas chromatograph fitted with HP-5 phenylmethyl siloxane columns of 30m length and 250μm internal diameter (0.1μm film thickness), pulsed splitless inlets (at 275°C), and flame ionization detectors (at 310°C). The injection volume was 1μl and the temperature program began by holding at 140°C for 0.55min, ramping at 100°C/min to 190°C, then slowing to 45°C/min to 320°C and holding for 1 min.

Individual CHC profiles were analyzed by quantifying the area under nine peaks corresponding to those used in previous studies (e.g. Chenoweth & Blows 2005; Delcourt et al. 2010). These peaks have been previously identified in order of their retention times as: (Z,Z)-5,9-$C_{24:2}$; (Z,Z)-5,9-$C_{25:2}$; (Z)-9-$C_{25:1}$; (Z)-9-$C_{26:1}$; 2-Me-C_{26}; (Z,Z)-5,9-$C_{27:2}$; 2-Me-C_{28}; (Z,Z)-5,9-$C_{29:2}$; and 2-Me-C_{30} (Howard et al. 2003). The relative abundance of each hydrocarbon was calculated by dividing the area under each peak by the total area of all nine peaks for that individual. Expressing each CHC as a relative abundance corrects for technical error associated with quantifying absolute abundances and is less prone to experimental error than the use of internal standards (Blows & Allan 1998; Savarit & Ferveur 2002). These proportions were transformed into log-contrasts, using (Z,Z)-5,9-$C_{24:2}$ as the common arbitrary divisor, to break the unit-sum

constraint inherent in compositional data, and thereby permit multivariate analyses (Atchison 1986). The resulting 8 log-contrast CHCs were used in subsequent analyses.

Phenotypic and Genetic Analyses:

A total of 10 multivariate outliers (0.5% of the total data set) were identified and removed using the multivariate Mahalanobis distance technique implemented in the software package JMP (version 9.0.0; SAS Institute Inc., Cary, NC). Log-contrast CHC values were subsequently standardized globally {~N[0,1]} prior to analyses.

Standardized phenotypic sexual selection gradients on the eight log-contrast CHCs were estimated using standard first and second-order polynomial regression on relative male mating success (Lande & Arnold, 1983). Experimental block and gas chromatography channel were included as fixed effects in the models. The overall importance of CHCs in explaining variation in male mating success was given by the adjusted coefficient of determination (r^2_{adj}). Because mating success is binomially distributed, significance testing employed a generalized linear model with a logistic link function, fit via maximum likelihood, implemented in the GENMOD procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC). The significance of overall linear and nonlinear selection was determined through likelihood ratio tests employing a sequential model building approach (Draper & John 1988; Rundle & Chenoweth 2011).

The additive genetic (i.e. sire-level) variance-covariance matrix (**G**) for the eight log-contrast CHCs was estimated for successful and unsuccessful males together, using restricted maximum likelihood, and employing the following multivariate mixed model:

$$Y_{ijlcm} = \mu + \mathbf{S}_i + \mathbf{D}_{j(i)} + B_l + C_c + \varepsilon_{ijlcm.} \qquad \text{(Eqn. 1)}$$

Fixed effects included the intercept (μ), experimental block (B), and gas chromatography channel (C). Sire (**S**), and dam nested within sire (**D**) were random effects. Statistical support for the genetic dimensions underlying **G** was evaluated using a series of nested likelihood ratio tests employing the factor analytic modeling approach implemented in the MIXED procedure of SAS and described in Hine and Blows (2006). In these analyses, the dam effect was fixed at eight dimensions, corresponding to the number of eigenvectors with nonzero eigenvalues at this level.

As a direct test of whether **G** differed when estimated using successful vs. unsuccessful male offspring, a fixed effect of success (chosen or rejected) was added to Eqn. 1 and then a likelihood ratio test was used to compare the fit of this model to one that allowed separate covariance matrices to be estimated at the sire level (as implemented using the 'group' statement in the SAS MIXED procedure). In these analyses, the dam effect was again fixed at eight dimensions. A

difference in **G** between high and low fitness sons may arise due to a difference in the genetic variances of these eight log-contrast CHCs, and/or differences in their covariance structure. Therefore, to further explore the difference in the genetic basis of CHCs between these groups, we took advantage of the fact that sires in our data set had sons that were both sexually successful and sexually unsuccessful to provide a direct test for the presence of a sire × mating success interaction following McGuigan and Blows (2009). Analogous to a genotype × environment interaction, a significant sire × mating success interaction demonstrates the presence of genetic variation in the effect of using successful vs. unsuccessful sons in estimating **G** (i.e. that sire-level reaction norms vary). Such an interaction is necessary for genetic variance to differ between high and low fitness males. To test for this interaction, successful and unsuccessful males were analyzed simultaneously using the following multivariate mixed model:

$$Y_{ijlcm} = \mu + \mathbf{S}_i + \mathbf{D}_{j(i)} + \mathbf{I}_{ik} + M_k + B_l + C_c + \varepsilon_{ijlcm}. \qquad \text{(Eqn. 2)}$$

Terms are as in Eqn 1 with the addition of fixed effect of male mating success (M) and a random effect (**I**) representing the interaction between the sire effect and male mating success. The covariance matrix **I** accounts for differences in the genetic basis of the log-contrast CHCs, the first eigenvector of which ($\boldsymbol{i}_{max}$) estimates the trait combination for which genetic values differed most between the two groups of males. Statistical support for the sire × success interaction was evaluated using a series of nested likelihood ratio tests again employing the factor analytic modeling approach (Hine & Blows, 2006). Unconstrained co-variance matrices were fit at all other levels in all cases.

Although statistical support for **I** is consistent with differences in genetic variance among fitness classes, it not sufficient to demonstrate this for the same reason that a G × E interaction does not necessitate genetic variance to differ between environments. A direct test is possible for a single trait, however, by employing a likelihood ratio test to compare the fit of a model with a single sire-level genetic variance to one that allows separate estimates for successful and unsuccessful classes at this level. This test was applied separately to the phenotypic traits described by each of the first three eigenvectors from a diagonalization of the pooled **G** ($\boldsymbol{g}_{max}$, $\boldsymbol{g}_2$, and $\boldsymbol{g}_3$ respectively; Table 2.1), representing the three statistically supported genetic dimensions and accounting for 96.8% of the total genetic variance in CHCs. These traits were calculated by scoring each male's multivariate CHC phenotype using CHC$\boldsymbol{g}_n = \boldsymbol{g}_n{}^{\mathrm{T}}\mathbf{Z}$, where **Z** is a row vector of the eight observed log-contrast CHC values for an individual and $\boldsymbol{g}_n$ is the *n*th eigenvector of **G** (i.e. $\boldsymbol{g}_{max}$, $\boldsymbol{g}_2$ or $\boldsymbol{g}_3$) (McGuigan et al. 2011). The same method was used to generate phenotypic scores for an additional biologically relevant trait, CHC$\boldsymbol{\beta}$, representing the combination of male log-contrast CHCs most strongly associated with male mating success and calculated by applying to each male's CHC phenotype the vector of linear sexual selection gradients (i.e. $\boldsymbol{\beta}$) from the phenotypic analysis of mating success above. Likelihood ratio tests were used to test whether genetic variance in CHC$\boldsymbol{g}_{max}$, CHC$\boldsymbol{g}_2$, CHC$\boldsymbol{g}_3$, and CHC$\boldsymbol{\beta}$ differed between high and low fitness males.

Results

Consistent with previous studies of this (Delcourt et al. 2010) and other populations of *D. serrata* (Chenoweth & Blows 2005; Hine et al. 2011; Hine, Chenoweth, & Blows 2004), phenotypic analysis revealed that male log-contrast CHCs were under significant directional sexual selection overall ($\chi^2 = 203.5$; d.f. = 8; $P < 0.001$). Variation in male CHCs explained 9.1% of the variance in male mating success ($r^2_{adjusted}$) and sexual selection was significant individually on five of the eight log-contrast CHCs (Table 2.1). Directional sexual selection was also strong, with three standardized gradients exceeding the median absolute value of 0.18 in Kingsolver et al.'s (2001) review of the strength of phenotypic selection in natural populations. Although the addition of nonlinear selection was significant overall ($\chi^2 = 80.02$; d.f. = 36; $P < 0.001$), only three of the 36 nonlinear gradients were individually significant and the inclusion of all nonlinear selection explained only an additional 2.1% of the variance in mating success (i.e. an increase in $r^2_{adjusted}$ from 9.1% to 11.2%). A nonparametric fitness function for CHC$\boldsymbol{\beta}$, estimated via a univariate cubic spline (Schluter 1988), also provided no indication of a fitness optimum within the range of phenotypic values (Fig. 2.1)

Factor-analytic modeling of the genetic covariance matrix for all males (i.e. irrespective of mating success; pooled **G**) revealed statistical support for three underlying genetic dimensions, accounting for 97% of total genetic variance in the eight log-contrast CHCs (reducing from 3 to 2 dimensions significantly worsened the fit of the model: $\chi^2 = 24.4$; d.f. = 6; $P < 0.001$). However, the genetic basis of these traits differed between successful and unsuccessful males, as indicated by a significantly better fit of a model that permitted separate, as opposed to a single,

sire-level covariance matrix to be estimated for these two groups (χ^2 = 154.9; d.f. = 33; P < 0.001). Analysis of unsuccessful males alone revealed statistical support for three dimensions of **G**, accounting for 95% of the genetic variance (Table 2.2). Consistent with reduced genetic variance among them (and therefore less power to detect it given similar samples sizes), only two dimensions of **G**, accounting for 86% of genetic variance in CHCs, were statistically supported for successful males (Table 2.2).

Visual inspection of **G** for unsuccessful (Table 2.3) and successful (Table 2.4) males reveals that for seven of eight log-contrast CHCs, unsuccessful males have greater genetic variance than successful males, a difference that is significant overall (cumulative binomial probability of seven or more traits having greater genetic variance in unsuccessful than successful males = 0.035). This is reflected in the sum of the eigenvalues of these matrices (i.e. the trace of **G**), which reveals that total genetic variance of these traits is 1.24 times higher for unsuccessful as compared to successful males. While some differences in the genetic co-variance structure of these traits are apparent in a visual comparison of the two matrices (Tables 2.3 and 2.4), including their dominant axis ($\boldsymbol{g}_{max}$; Table 1), in order to better characterize these differences, and to provide a direct test for a difference in the genetic basis of CHCs between these groups, I estimated the sire × mating success interaction (**I**).

Factor analytic modeling revealed statistical support for the first two genetic dimensions of **I** ($\boldsymbol{i}_{max}$ and $\boldsymbol{i}_2$), accounting for 55% and 35% of the total genetic variance at this level, respectively (reducing from 2 to 1 dimensions, χ^2 = 17.24; d.f. = 7; P < 0.016). Significance of the variance

contained in **I** indicates that sire-level genetic variances in CHCs differ when estimated using their successful vs. unsuccessful sons, analogous to the interpretation of a genotype × environment interaction (Figure 2.2). Such an interaction is necessary for genetic variance to differ among unsuccessful and successful males, although does not provide a direct test for a difference in variance. The trait combination described by the vector $\boldsymbol{i}_{max}$ (Table 2.1) is associated with $\boldsymbol{g}_{max}$ (vector correlation of 0.782), indicating that the trait combination describing the greatest difference in the genetic basis of CHCs among groups lies in the multivariate trait combination for which genetic variance in the eight log-contrast CHCs is greatest.

A direct test for a difference in CHC genetic variance between successful and unsuccessful males is possible in a univariate framework. I applied this test separately to the three phenotypic traits described by the first three eigenvectors of the pooled **G** ($\boldsymbol{g}_{max}$, $\boldsymbol{g}_2$, and $\boldsymbol{g}_3$), termed CHC$\boldsymbol{g}_{max}$, CHC$\boldsymbol{g}_2$ and CHC$\boldsymbol{g}_3$ respectively. These trait combinations capture the greatest portion of genetic variance in CHCs among all males (accounting for 66%, 22%, and 8.8% total genetic variance respectively) and represent the three statistically supported genetic dimensions underlying the pooled **G** (Table 2.2). Genetic variance was greater among unsuccessful than successful males for all three traits, although the difference was concentrated primarily along $\boldsymbol{g}_2$ (1.13, 1.80 and 1.03 times more genetic variance in unsuccessful than successful male for CHC$\boldsymbol{g}_{max}$, CHC$\boldsymbol{g}_2$ and CHC$\boldsymbol{g}_3$ respectively). However, none of these differences were significant when tested individually (CHC$\boldsymbol{g}_{max}$: $\chi^2 = 0.711$; d.f. = 1; P = 0.39; CHC$\boldsymbol{g}_2$: $\chi^2 = 1.61$; d.f. = 1; P = 0.20; CHC$\boldsymbol{g}_3$: $\chi^2 = 0.03$; d.f. = 1; P = 0.87). Finally, for CHC$\boldsymbol{\beta}$, the trait combination under directional sexual selection, relatively little genetic variance existed in the population as a whole for this

trait combination ($V_A = 2.52x10^{-6}$), although it was 1.41 times greater in unsuccessful than successful males. Again, however, this difference was not significant ($\chi^2 = 2.24$; d.f. = 1; P = 0.12).

Stabilizing selection on CHC genetic variance appeared to be concentrated along $\boldsymbol{g_2}$. Although this phenotypic trait combination (CHC$\boldsymbol{g_2}$) was also a target of directional ($\beta = 0.079$, $\chi^2 = 11.41$, P <0.001) and stabilizing ($\gamma = -0.04$, $\chi^2 = 8.95$, P = 0.003) sexual selection through female mate choice, this selection was relatively weak and accounted for little of the variance in male mating success ($r^2_{adjusted}$, linear selection = 0.7%, linear + nonlinear = 1.1%). Significance, therefore, likely reflects the substantial statistical power associated with phenotyping 1978 males.

Discussion

The often observed lack of contemporary evolution (Kingsolver & Diamond 2011; Svensson & Gosden 2007), despite directional selection on phenotypic traits (Endler 1986; Hereford et al. 2004; Hoekstra et al. 2001; Kingsolver et al. 2001), implies a limit to trait evolution. Artificial selection experiments suggest the importance of opposing selection, arising through the pleiotropic effects of alleles underlying the target traits on other components of fitness. However, empirical evidence from unmanipulated populations for the genetic basis of these evolutionary limits is generally lacking. Ascertaining these limits has proven empirically difficult and remains a central issue in evolutionary genetics. Opposing natural selection will generate net stabilizing selection around a fitness optimum, although characterizing these optima through a

phenotypic analysis is challenging because the pleiotropic fitness costs may arise through any number of unidentified traits. Using a genetic analysis, however, the signature of stabilizing selection can be detected through asymmetries in genetic variance of traits for high and low fitness individuals, allowing the existence of an evolutionary optimum to be inferred (McGuigan et al. 2011). Here, I have used this approach to demonstrate a genetic limit to the exaggeration of a suite of male sexual displays (CHCs) in *D. serrata.*

Consistent with past studies, CHCs were under strong directional sexual selection via female mate preferences, with the genetic (co)variance structure of these sexual displays differing among high and low fitness individuals. Characteristic of stabilizing selection, genetic variance was greater among low than high fitness males for seven of the eight log-contrast CHCs, representing 1.24 times more genetic variance in the former as compared to the latter group. A difference in seven of the eight traits is significantly more than would be expected by chance (binomial probability, $P = 0.035$). A significant sire × mating success interaction was also detected, indicating that the genetic basis of CHCs differed between high and low fitness groups. While such an interaction is not direct evidence for a difference in genetic variance (contra McGuigan & Blows 2009), for the same reasons that a genotype × environment interaction (i.e. non-parallel reaction norms) is not sufficient for genetic variance to differ between environments, it is a necessary condition and consistent with a difference.

Differences in genetic variance were concentrated primarily along the second genetic dimension (i.e. eigenvector) of CHCs, with genetic variance being 1.8 times greater among unsuccessful

than successful males for this trait combination. Statistical support for this difference was lacking, however, in separate tests of this and other trait combinations (i.e. CHC$\boldsymbol{g}_{max}$, CHC$\boldsymbol{g}_2$, CHC$\boldsymbol{g}_3$, and CHC$\boldsymbol{\beta}$), although genetic variance in CHC$\boldsymbol{\beta}$ was extremely low overall. In general, such tests can only accommodate univariate traits, and therefore, address only a subset of the total genetic variance, reducing their power.

An alternative approach to uncovering a fitness optimum is through the genetic covariance of measured traits with fitness, allowing stabilizing selection on the genetic variance of these traits to be estimated directly (McGuigan et al. 2011). This approach, however, requires estimates of lifetime fitness for many replicate individuals within the context of a known pedigree, which is empirically challenging to obtain. An advantage of the current approach is that it requires only a broad classification of individuals into high and low fitness groups. Such classification can be relatively straightforward to obtain in species such as *Drosophila,* in which mating success is a substantial component of male fitness and can be assayed under lab conditions. Also, because mating success is likely to depend on an individual's condition, mutations that are deleterious to overall fitness are likely to be deleterious to mating success as well (Whitlock & Agrawal 2009); males of low mating success should therefore also carry alleles deleterious to non-sexual fitness as well. Nevertheless, the outcome of a single binomial mate choice trial is not a particularly sensitive measure of male mating success, and is likely to have underestimated the true variance (Andersson 1994; Briscoe et al. 1992; McGuigan & Blows 2009), thereby reducing the estimated difference in genetic variance among groups. Replicate measures of mating success for

individual males is empirically feasible in *Drosophila* (e.g., Rundle, Odeen, & Mooers 2007) and may increase the power to detect a difference.

The general inability of sexual selection to increase male mating success in unmanipulated populations (Hall, Lindholm, & Brooks 2004; McGuigan, Van Homrigh, & Blows 2008) implies that unconditionally beneficial alleles are not segregating for male sexual displays, and emphasizes the potential importance of opposing selection in limiting the exaggeration of such traits. In *D. serrata* in particular, artificial selection on CHCs in the direction of female mate preferences has been shown to increase male mating success. However, trait responses were halted after a number of generations, despite an increase in genetic variance for this combination of CHCs as the new selective limit was approached (Hine et al. 2011). After the relaxation of artificial selection, trait values rapidly decayed towards their initial values, implicating opposing selection as generating this new evolutionary limit. More importantly, the response of males to artificial selection, and the increase in genetic variance during this response, indicate that alleles conferring an increase in CHC attractiveness, and hence male mating success, segregate at low frequency in the ancestral population. These alleles are presumably held at a low frequency by opposing natural selection on them. Consistent with this, I have demonstrated the signature of stabilizing selection on the genetic variance underlying CHCs in an unmanipulated *D. serrata* population.

If male CHCs are at an evolutionary optimum generated by opposing selection, in the absence of a direct benefit of mate choice, costly female preferences for these traits may depend on the

indirect benefits females gain by discriminating against males carrying a greater number of deleterious mutations. The combination of CHCs in *D. serrata* preferred by females is unusually condition-dependent relative to other possible combinations (Delcourt & Rundle 2011). Consequently, males expressing higher values of this trait may carry fewer deleterious mutations and directional female mate preferences may, therefore, contribute to stabilizing selection on genetic variance underlying CHCs. Consistent with this, despite strong direction selection along $\boldsymbol{\beta}$, significant stabilizing sexual selection was observed on CHC$\boldsymbol{g_{max}}$ (γ = -0.005; P = 0.017) and CHC$\boldsymbol{g_2}$ (γ = -0.040; P = 0.002). This selection is weak, however, consistent with stabilizing selection on CHC genetic variance also arising from pleiotropic effects on other unmeasured traits.

Essentially no standing genetic variance lies in the direction of sexual selection ($1.7x10^{-4}$ % of the total V_A along CHC$\boldsymbol{\beta}$, with $\boldsymbol{\beta}$ is oriented 91.5° and 87.3° from $\boldsymbol{g_{max}}$ and $\boldsymbol{g_2}$ respectively), consistent with previous results from this (Delcourt et al. 2010; Hine et al. 2004) and other species (Hall et al. 2004; Hunt et al. 2007; McGuigan et al. 2008). The lack of genetic variance in the direction of sexual selection is consistent with the depletion of standing genetic variation due to strong and persistent female mate preferences, and suggests that at mutation-selection balance the maintenance of costly mate preferences may depend on a female's ability to discriminate against males carrying novel deleterious mutations every generation (Tomkinks et al. 2004; Whitlock 2000). Although it has been demonstrated that females can discriminate against males carrying large effect deleterious mutations (Maclellan, Whitlock, & Rundle 2009; Sharp & Agrawal 2008), and those which have been artificially induced (Radwan 2004), there is

little evidence to indicate whether females discriminate against naturally arising deleterious alleles.

In summary, I have shown that despite directional phenotypic selection on CHCs via one component of male fitness (mating success), the genetic variance underlying these traits is subject to stabilizing selection. This suggests that females are discriminating against males residing further from their fitness optima and which are inferred to be carrying more deleterious mutations, thus aligning natural and sexual selection processes. McGuigan & Blows (2009) provide similar results in an unmanipulated population of *D. bunndanda*, emphasizing the importance of characterizing selection at the genetic level. The commonly observed failure of directional selection to produce a prolonged evolutionary response may be explained by extensive pleiotropy generating stabilizing selection on trait genetic variances. Additional empirical studies of this nature will be needed in order to assess the generality of these results and to provide insight into the nature and maintenance of genetic variance in traits under selection.

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Tables

Table 2.1: The multivariate trait combination describing the standardized sexual selection gradient $\boldsymbol{\beta}$, the first three eigenvectors of **G** ($\boldsymbol{g_{max}}$, $\boldsymbol{g_2}$, and $\boldsymbol{g_3}$) for the pooled data set (i.e. successful and unsuccessful males together), $\boldsymbol{g_{max}}$ separately from the analyses of low and high fitness males (i.e. unsuccessful and successful respectively), and the first eigenvector of **I** ($\boldsymbol{i_{max}}$).

		Pooled			Unsuccessful	Successful	
CHC	$\boldsymbol{\beta}$	$\boldsymbol{g_{max}}$	$\boldsymbol{g_2}$	$\boldsymbol{g_3}$	$\boldsymbol{g_{max}}$	$\boldsymbol{g_{max}}$	$\boldsymbol{i_{max}}$
(Z,Z)-5,9-$C_{25:2}$	0.068*	0.567	-0.356	0.015	0.551	0.520	0.027
(Z)-9-$C_{25:1}$	-0.077*	0.232	-0.242	-0.849	0.184	0.310	0.165
(Z)-9-$C_{26:1}$	0.011	0.267	0.493	-0.325	0.261	0.304	0.307
2-Me-C_{26}	-0.045	0.223	-0.555	0.191	0.217	0.250	0.199
(Z,Z)-5,9-$C_{27:2}$	-0.221**	0.407	0.375	0.057	0.420	0.410	0.370
2-Me-C_{28}	0.060	0.391	-0.097	0.276	0.405	0.382	0.353
(Z,Z)-5,9-$C_{29:2}$	0.539**	0.226	0.263	0.097	0.199	0.218	0.731
2-Me-C_{30}	-0.234**	0.366	0.208	0.219	0.408	0.342	0.207

*P < 0.02

**P < 0.0001

Table 2.2: Model fit statistics of the number of effective dimensions of the genetic (co)variance matrix (**G**) separately for unsuccessful and successful males. The percent of the total genetic variance in CHCs (% variance) was calculated from the full (i.e. eight-dimensional) factor analytic model.

		Unsuccessful Males			Successful Males		
Dimension	d.f.	%variance	-2LL	P [(1)]	%variance	-2LL	P [(1)]
0	-	-	18230.74	-	-	17906.04	-
1	8	63.5	18143.28	< 0.001	70.1	17854.98	< 0.001
2	7	23.4	18105.57	< 0.001	16.1	17810.88	< 0.001
3	6	8.2	18082.85	< 0.001	9.5	17799.26	0.071
4	5	3.5	18077.93	0.425	3.7	17796.09	0.674
5	4	1.2	18076.27	0.798	0.7	17794.88	0.878

[(1)] Results of a likelihood ratio test (-2LL), with degrees-of-freedom as indicated (d.f.), of whether excluding the current factor significantly worsens the fit of the model.

Table 2.3: Genetic covariance matrix (**G**) for eight log-contrast CHCs for unsuccessful males, estimated as four-times the sire-level covariance matrix. Genetic variances are along the diagonal (in bold), with co-variances below and correlations above (in italics).

	(Z,Z)-5,9-$C_{25:2}$	(Z)-9-$C_{25:1}$	(Z)-9-$C_{26:1}$	2-Me-C_{26}	(Z,Z)-5,9-$C_{27:2}$	2-Me-C_{28}	(Z,Z)-5,9-$C_{29:2}$	2-Me-C_{30}
(Z,Z)-5,9-$C_{25:2}$	**1.508**	*0.587*	*0.264*	*0.622*	*0.644*	*0.870*	*0.393*	*0.757*
(Z)-9-$C_{25:1}$	0.561	**0.605**	*0.179*	*0.418*	*0.249*	*0.358*	*-0.124*	*0.244*
(Z)-9-$C_{26:1}$	0.279	0.120	**0.742**	*-0.239*	*0.884*	*0.740*	*0.788*	*0.705*
2-Me-C_{26}	0.657	0.280	-0.177	**0.741**	*0.103*	*0.705*	*-0.036*	*0.337*
(Z,Z)-5,9-$C_{27:2}$	0.755	0.185	0.727	0.085	**0.911**	*0.742*	*0.875*	*0.919*
2-Me-C_{28}	0.922	0.240	0.304	0.524	0.612	**0.746**	*0.565*	*0.902*
(Z,Z)-5,9-$C_{29:2}$	0.287	-0.057	0.404	-0.018	0.497	0.290	**0.354**	0.750
2-Me-C_{30}	0.809	0.165	0.529	0.252	0.764	0.678	0.388	**0.758**

Table 2.4: Genetic covariance matrix (**G**) for eight log-contrast CHCs for successful males, estimated as four-times the sire-level covariance matrix. Genetic variances are along the diagonal (in bold), with co-variances below and correlations above (in italics).

	(Z,Z)-5,9-$C_{25:2}$	(Z)-9-$C_{25:1}$	(Z)-9-$C_{26:1}$	2-Me-C_{26}	(Z,Z)-5,9-$C_{27:2}$	2-Me-C_{28}	(Z,Z)-5,9-$C_{29:2}$	2-Me-C_{30}
(Z,Z)-5,9-$C_{25:2}$	**1.234**	*0.630*	*0.413*	*0.733*	*0.679*	*0.839*	*0.522*	*0.714*
(Z)-9-$C_{25:1}$	0.597	**0.728**	*0.579*	*0.601*	*0.424*	*0.516*	*0.366*	*0.400*
(Z)-9-$C_{26:1}$	0.356	0.384	**0.602**	*0.141*	*0.866*	*0.993*	*0.872*	*0.759*
2-Me-C_{26}	0.563	0.354	0.075	**0.478**	*0.362*	*0.783*	*0.311*	*0.502*
(Z,Z)-5,9-$C_{27:2}$	0.652	0.313	0.580	0.216	**0.747**	*0.841*	*0.960*	*0.949*
2-Me-C_{28}	0.715	0.337	0.349	0.415	0.557	**0.587**	*0.763*	*0.928*
(Z,Z)-5,9-$C_{29:2}$	0.294	0.159	0.344	0.109	0.421	0.297	**0.258**	*0.867*
2-Me-C_{30}	0.565	0.243	0.420	0.247	0.584	0.507	0.314	**0.508**

Figures

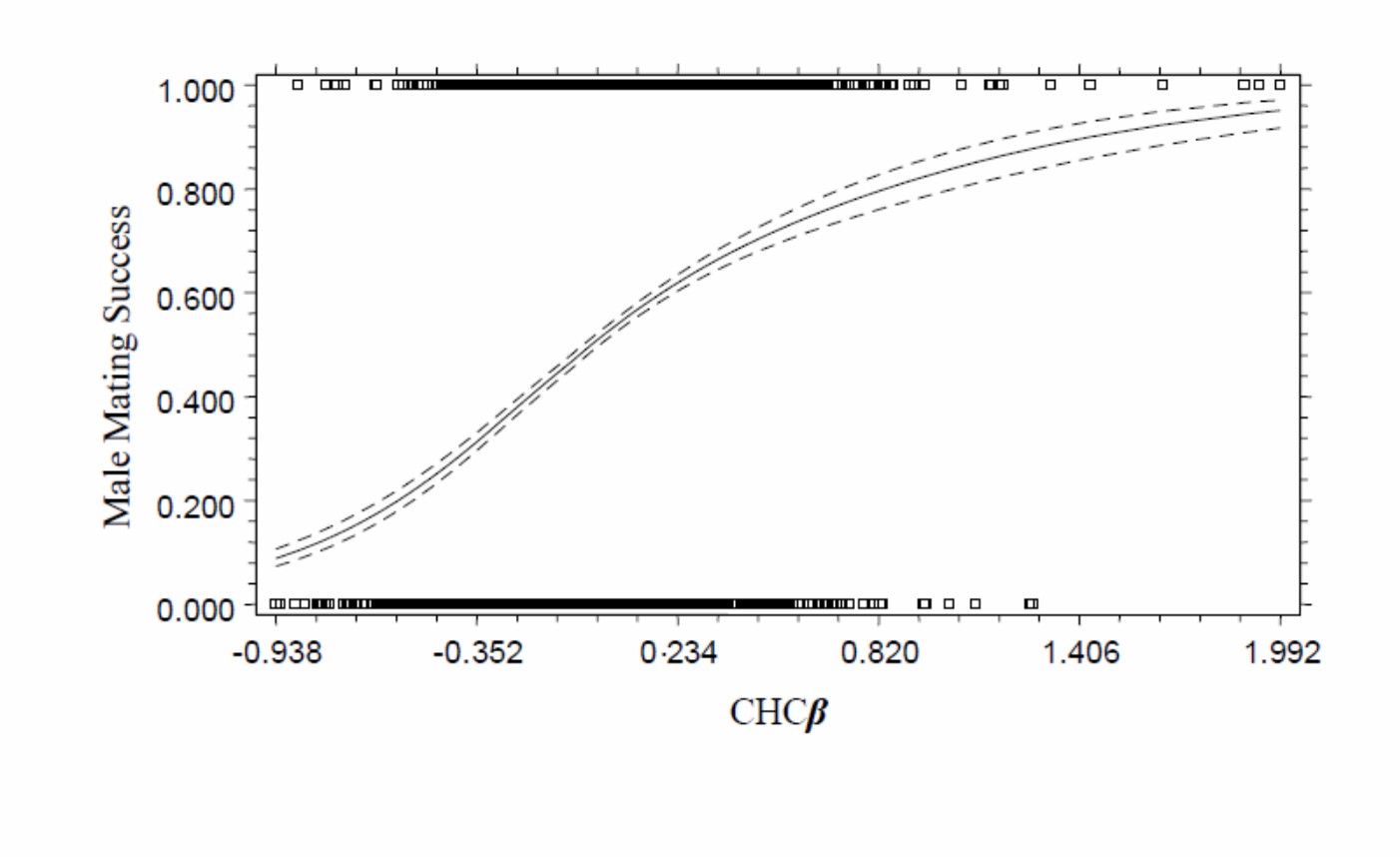

Figure 2.1: A nonparametric fitness function ±1 standard error (generated from 100 bootstrap replicates), depicting sexual selection on the phenotypic trait CHC***β***, fit using a univariate cubic spline with smoothing parameter λ = -1 to minimize the general cross-validation score (Schluter 1988). Individual points are the mating success scores for separate sons from the breeding design as determined in the mate choice assays.

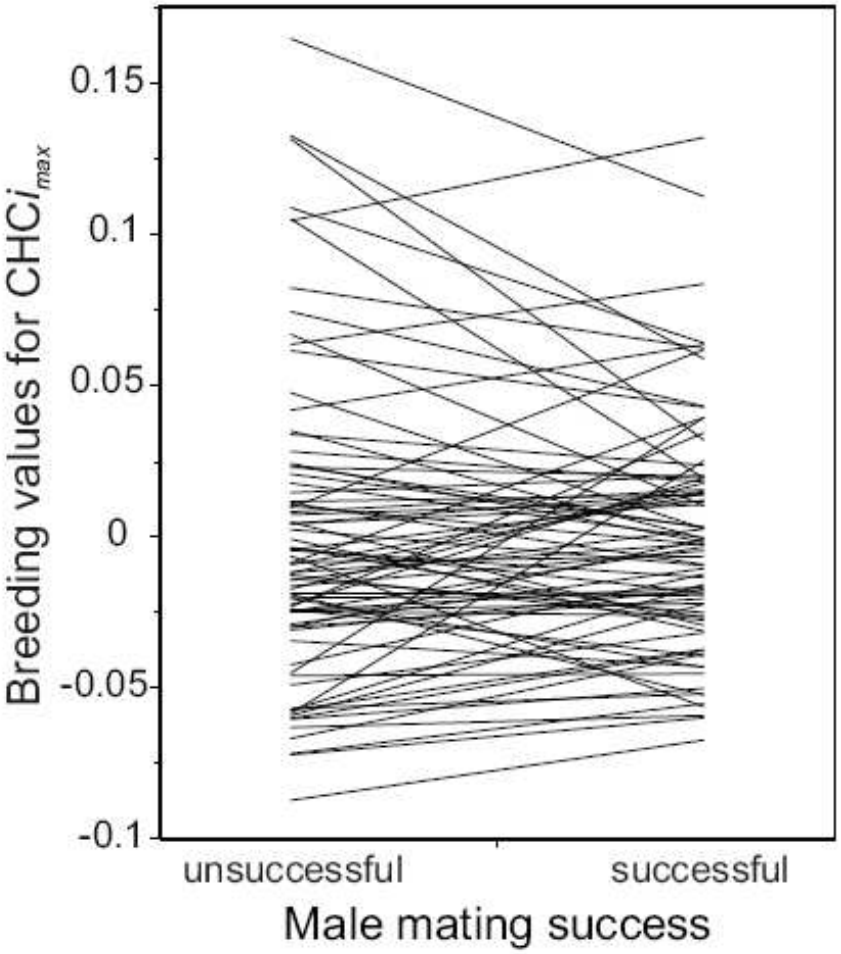

Figure 2.2: The interaction between male mating success and sire breeding values for the first eigenvector of **I**, $\boldsymbol{i}_{max}$. The crossing reaction norms indicate that sire breeding values for genetic variance differ when estimated from their successful and unsuccessful sons, indicative of a sire × mating success interaction.