The Evolutionary Dynamics of Intralocus Sexual Conflict

Submitted by:

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Declaration

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.

Signature……..

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Abstract

Males and females often have divergent evolutionary interests, sparking two forms of sexual conflict: 1) interlocus sexual conflict (IRSC), an antagonistic interaction between the sexes that is mediated by different loci in each sex; 2) intralocus sexual conflict (IASC), where genes have opposite fitness consequences depending on the sex expressing them. Both forms of conflict appear to be common, yet there are large gaps in our understanding of their evolutionary dynamics.

I focus on IASC and begin by synthesising theoretical concepts and empirical findings to better understand its evolutionary dynamics in a critical review of the topic (**Chapter 1**). I take a multifaceted approach by considering the maintenance, resolution, and consequences of this evolutionary feud. I then explore the extent of sexually antagonistic genetic variation for fitness in a large-scale study of *Drosophila melanogaster*, using hemiclonal analysis (**Chapter 2**). I compare results to data collected from the same population five years previously and show that the strength of the conflict has declined over time. Next, I show that subtle changes in temperature during the adult life-stage can dramatically affect sex-specific fitness and alter the direction of the conflict, which could contribute to the maintenance of IASC in natural populations (**Chapter 3**). I also present a new theoretical model that incorporates IASC into traits that are involved in IRSC arms races (**Chapter 4**). Surprisingly, IASC can have dramatic and contrasting effects on sexually antagonistic coevolution: stabilising arms races or drawing the sexes into repeated bouts of arms race escalation and stasis. Finally, I extend IASC theory to explore an analogous conflict between castes in social animal societies (**Chapter 5**) and suggest unique research opportunities to be capitalised upon in species with a division of labour. I summarise the work in this thesis by highlighting the broad and varied biological consequences of such a pervasive conflict (**Chapter 6**).

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Author's Declarations

All of the chapters presented in this thesis were written by Tanya M. Pennell (TMP), with comments from Edward H. Morrow (EHM). Further contributors for each chapter are detailed below.

Chapter 1

A version of this chapter was published as a review article (Pennell, T.M, Morrow, E.H. 2013. Two sexes, one genome: the evolutionary dynamics of intralocus sexual conflict. *Ecology and Evolution*. 3: 1819–1834). TMP and EHM are grateful to two anonymous reviewers.

Chapter 2

The fly populations and hemiclonal lines were set up my TMP, with technical support from Ilona Flis (IF). Data in this chapter was collected by TMP, IF and Fiona Ingleby. Statistical analyses by TMP.

Chapter 3

The fly populations and hemiclonal lines were set up my TMP. Fitness data collection by TMP, behavioural data collection by TMP and Virginia Mahieu. Statistical analyses by TMP. A version of this chapter is under review with *Evolution*.

Chapter 4 (and Appendix 1)

The theoretical concept was formed by TMP. The quantitative genetic model was developed by TMP and Sander van Doorn (SvD). All aspects of mathematical analysis were conducted by SvD. A version of this manuscript is under review with *Proceedings of the National Academy of Sciences*.

Chapter 5

This chapter was co-authored by EHM, Luke Holman and Jeremy Field.

Chapter 1: Introduction

1.1 - Two Sexes, One Genome: Intralocus Sexual Conflict

The evolutionary interests of males and females are often worlds apart. This is thought to be a result of gamete dimorphism, causing the sexes to occupy distinct reproductive roles and experience contrasting selection pressures (Trivers 1972; Parker 1979). In an ideal scenario, the sexes will adapt accordingly by expressing different trait values; however, independent evolution is constrained by shared molecular "machinery" controlling trait expression in both sexes (i.e., alleles have similar additive effects in each sex). This creates high intersexual genetic correlations (r_{MF}), which may make it impossible for the sexes to reach their own trait fitness optima (Lande 1980). In such instances, an evolutionary tug-of-war over allelic expression may proceed. This is a phenomenon known as intralocus sexual conflict (hereafter referred to as "IASC"). Below, I discuss empirical evidence for the existence of IASC in natural and laboratory-based populations (section 1.2), and present empirical estimates of standing genetic variation for IASC within a laboratory adapted population **(Chapter 2)**.

Outstanding questions concern the conditions that maintain IASC and the mechanisms capable of resolving it. A key question is whether evidence of on-going conflict is indicative of conflict that cannot be resolved, or simply a transient evolutionary stage before resolution. The literature provides some convincing evidence that genetic and behavioural innovations can allow the sexes to independently reach optimal trait values (see section 1.4). It is for this reason that dimorphic gene expression and sexual phenotype dimorphism are thought to have evolved (Lande 1980). In contrast, genetic barriers and stochastic selection pressures (see sections 1.5 and 1.6) may ensure that the sexes remain constrained by intersexual genetic correlations, thereby preventing resolution. Consequently, the potential for resolution (or its impediment) is likely to be population- and/or trait specific, and knowledge of why (or why not) conflicts are resolved is critical to understanding their evolutionary dynamics. In **Chapter 3** I explore how the environment affects the dynamic of IASC through empirical observations, finding that minor shifts in temperature can reduce the

strength of conflict for certain genotypes. This is likely to have implications for conflict resolution as it creates inconsistent selection for sex-specific gene expression.

I also explore the links that exist between intra- and interlocus sexual conflict (IRSC: sexual conflict that occurs over the outcome of male–female interactions rather than shared traits; Rice and Holland 1997), as they appear to be closely associated through reciprocal interactions (see Box 1.1). Historically, IASC was overshadowed, as attention was drawn to IRSC and the co-evolutionary arms-races that follow, potentially driving speciation and diversification (Chapman *et al.* 2003a; Arnqvist and Rowe 2005; Tregenza *et al.* 2006); however, interactions between these two forms of sexual antagonism could have important evolutionary consequences, which have not been reviewed before, nor investigated empirically. There are several possibilities, including the potential for IRSC to alter selection on traits that are shared between the sexes, thereby fuelling IASC. On the other hand, IASC may prevent a trait from evolving in one sex, which could stall arms-races that result from IRSC. Resolved conflict could also present an opportunity for a trait to become exaggerated in one sex, potentially causing IRSC if a novel and harmful interaction between the sexes is formed. These predictions were tested with a quantitative genetic model of traits involved in intra- and interlocus sexual conflict in **Chapter 4**. This model supports the idea that IASC can slow down inter-locus arms races, but also introduces a new hypothesis that IASC can alter the direction of arms-races. This has additional consequences for IASC resolution as it can create inconsistent selection mechanisms for sex-biased gene expression, similar to the effects of a changing physical environment.

IASC concerns conflict between the sexes, but this form of genomic conflict could exist between individuals of the same sex that exhibit other forms of polymorphism (e.g. vocal versus sneaker frogs, or colour polymorphic beetles). I draw upon the example of polymorphism between queen and worker castes in social animal societies, which are distinguished by their division of labour and reproduction. The two castes share a genome but require alternative expression

of genes to produce different phenotypes, potentially sparking an analogous conflict to IASC (intralocus caste conflict: IACC). I review the underlying evolutionary principles connecting IACC and IASC and the empirical evidence for IACC. I also suggest a framework for IACC research based on IASC, and highlight the practical difficulties of studying this analogous conflict in different systems that lend themselves to contrasting experimental approaches in **Chapter 5.**

Finally, in **Chapter 6** I discuss the widespread evolutionary significance of IASC and suggest fruitful avenues for future research in light of theoretical and empirical work presented in this thesis. I also discuss the broader biological consequences of sexual conflict. For example, IASC has been implicated in processes of speciation (Rice and Chippindale 2002), and in maintaining alleles involved in disease (Gilks *et al.* 2014).

Box 1.1: Interactions Between Intra- and Interlocus Sexual Conflict

The first potential interaction to consider is how interlocus sexual conflict (IRSC) may be able to ignite IASC (Figure 1.1a). Consider male mating rate as an example. Often, as mating frequency increases, male fitness is expected to increase accordingly; however, females are expected to incur relatively greater costs from multiple mating compared with males (Thornhill and Alcock 2001). This includes time and energy costs, as well as increased risk of pathogen/parasite infection, predation, and injury. Therefore, by increasing male mating rate, this could consequently promote IRSC and therefore create positive selection for females to reduce the effects of male harassment. Genes involved in mating resistance, however, could be intersexually genetically correlated. This may consequently spark IASC over resistance traits. Innocenti and Morrow (2010) also suggest another possible link between inter- and intralocus sexual conflict. They identified transcripts from sex-limited tissues that are thought to be mediating IASC, such as those expressed in accessory gland and sperm-storage organs. The authors suggest a link between the two forms of sexual antagonism because these tissues are also thought to be important in mediating male–female coevolutionary arms races that stem from IRSC (Chapman *et al.* 2003a; Pitnick *et al.* 2009).

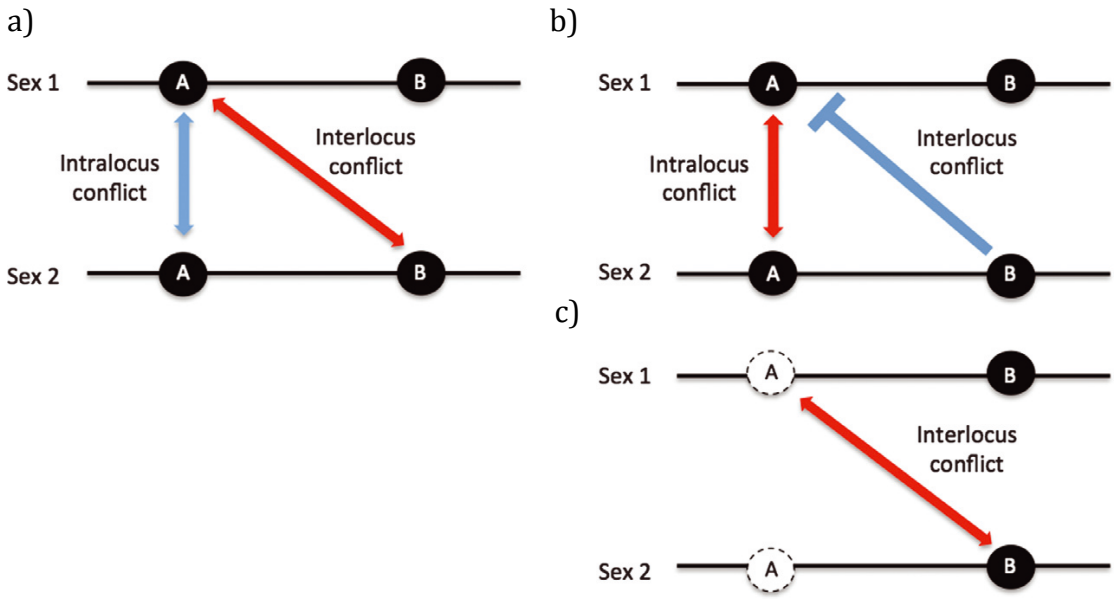
Second, if IASC over a trait remains unresolved, then counter adaptations in response to IRSC may be inhibited (Figure 1.1b). In the case described above, males would be permitted to evolve toward their optimal fitness value for mating frequency, while the female resistant trait (and therefore mating rate) may be trapped at a suboptimal value. This could explain why counter adaptations in some female traits are not apparent, even though they are expected to arise. This may lead to false assumptions that females benefit from high (observed) mating frequencies, when in fact they do not.

A third interaction to consider is that which stems from resolved conflict, that is, if mechanisms arise to resolve conflict (enabling males and females to evolve

Box 1.1 Continued

independently of each other) this may allow a male trait to become exaggerated to a point where it reduces female fitness due to harmful interactions (Figure 1.1c). For example, many male sperm traits are under the control of duplicate genes that are expressed solely in males (Wyman *et al.* 2012). As mentioned previously, this may have evolved as a way to resolve IASC. These sperm-related genes, however, are often found to be rapidly evolving under positive selection (Swanson and Vacquier 2002), which is possibly due to coevolutionary arms races between the sexes that result from IRSC. The release from IASC may thus have contributed toward these arms races. Consequently, female fitness may be reduced by IRSC in a way that is comparable to the reduction in fitness caused by IASC. This also raises questions regarding whether resolving IASC ultimately achieves net fitness benefits within a population.

Figure 1.1 - The Possible Interactions Between Intra- and Interlocus Sexual Conflict: loci are represented by letters (A/B) surrounded by circles (closed = existing conflict, open = resolved conflict). Selectional forces and responses to selection are represented by red and blue arrows, respectively - a) IRSC selects on a shared trait to cause IASC; b) IASC can prevent a trait from coevolving in response to selection caused by IRSC; c) Resolved IASC can allow a trait to coevolve in response to IRSC, thereby enabling an intersexual arms race.



1.2 - An Ongoing Conflict

IASC is receiving an increasing amount of attention from evolutionary biologists, taking the form of various studies – both at the phenotypic and genetic level (Rice and Gavrilets 2014). A large body of evidence for ongoing IASC comes from correlative studies in particular. This includes hemiclonal analysis, a method developed by Rice (1996) for use in the fruit fly *Drosophila melanogaster*, where the direct effects of genome-wide allelic variation on sex-specific fitness can be observed via the production of "hemiclones". Here, distinct crosses force the inheritance of whole haplotypes intact, creating many individuals of both sexes that share the same haplotype (see section 1.9 for a methods description). This permits experiments to measure the fitness of a genome in relation to which sex it is expressed in. Studies that have used this quantitative genetic approach have repeatedly demonstrated negative r_{MF} for fitness within populations, which is a signature of ongoing IASC because it indicates that the average additive effects of genes are sexually antagonistic, thereby causing opposite fitness effects in each sex (Rice 1998; Chippindale *et al.* 2001; Gibson *et al.* 2002; Pischedda and Chippindale 2006; Long and Rice 2007; Delcourt *et al.* 2009; Bedhomme *et al.* 2008; Innocenti and Morrow 2010; Hesketh *et al.* 2013). The value of r_{MF} is calculated as the ratio of the additive genetic covariance for fitness between the sexes, to the geometric average of male and female specific additive genetic variance for fitness. Alternatively, isofemale lines can be created through quantitative genetic breeding designs, which has been used to express the same set of genes in both sexes and to demonstrate negative r_{MF} for fitness in both *Drosophila* (Punzalan *et al.* 2014) and seed beetle *Callosobruchus maculatus* (Berger *et al.* 2014) populations.

Further evidence of ongoing conflict comes from studies showing reduced fitness of opposite-sex offspring. Similar to hemiclonal analysis, these correlative studies illustrate how a fit male genotype can be less fit when expressed in a female – and vice versa. IASC has been demonstrated in this way in a laboratory study of ground crickets *Allonemobius socius*, where higher fitness males were shown to sire high fitness sons, but low fitness daughters (Fedorka and Mousseau 2004). Later studies of wild mountain goat *Oreamnos americanus* and

red deer populations *Cervus elaphus* further demonstrate that opposite-sex offspring suffer declines in fitness (Foerster *et al.* 2007; Mainguy *et al.* 2009). Pischedda and Chippindale (2006) opted for a different approach, using hemiclonal analysis to produce high and low fitness hemiclones, and then subsequently measuring the fitness of offspring from both males and females. Consistent with IASC theory, there was a negative correlation between the fitness of parents and their opposite-sex offspring. Furthermore, there is evidence that IASC can exist in humans, with negative correlations found between the sexes for traits associated with reproductive success (Garver-Apgar *et al.* 2011; Stulp *et al.* 2012; Morrow 2015).

Artificial selection regimes can also be applied to demonstrate ongoing conflict. Mokkonen *et al.* (2012) artificially selected on male testosterone levels in bank voles *Myodes glareolus*, leading to increased male reproductive success, but declines in female reproductive success. Earlier work by Morrow *et al.* (2008) enforced gender-limited selection independently in each sex through experimental constraints on reproductive success in *D. melanogaster*. This resulted in a decline in the net adult fitness of the non-selected sex relative to the selected sex. Prasad *et al.* (2007) found parallel evidence in the same system, by imposing gender-limited selection in a different way – the X and autosomal chromosomes were experimentally forced to co-segregate as haplotypes and thus to be transmitted from father to son. This novel method prevented female-specific selection in most of the haploid genome, which could then be expressed in males and females, and the sex-specific fitness consequences of male-limited evolution characterised.

The overall picture is that IASC is taxonomically widespread, existing in insects and vertebrates, including humans. This raises a fundamental question of why this conflict is so prevalent. This can be answered through gaining an understanding of its dynamic, which I explore in the subsequent sections of this chapter.

1.3 - The Genomic Location of Sexually Antagonistic Alleles

It is evident from the studies cited above that IASC is widespread among organisms with separate sexes. Yet, the genomic distribution and fitness effects of antagonistic loci remain largely unknown. Theory suggests that such an allele can exist on any chromosome (autosome or sex chromosome) when its fitness benefits to one sex outweigh the costs imposed on the opposite sex (Rice 1984; but see Fry 2010); however, for XY systems, it is predicted that there are more sexually antagonistic alleles on the X chromosome than elsewhere (Gibson *et al.* 2002; Lindholm and Breden 2002; Fitzpatrick 2004; Tower 2006; Innocenti and Morrow 2010; but see Fry 2010). Specifically, male-benefit recessive alleles and female-benefit dominant alleles are expected to accumulate here. If we consider X-linked recessive alleles that are male benefit, they are always expressed in males (because males are hemizygous in XY systems), but expressed in only half of all females (those that are homozygous for this allele). Consequently, there is weak selection against them in females, because the benefits are exposed to selection more frequently than the costs (Rice 1984). Similarly, female-benefit dominant alleles will also be selected to accumulate on the X chromosome, because they are expressed two thirds of the time in females, but only one third of the time in males (Rice 1984). Following Rice's theory, the patterns of expression that occur on the X chromosome could also enable a sexually antagonistic allele to be selected for, even if the costs imposed on one sex exceed the benefits to the other. Under these circumstances, they could cause net fitness loss within a population. It may therefore be expected that sexually antagonistic alleles of greatest fitness effect may be found on the X chromosome, rather than autosomes. This could explain observations by Pischedda and Chippindale (2006) and Foerster *et al.* (2007), who found that high fitness sires had low fitness daughters, whereas there was no correlation between sire and son fitness. We might expect such a pattern to arise if the most significant antagonistic fitness effects are caused by X-linked alleles, which consequently will not be inherited from father to son.

Rice (1984) modelled changes in the frequency of X-linked sexually antagonistic alleles over time. Due to the fitness costs imposed on the opposite sex, such

alleles never reached fixation within a population, but were instead maintained at a stable equilibrium frequency. Recently, Dean *et al.* (2012) characterised the dynamics of an X-linked sexually antagonistic allele empirically, which before now had only ever been predicted by theory. They artificially created a male-benefit sexually antagonistic allele that resided on the X chromosome and reduced female fitness when expressed in a homozygous state. After 23 generations, this allele increased in frequency from 3% to 8%. Additional populations were created where the initial frequency of the antagonistic allele was at a higher percentage (35–85%). After three generations, the frequency of the allele declined. This novel approach has provided a valuable insight into the maintenance of IASC, showing that the X chromosome is capable of harbouring sexually antagonistic alleles at an equilibrium frequency, much like Rice (1984) had anticipated.

A model by Mullon *et al.* (2012) also considered how genetic drift might differentially affect the maintenance of antagonistic alleles on the autosomes and sex chromosomes. For XY systems, it is often assumed that genetic drift affects the X chromosomes to a much greater extent due to their smaller effective population size (Vicoso and Charlesworth 2009). It could therefore be expected that the X chromosome might actually harbor fewer sexually antagonistic alleles, due to selection being less efficient in the face of drift; however, Mullon *et al.* (2012) argue that genetic variation at sexually antagonistic loci is actually more likely to be maintained on the X chromosomes than the autosomes; this is due to increased reproductive variance in males, which subsequently increases the effective population size of the X. The opposite is thought to be true in ZW systems, where females are the heterogametic sex. Under these circumstances, the Z chromosome will have a low effective population size compared to the autosomes because of the lower reproductive variance in females (Mullon *et al.* 2012). Consequently, there may be a contrast between the genomic location of sexually antagonistic loci in XY and ZW systems, with the sex chromosomes harbouring more sexually antagonistic alleles in XY systems.

A better insight into the genetic basis of IASC could be achieved through the application of molecular and genomic tools. Recent technological advancements in sequencing methods are laying the foundations for such fine-scale genomic studies (Davey *et al.* 2011), which will allow the location and function of sexually antagonistic genes to be identified. This would be an important development, as genetic studies of this kind are currently scarce (Williams and Carroll 2009). Combined research by Smith *et al.* (2011a) and Rostant *et al.* (2015) however, has identified an allele involved in DDT resistance that is sexually antagonistic. The allele identified (autosomal gene, cyp6g1) confers DDT resistance when upregulated by the insertion of a transposable element (DDT-R). Previously, females that expressed cyp6g1 were found to have higher fitness, even in the absence of DDT (McCart *et al.* 2005). Nevertheless, before the use of DDT as an insecticide, the DDT-R allele existed in natural populations at low frequency. This raises questions concerning why the DDT-R allele did not rise to high frequency in spite of fitness benefits to females. Smith *et al.* (2011a) suggested this might be a result of sexual antagonism, as they found some evidence (although inconsistent) for a fitness cost to males of up-regulating cy6bg1. This was recently confirmed by an experimental evolution study by Rostant *et al.* (2015), who showed that the frequency of the DDT-R allele was maintained at the same equilibrium frequency as expected by their theoretical model of the DDT-R allele if evolving under IASC.

In order to identify more extensive patterns of intralocus conflict, the application of modern genomic tools may be useful in some organisms. This could facilitate the identification of correlations between genes, sex, and fitness, which could potentially provide strong evidence for the occurrence of IASC if followed up by mechanistic studies. Innocenti and Morrow (2010) made some progress toward identifying the molecular basis of sexually antagonistic genome-wide variation. They fitted a regression model to test for associations between gene expression, fitness and sex in *D. melanogaster*. Use of the FlyAtlas database (a resource developed by Chintapalli *et al.* 2007) also allowed the authors to identify tissue-specific patterns of sexually antagonistic transcripts. A total of 8% of *D. melanogaster* transcripts were shown to be sexually antagonistic, with

enrichment in all tissues except for the gonads. The pattern described may result from the gonads' specific regulatory mechanisms and a lack of correlation between the genes expressed here and those expressed in other tissues. These results are interesting because they imply ongoing sexual antagonism through almost the entire body. Also, the proportion of transcripts shown to be sexually antagonistic relative to the proportion that was related in some way to adult fitness was large (~60%). It is also likely to be a conservative estimate, as conflict over different traits may arise at other life stages due to dramatic changes in selection pressures throughout development.

1.4 - Conflict Resolution Mechanisms

Conflict resolution is an active topic for biologists studying IASC. This owes to the uncertainty of whether traits experiencing IASC will eventually reach resolution, or whether they will remain in this state indefinitely. In order to address this question, we need to consider the possible mechanisms of resolution. This will help us to dissect traits on an individual basis to predict their evolutionary fates and the consequences for whole-organism fitness.

An abundance of theoretical work suggests that conflict could be resolved via a number of mechanisms, which together or in isolation, would relieve the gender load that arises when the sexes are displaced from their fitness optima. Sexual dimorphism is suspected to represent conflict resolution and is thought to be caused by underlying changes in the genetic architecture of a particular trait, which then permits males and females to evolve along their own independent trajectories. This occurs when underlying genetic changes cause the intersexual genetic correlation (r_{MF}) to deviate from 0. In fact, a negative correlation between r_{MF} and sexual dimorphism was identified across most traits in the fly, *Prochyliza xanthostoma* (Bonduriansky and Rowe 2005a).

To test whether sexual dimorphism represents a robust resolution to IASC, Tigreros and Lewis (2011) applied artificial selection to a dimorphic trait (body size) in opposing directions to each sex. They were able to demonstrate that once dimorphism evolves, it can be irreversible under short-term selection; thus

signifying a resistant resolution to sexual conflict. It may then be reasonable to assume that as the evolution of sexual dimorphism is biologically widespread (Darwin 1871), then perhaps conflict resolution is too.

This might hold true to some extent; however, sexual antagonism has in fact been found to affect even highly dimorphic traits (Pischedda and Chippindale 2006; Long and Rice 2007; and see Bedhomme *et al.* 2008). Furthermore, a review of selection estimates for 89 traits taken from 34 species reinforces these findings (Cox and Calsbeek 2009). As Cox and Calsbeek (2009) state, if the extent of dimorphism does not match up to the fitness peaks of the two sexes, then sexual dimorphism will not be an indication of permanent conflict resolution. More support is provided by Innocenti and Morrow (2010), who identify existing conflict over traits with sexually dimorphic gene expression. In their study, almost 92% of the genes identified were found to be sex biased in expression, and only 8% of these were actually shown to be sexually antagonistic. As conflict may be absent for many of these dimorphic transcripts, this could be an indication of widespread conflict resolution. To predict whether these patterns have evolved under positive selection in response to IASC between the sexes, it is necessary to assess the fitness consequences of sex-specific expression levels. Indeed, a look at genome-wide transcription profiles reveals that a considerable amount of sex-biased gene expression is related to sex-specific functions with positive fitness effects (Connallon and Clark 2011a). Therefore, we could envisage that the dimorphic gene expression patterns shown by Innocenti and Morrow (2010) might have evolved as a mechanism to resolve conflict; however, for some genes identified in this study, sex-specific transcription did not always predict sex-specific functions or fitness consequences. This highlights how some transcriptional differences between the sexes may not have evolved directly in response to IASC. These studies provide consistent evidence that although sexual dimorphism could theoretically permit resolution, its use as a signature of resolved conflict should be avoided.

Nevertheless, there are many theoretical examples of how sex-specific gene expression (which may lead to sexual dimorphism) could resolve IASC. One way

to achieve this is via sex-specific hormonal cascades or modifiers (Rice 1984). For example, secondary sexual trait expression is determined by testosterone levels in vertebrates (Mougeot *et al.* 2004; Blas *et al.* 2006) and titers of juvenile hormone in insects (Emlen *et al.* 2006), both of which differ between the sexes. These hormone levels will subsequently affect the induction of intracellular signaling that leads to changes in gene transcription. Concentrations of regulatory proteins that target specific genes can also affect the level of gene transcription. These regulatory proteins play an important role in *D. melanogaster* and *Caenorhabditis elegans*, for example, by initiating sex-specific developmental pathways (Yi and Zarkower 1999; Yi *et al.* 2000). There are outstanding questions regarding the birth of such gene expression patterns, as dimorphism could either result from the repression or gain of gene expression in one sex relative to the other (Williams *et al.* 2008). Nevertheless, there are a handful of studies addressing this question, where the authors have been able to identify genes involved in regulating sexual dimorphism, and predict an ancestral state of monomorphic expression for some traits (Emlen *et al.* 2007; Williams *et al.* 2008; Moczek and Rose 2009; Williams and Carroll 2009; Khila *et al.* 2012). A phylogenetic analysis of wing pattern evolution in butterflies also found evidence that for some traits, sex-limited gene expression occurred simultaneously as the trait arose in a lineage; whereas, for other traits there was an ancestral state of dimorphic expression, followed by the subsequent loss of expression in one sex (Oliver and Monteiro 2011).

An additional mechanism for controlling sex-specific gene expression is through alternative splicing (McIntyre *et al.* 2006). Here, sex will determine the final protein form that is produced from a shared coding region in the genome. This is a post-transcriptional process, where the RNA produced from a single gene is spliced in alternate ways through the joining of different exon combinations. McIntyre *et al.* (2006) conducted a genome-wide analysis of alternative splicing in *D. melanogaster*, discovering that at least 12% of all genes are spliced in this sex-specific manner. Although empirical data are lacking, it is possible that the patterns of sex-biased alternative splicing described here may have evolved to resolve IASC.

The translocation of genes to sex chromosomes could also facilitate sex-limited gene expression (Charlesworth and Charlesworth 1980; Rice 1984; Bachtrog 2006). It is thought that some male-benefit, female-detriment genes have been translocated from autosomes to the Y chromosome for example (Bachtrog 2006), consequently enabling males to evolve independently of females (in species where females are the homogametic sex). In order for this to resolve conflict, however, females must not require the translocated gene for functional purposes. As this is unlikely for most genes, a favourable alternative hypothesis is that genes are duplicated, translocated, and then undergo sex-limited gene expression (Ellegren and Parsch 2007; Baur *et al.* 2008; Connallon and Clark 2011b; Gallach and Betrán 2011). In this scenario, duplications initially produce additional gene copies with identical function, but they can later be released from the ancestral function by evolving freely through mutation and selection (Wyman *et al.* 2012). When followed by sex-limited expression, this can subsequently allow the sexes to diverge in their trait values. The duplications produced can also be translocated to non sex-chromosome locations (Mank 2009), with sex-specific modifiers evolving to control their expression on autosomes too. A recent analysis of gene expression by Wyman *et al.* (2012) found that these duplicate pairs are typically male-biased in expression. This is suggested to be a product of sperm competition, as this can create strong sexual selection pressures on male traits, such as those related to ejaculate function (and may also have implications for IRSC; see Box 1.1).

Genomic imprinting presents another possible mechanism to alleviate IASC through sexual dimorphism (Day and Bonduriansky 2004; Patten and Haig 2008). Imprinting relies on changes to DNA methylation patterns that occur during gametogenesis in parents and affect the expression of genes in offspring. The best-known examples are igf and igf2, growth factors that are known to be silenced when inherited paternally (Barlow 1995; Ferguson-Smith and Surani 2001). Simulations indicate that this pattern could arise due to IASC, as long as the benefits of imprinting an antagonistic allele in one sex outweigh the costs of doing so in the other (Day and Bonduriansky 2004). The possibility for an

imprinting modifier allele to invade a population in this way is also heavily dependent on dominance, as shown in simulations by Cleve and Feldman (2007). Their study built upon a previous model by Day and Bonduriansky (2004), where only additive variation for fitness was considered. Despite these findings, for imprinting to fully resolve conflict it would be necessary for parents to imprint genes in a gamete karyotype-specific manner. For example, males should imprint genes so that male-benefit sexually antagonistic alleles are switched off in X-bearing sperm only. This would enable males to increase the fitness of sons, without detrimentally affecting the fitness of daughters. This mechanism would include imprints on autosomes that were dependent on whether they are found in X or Y sperm. Despite this theoretical requirement for resolution, the occurrence of imprinting in this manner is yet to be proven empirically. So far, 80 genes are recognised as being imprinted in mammals (Morison *et al.* 2005), although others propose that this figure could actually exceed 600 (Luedi *et al.* 2005). Imprinting therefore presents another potential mechanism with capabilities of resolving conflict on a genome-wide scale, but one that lacks empirical support.

Sexual dimorphism has also been shown to increase for some traits as a result of condition dependence, by weakening r_{MF} (Bonduriansky and Rowe 2005b). Condition dependence is expected to evolve for traits that are under strong sexual selection, which as a result become exaggerated to a point where they are costly to produce – hence the expression of these traits comes to reflect condition (Rowe and Houle 1996). If the level of condition dependence of a trait becomes unequal between the sexes, then this may permit the elaboration of a trait in only one sex, consequently exaggerating the degree of sexual dimorphism; however, Bonduriansky and Rowe (2005b) do not quantify the fitness consequences of sexual dimorphism through condition dependence; therefore, its ability to resolve conflict was not clear. It is also necessary to explore the genetic mechanisms facilitating this as it is also unclear how trait r_{MF} affects the potential for condition dependence (Bonduriansky and Rowe 2005b).

Rather than confronting the genetic basis of IASC, some species appear to have evolved an alternative way to mask the effects of sexually antagonistic genes – sex ratio adjustment (SRA). It is conceivable that this strategy presents a means of partially resolving IASC when it is not possible to achieve sex-limited gene expression via changes to trait genetic architecture. A study conducted in the wild and follow-up laboratory investigation revealed how side blotched lizards, *Uta stansburiana*, are likely to choose sperm depending on the phenotype of their mate (Calsbeek and Sinervo 2004; but see Calsbeek *et al.* 2015). This enables females to select the sex of their offspring as a remarkable way to diffuse IASC. For instance, females mated to larger males produce more sons because size is positively correlated to male fitness, but negatively correlated to female fitness. In accordance, a small sire results in increased production of daughters. Both sexes benefit from this since it presents an opportunity to maximize the fitness of their progeny in the face of antagonistic alleles. There are parallel findings in brown anoles, *Anolis sagrei* (Calsbeek and Bonneaud 2008; Cox and Calsbeek 2010); fruit flies, *D. melanogaster* (Connallon and Jakubowski 2009), and barn owls, *Tyto alba* (Roulin *et al.* 2010).

Katsuki *et al.* (2012) focused on SRA in broad-horned flour beetles, *Gnatocerus cornutus*. Interestingly, the sex of offspring produced by a female depended on her own fitness, rather than that of her mate. A low fitness female produced opposite-sex offspring, whereas higher fitness females increased the production of daughters. Why the fitness of their mate had no effect on offspring ratio was not clear, but by basing offspring ratio on recognition of their own fitness, females could increase their inclusive fitness and that of their mates. Although lacking any pertinent evidence, they suggest females could alter their hormone levels to determine offspring sex.

A simple model was also developed by Blackburn *et al.* (2010) to explore the circumstances under which SRA could evolve. Providing that sufficient genetic variation exists at SRA loci, then SRA is expected to evolve rapidly. They note that while they only looked at a single gene, SRA is equally likely to evolve in the

presence of many sexually antagonistic genes if it results in a net increase in fitness.

As well as allowing us to understand the selection pressures leading to sex ratio adjustment, these studies reinforce the argument that IASC can in fact have evolutionarily important outcomes. Nevertheless, to obtain a more complete picture, the proximate mechanisms leading to SRA require much greater empirical attention.

It would seem that IASC could be eliminated through both genetic and strategic innovations; however, this is not to say that sexual antagonism for every trait may be so easily resolved. In particular, there is much to learn about the genetic mechanisms behind the evolution of sexual dimorphism and how these work to alleviate IASC (Rhen 2000; Rice and Chippindale 2001, 2002; Day and Bonduriansky 2004; Bonduriansky and Rowe 2005b); especially in the face of strong intersexual genetic correlations (Lande 1980) or when pleiotropic genes are involved (Badyaev 2002; Ellegren and Parsch 2007; Van Doorn 2009). Moreover, despite expectations that sex-biased gene expression could rapidly evolve to diminish sexual conflict (Reeve and Fairbairn 1996, 2001; Van Doorn 2009), others describe this conclusion as premature (Stewart *et al.* 2010). This is supported by evidence that low levels of sexual antagonism can exist for traits that appear to be sexually dimorphic (Harano *et al.* 2010). Perhaps, IASC in some traits can only ever be partially resolved, with a simmering level of sexual antagonism always maintaining fitness levels below optima for the sexes. To understand, this requires a look at the potential barriers to conflict resolution, for which there is some convincing evidence.

1.5 - Barriers to Conflict Resolution

As previously mentioned, r_{MF} is negatively correlated with many sexually dimorphic traits (Bonduriansky and Rowe 2005a), owing to the fact that when the sexes share the same genetic architecture for a trait it becomes difficult for them to become sex limited in expression and thus to become sexually dimorphic. Measurements indicate that r_{MF} for many traits is high (Lande 1980;

Meagher 1994; Roff 1997; Merilä *et al.* 1998; Delph *et al.* 2004; Mank 2007; Chenoweth *et al.* 2008), which also implies that it could be difficult to resolve IASC through sexual dimorphism. While some propose that mutations with sex-biased effects could accumulate given enough time, which would weaken the r_{MF} and permit the evolution of sex-limited gene expression (van Doorn 2009), others contend this. Stewart *et al.* (2010) state that the evolution of some mechanisms to achieve sex-limited gene expression (gene duplication, alternative splicing) will be very slow unless the gene is already controlled by a sex-specific DNA regulatory binding site, or if a duplicated gene can be translocated to where it can be regulated in such a way. In contrast, changes involving sex-specific gene regulation might resolve IASC in a far shorter timeframe (Ellegren and Parsch 2007).

The effectiveness of gene duplication in relieving IASC could also be lessened if it consequently disrupts existing gene networks after translocation (Force *et al.* 1999; Gu *et al.* 2004; Huminiecki and Wolfe 2004; Gallach and Betrán 2011). It could also prove to be a poor resolution, as any mutations that arise will not be exposed to selection in the non-expressing sex. This could cause mutations to accumulate in this gene, which may consequently be deleterious when expressed in the opposite sex. In other words, the mutational load will be doubled as the gene is only exposed to selection half of the time (Morrow *et al.* 2008). Furthermore, after duplication and translocation, genes could be indirectly selected via covariance with other genes, causing IASC to reoccur in a trait where it was once temporarily resolved (Hosken 2011).

Pleiotropic interactions between those genes involved in sexual antagonism, and those that are not, could be a common impediment to conflict resolution (Badyaev 2002; Ellegren and Parsch 2007; van Doorn 2009). Harano *et al.* (2010) suggest a role for pleiotropy in mediating IASC in *G. cornutus*. Here, resolved conflict appears to be depicted by the stark contrast between a male's exaggerated mandible size and a female's absence of this exaggeration. To explore this further, Harano *et al.* (2010) used artificial selection to increase male mandible size; but while there was no correlated response in female

mandibles, female fitness declined simultaneously as male fitness increased. A proximate explanation for the reduction in female fitness is that a reduction in female abdomen size, which also occurred in response to selection on male mandible size, affected egg production, and lifetime reproductive success. This provides some support for the idea that there may be genetic covariance between mandible size in males and a trait that is sexually antagonistic. Despite conclusions made by Harano *et al.* (2010), it should also be considered that similar effects on female fitness might also result from IRSC; for example, an increase in male mandible size may have promoted a harmful interaction between the sexes that directly reduced female fitness.

The scale of pleiotropic effects is not fully resolved, but Fitzpatrick (2004) found a majority of genes to be pleiotropic in *D. melanogaster*. Genes were randomly sampled from FlyBase (http://www.flybase.net) and categorized as being pleiotropic if they contributed to two or more traits (e.g. if a single gene was associated with different structures, behaviours or molecular processes). Of the genes studied here, 78% were deemed pleiotropic, and most were putatively sexually selected but not preferentially sex-linked. Under the premise that this pattern reflects that found across the genome, pleiotropy could present a significant obstacle to whole-genome conflict resolution. Mank *et al.* (2008) provide further evidence for pleiotropy as a constraint to resolution, although using tissue specificity in expression as a proxy for actual pleiotropy, with tissue-specific genes deemed less pleiotropic than non-specific genes. The specificity of genes was then compared to the level of sex-biased expression. A consistent relationship was identified between sex-biased gene expression and tissue specificity in both mice and chickens. This is expected to represent resolved conflict, as these genes may have been able to achieve sex-biased gene expression due to lack of pleiotropic constraint. The results also suggest that most pleiotropic genes are those experiencing sexual antagonism, which is supported by the link between pleiotropy and absence of sex-biased expression; however, the validity of this proxy is debatable since genes can be expressed in multiple tissues and serve the same function in each of them. Conversely, a gene that is expressed in only one tissue may function in completely different ways

throughout development. Also, as mentioned previously, caution should be taken when using sex-biased expression as a proxy for resolved conflict.

It is clear that IASC could be more easily resolved for some traits than others, and that a gender load may always exist due to underlying genetic architecture. As discussed, there are multiple genetic obstacles that contribute toward making genome-wide resolution practically impossible, especially as many genes serve multiple functions as well as the antagonistic trait (Ellegren and Parsch 2007). There is, however, an important gap in our knowledge of the genetic basis of sexual antagonism. This could be filled through studies that focus on the genes underlying this conflict and the genetic architecture of sexually dimorphic traits that appear to represent conflict resolution. This is relevant because there is no clear evidence for how sex-specific regulation evolves for genes that are under sexually antagonistic selection (Mank 2009).

1.6 - The Dynamics of Conflict Resolution

Mank *et al.* (2011) took an interesting perspective on IASC, linking sex-chromosome evolution to dosage compensation and sexual antagonism. Sex chromosome evolution may be a product of sexual antagonism, allowing sex-limited expression of genes to diffuse conflict; however, a consequence could be that some genes on the X chromosome are hyper-transcribed in the heterogametic sex in an attempt to compensate for having only one X chromosome. This in itself sets the stage for IASC, as it can result in overexpression of genes in the homogametic sex and subsequent counter-adaptations to reduce transcription levels, which could be an important factor when considering the maintenance of sexual antagonism and prevention of resolution.

Heterogeneity in sex-specific optima (van Doorn 2009) could also weaken selection for conflict resolution, because the fitness consequences of possessing an allele would become variable over space and time in each sex. For instance, sexual conflict environment could alter the selection pressures acting on antagonistic alleles and stall conflict resolution (Brommer *et al.* 2012). This

could occur if a female trait to minimise the cost of mating (i.e., arising from IRSC) increased fitness in environments with a high exposure to males, but caused a decrease in fitness in low exposure environments (Brommer *et al.* 2012). The physical environment could also affect trait optima for the sexes (Mokkonen *et al.* 2012), with heterogeneous conditions potentially causing parallel selection pressures to those found by Brommer *et al.* (2012).

Condition dependence could work in a similar way. Although Bonduriansky and Rowe (2005b) found that condition dependence could resolve conflict, they note that this may depend on the function, costs, and genetic architecture of the sexually antagonistic trait. They also showed that intersexual genetic correlations for condition dependence could evolve, which may in fact cause sexual conflict itself. From another perspective, perhaps this alters the dynamics of selection for any kind of conflict resolution. Intersexual genetic correlations for condition dependence, for example, will mean that any selection on a trait will be dependent on both male and female condition, and how gene expression and fitness is subsequently affected. Therefore, such variable selection pressures for sex-limited gene expression could maintain sexually antagonistic alleles and render conflict resolution less probable. This is comparable to the variable selection pressures caused by environmental heterogeneity. This is an interesting avenue for future research, particularly as there is no clear evidence for whether condition dependence could eliminate or exaggerate IASC (Bonduriansky and Rowe 2005b).

Condition dependence and environmental heterogeneity appear to maintain sexually antagonistic alleles within a population. As this should theoretically create selection for conflict resolution, it therefore seems paradoxical that they could also act to prevent resolution altogether; however, if a trait is condition dependent, or affected by environmental heterogeneity, then at one time IASC and selection for resolution may be strong, yet at other times IASC and selection for resolution could weaken. Such variable selection against IASC could perhaps prevent resolution from evolving at all for some traits. We now consider some other examples of where this could apply.

In an effort to discover the conditions under which sexually antagonistic alleles can be maintained, Arnqvist (2011) used simulations to explore the effects of assortative mating by fitness. In the presence of antagonistic alleles, this translates into disassortative mating by genotype. Based on the conditions that sexually antagonistic variation was polygenic (Patten *et al.* 2010) and fitness exhibited sex-specific dominance (Fry 2010), matings that occurred between individuals of similar fitness were shown to maintain sexually antagonistic alleles in these simulated populations (Arnqvist 2011). As assortative mating based on phenotype is almost ubiquitous in nature, and often correlates with genetic quality, it could therefore maintain IASC in many species (Arnqvist 2011).

Further theoretical work suggests that population size could also influence the maintenance of sexually antagonistic variation (Connallon and Clark 2012). The incorporation of the effects of recurrent mutation and genetic drift into population genetic models of sexual antagonism illustrates this well. One property of antagonistic selection is that it is rendered ineffective in the face of genetic drift (Connallon and Clark 2012). By accounting for the fact that smaller populations are more susceptible to the effects of genetic drift, this means that sexually antagonistic alleles are less likely to occur under these circumstances. Sexually antagonistic alleles are therefore expected to be maintained in larger populations because antagonistic selection is able to override the effects of genetic drift, thus increasing the mean heterozygosity and contribution to fitness variance of these antagonistic loci (Connallon and Clark 2012). Interestingly, an independently derived theory also predicts that IRSC will be greater in larger, higher density populations (Gavrilets 2000), a prediction with some empirical support (Martin and Hosken 2003). Given the numerous potential links between intra- and interlocus sexual conflict (Box 1.1), population size may play a key role in the maintenance of sexually antagonistic alleles.

If the dynamics of population size or mating habits are constantly changing, then this may act to prevent conflict resolution, much like environmental

heterogeneity or condition dependence could. Thus, although these processes are able to increase the level of sexual antagonism at times, at any point when their dynamics change, selection for conflict resolution could be reduced. This could lead to perpetual sexual antagonism without resolution ever evolving. Studying sexual conflict in species that experience stochastic environmental selection pressures and changing population dynamics could help us to understand how the intensity of sexual antagonism could change in this way, and ultimately how this may hinder or promote the evolution of conflict resolution.

1.7 - Study Species: *Drosophila melanogaster*

The intriguing topic of conflict resolution remains somewhat of a black box for IASC research. It is a topic that I shed light on in the following chapters through empirical and theoretical observations. **Chapters 2, 3** and **4** focus on the timescale of resolution and how selection from the physical and social environment can constrain the evolution of sex-biased gene expression. All laboratory work conducted during my PhD consisted of fruit fly-based research, using *Drosophila melanogaster* as the focal study species. Empirical results obtained using *D. melanogaster* are presented in **Chapters 2** and **3**. This section describes this study system in more detail.

D. melanogaster belongs to the family Drosophilidae (consisting of more than 4000 species) and can be found on every continent of the world. Members of this family lay eggs on rotting vegetable/fruit matter. The *D. melanogaster* embryonic developmental stage lasts approximately 24 hours, after which they transform into larvae that consume rotting fruit matter as they grow. This life stage lasts approximately 96 hours, and during this time they undergo three molts: first, second and third instar stages. After each molt the larva take on a progressively larger form. After the third instar stage, pupal development occurs for 96-120 hours. After this time, the flies emerge as adults. Males are sexually mature approximately 8 hours after adult eclosion, whereas females can be mated immediately after adult eclosion by mature males. Prior to mating, the sexes partake in a courtship ritual where a male orientates towards a female, followed by wing vibration (see Figure 1.2c). This display allows both the male and

female to assess each other as potential mating partners based on visual, olfactory, mechanosensory and auditory cues (Spieth 1974). Copulation itself lasts for 15 minutes on average. Both sexes multiply mate, and females can store sperm. This mating system also creates sperm competition between males (Parker 1970).

Adult males and females of *D. melanogaster* exhibit sexual dimorphism (see Figure 1.2a-b), differing in size, pigmentation, number of abdominal segments, structure of genitalia, and the presence of sex-combs (Cowley *et al.* 1986). Less conspicuous sexual dimorphism in *Drosophila* has been shown in pheromone signals, with the two sexes showing distinct hydrocarbon profiles (Foley *et al.* 2007; Ingleby *et al.* 2014).

Fruit flies have been used extensively in the laboratory and have become arguably the most recognised organism in developmental and evolutionary biology research. It was first used for genetic analysis by Morgan (1910), and has since been used to contribute to major principles of genetics. This owes to its short generation time, and the ease of which it can be maintained within and shared between laboratories. Genetic mutants of *D. melanogaster* are both widely available and easy to induce experimentally, contributing to its success as a model organism.

There are benefits of using laboratory-based organisms such as *D. melanogaster*, particularly for sexual conflict research. Foremost, identifying the sex-specific fitness effects of genes is crucial to understanding this conflict and this is often most feasible to do so in the laboratory. Typically, wild studies of fitness have practical limitations, including difficulties of identifying the relatedness of individuals, tracking their movement, the long generation times of study species, limited control over experimental conditions, and difficulties of replicating experiments. There are also caveats of measuring the fitness of wild-caught populations immediately after introduction to the laboratory, as this does not reflect standing genetic variation under conditions to which the population has adapted. Studies of laboratory-adapted populations offer a potential solution, as

they provide a means of measuring fitness in a situation that mimics a population in its natural environment. It is often argued that the benign conditions of the laboratory, however, do not reflect the stochastic nature of a wild environment; but it is the simplicity of such studies that enable us to reveal fundamental processes of evolution. We can also test the effect of tightly controlled variables, to build an understanding of evolutionary dynamics that can be applied to wild populations.

The laboratory-adapted population I studied throughout my PhD (LH_M) was ideal for it's purpose due to reasons explained above (see section 1.8). This population has been maintained in laboratories for over two decades, and has been shared between laboratory groups, enabling an accumulation of insightful sexual conflict research on a single population over time (e.g. Chippindale *et al.* 2001; Gibson *et al.* 2002; Rice *et al.* 2005; Long and Rice 2007; Delcourt *et al.* 2009; Innocenti and Morrow 2010; Abbott *et al.* 2013; Innocenti *et al.* 2014). In addition, a powerful technique for quantitative genetic analysis – hemiclonal analysis – is feasible is *D. melanogaster* and this provides a means of testing the additive effects of genes on sex-specific fitness with the capacity to replicate experiments on a large-scale (see section 1.9).

Figure. 1.2 - Photographs of *D. melanogaster*: a) adult male and b) female and c) their courtship ritual (courtesy of Qinyang Li)

a)

b)

c)

1.8 – LH_M and Competitor Stock Populations

LH_M Population

LH_M is a large outbred population of *D. melanogaster*, so named because it is a population maintained at a moderate or medium density, founded by Larry Harshman. In 1991, 400 inseminated females were collected from an orchard near Modesto, California. The population has since been maintained at a large effective population size (>1800 breeding individuals) for more than 500 generations. We followed the standard protocol for rearing LH_M (Rice *et al.* 2005).

We maintained flies at a constant temperature of 25°C, humidity of 65%, and a 12:12 hour light:dark photoperiod. Every generation, adult flies (2-3 days after adult eclosion) were kept at a 1:1 sex ratio (16 males and 16 females per 25 mm vial). This adult density created competitive conditions and enabled behavioural interactions to contribute to adult fitness. Within each vial there was 3ml of cornmeal-molasses-agar food and 6mg of dried baker's yeast. After 2 days, adult flies were transferred into fresh vials, containing the agar food mixture without yeast supplement. After 18hrs, during which females were able to oviposit, vials were cleared of flies and the number of eggs was reduced to 150. This maintains the larval density at a moderate level within each vial, thereby reducing but not eliminating competition for food and space.

Competitor Flies

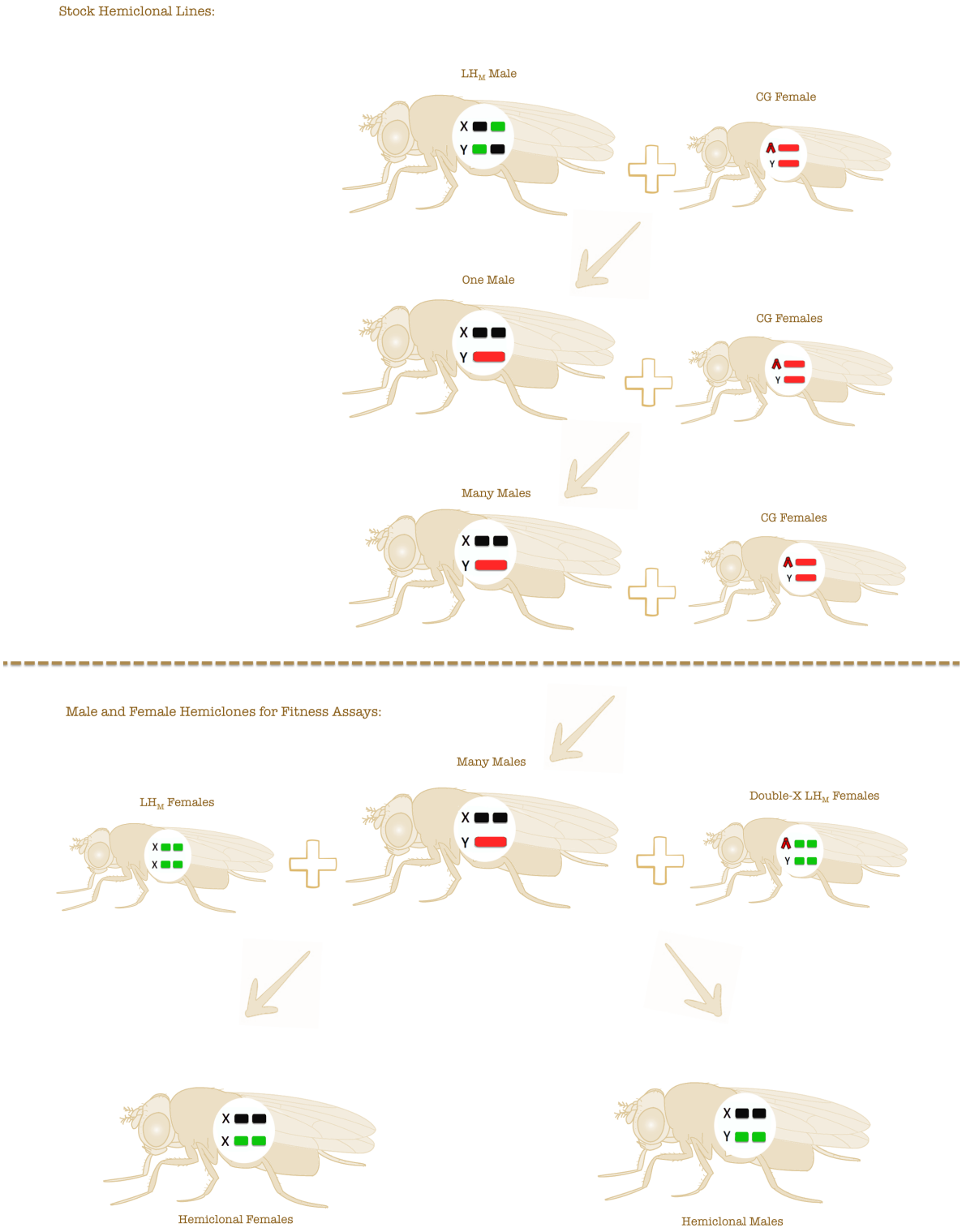
We used competitor flies in fitness assays (**Chapters 2** and **3**), which differ from LH_M at an eye colour locus but are otherwise genetically identical: the LH_M population are homozygous for the red eye-colour allele (*bw+, bw+*), whereas competitor flies are homozygous for the brown-eye colour allele (bw^-, bw^-). Competitor flies were generated following nine generations of backcrossing to LH_M.

1.9 - Hemiclonal Analysis as a Genetic Tool

A key aspect of my experiments (**Chapters 2** and **3**) was the use of hemiclonal analysis, which is a quantitative genetic technique that enables additive genetic variation for sex-specific fitness to be estimated. Here, random individuals are taken from a source population and their genomes are expressed in random genetic backgrounds from the same population, creating many individuals of the same haplotype (illustrated in Figure 1.3) – analogous to fertilizing a set of clonal eggs with many sperm.

Hemiclones are produced through three distinct crosses, involving so-called "clone-generator" females and wild-type males. These females possess a compound or double-X chromosome, where the two copies are physically fused together, and a translocation of the major autosomes 2 and 3. The resulting heterozygous genotype controls transmission of the male-derived complement, producing individuals that are identical across more than 99.5% of their genomic haplotype. By generating multiple hemiclonal lines from one population, this provides a "snapshot" of the standing genetic variation and permits further experiments to measure the fitness of a genome in relation to the sex it is expressed in. Note that given the patterns of inheritance of a hemiclonal genotype, the variation across lines does not include any non-additive dominance variation or maternal effects, although some epistatic interactions remain (Rice *et al.* 2005). It is also notable that the hemiclonal flies produced are as outbred (having the same levels of homozygosity) as any other individual in the base population and this allows us to explore the quantitative genetics of traits using individuals that are genetically and phenotypically indistinguishable from flies within the base population, but with known levels of relatedness. It is a technique that is, for now at least, confined to the *D. melanogaster* model system (Rice 1998; Chippindale *et al.* 2001; Gibson *et al.* 2002; Pischedda and Chippindale 2006; Long and Rice 2007; Bedhomme *et al.* 2008; Innocenti and Morrow 2010), as there is limited scope for its application in other species. This owes to the fact that many systems lack the genetic tools necessary to force the inheritance of whole haplotypes intact (Abbott and Morrow 2011).

Figure 1.3 – Illustration of Hemiclonal Analysis: crosses to produce hemiclonal males and females (modified from Abbott and Morrow 2011). The compound X is represent by a red chevron, and the translocated autosomes 2 and 3 are represented by long red bars. Wild-type chromosomes from LH_M are represented by short green bars and the target hemiclonal haplotype by short black bars.



1.10 – Introduction Summary

There has been an accumulation of theoretical and empirical research on IASC in recent years, which has dramatically improved our understanding of its evolutionary dynamic and biological consequences. The following chapters fill important gaps that have not been covered in the literature, with an emphasis on exploring how the strength of sexual conflict changes over time (**Chapter 2**), how the physical (**Chapter 3**) and social (**Chapter 4**) environment can alter conflict outcome and resolution, and the broader implications of this evolutionary feud (**Chapter 6**). My main approach to conducting empirical tests of IASC theory is to use quantitative genetic analyses with hemiclonal lines of *D. melanogaster* (**Chapters 2** and **3**). As an extension of IASC theory, I also apply concepts of IASC to an analogous conflict between castes in social insects (**Chapter 5**). This demonstrates the multidisciplinary application of sexual conflict theory to understand trait evolution in other contexts.

Chapter 2: Standing Genetic Variation for Intralocus Sexual Conflict and the Timescale of Conflict Resolution

2.1 – Abstract

Evolutionary theory predicts the depletion of genetic variation for fitness traits, as populations converge at a fitness peak. Nonetheless, numerous studies have identified extensive amounts of standing genetic variation for such traits in both natural and laboratory-adapted populations. The maintenance of fitness variation has been explained by various mechanisms, including high mutation rates, frequency-dependent selection and immigration. The presence of intralocus sexual conflict provides an additional mechanism, where the sexes select for different alleles at given fitness-related loci. Sexually antagonistic genetic variation for fitness has indeed been shown in several populations, suggesting its prevalence in dioecious species. We extend these findings by exploring the genetic basis of sex-specific fitness variation in our laboratory-adapted population of *D. melanogaster* with a sample of 223 genomes. We also compare these findings to estimates obtained from a smaller sample from the same population five years prior, showing that the strength of sexual antagonism has declined significantly. This comparison provides novel insights into how the strength of conflict can change over time and its consequence for the maintenance of fitness variation.

2.2 – Introduction

Fitness is a measure of an individual's reproductive performance, which we define as the number of offspring produced within an individual's lifetime. An essential requirement for evolution is that there must be heritable genetic variation for fitness within a population from which selection can act. This is according to Fisher's Fundamental Theorem of Natural Selection (Fisher 1930), stating 'the rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time'. Uncovering the genetic basis of fitness variation is therefore crucial to understanding evolution, and predicting population-specific responses to selection.

The maintenance of fitness variation within a population is a particular area of interest and debate, since selection is expected to erode genetic variation for fitness, as the population converges at a fitness peak (Charlesworth 1987; Fisher 1930). In contrast, genetic variation for non-fitness traits is expected to be much higher due to lack of direct selection. Yet, heritable variation for fitness related traits does exist within populations (Mousseau and Roff 1987; Burt 1995; Fowler *et al.* 1997; Merilä and Sheldon 2000; Innocenti and Morrow 2010) and various reasons for this have been proposed, including: high mutation rates (Haag-Liautard *et al.* 2007; Lynch and Walsh 1998); frequency dependent selection (Trotter and Spencer 2007); immigration (Charlesworth 1987); condition dependent selection (Rowe and Houle 1996); genetic trade-offs (Andersson and Iwasa 1996); and disruptive selection (Mather 1955; Kingsolver *et al.* 2001). Other theory suggests a role for intralocus sexual conflict (IASC) in maintaining a large proportion of genetic variation for fitness traits (Rice, 1984), which we aim to explore in this study.

As described in **Chapter 1**, IASC is a form of sexual antagonism that arises as a consequence of the sexes shared genome: when sex-specific gene expression is incomplete, the sexes can be constrained from reaching their respective phenotypic optima. This limitation creates direct selection on each sex for different alleles at the same locus - hence genetic variation for fitness is maintained. This disparity between the sexes has been revealed through observations that a given genotype may have opposite fitness effects when expressed in males and females (Chippindale *et al.* 2001; Gibson *et al.* 2002; Fedorka and Mousseau 2004; Bonduriansky and Rowe 2005a; Pischedda and Chippindale 2006; Brommer *et al.* 2007; Foerster *et al.* 2007; Long and Rice 2007; Prasad *et al.* 2007; Bedhomme *et al.* 2008; Bilde *et al.* 2009; Mainguy *et al.* 2009; Svensson *et al.* 2009; Harano *et al.* 2010; Innocenti and Morrow 2010; Garver-Apgar *et al.* 2011; Stulp *et al.* 2012; Hesketh *et al.* 2013; Mills *et al.* 2012; Mokkonen *et al.* 2012; Berger *et al.* 2014; Tarka *et al.* 2014).

The resolution of IASC can be achieved through changes in underlying genetic architecture of fitness-related traits that permit sex-biased gene expression. Differential gene expression can be orchestrated through mechanisms such as: genomic imprinting (Day and Bonduriansky 2004), gene duplication (Connallon and Clark 2011b; but see Hosken 2011), alternative splicing (McIntyre *et al.* 2006) and sex-specific gene expression modification (Ellegren and Parsch 2007; described in section 1.4; Pennell and Morrow 2013). As conflict is resolved or partially resolved within a population, genetic variation for fitness is expected to decline because traits within each sex are released from genetic constraints and can evolve toward their fitness optima. This leads to the testable, but so far untested, prediction that the heritability of fitness will decline as IASC is mitigated.

In this study, we aim to quantify genetic variation for fitness in a laboratory-adapted population of *D. melanogaster*, and explore to what degree the variation is sexually antagonistic or sexually concordant. We employ hemiclonal analysis, a method that enables standing genetic variation to be captured from a source population and expressed in many individuals of both sexes (described in section 1.9). This experimental design enables sex-specific additive genetic variance for fitness and between-sex covariance to be estimated. We compare these findings (recorded during 2012) to a study of the same population from 2007 (Innocenti and Morrow 2010), to test whether the amount of sexually antagonistic genetic variation for fitness has changed during a 5-year period. According to theory (Rice 1984), the conflict should become resolved over time as sex-specific gene regulation evolves. We also explore the correlation between sexually antagonistic variation and the heritability of fitness. Direct empirical comparisons of IASC in a single population over time are currently absent; therefore this study offers new insight into the maintenance of genetic variation for fitness, the dynamic of intralocus sexual conflict and the timescale of conflict resolution.

2.3 – Methods

Creating 223 Hemiclonal Lines

Hemiclonal analysis was used to sample 223 haplotypes from LH_M (see section 1.8), which were then expressed in multiple individuals of both sexes with different random genetic backgrounds from the source population (see section 1.9). This design enabled us to explore the additive effects of genes on sex-specific fitness since within each hemiclonal line, males and females share one near complete haploid genome in common (with the exception of the fourth dot chromosome). Below is a description of how the 223 hemiclonal lines were created.

The 223 haplotypes were sampled from LH_M and maintained as heterozygous stock hemiclonal lines by crossing with clone generator (CG) females [C(1)DX, *y*, *f*; T(2;3) *rdgC st in ri* p^P *bw*] (Chippindale *et al.* 2001; Rice *et al.* 2005). All crosses and assays were conducted in identical conditions to that described for the LH_M stock (section 1.8). To create stock hemiclone males 10 CG females were first crossed to each male sampled from LH_M. The hemiclonal haplotype was further amplified via another cross with CG females (1 hemiclonal male from the previous cross with 10 CG females). This was followed by further hemiclone amplification, where 5 hemiclone males from the previous cross were crossed with 10 CG females.

We expressed hemiclonal haplotypes as males and females, which were assayed for sex-specific fitness. Hemiclonal haplotypes were expressed as males by mating 16 stock hemiclonal males with 16 virgin double-X LH_M females [C(1)DX, *y*, *f*]. The double-X LH_M population was created by backcrossing the CG double-X into LH_M for 2 generations. Hemiclonal haplotypes were expressed as females by mating stock hemiclonal males with virgin LH_M females (16 hemiclonal males and 16 LH_M females). Figure 1.3 illustrates the hemiclonal crosses described above.

Assays of Sex-Specific Fitness

Assays were designed to give a measurement of total adult lifetime fitness for 223 hemiclonal haplotypes when expressed as either male or female (dataset hereafter referred to as "*H223*"). All adult flies were assayed 2 days after adult eclosion, under competitive conditions that closely match those experienced by adults in the base population (see section 1.8). The fitness assay protocol for males and females in each hemiclonal line is illustrated in Figure 2.1.

For each male assay, 5 red-eyed hemiclonal males (bw^+/bw^+) from each line were combined in a yeasted vial containing standard agar food mixture, with 10 competitor brown-eyed males (*bw-/bw-*) and 15 virgin brown-eyed females (*bw-/bw-*). After 2 days, each brown-eyed female was isolated into an individual test tube (containing 3ml of food) and left to oviposit for 18 hours. On Day 12, progeny from each brown-eyed female was scored for eye colour. Due to dominance of the wild-type allele, hemiclonal males were assigned paternity to progeny with wild-type red eyes, giving an average fitness score (proportion of offspring sired) for the 5 hemiclonal males that were assayed per line. This assay was replicated 5 times, representing a total of 25 hemiclonal males per line. Relative fitness measures were calculated by averaging the fitness across replicates, obtained by dividing the proportion of offspring sired by hemiclonal males (mean proportion calculated from the 5 hemiclonal males per replicate) by the maximum mean proportion across all hemiclonal lines and replicates.

The female fitness assays followed a similar protocol. Here, 5 virgin red-eyed (bw^+/bw^+) hemiclonal females were combined in vials with 10 competitor brown-eyed females (bw^-/bw^-) and 15 brown-eyed (bw^-/bw^-) males for 2 days. After 2 days, the 5 red-eyed hemiclonal females were isolated into individual test tubes and left to oviposit for 18hrs. The number of eggs per female was counted to provide a measure of fecundity. By averaging this measure across all 5 females this provided an average female fitness score for that line. This assay was replicated 5 times, representing a total of 25 females per hemiclonal line. Relative fitness measures were calculated by averaging fitness across replicates, obtained by dividing the average number of eggs per female (mean number of

eggs calculated from the 5 hemiclonal females per replicate) by the maximum mean number of eggs across all hemiclonal lines and replicates.

Statistical Procedures

1) The Genetic Basis of Fitness in LH_M

We explore the extent of sexual antagonism in our population using two approaches. First, relative male and female fitness data (*H223*) was analysed in R v.3.1.2 (R Core Team 2014) by fitting a linear mixed model, using Bayesian methods and Markov Chain Monte Carlo sampling techniques (MCMCglmm: Hadfield 2010). In this model, Y = S + L + R + ε, where Y is relative fitness; S (sex) is a fixed effect; L (line) is a 2×2 matrix that specifies sex-specific variances among lines and their covariance; R (replicate) is a 2 x 2 matrix that specifies sex-specific variances among replicates and their covariance; and ε is a matrix of sex-specific, within-line residual variances. Flat priors for the correlation were used.

Using this model, total phenotypic variance for fitness was partitioned into sex-specific genetic components and their correlation, from which the intersexual genetic correlation (r_{MF}) for fitness could be estimated. This correlation indicates how selection on a trait in one sex will respond to selection in the opposite sex. If r_{MF} is greater than zero, the average additive effects of genes in a population can be described as sexually concordant. On the other hand if r_{MF} is less than zero, the average additive effects of genes can be described as sexually antagonistic, which is a signature of on-going IASC:

$$r_{MF} = \frac{COV_{Amf}}{\sqrt{V_{Am} * V_{Af}}}$$

where COV_{Amf} is the covariance of male and female additive genetic variance for fitness, V_{Am} is male additive genetic variance for fitness and V_{Af} is female additive genetic variance for fitness. As hemiclones share only half of their genome in

common (Rice *et al.* 2005), sex-specific additive genetic variances and covariances were multiplied by two.

We calculated narrow sense heritability (h^2) for each sex, which is important for predicting how a trait such as fitness could respond to selection:

$$h^2 = \frac{V_A}{V_G + V_R}$$

where V_A is sex-specific additive genetic variance, V_G is total genetic variance (male and female), and V_R is residual variance. A high h^2 indicates both high resemblance between parents and offspring, and the existence of additive genetic variation on which selection could act.

Secondly, an alternative and more detailed view of the dynamic of IASC was gained by projecting average hemiclonal line scores of sex-specific relative fitness along axes that described sexually antagonistic and sexually concordant variation for fitness (Berger *et al.* 2014). For each treatment, line fitness scores were relativised within each sex and projected along axes that described the direction of genetic variation: the first axis, with a gradient of 1, described sexually concordant genetic variation ($r_{MF} = 1$). The second axis was orthogonal to the first (gradient = -1), and described sexually antagonistic genetic variation ($r_{MF} = -1$).

To achieve this, the two-dimensional Cartesian coordinate system describing the relative fitness of male and female hemiclonal lines was rotated clockwise by 45°:

$$X' = x\cos(\theta) - y\sin(\theta)$$

$$Y' = x\sin(\theta) + y\cos(\theta)$$

where X' and Y' are the values of sexually antagonistic and concordant fitness, x and y are male and female line fitness, and θ is the angle by which the coordinate

system has been rotated (45°). This method provided a percentage value of the contribution of sexually antagonistic and sexually concordant variation to fitness in LH_M.

To gain confidence intervals for the estimate of the percentage of sexually antagonistic variation obtained using the Berger *et al.* (2014) method above, we used bootstrapping. Here, we randomly sampled 5 replicates of sex-specific fitness estimates (with replacement) from 223 lines, a total of 10000 times. For each sample, the average sex-specific fitness estimate for each line was projected along the axes described above. This gave 10000 estimates of the percentage of sexually antagonistic variation in *H223*, from which confidence intervals could be estimated. This is a robust procedure for constructing confidence intervals for datasets (Efron and Tibshirani 1993).

2) Comparing the Standing Genetic Variation for Fitness in LH_M Between Years

The LH_M population has been maintained under homogenous laboratory conditions (see section 1.8) since 1991, although it has been moved between laboratories during this time. Prior to 2007, the population was moved from a laboratory in the United States to Sweden, and it was later transferred to the United Kingdom in 2011, after which the *H223* data was collected in 2012. Despite efforts to maintain the LH_M population under the same conditions during this time, fluctuations within and between laboratories were possible (e.g. stability of incubator temperature and humidity, and the precise chemical composition of food ingredients).

Measurements of sex-specific relative fitness in LH_M were collected in the Swedish laboratory in 2007 from 100 hemiclonal lines (Innocenti and Morrow 2010), hereafter referred to as "*H100*". This means that 5 years passed between the *H100* and *H223* studies. As well as the *H100* and *H223* datasets differing in the number of hemiclonal lines sampled from the population, they also differ in the number of replicates of sex-specific fitness estimates that were obtained per hemiclonal line: *H223* consists of 5 average measures of relative fitness for each sex and hemiclonal line; whereas *H100* consists of 4 average measures of female

relative fitness and 6 of male relative fitness, per hemiclonal line. However, the fitness assay protocol for *H100* was identical to that described for *H223*.

To test whether the amount of sex-specific and sexually antagonistic genetic variation had changed within a 5-year period, we independently analysed the *H100* dataset from 2007 and compared estimates of h^2 and r_{MF} to those obtained from *H223* in 2012. To obtain these estimates, the same linear mixed model that was applied to *H223* was also applied to *H100*, which gave posterior distributions of sex-specific variances and their covariance.

We also gained an alternative view of the dynamic of IASC by projecting average hemiclonal lines scores of sex-specific relative fitness along axes that described the amount of sexually antagonistic and sexually concordant variation for fitness, using the same Berger *et al.* (2014) method described above for the *H223* analyses. Confidence intervals for the point estimate of sexually antagonistic variation were obtained by bootstrapping. We randomly sampled replicates (with replacement) of sex-specific fitness estimates from the 100 lines (4 replicates for females, 6 replicates for males, in line with the original dataset), 10000 times. For each sample, the average sex-specific fitness estimate for each line was projected along the axes described directly above. This gave 10000 estimates of the percentage of sexually antagonistic variation in *H100*, from which confidence intervals could be estimated.

2.4 – Results and Discussion

1) The Genetic Basis of Fitness in LH_M

After measuring sex-specific fitness within 223 hemiclonal lines (Figure 2.2), a linear mixed model was used to partition total phenotypic variance into sex-specific variance and their covariance. Within the *H223* dataset, we found high and significant narrow-sense heritability (h^2) for female fitness (Table 2.1) in accordance with some studies (e.g. in *Drosophila*: Pischedda and Chippindale 2006, Long *et al.* 2009, Innocenti and Morrow 2010; and birds: Merilä and

Sheldon 2000, Teplisky *et al.* 2009), but not others (e.g. in birds: McCleery *et al.* 2004; and mammals: Kruuk *et al.* 2000, Coltman *et al.* 2005, Foerster *et al.* 2007). In contrast, h^2 for male fitness was lower but remained significantly different from zero (Table 2.1). These results are consistent with other research (e.g. in *Drosophila*: Pischedda and Chippindale 2006, Innocenti and Morrow 2010; birds: Merilä and Sheldon 2000, Teplisky *et al.* 2009; and mammals: Foerster *et al.* 2007), where the h^2 of fitness in males was lower than that of females. This pattern can arise due to higher residual variance in males, as they can be more sensitive to environmental effects (e.g. stochasticity in mating success) compared to females (Price and Schluter 1991), thereby lowering estimates of h^2 even when additive genetic variances are high. However, in the current study residual variance was not higher in males. Instead, lower h^2 in males can be attributed to lower genetic variance for fitness, which could result from stronger directional selection (natural or sexual selection) in males that has eroded genetic variation (Kimura 1958).

To explore the strength of sexual antagonism due to IASC in LH_M, sex-specific additive genetic variance and covariance for fitness was drawn from the linear mixed model and used to estimate r_{MF}. We found r_{MF} to be positive but not significantly different from zero (Table 2.1), which at face value suggests that sexually antagonistic genetic variation for fitness does not contribute to a large majority of the overall standing genetic variation for fitness in our population. This contrasts to previous studies where r_{MF} was found to be negative in LH_M, which is indicative of strong IASC (Chippindale *et al.* 2001; Gibson *et al.* 2002; Innocenti and Morrow 2010).

By projecting hemiclonal line scores of sex-specific relative fitness along axes that described sexually antagonistic and sexually concordant variation for fitness (Berger *et al.* 2014), we found that approximately half (54.0%; CI: 46.5-61.5) of the total variance in fitness variation in *H223* was sexually antagonistic, representing on going conflict; with the remaining 46% (CI: 38.5-53.4) of fitness variation being sexually concordant, suggesting widespread conflict resolution (Figure 2.4).

2) Comparing the Genetic Basis of Fitness in LH_M Between Years

A linear mixed model was applied to *H100* and the posterior distribution was used to provide point estimates of h^2 and r_{MF} for this dataset. We found that h^2 of female fitness was high and significant in *H100*, in line with that found in *H223* (Table 2.1). The large and overlapping credible intervals for both estimates suggests that the h^2 of female fitness has not changed significantly over time. Similarly, the h^2 of male fitness was low but significant for both *H223 and H100* and credible intervals were overlapping (Table 2.1), suggesting that h^2 of male fitness has not changed significantly between years. However, there was a trend for higher heritabilities in the *H100* dataset (Table 2.1).

In line with previous findings (Innocenti and Morrow 2010), r_{MF} in *H100* was negative and significantly different from zero (Table 2.1). Although the 95% credible intervals for r_{MF} in *H100* and *H223* overlap, the point estimate for r_{MF} in *H100* lies outside of the credible interval for r_{MF} in *H223* (Figure 2.3).

Fitness measures taken from the bootstrapped *H100* and *H223* were also projected along axes that described sexually antagonistic and sexually concordant variation for fitness between hemiclonal lines (Berger *et al.* 2014). Using this method, we found that the average percentage of sexually antagonistic fitness variation in *H100* was 62.4% (CI: 54.2-70.7). Again, although the confidence intervals for the estimates of the percentage of sexually antagonistic variation obtained from *H100* and *H223* overlap, the point estimate for *H100* lies outside of the 95% confidence interval for *H223* (Figure 2.4). This result indicates that 13.5% of the genetic variation that was sexually antagonistic in LH_M has become sexually concordant since Innocenti and Morrow (2010) sampled genotypes from the population.

3) Conclusions

Although IASC contributes to a high proportion of fitness variation in *H223*, there is evidence that the extent of this conflict has declined in the past five years (>100 generations). This is supported by observations that point estimates for

values of antagonism were higher for *H100* and found outside of the 95% confidence intervals for values of antagonism obtained from *H223* (Figures 2.3 and 2.4).

Past estimates of sex-specific fitness in LH_M spanning over a decade suggests that IASC accounted for a large proportion of fitness variance within the population during this timeframe (Chippindale *et al.* 2001; Gibson *et al.* 2002; Innocenti and Morrow 2010). For example, Chippindale *et al.* (2001) sampled 40 genotypes from LH_M and found a significant negative intersexual genetic correlation for fitness, and Gibson *et al.* (2002) sampled 20 X-chromosomes from LH_M and identified a negative correlation for fitness of males and females that shared the same X chromosome. Innocenti and Morrow (2010) also identified a significant negative intersexual genetic correlation for fitness in the population after sampling 100 genotypes from LH_M, more than 7 years later. Here we present evidence that the previous conflict has declined by more than 13% within 5 years since Innocenti and Morrow (2010) assayed sex-specific fitness in the population. This raises a question of why the strength of conflict has changed in recent years.

Genetic Mechanisms to Resolve Conflict

One answer could be that mechanisms evolved that enabled sex-biased gene expression to reduce r_{MF} for fitness-related traits, partially resolving IASC in LH_M: such as genomic imprinting (Day and Bonduriansky 2004), gene duplication (Connallon and Clark 2011; but see Hosken 2011), alternative splicing (McIntyre *et al.* 2006) and sex-specific gene expression modification (Ellegren and Parsch 2007; described in section 1.4 and Pennell and Morrow 2013). Genomic imprinting could reduce IASC if alleles inherited from the opposite-sex parent are silenced (Day and Bonduriansky 2004), which has been observed in different taxa, including insects (Ferguson-Smith *et al.* 2001). However, evidence for parent of origin imprinting is lacking in *Drosophila* (Coolon *et al.* 2012) and little is known about the timescale over which imprinting patterns might arise (Patten *et al.* 2014). Genes can also be duplicated and translocated to other chromosomal locations for sex-specific expression, after which they undergo sub-

functionalisation. There is evidence for the involvement of duplicate genes in sex-biased expression in *Drosophila*, often with specific roles in male reproductive function (Gallach *et al.* 2010; Wyman *et al.* 2012). Similarly, alternative splicing (Telonis-Scott *et al.* 2009; Hartmann *et al.* 2011) and hormonal regulation (Kopp *et al.* 2000; Fagegaltier *et al.* 2014) of sex-biased genes exist in *Drosophila*. Although little is known about the timeframe over which these mechanisms arise, they are expected to require long periods of time (Stewart *et al.* 2010). The long timeframe is imposed because genes are likely to first require duplication before the evolution of DNA regulatory sequence changes that can respond to a sex-specific regulatory signal (Stewart *et al.* 2010). Alternatively, the evolution of new *cis*-acting regulatory sequences will be required that control post-transcriptional RNA editing (Stewart *et al.* 2010). On the contrary, the evolution of sex-biased expression could be fast-tracked if a gene is already under the influence of a regulatory binding site, or if it were translocated to such a location. Even so, the timeframe for conflict resolution could be extended because there is risk of disrupting gene networks through the translocation of duplicate genes to new locations, and most genes mediating conflict are predicted to be under pleiotropic constraint (Mank *et al.* 2008; described in section 1.5 and Pennell and Morrow 2013).

Although it is unclear how genetic constraints are overcome (Lynch and Walsh 1998), results presented in this chapter corroborate the theory (Lande 1980; 1987; Reeve and Fairbairn 2001) that conflict resolution is slow even under a constant environment. The fact that over 13% of the existing IASC was reduced within 5 years in this study also suggests that whichever mechanism(s) arose might have facilitated the sex-biased expression of many genes simultaneously. This is predicted by Mank *et al.* (2013) who suggest that hormonal regulation of a few genes can then transmit sex-biased to thousands of other genes under their regulation.

Data qualitatively demonstrating conflict resolution using fitness estimates within laboratory adapted or natural populations are currently absent, but there is evidence of both slow and fast evolution of sex-biased gene expression, which

indirectly indicates the potential for both slow and fast resolution of IASC. Constraints on the evolution of sex-biased expression was demonstrated in a recent study by Hollis *et al.* (2014), where a laboratory adapted population of *Drosophila* was released from male-specific selection through enforced monogamy and subsequently gene expression rapidly evolved (within 65 generations) to become female-biased. This demonstrates that previous barriers to sex-biased expression existed, which could not be overcome through long-term adaptation. Griffin *et al.* (2013) also suggest that conflict resolution is slow: the same genes that had genetic constraints imposed by intersexual genetic correlations in one population were associated with high IASC in another population. This result indicates that it could be difficult to break down intersexual genetic correlations for certain traits shared within a species, even in populations that have been separated by hundreds of generations and that evolved under different environmental conditions. The latter study however, considered univariate selection only, which is perhaps unrealistic because selection operates at a multivariate level.

Contrasting evidence however, suggests that the evolution of sex-biased gene expression might occur over shorter timescales. For example, Delph *et al.* (2011) used artificial selection to break down a high intersexual genetic correlation for flower size in the dioecious plant, *Silene latifolia*, in less than five generations. Other selection experiments have shown similar outcomes: sexual dimorphism arose after 12 generations of adaptation to a novel environment in *Drosophila* (Chenoweth *et al.* 2008), 33 generations of disruptive selection in the butterfly *Bicyclus anynana* (Zwaan *et al.* 2008), and 100 generations of female-specific selection in domesticated chickens (Moghadam *et al.* 2012). Such quick responses to these novel selection pressures might be explained by mechanisms that existed before selection was applied, rather than the evolution of new sex-specific alleles or modifier loci. In comparison, the apparent slow evolution of conflict resolution in $LH_{M,}$ more likely involves the evolution of new sex-specific alleles or modifier loci that are thought to arise over longer timescales.

Other Evolutionary Explanations for Reduced IASC

It is also necessary to explore other evolutionary explanations for the apparent resolution of IASC in LH_M. One possibility is that minor changes in the laboratory environment altered sex-specific selection pressures so that they became more sexually concordant in recent years, thereby reducing IASC without the need for sex-biased gene expression to evolve. Although we maintained the LH_M population under the same laboratory conditions as Innocenti and Morrow (2010), it is difficult to account for subtle variation in factors such as variability or absolute differences in incubator temperature, humidity, light intensity and nutrition that might differ between laboratories. Research has shown that extreme changes in temperature and nutrition can alter the strength of IASC in other populations (Delcourt *et al.* 2009; Berger *et al.* 2014; Punzalan *et al.* 2014), and I present data in **Chapter 2** where very minor changes in temperature altered the strength of IASC in a subset LH_M genotypes: genotypes that were previously sexually antagonistic under standard temperature conditions became sexually concordant at treatment temperatures that differed by 2°C. However, in what way subtle changes in the environment might affect sex-specific selection within a whole population over time requires further investigation. It is in fact likely that these variable selection pressures act to hinder conflict resolution because selection for sex-biased gene expression becomes inconsistent. Additionally, these minor fluctuations are also likely to occur within a single laboratory during the course of an experiment and even within a single generation, but it is unknown how these changes in environment compare to between-laboratory variation and subsequently whether this is likely to explain why IASC has declined only recently (Rice and Chippindale 2001; Gibson *et al.* 2002; Innocenti and Morrow 2010).

Another factor to consider is the effect of genetic drift: random changes in allele frequencies over time, which can override forces of sex-specific selection and reduce the contribution of sexually antagonistic alleles to fitness variance (Connallon and Clark 2012; Hesketh *et al.* 2013). This could mean that sexually antagonistic alleles are only ever transiently maintained in a population

(Connallon and Clark 2012). However, the effect of genetic drift is intensified in smaller populations and is expected to affect large, outbred populations such as LH_M (1800 individuals) to a lesser extent.

The relative stability of laboratory conditions could also exaggerate the importance of sexually antagonistic alleles to fitness variance, because unconditionally deleterious mutations (which have sexually concordant effects) should be removed from a population under a constant environment (Chapman *et al.* 2003b). Although this might have increased the strength of IASC in LH_M when the population was introduced into the laboratory in 1991, this does not explain why the conflict declined only relatively recently (in the past five years), having apparently been maintained for over a decade. As discussed above, the most likely alternative hypothesis is that the constant environment of the laboratory enabled conflict resolution over time (Pennell and Morrow 2013).

Future Directions

Determining factors responsible for affecting estimates of the strength of IASC is difficult, especially when these estimates represent a 'snapshot' of standing genetic variation for fitness at any one time. An accumulation of data on a single population can help to disentangle these effects, as multiple snapshots can be taken at different time points during evolution. The study presented in this chapter is the first of its kind, where genetic variation for fitness and the level of IASC has been explored within a single population during long term adaptation to the laboratory, and where identical statistical methods have been applied to different datasets. We can also count on the reliability of this data because both the *H100* and *H223* datasets are large, and likely to capture all of the standing genetic variation for fitness in LH_M. If LH_M is in the process of resolving much of its existing IASC, then the prediction is that the contribution of sexually antagonistic alleles to fitness will decline further in the future, as mechanisms to achieve sex-biased gene expression arise and fix within the population – something that will remain to be tested. Understanding conflict resolution is fundamental for building a picture of the dynamic of IASC, and predicting adaptive responses to selection in each sex.

With regard to the expectation that IASC maintains fitness variation, it is also notable that populations with higher levels of conflict are expected to also have higher sex-specific heritabilities for fitness. As conflict becomes resolved, this should reduce the amount of additive variation for fitness within each sex, thereby reducing the heritability of fitness as each sex converges towards a fitness peak. Some support is provided in this study, as despite sex-specific heritability estimates overlapping between *H100* and *H223*, there was a trend for higher estimates in *H100* where IASC was also stronger. This has not been the focus of other studies of IASC, but could allow us to understand how IASC contributes to variance in fitness. Additionally, although IASC could maintain heritable genetic variation for fitness, which is a crucial requirement for selection, this does not provide an indication of trait evolvability. Instead, traits might experience long-term constraints of sexual antagonism. This might seem paradoxical because it appears as if the same mechanism that maintains the building blocks for evolution, also acts to constrain it. A difficulty for future studies will be teasing apart the effect of sexual antagonism on the maintenance of additive fitness variation from other evolutionary processes, such as those discussed in the introduction of this chapter.

Finally, if it has taken over a decade of adaptation to benign laboratory conditions for mechanisms of sex-biased gene expression to resolve IASC in LH_M, then this could have implications for conflict resolution in natural populations. The variable conditions experienced by populations outside of the laboratory are predicted to impede the evolution of sex-biased gene expression further, imposing an even longer timeframe for conflict resolution (see section 1.6 and Pennell and Morrow 2013). Resolution for some traits in natural populations could therefore be extremely slow or even improbable.

Table 2.1 – *H223* and *H100* Heritabilities and Intersexual Genetic Correlations for Fitness: a linear mixed model was applied to each dataset, which partitioned phenotypic variance for male and female adult fitness and their covariance. The posterior distributions were used to estimate sex-specific heritabilities h^2 and intersexual genetic correlations for fitness r_{MF}.

	Variance	h^2	**95% CI**
H223			
Female	0.008	0.222	0.155; 0.300
Female Residual	0.032		
Male	0.002	0.071	0.031; 0.116
Male Residual	0.025		
H100			
Female	0.015	0.347	0.229; 0.477
Female Residual	0.028		
Male	0.004	0.095	0.034; 0.155
Male Residual	0.040		
	Covariance	r_{MF}	**95% CI**
H223			
Male-Female	0.0056	0.135	-0.235; 0.438
H100			
Male-Female	-0.0033	-0.415	-0.73; -0.060

Figure 2.1 – Fitness Assay Protocol: a) female and b) male assay of lifetime adult fitness for each hemiclonal line. Red-eyed flies represent target hemiclones from a single line. Brown-eyed flies represent non-target flies. Replicated 5 times for each sex and hemiclonal line, providing a total of 5 values of average egg number (representing 25 hemiclonal females) and 5 values of average proportion of offspring sired (representing 25 hemiclonal males) per line.

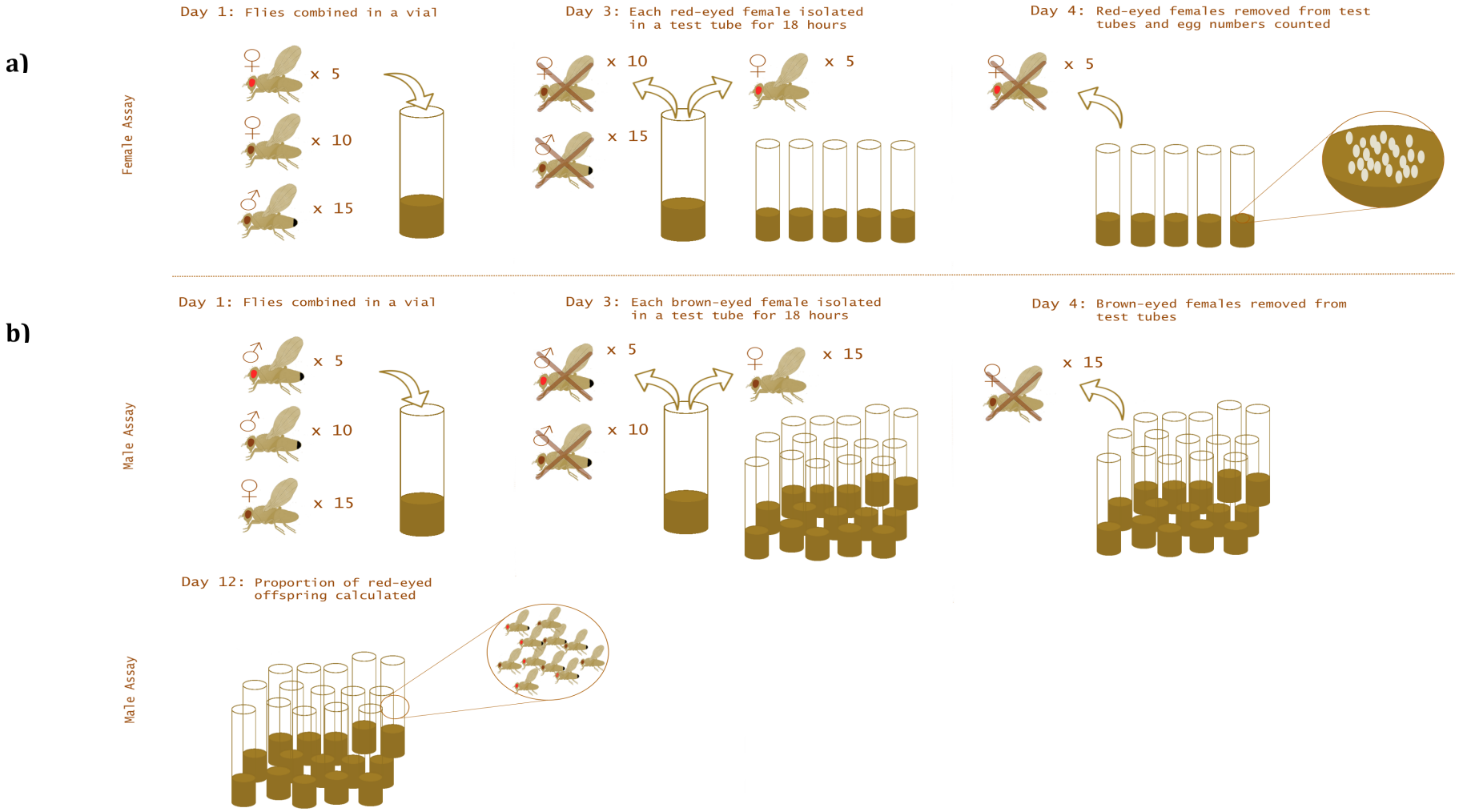


Figure 2.2 – *H223* and *H100* Sex-Specific Relative Fitness: open black circles and filled grey circles represent average male and female relative fitness measures of 223 hemiclonal lines (*H223*) and 100 hemiclonal lines (*H100*), respectively. Black and grey broken lines represent 95% confidence ellipses for average line sex-specific fitness in *H223* and *H100* respectively.

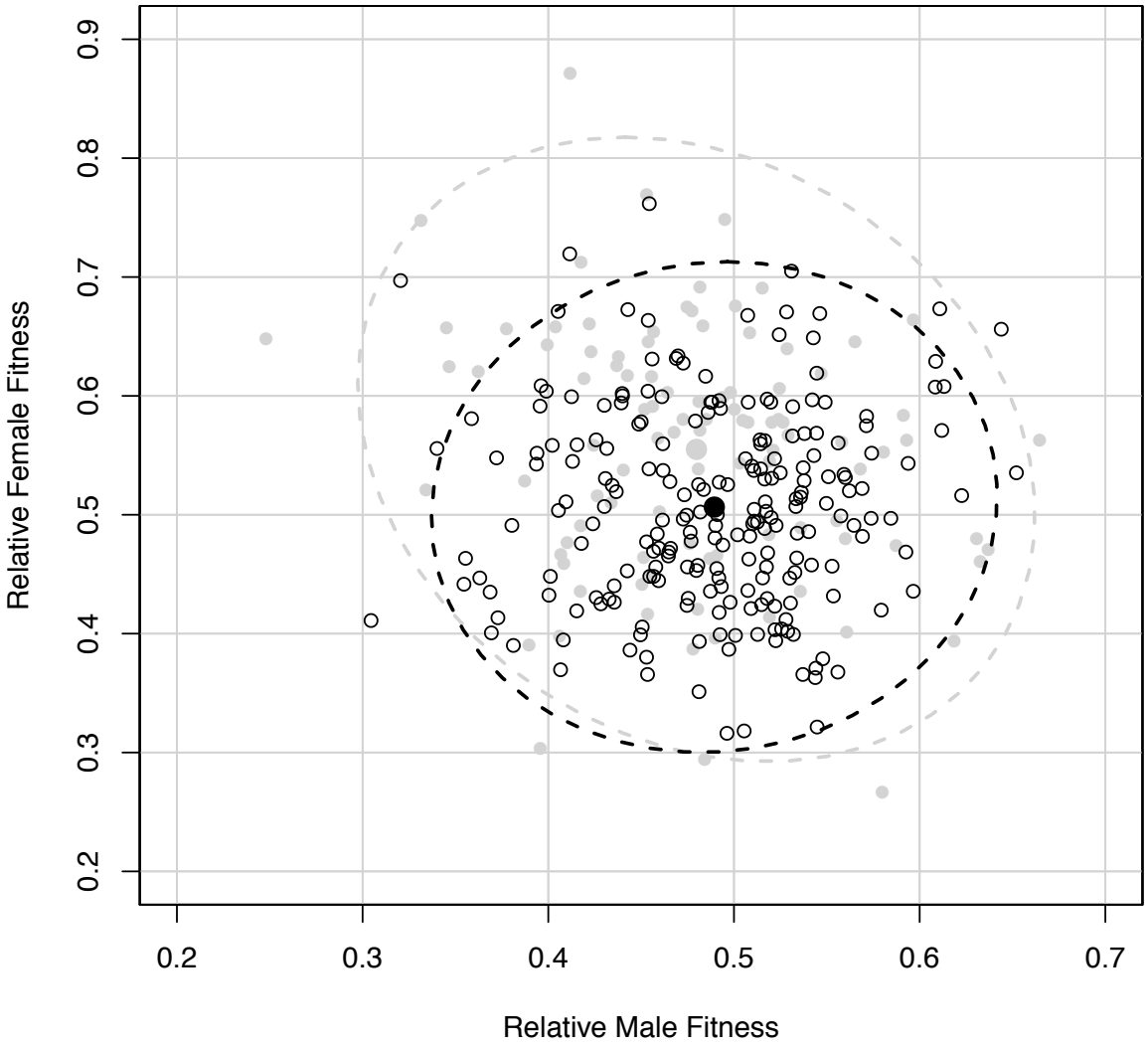


Figure 2.3 – Posterior Distributions of r_{MF} Values Obtained from *H223* and *H100*: black and grey density curves represent values of r_{MF} calculated from the posterior distributions of linear mixed models applied to *H223* and *H100* datasets, respectively. Black and grey circles and horizontal bars represent point estimates and 95% credible intervals for r_{MF} values obtained from *H223* and *H100*, respectively.

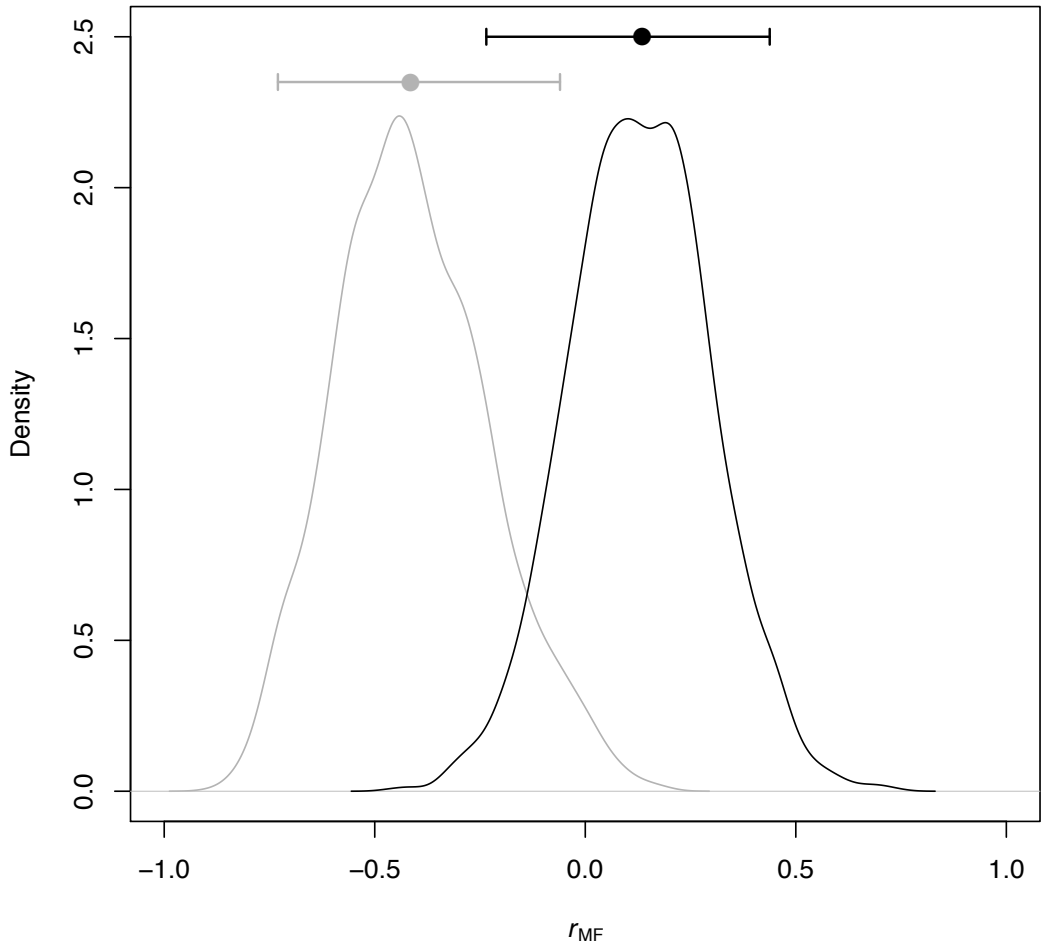
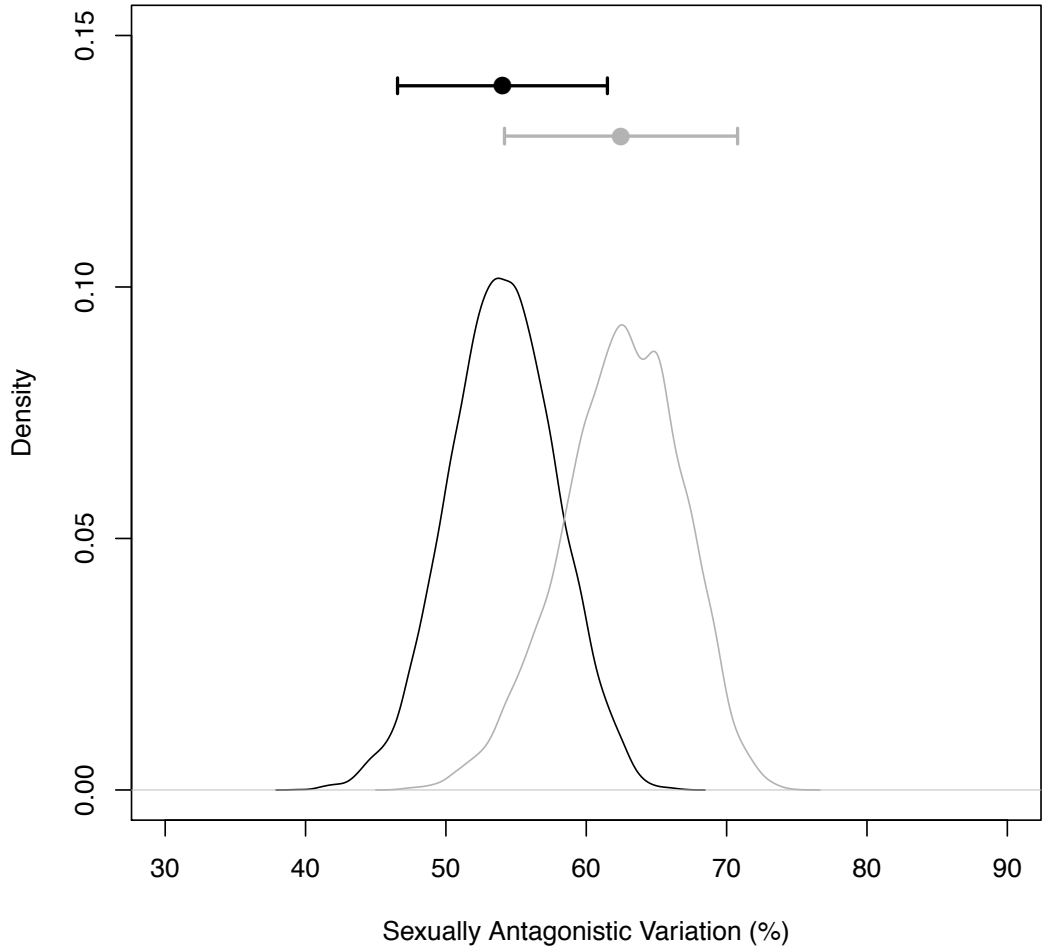


Figure 2.4 – Density Distributions of Estimates of Sexually Antagonistic Variation Obtained by Bootstrapping *H223* and *H100*: the same number of replicates that were in the original datasets, were drawn at random with replacement, a total of 10000 times (5 replicates for both sexes in *H223*; 6 replicates for males and 4 replicates for females in *H100*). Black and grey density curves represent estimates of the percentage of sexually antagonistic variation obtained by bootstrapping *H223* and *H100*, respectively. Black and grey circles and horizontal bars represent point estimates and 95% confidence intervals for estimates obtained from *H223* and *H100*, respectively.



Chapter 3: Direction of Intralocus Sexual Conflict Shifted by Sex-Specific Temperature Effects on Fitness

3.1 - Abstract

Males and females often require the expression of different phenotypes from a shared genome. As a result, sex-specific selection can cause alleles that have opposite fitness effects in the sexes to be maintained in a population – a pervasive constraint on adaptation known as intralocus sexual conflict (IASC). This is characterised by a negative intersexual genetic correlation (r_{MF}) for fitness. Resolution of IASC is predicted to evolve after strong and consistent selection for sex-specific gene expression, which breaks down r_{MF}, but is hindered by genetic barriers such as epistasis, pleiotropy and genetic drift. Differences in sex-specific selection brought about by a changing environment could act as an additional barrier by reducing r_{MF} before sex-specific gene expression can evolve. We show that very minor changes in temperature during adult stages can indeed alter sex-specific fitness and change the relative strength of IASC for given genotypes of *Drosophila melanogaster*. IASC became female-biased at warmer temperatures, but male-biased at cooler temperatures. Moreover, we show changes in sex-specific behavioural phenotypes that may contribute to sex-specific fitness at different temperatures. These results indicate that even relatively small and transitory fluctuations in ambient temperature experienced during an individual's lifetime, could have important consequences for the maintenance of IASC in natural populations where environmental variation is common.

3.2 - Introduction

The maximisation of fitness in each of the two sexes is often achieved through the expression of different phenotypes; however, a shared genome could act as a pervasive constraint on sex-specific gene expression (Lande 1980; Rice 1984; Arnqvist and Rowe 2005; Bonduriansky and Chenoweth 2009). Consequently, sex-specific selection causes alleles that have opposite fitness effects in each sex to be maintained in a population - known as intralocus sexual conflict (IASC) -

which is characterised by a negative intersexual genetic correlation (r_{MF}) for fitness within a population (Chippindale *et al.* 2001; Foerster *et al.* 2007; Delcourt *et al.* 2009; Innocenti and Morrow 2010; Brommer *et al.* 2012; Hesketh *et al.* 2013; Berger *et al.* 2014; Punzalan *et al.* 2014). The resolution of IASC requires strong and consistent selection for sex-specific gene expression, which breaks down r_{MF} (Lande 1980). The long timeframe required for resolution is imposed by genetic barriers such as epistasis, pleiotropy and genetic drift (van Doorn 2009; Pennell and Morrow 2013; **Chapter 1**). Changes in sex-specific selection in response to a changing environment is also likely to impede conflict resolution as it creates inconsistent selection for mechanisms that facilitate sex-biased gene expression (Pennell and Morrow 2013; **Chapter 1**).

There is empirical evidence that r_{MF} for fitness could change when populations are exposed to 'novel' environments (Delcourt *et al.* 2009; Berger *et al.* 2014; Punzalan *et al.* 2014), where experimental treatments represented substantial deviation from conditions to which the populations were adapted: including stressful temperature conditions in *Callosobruchus maculatus* (Berger *et al.* 2014), and novel food environments in *D. serrata* (Delcourt *et al.* 2009; Punzalan *et al.* 2014). In these studies focal individuals were exposed to novel environments from the embryo stage onwards, and therefore incorporate environmental effects on both developmental and adult processes.

Natural populations frequently experience more subtle changes in the environment, either within a single locale or over a species range, yet it is unclear how these minor deviations could impact r_{MF} for fitness and consequently the dynamic of IASC resolution. We focus on the effects of small changes in temperature, which is expected to fluctuate within a microclimate during an individual's lifetime (as opposed to drastic changes in the environment brought about by climate change). Variation in temperature is likely to influence a large number of physiological processes and the expression level of a large number of genes, it is therefore a parameter that is well suited to examining how intersexual genetic correlations are affected by changes to the environment.

Finally, temperature can be easily manipulated along a gradient in the laboratory to test its effects.

We assayed sex-specific fitness of hemiclonal lines, sampled from our laboratory-adapted population of *D. melanogaster*, and determined r_{MF} at temperature treatments that represented small, non-overlapping deviations (separated by 2°C) from the standard temperature to which the flies had adapted for hundreds of generations. In contrast to the studies above (Delcourt *et al.* 2009; Berger *et al.* 2014; Punzalan *et al.* 2014), we limited experimental treatments to adult stages only, which allowed the effects of temperature on adult competitive ability and reproduction to be separated from the more pervasive effects of temperature on individual development and development of offspring. This is a key component of this study, as changes to sex-specific selection within an individual's lifetime could determine the outcome of IASC. We also quantified three adult behavioral phenotypes in each sex, using video playback of paired-mating trials, to test whether they correlate with sex-specific fitness at each temperature: courtship latency, copulation latency and copulation duration.

3.3 - Methods

Base Population

LH_M is a large, outbred population of *D. melanogaster,* which has been maintained in the laboratory for over 500 non-overlapping generations under standardised environmental conditions of 25°C, 12:12 light:dark cycle, 65% humidity and yeast-agar-molasses media (see section 1.8).

Creating Hemiclonal Lines

We used cytogenic cloning to create 223 hemiclonal lines sampled from LH_M (see **Chapter 2** for a detailed methods description), where males and females within a line express identical wild-type (LH_M) haplotypes in different random wild-type (LH_M) genetic backgrounds (Chippindale *et al.* 2001; Rice *et al.* 2005).

Briefly, 223 males were sampled from LH_M and crossed with "clone generator" (CG) females. The CG females possess a double-X and a translocation of the major autosomes 2 and 3, which forces the transmission of the entire LH_M haplotype from father to son (with the exception of the fourth dot chromosome). Thus we were able to create 223 lines with multiple 'stock hemiclonal males', which within each line possess the same LH_M haplotype. To produce female or male hemiclonal flies for fitness assays, stock hemiclonal males were crossed with virgin LH_M or double-X-LH_M females, respectively. Within each line, individual hemiclonal flies of both sexes therefore share one nearly complete genomic LH_M haplotype, where the other haplotype is a random sample from the LH_M base population (see section 1.9 for more information on hemiclonal analysis).

Choosing Sexually Antagonistic Genotypes

Total adult lifetime fitness for 223 hemiclonal haplotypes when expressed as either male or female was obtained based on previously published protocols (**Chapter 2**), which closely match the rearing conditions of the LH_M base population (section 1.8). A sub-set of 14 hemiclonal lines were selected for the current study based on the criteria that they harboured high levels of sexually antagonistic genetic variation for fitness at 25°C (the standard laboratory temperature conditions), characterised by a negative correlation between male and female fitness. To choose these lines, fitness values were first relativised within each sex by dividing each raw fitness value within a sex by the maximum raw fitness value within a sex (across replicates). The average relative male and female fitness values of each line were then ranked separately from highest to lowest. A total of 7 lines were selected with a female bias in fitness, hereafter referred to as 'feminised' genotypes. These lines showed the largest difference in fitness rank between males and females, where females were the higher-ranking sex. Similarly, 7 lines were selected with a male bias in fitness, referred to as 'masculinised ' genotypes. These lines were chosen in the same way, but where males were the higher-ranking sex. A total of 3 lines where variance in relative fitness was greater than 0.05 in either sex were excluded from the selection procedure.

Line selection allowed us to choose lines showing large fitness differences between the sexes, in order to increase power to detect changes in sexual antagonism when treatments were applied (Rice and Chippindale 2001). For the current experiment, sex-specific adult fitness was quantified again under the standard temperature (25°C) that the LH_M population has adapted to, as well as at two further treatment temperatures (23°C and 27°C).

Fitness Assays at 23°C, 25°C and 27°C Using Selected Genotypes

All hemiclones used in the following fitness assays were reared under standard temperature conditions (25°C). Independent crosses were carried out for each treatment to obtain male and female hemiclones from the 14 lines previously selected to represent 'masculinised ' and 'feminised' genotypes. All non-target flies used in fitness assays carried a brown-eye marker (homozygous for the brown eye-colour allele: bw^-/bw^-) that was introgressed into an LH_M background. Hemiclonal flies had wild-type red eyes (bw^+/bw^+)

Male fitness assays were carried out as follows: for each treatment, 5 hemiclonal males from each line were combined with 10 brown-eyed competitor males (bw^-/bw^-) and 15 brown-eyed virgin females (bw^-/bw^-) in a yeasted vial containing standard agar food mixture. Vials were immediately transferred to the treatment temperature (23°C, 25°C or 27°C). After 2 days, females were isolated into individual test-tubes (with 3ml of food) and left to oviposit in their respective treatment temperature for 18 hours. After this time, all test tubes were returned to the standard conditions, where temperature was maintained at 25°C. On Day 12 after egg-laying, progeny from each female was scored for eye-colour. Hemiclonal males were assigned paternity to progeny with wild-type red eyes (bw^+/bw^-), giving an average fitness score (number of offspring sired out of total progeny) for the 5 hemiclonal males that were assayed per line. This assay was replicated 6 times for each treatment, representing a total of 30 hemiclonal males per line and per treatment. Relative fitness measures were calculated within treatment and across replicates.

The female fitness assays followed a similar protocol to the male fitness assays. Here, for each treatment and line, 5 virgin hemiclonal females were combined in vials with 10 brown-eyed competitor females (bw^-/bw^-) and 15 brown-eyed males (bw^-/bw^-) for 2 days. These were immediately transferred to their respective treatment temperatures (23°C, 25°C or 27°C). After 2 days, the 5 hemiclonal females were isolated in individual test-tubes and left to oviposit in their treatment temperature for 18hrs. After this time, test-tubes were transferred to 25°C. On day 12, the number of offspring that had emerged was counted to provide a fecundity score for each female. By averaging this measure across all 5 females this provided an average female fitness score for that line and treatment. As with the male assay, this assay was replicated 6 times, representing a total of 30 hemiclonal females per line and per treatment. Relative fitness measures were calculated within treatment and across replicates.

Data loggers (Tinytag Talk 2; model: TK-4014-MED; Gemini Data Loggers UK) were placed inside incubators during each assay, where temperature was recorded at 5 minute intervals to a resolution of 0.05°C. This provided real-time estimates of temperature frequency distributions when applying each treatment (Figure 3.2).

Behavioral Assays at 23°C, 25°C and 27°C Using Selected Genotypes

Independent crosses were carried out again for each treatment to obtain male and female hemiclones from the 14 lines previously selected to represent 'masculinised ' and 'feminised' genotypes.

The behaviour of males and females from each line was assayed by analysis of 1hr long video footage of paired mating trials, conducted during the light phase of the light:dark cycle. For each treatment, 1 hemiclone male from each line was combined in a yeasted test tube containing 3ml standard agar food mixture, with 1 virgin female (bw^-/bw^-). Test tubes were immediately transferred to incubators set to the treatment temperature (23°C, 25°C or 27°C) and video cameras (Panasonic HC-V520) were set to begin recording for 1 hour. This was

replicated 6 times for each hemiclonal line at each treatment temperature. Treatments were carried out in succession throughout the day. On different days, the order of treatments was changed.

For female mating trials, at each treatment temperature 1 hemiclone female from each line was combined in a yeasted test tube containing 3ml standard agar food mixture, with 1 male (bw^-/bw^-). Test tubes were immediately transferred to incubators set to the treatment temperature (23°C, 25°C or 27°C) and video cameras (Panasonic HC-V520) were set to begin recording for 1 hour. This was replicated 6 times for each hemiclonal line at each treatment temperature.

Videos were played back (QuickTime Player 7) and three behaviours were recorded during each mating trial: courtship latency, defined as the time elapsed since the beginning of the trial until the male initiated first courtship (wing vibration); copulation latency, defined as the time elapsed from the beginning of the first courtship to the beginning of copulation; and copulation duration, defined as time elapsed from when the genitalia were engaged to when they were separated.

Statistical Analyses

Sex-Specific Fitness of Selected Genotypes at 23°C, 25°C and 27°C

The following method was applied separately to each treatment dataset. Across the 14 lines, relative fitness measures were calculated by dividing each raw fitness value by the maximum value within a sex and across replicates. We used these values to quantify the level of sexually antagonistic genetic variation in our chosen lines at each temperature. Relative male and female fitness data were analysed by fitting a linear mixed model using the 'MCMCglmm' R package (Hadfield 2010) in R v.3.1.2 (R Core Team 2014), with Bayesian inference and Markov chain Monte Carlo sampling techniques. In this model, $Y = S + L + R + \varepsilon$, where Y is relative fitness; S (sex) is a fixed effect; L (line) is a 2×2 matrix that specifies sex-specific variances among lines and their covariance; R (replicate) is a 2 x 2 matrix that specifies sex-specific variances among replicates and their

covariance; and ε is a matrix of sex-specific, within-line residual variances. Flat priors were used. Using this model, total phenotypic variance was partitioned into sex-specific genetic components and their correlation, from which the intersexual genetic correlation (r_{MF}) for fitness could be estimated (Table 3.1). As hemiclones share only half of their genome (section 1.9), sex-specific line variances and covariances were multiplied by two. To calculate r_{MF}, the between-sex covariance for fitness was divided by the product of sex-specific additive genetic variances for fitness.

Next, we characterised the relative contribution of sexually antagonistic and sexually concordant genetic variation for fitness at each temperature, using a method analogous to principle component analysis (see Berger *et al.* 2014 for details). Bootstrapping was first applied to each treatment dataset, so that 6 replicates were drawn at random (with replacement) from each line and sex, a total of 10000 times. For each of the samples, sex-specific line relative fitness scores were then projected along axes that described the direction of genetic variation (described in **Chapter 2**): the first axis, with a gradient of 1, described sexually concordant genetic variation (r_{MF}= 1). The second axis was orthogonal to the first (gradient = -1), and described sexually antagonistic genetic variation (r_{MF}= -1). The bootstrapping method enabled us to obtain a point estimate of the percentage of sexually antagonistic variation and 95% confidence intervals for each treatment (Figure 3.1).

To explore the contribution of the selected genotypes to IASC across temperatures, the sex-bias in fitness was calculated separately for masculinised and feminised genotypes. For each line, average female relative fitness was subtracted from average male relative fitness. Values greater than zero indicated a male bias in fitness, and values less than zero indicated lines that were female biased in fitness. The sex-bias in fitness was compared separately for feminised and masculinised genotypes across each treatment temperature (Figure 3.2), using Tukey HSD pairwise comparison in R.

The changes in sex-specific relative fitness underlying the sex-bias in fitness were explored separately for masculinised and feminised groups by comparing

the relative fitness values of lines across temperatures with a linear mixed effects model, using the 'nlme' (Pinheiro *et al.* 2015) package in R and the lme function. The model included temperature as a fixed effect, with line and replicate as random effects. Post-hoc analysis with 'multcomp' package (Hothorn *et al.* 2008) and the glht function was used to conduct Tukey all-pair comparisons of relative fitness across temperatures (Figure 3.3). Raw fitness values (number of progeny produced) were also compared across temperatures, separately for males and females with masculinised and feminised genotypes, using the same methods described above (Table 3.2).

Sex-Specific Behaviours of Selected Genotypes at 23°C, 25°C and 27°C

For courtship latency, the maximum time (3600 seconds) was given if no courtship occurred. For both copulation latency and copulation duration, trials were excluded from the analysis if no copulation occurred. For each behaviour, the data were analysed by fitting a linear model (lme function in package 'nlme': Pinheiro *et al.* 2015) with behaviour as the response variable, temperature as a fixed effect, and line and replicate as random effects. Post-hoc analysis with 'multcomp' package (Hothorn *et al.* 2008) and the glht function was used to conduct Tukey all-pair comparisons across temperatures.

3.4 - Results

Quantifying the Strength of IASC Across Temperatures

We found that point estimates of r_{MF} for fitness across the 14 selected lines were negative at all three temperatures in the current experiment (Table 3.1), but credible intervals were large and overlapping zero (Table 3.1), in part due to the small number of genotypes modelled.

The direction of sex-specific selection was explored further by projecting relative male and female fitness scores along axes that described sexually concordant and sexually antagonistic variation (Berger *et al.* 2014). We found that 63% (95% CI: 44 - 78), 51% (95% CI: 34-70) and 61% (95% CI: 49-73) of the genetic

variation for fitness was sexually antagonistic at 25°C, 23°C and 27°C respectively, whereas the remaining variation was sexually concordant (Figure 3.1). This provides an alternative view on the contribution of sexual antagonism to overall fitness variance, where if r_{MF} = 0 then 50% of the fitness variance would be sexually antagonistic and 50% would be sexually concordant. The contribution of sexually antagonistic variation to overall fitness variation was lowest at 23°C, but still explained a large proportion of fitness variance at all three temperatures.

Exploring the Direction of IASC Across Temperatures

There was a significant effect of temperature on the sex-bias in fitness for masculinised and feminised genotypes (Table 3.2). At 25°C, the masculinised genotypes showed a male bias in sex-specific fitness and feminised genotypes showed a female bias in sex-specific fitness (Figure 3.2). As a general pattern, masculinised genotypes increased in male-biased fitness at cooler temperatures but this sex-bias in fitness disappeared at warmer temperatures (Figure 3.2). Although the difference in sex bias of relative fitness between temperatures was not statistically significant when comparing 25°C with either 23°C or 27°C, the difference was significant between 23°C and 27°C (Tukey HSD; P=0.012). In comparison, feminised genotypes showed greater female bias in fitness at warmer temperatures, but a loss of sex specific fitness bias at cooler temperatures (Figure 3.2). For these genotypes the sex bias in fitness was significantly lower at 23°C when compared to both 25°C (Tukey HSD; P=0.02) and 27°C (Tukey HSD; P=0.003). Although the point estimate for female bias in fitness was higher at 27°C compared to 25°C, this difference was not statistically significant.

Explaining Sex-Specific Temperature Effects on Relative Fitness

We investigated changes in the absolute values of fitness and relativised fitness measures for masculinised and feminised genotypes of each sex to uncover why the sex-bias in fitness (Figure 3.2) changed across temperatures. For masculinised and feminised male genotypes there was a significant effect of

temperature on both absolute fitness and relative fitness (Table 3.2). For masculinised female genotypes there was no significant effect of temperature on absolute fitness, but this effect was significant for feminised female genotypes (Table 3.2). For masculinised and feminised female genotypes there was a significant effect of temperature on relative fitness (Table 3.2).

For masculinised genotypes, the significant increase in male-bias of relative fitness at 23°C compared to 27°C can be attributed to an increase in the relative fitness of males and a decrease in the relative fitness of females. Males with masculinised genotypes sired significantly more offspring at 23°C compared to 25°C (Tukey HSD, P=<0.001) and 27°C (Tukey HSD, P=0.002; Figure 3.4). When male fitness data was relativised, the difference between 23°C and both 25°C (Tukey HSD, P= 0.011) and 27°C (Tukey HSD, P=<0.001) remained significantly different (Figure 3.3). In contrast, the number of offspring produced by females with masculinised genotypes was not significantly different at 23°C compared to 27°C (Tukey HSD, P = 0.8547); however, relative female fitness was significantly lower at 23°C compared to 27°C (Tukey HSD, P=0.0001; Figure 3.3). This difference is likely due to a lower variance and maximum reproductive output at 23°C (Table 3.3).

The loss of male-bias in relative fitness for masculinised genotypes at 27°C was caused by a marginally non-significant increase in female relative fitness at 27°C, compared to 25°C (Tukey HSD, P=0.0505; Figure 3.3). This was not caused by an increase in the number of offspring produced by females at 27°C (Tukey HSD, P= 0.106; Figure 3.5), but by reduced variance and lower maximum reproductive output at 27°C compared to 25°C (Table 3.3).

For feminised genotypes, the significant loss in the sex-bias of fitness at 23°C (compared to both 25°C and 27°C) was driven by an increase in the relative fitness of males. The proportion of offspring sired by males with feminised genotypes was greater at 23°C compared to 25°C (Tukey HSD, P=2.12x10^{5}), but there was no significant difference between 23°C and 27°C (Tukey HSD, P=0.995; Figure 3.4). Once relativised, the difference remained significant between 23°C

and 25°C (Tukey HSD, *P*=0.028; Figure 3.3). In comparison, females with feminised genotypes produced fewer offspring at 23°C compared to 25°C (Tukey HSD, *P*=0.028; Figure 3.5), but this did not remain significantly different once relativised (Tukey HSD, *P*=0.805; Figure 3.3).

The non-significant increase in female-bias in relative fitness for feminised genotypes at 27°C compared to 25°C can be explained by a significant trend for higher female relative fitness at 27°C compared to 25°C (Tukey HSD, *P*=0.007; Figure 3.3). This is not explained by an increase in the number of offspring produced (Tukey HSD, *P*=0.106; Figure 3.5), but rather by a decrease in the variance and maximum number of offspring produced by all females at 27°C (Table 3.3), compared to 25°C.

Exploring Behavioural Phenotypes Across Temperatures

There was a significant effect of temperature on female courtship latency (Table 3.4): Females with masculinised and feminised genotypes had a significantly longer courtship latency at 27°C (Figure 3.6), compared to 25°C (Tukey HSD, *P*=0.025). However, for females with masculinised and feminised genotypes there was no significant difference in copulation latency or copulation duration across temperatures (Table 3.4).

For males with masculinised and feminised genotypes there was no significant difference in courtship latency or copulation latency across temperatures (Table 3.4). However, there was a significant effect of temperature on male copulation latency (Table 3.4): for males with masculinised and feminised genotypes there was a significantly longer copulation duration at 23°C (Figure 3.7), compared to 25°C (Tukey HSD, *P*=0.035) and 27°C (Tukey HSD, *P*=0.015).

3.5 - Discussion

IASC explained a substantial proportion of fitness variation across all treatments (Figure 3.1) but the balance of this conflict changed due to sex-specific effects of temperature on fitness (Figure 3.2): males were more successful at cooler temperatures, creating a male bias in fitness; whereas females were more

successful at warmer temperatures, creating a female bias in fitness. Moreover, although all genotypes (hemiclonal lines) showed primarily sexually antagonistic fitness effects at 25°C, some of the genotypes became sexually concordant at either 23°C or 27°C and other genotypes became more sexually antagonistic at these temperatures. This balance of fitness effects meant that sexual antagonism was still strong at all three temperatures (Figure 3.2), but there was a trend for stronger sexual antagonism at the temperature at which the population is maintained (25°C). One way this might arise is if under novel environments alleles that have conditional fitness effects become more important for fitness, which lowers the contribution of sexually antagonistic alleles to fitness variance (Hoffmann and Merilä 1999; Tomkins *et al.* 2004; Martin and Lenormand 2006; Long *et al.* 2012; Long *et al.* 2013). This was demonstrated by Berger *et al.* (2014) who showed a reduction in IASC driven by losses in fitness in both sexes caused by stress in a novel environment. Our results however do not fit with this hypothesis, as we show that the effects of novel temperatures were not caused by stress responses, but rather by increases in sex-specific fitness. This changed the balance of sex-specific fitness effects of previously sexually antagonistic genotypes so that they became sexually concordant. Other studies showed variable changes in the strength of conflict in novel environments (Berger *et al.* 2014; Punzalan *et al.* 2014), which might also be due to changes in sex-specific selection, but these were not quantified.

In contrast to comparable studies on the environmental effects on IASC (Declourt *et al.* 2009; Berger *et al.* 2014; Punzalan *et al.* 2014), we separated the effects of temperature on adult survival and reproduction from processes of individual development and the development of offspring. This is a key distinction because we show that changes in sex-specific selection can occur within the adult life stage to affect the outcome of IASC. Collectively, our results highlight the transient nature of sexual conflict and emphasise the important role of environmental stochasticity in influencing its outcome.

In our study, treatments were largely non-overlapping but separated by only 2°C (Figure 3.2), yet this small temperature difference was sufficient to produce

distinct patterns in sex-specific fitness across treatments. Most research however, focuses on the effects of extreme temperature on fitness-related phenotypes in *Drosophila*, such as survival and recovery rate, rather than the effects of discreet temperature variation. We can infer from these studies that the sexes often respond differently to changes in temperature, as we find here. For example, there are cases where males were reported to be less resistant to heat stress than females (Krebs and Loeschcke 1994; Dahlgaard *et al.* 1998), and others where males were the more resistant sex (Williams *et al.* 2012; Condon *et al.* 2015). There is also evidence that males take longer to recover from extreme cold temperatures (David *et al.* 1998; Condon *et al.* 2015). As well as sexually dimorphic patterns of temperature responses, there is evidence for sex-specificity in adult temperature preference in some *Drosophila* species (Yamamoto 1994); however such preferences are not evident in other species of this genus, including *D. melanogaster* (Yamamoto and Ohba 1982; Krstevska and Hoffmann 1994; Yamamoto 1994; Sayeed and Benzer 1996).

Two previous studies also measured sex-specific responses to selection for desiccation resistance in the LH_M population (Chippindale *et al.* 1998; Kwan *et al.* 2009). They observed differential pathways of best response for males and females regarding development, behaviour, and fertility schedules (Chippindale *et al.* 1998; Kwan *et al.* 2009). Their results were also consistent with the idea that intersexual genetic correlations can constrain the sexes from reaching new fitness optima and therefore spark new conflicts under different selective regimes.

Slight deviations around optimal temperature preferences are known to invoke physiological changes, involving distinct chemical pathways in ectotherms, that potentially affect fitness (Dillon *et al.* 2009). The differential effects of temperature on male and female fitness found here occurred during the adult stage of development, as focal flies had no prior exposure to treatment temperatures. These effects are likely to arise due to sex-specific physiological responses to temperature (Hariharan *et al.* 2014), which could affect reproductive behaviour or other aspects of reproduction, such as gamete

function pre- and post-embryogenesis (Ashburner *et al.* 2005). We quantified three behaviours in males and females from each of the selected lines, when exposed to either 23°C, 25°C, or 27°C: courtship latency, copulation latency, and copulation duration. We found that females with masculinised and feminised genotypes had a significantly longer courtship latency at 27°C, the temperature at which females perform the best (Figure 3.2), compared to 25°C. This could reflect temperature responses in females that resulted in a reduced ability to attract males. The cuticular hydrocarbon profile of females seems a likely candidate because it has been shown that changes in the composition of these chemical cues affects the likelihood that a male will attempt courtship in *Drosophila* (Siwicki *et al.* 2005). Temperature effects on female hydrocarbon profiles have also been demonstrated in *D. simulans* (Ingleby *et al.* 2013).

It should be noted that the behavioural assays conducted in our study were not under competitive conditions, whereas fitness assays took place under competitive conditions that the population had adapted to. It is possible that reduced courtship by males is advantageous to females in a competitive environment because it decreases the likelihood that mating occurs, which in turn benefits females by reducing costs associated with mating above their optimal threshold level (Arnqvist and Nilsson 2000). Both mating (Fowler and Partridge 1989; Chapman *et al.* 1995) and courtship (Partridge and Fowler 1990; Friberg and Arnqvist 2003) have been shown to directly decrease female fitness. Alternatively, behavioural effects in males might have led to decreased courtship activity towards females at higher temperatures (Patton and Krebs 2001; but see Best *et al.* 2012 who suggest that male courtship intensity should increase with temperature); however, we did not observe any significant effect of temperature on courtship latency in males. This outcome might have been affected by our study design, as hemiclonal males used in the male assays were isolated for 2 days prior to the observations, whereas males used for the female assays were not. This appears to have had an effect, as hemiclonal males were quick to initiate courtship, and there was little variation between males (Figure 3.7; but see Dukas 2005 who suggests that inexperienced males should be slower to initiate courtship). Another possibility is that females were better able to evade

courting males, as higher temperatures might have increased locomotory activity. It is known that males attempt courtship more frequently on slower moving females (Cook 1979) and that higher temperature increases locomotion (Gibert *et al.* 2001). Other research suggests that female mating rate increases with temperature, even when they are exposed to higher temperature prior to mating (Best *et al.* 2012); however, in this particular study the treatment temperatures did not exceed 25°C, which the flies were adapted to.

In addition to temperature effects on female behavioural phenotypes, copulation duration was significantly greater for males with masculinised and feminised genotypes at 23°C, the temperature at which males perform the best (Figure 3.2), compared to 25°C. It is likely that males are able to transfer greater quantities of seminal fluid with longer copulations (Gilchrist and Partridge 2000), which might translate into higher success in sperm competition. These changes in male and female behavioural phenotypes could therefore contribute to the temperature effects on sex-specific fitness that were identified in this study.

Changes in sex-specific selection for given genotypes across subtle and transient temperature manipulations are particularly relevant in the context of IASC resolution (see section 1.6). To mitigate conflict requires mechanisms that facilitate sex-biased gene expression, such as genomic imprinting (Day and Bonduriansky 2004), gene duplication (Connallon and Clark 2011; but see Hosken 2011), alternative splicing (McIntyre *et al.* 2006) and sex-specific gene modification (Ellegren and Parsch 2007). The time taken for such mechanisms to arise and fix within a population is expected to be long (Stewart *et al.* 2010) and will be further constrained by epistasis and pleiotropy (Mank *et al.* 2008). The extensive timeframe required for sex-specific gene expression to evolve (Lande 1980) also means that it is vulnerable to disruption, i.e. if selection pressures change. In natural populations, temperature can be variable and sometimes unpredictable. If, as we find here in a laboratory-adapted population, minor temperature shifts can reduce the relative strength of IASC for certain genotypes in natural populations (indicated by a change from primarily sexually discordant fitness effects to primarily sexually concordant fitness effects), this would

weaken selection for IASC resolution. For some traits, this might mean that perpetual cycles of IASC arise due to environmental variability, but conflict is never fully resolved. Such apparently trivial environmental effects on IASC could therefore help explain the maintenance of sexually antagonistic variation that has been shown for fitness and fitness correlates in natural populations (Brommer *et al.* 2007; Foerster *et al.* 2007; Mainguy *et al.* 2009; Svensson *et al.* 2009; Tarka *et al.* 2014).

An alternative hypothesis, is that the selected hemiclonal lines represented genotypes that were sexually antagonistic, but conflict was not mediated by sexually antagonistic alleles at individual loci in each sex. Instead conflict might have been driven by sets of alleles with sex-limited effects. For example, some alleles in masculinised genotypes may have positive fitness effects in males but are neutral for female fitness, and alleles at different genetic loci have negative fitness effects in females but are neutral for male fitness. It is perhaps more likely that the fitness affects we identified were in fact driven by a combination of alleles with sexually antagonistic or sex-specific effects. Although a recent model (Morrow and Connallon 2013) predicted that the equilibrium frequency of alleles with sexually antagonistic effects would be higher than for alleles with sex-limited effects on fitness, the genetic basis of sexual antagonism can only be verified if the identity of the loci involved are known.

Table 3.1 - Intersexual Genetic Correlations (r_{MF}) for Adult Fitness: a mixed model was used to partition the phenotypic variance for male and female adult fitness, and estimate the covariance between them, at each temperature.

	r_{MF}	CI
23°C	-0.16	-0.68; 0.43
25°C	-0.29	-0.81; 0.27
27°C	-0.18	-0.78; 0.35

Table 3.2 - Fitness Analyses of Variance (ANOVA): the effect of temperature on mean raw and relative fitness estimates for masculinised and feminised male and female genotypes, and on the sex-bias of fitness for masculinised and feminised genotypes. Bold *P*-values indicate statistically significant results.

	Raw fitness (male)			**Relative fitness (male)**		
Masculinised:	df	*F*	*P*	df	*F*	*P*
Temperature	2; 116	16.3387	**<0.0001**	2; 116	8.7287	**0.0003**
Feminised:						
Temperature	2; 118	13.84604	**<0.0001**	2; 118	3.65238	**0.0289**

	Raw fitness (female)			**Relative fitness (female)**		
	df	*F*	*P*	df	*F*	*P*
Masculinised:						
Temperature	2; 118	1.8003	0.1698	2; 118	4.4484	**0.0137**
Feminised:						
Temperature	2; 118	6.0861	**0.0031**	2; 118	7.73104	**0.0007**

	Sex-bias of fitness		
	df	*F*	*P*
Masculinised:			
Temperature	2	5.352	**0.015**
Feminised:			
Temperature	2	8.4066	**0.002641**

Table 3.3 - Raw Data Overview: male and female mean raw fitness for masculinised and feminised genotypes, and variance and maximum values of male and female raw fitness for both genotypes across treatment temperatures.

	Mean	Variance	Maximum
Female fitness: number of offspring produced			
Feminised 23°C	30.73	9.47	34.30
Masculinised 23°C	25.95		
Feminised 25°C	38.09	40.83	43.17
Masculinised 25°C	29.47		
Feminised 27°C	36.49	26.12	39.12
Masculinised 27°C	28.36		
Male fitness: proportion of offspring sired			
Feminised 23°C	0.09	0.000184	0.12
Masculinised 23°C	0.11		
Feminised 25°C	0.06	0.000153	0.10
Masculinised 25°C	0.08		
Feminised 27°C	0.09	0.000153	0.11
Masculinised 27°C	0.09		

Table 3.4 - Behaviours Analyses of Variance (ANOVA): the effect of temperature on three behaviours (courtship latency, copulation latency and copulation duration) for males and females. Bold *P*-values indicate statistically significant results.

	Male			Female		
Effect of temperature on:	df	*F*	*P*	df	*F*	*P*
Courtship latency	2; 222	1.213474	0.2991	2; 239	3.37934	**0.0357**
Copulation latency	2; 207	0.89556	0.41	2; 217	1.207821	0.3008
Copulation duration	2; 209	4.7263	**0.0098**	2; 217	0.955	0.3863

Figure 3.1 - Density Distributions of Estimates of Sexually Antagonistic Variation Obtained by Bootstrapping Treatment Datasets: 6 replicates were drawn at random (with replacement) from each line and sex combination, a total of 10000 times from each treatment dataset. Blue, red and black density curves represent estimates of the percentage of sexually antagonistic variation obtained by bootstrapping the 23°C, 25°C and 27°C datasets, respectively. Blue, red and black circles and horizontal bars represent point estimates and 95% confidence intervals for estimates obtained from the 23°C, 25°C and 27°C datasets, respectively.

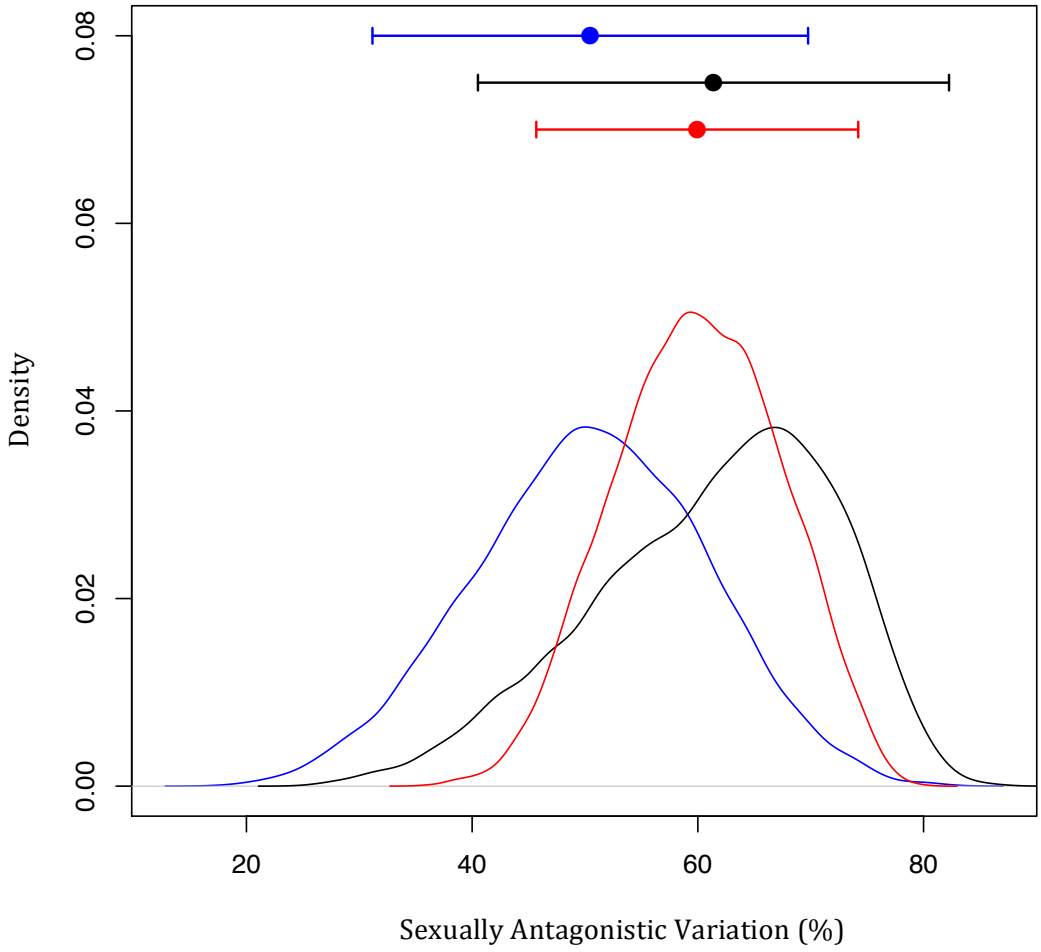


Figure 3.2 - Sex Bias in Relative Fitness: mean sex difference in relative fitness of masculinised and feminised genotypes and 95% confidence intervals, represented by closed and open circles respectively (0> indicates male fitness bias, 0< indicates female fitness bias). Letters indicate significant differences between temperatures based on Tukey HSD results for each genotype. Grey curves above each treatment temperature represent density distributions of temperature observations made using data loggers during each experiment (summary statistics for 23°C, 25°C and 27°C respectively - mean: 23.1, 25.4, 27.0; variance: 0.17, 0.27, 0.04; minimum: 21.7, 23.8, 26.1; maximum: 24.3, 26.7, 28.4; sample size: 6921, 4058, 6921).

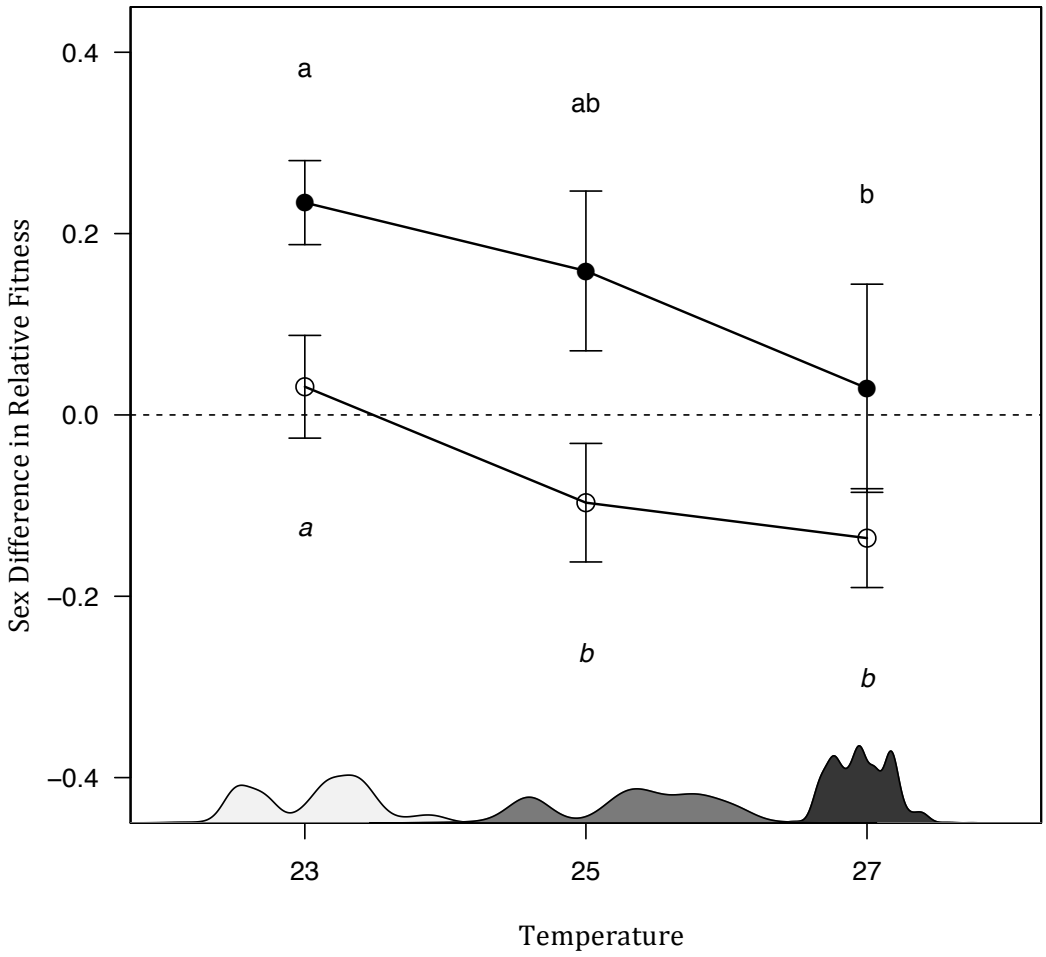


Figure 3.3 - Sex-Specific Relative Fitness: sex-specific relative fitness for masculinised and feminised genotypes at 25°C represented by closed and open circles respectively. Red and blue arrows indicate change in male and female relative fitness at warmer (27°C) and cooler (23°C) temperatures respectively. Stars (P=<0.05:*; P=<0.01:**; P=<0.001:***) and letters (ns: non significant) alongside horizontal and vertical grey lines represent the significance of differences in male and female relative fitness respectively between temperatures based on Tukey HSD results. For example, for feminised genotypes between 25°C and 27°C: there was no significant change (ns) in male relative fitness, but there was a significant increase in female relative fitness (**).

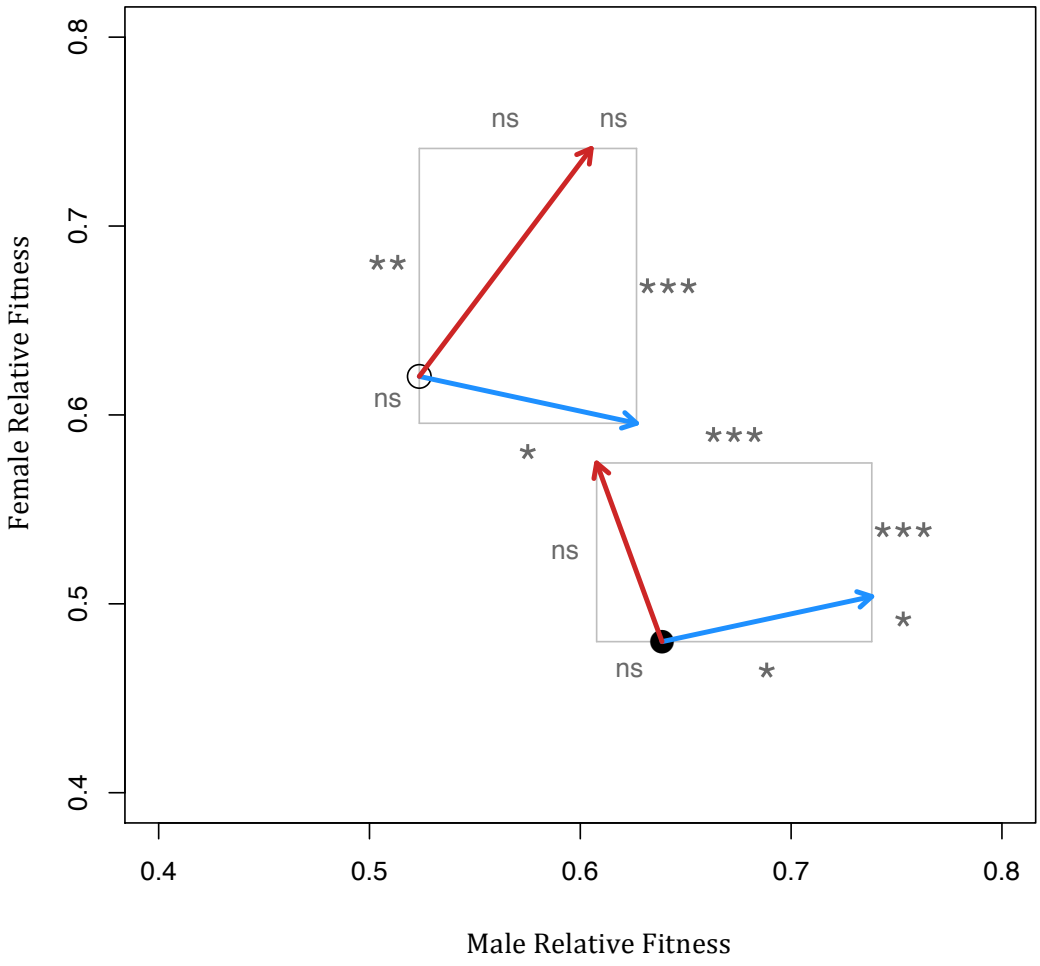
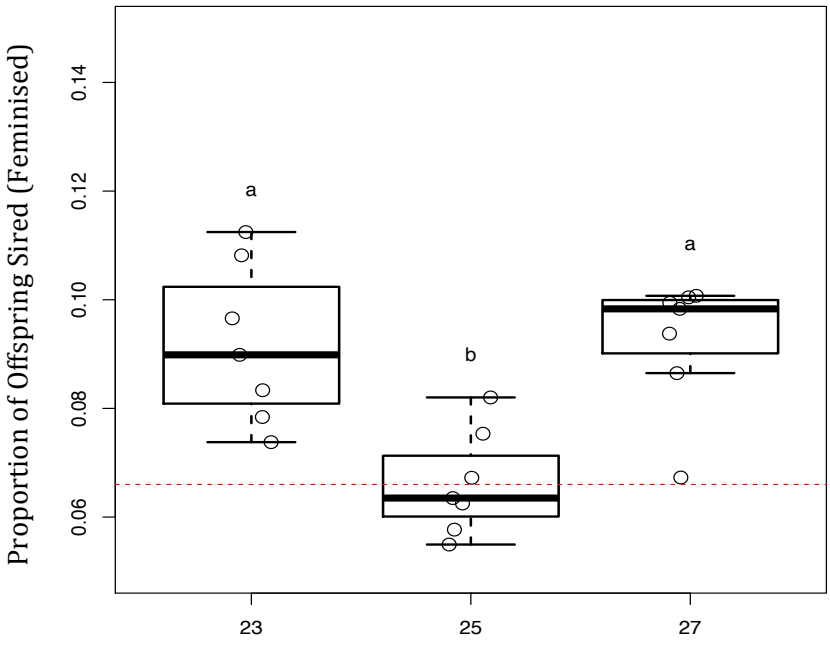


Figure 3.4 – Male Raw Fitness Across Temperatures: proportion of offspring sired per male with a) feminised and b) masculinised genotypes, with boxplots to represent median and interquartile range at each temperature. The red horizontal line indicates the expected proportion of offspring sired per hemiclonal male. Letters indicate significant differences based on Tukey HSD.

a)



b)

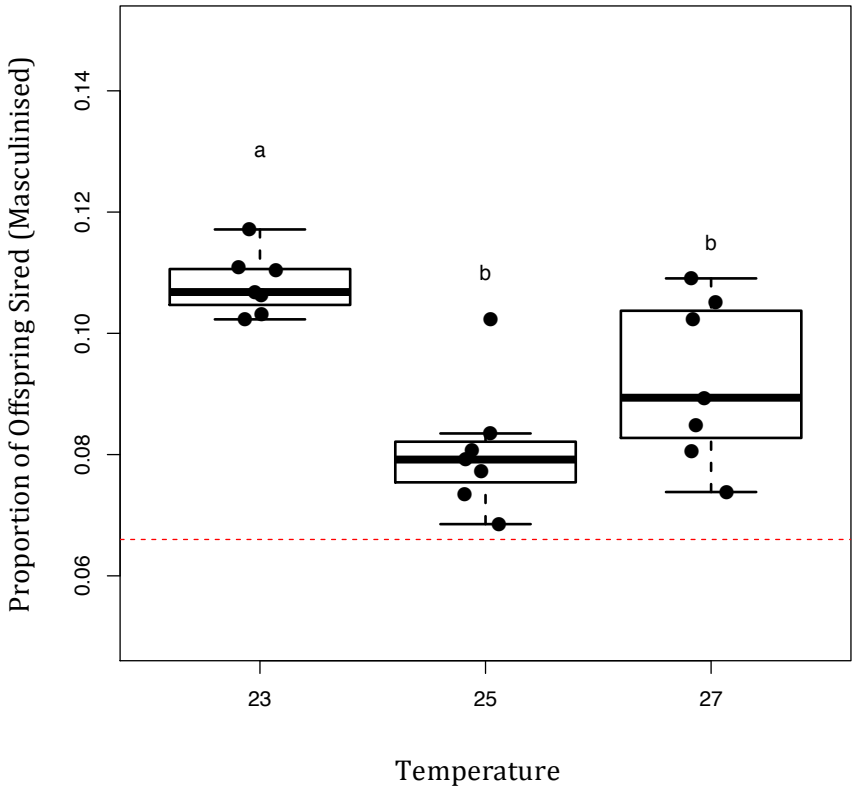
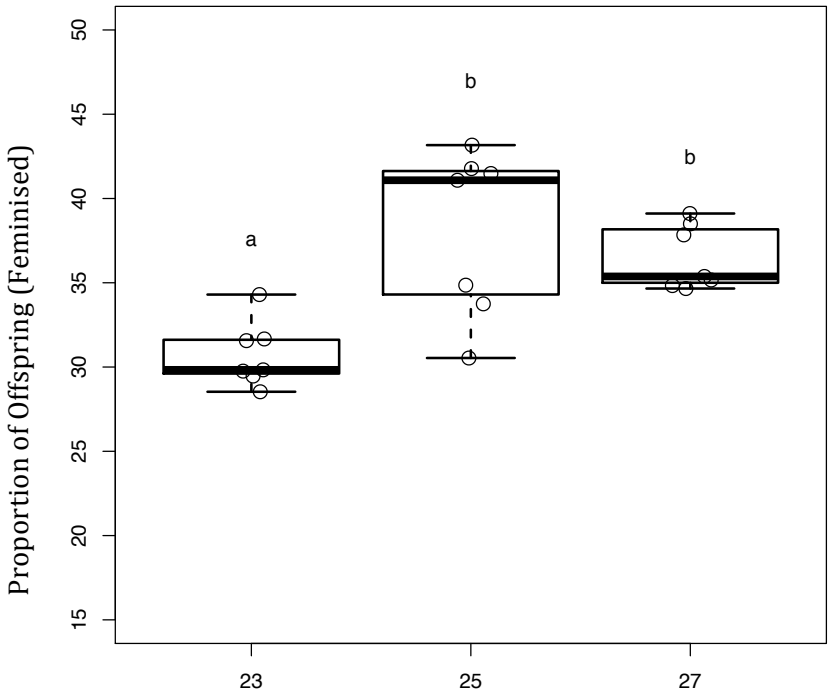


Figure 3.5 - Female Raw Fitness Across Temperatures: average number of progeny produced by females with a) feminised and b) masculinised genotypes, with boxplots to represent median and interquartile range at each temperature. Letters indicate significant differences based on Tukey HSD.

a)



b)

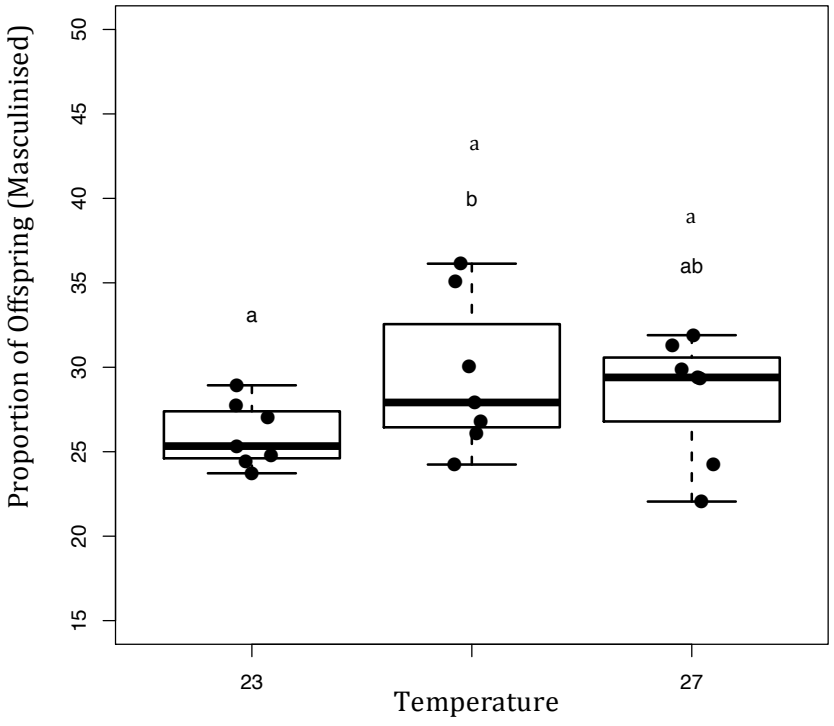
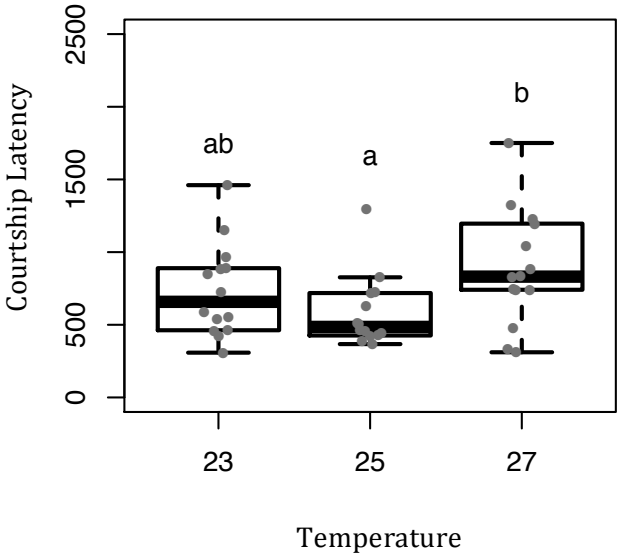
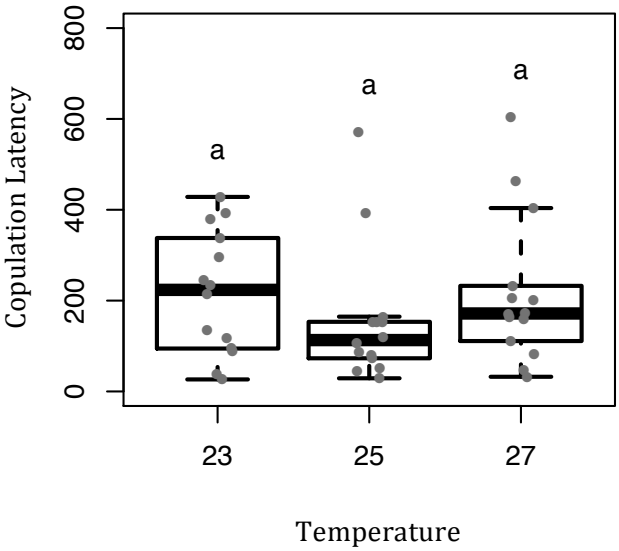


Figure 3.6 - Female Mating Trial Behaviours Across Temperatures: a) courtship latency, b) copulation latency and c) copulation duration (seconds) with boxplots to represent median and interquartile range at each temperature. Letters indicate significant differences based on Tukey HSD.

a)



b)



c)

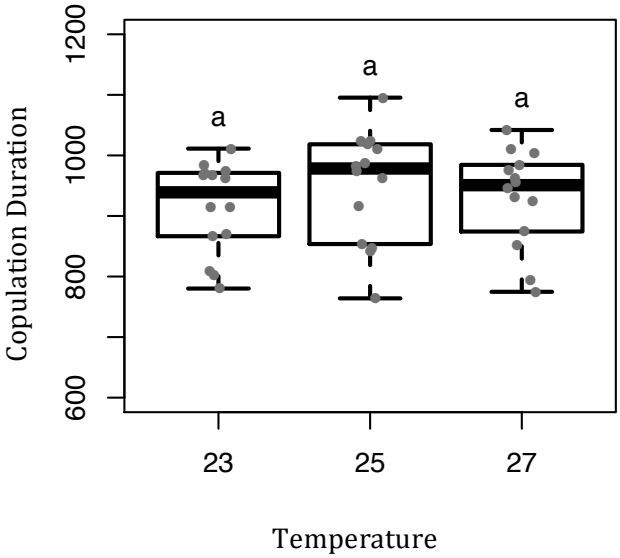
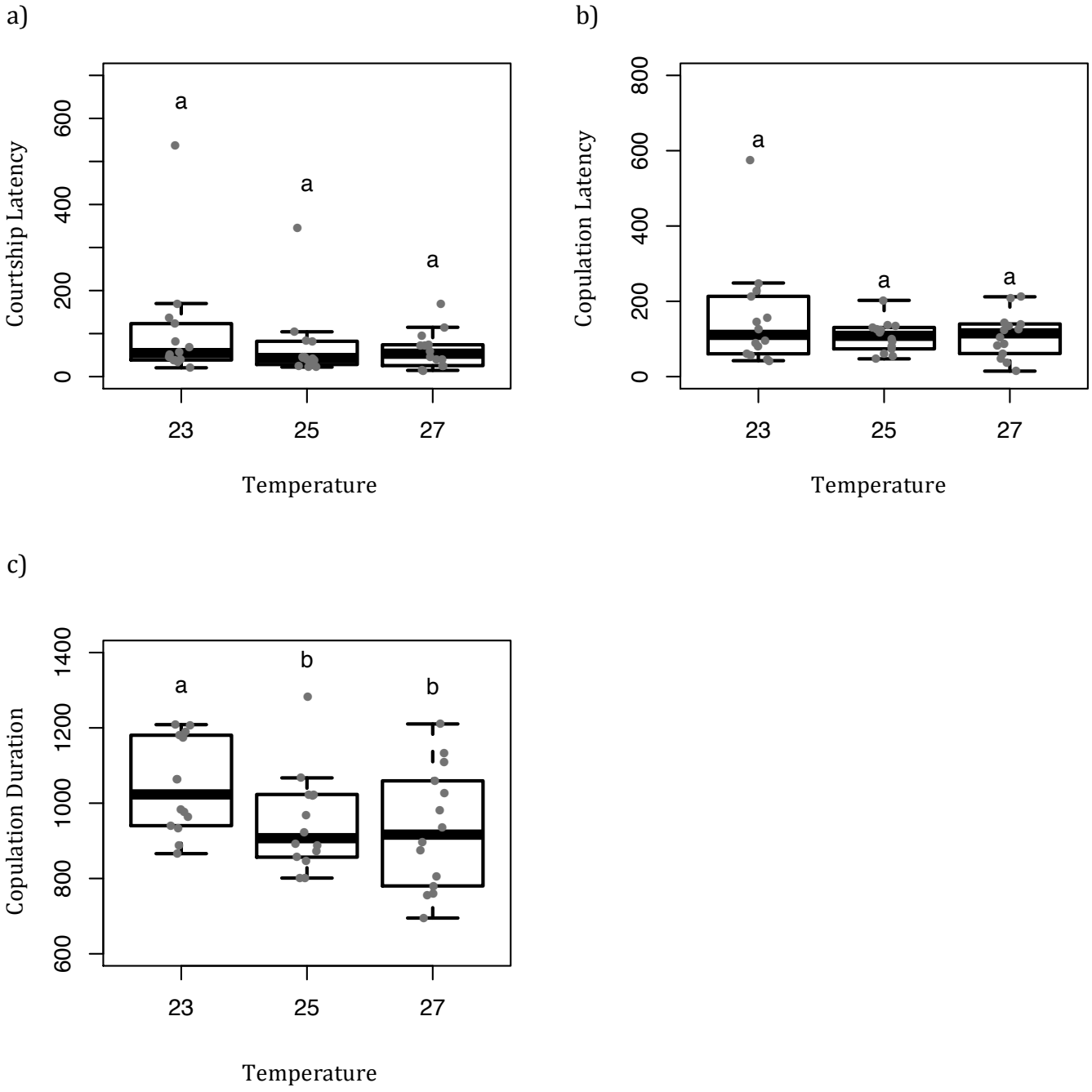


Figure 3.7 - Male Mating Trial Behaviours Across Temperatures: a) courtship latency, b) copulation latency and c) copulation duration (seconds) with boxplots to represent median and interquartile range at each temperature. Letters indicate significant differences based on Tukey HSD .



Chapter 4: Supporting a Truce, While Fuelling the Arms Race: Contrasting Effects of Intralocus Sexual Conflict on Sexually Antagonistic Coevolution

4.1 – Abstract

Evolutionary conflict between the sexes can induce arms races wherein males evolve traits that are detrimental to the fitness of their female partner and vice versa. This interlocus sexual conflict (IRSC) has been implicated as a cause of perpetual intersexual antagonistic coevolution with wide-ranging evolutionary consequences. However, theory suggests that the scope for perpetual coevolution is limited, if traits involved in IRSC are subject to pleiotropic constraints. Here, we consider a biologically plausible form of pleiotropy that has hitherto been ignored in treatments of IRSC, and arrive at drastically different conclusions. Our analysis is based on a quantitative-genetic model of sexual conflict, in which genes coding for IRSC traits have side effects in the other sex, due to incomplete sex-limited gene expression. As a result, the genes are exposed to intralocus sexual conflict (IASC), a tug-of-war between opposing male- and female-specific selection pressures. We find that the interaction between the two forms of sexual conflict has contrasting effects on antagonistic coevolution: pleiotropic constraints stabilise the dynamic of arms races if the mating traits are close to evolutionary equilibrium, but can prevent populations from ever reaching such a state. Instead, the sexes are drawn into a continuous cycle of arms races, causing the build-up of IASC, alternated by phases of IASC resolution (caused by shifts in sex-specific selection) that trigger the next arms race. This dynamic not only sparks IASC over new traits, but also creates inconsistent selection for sex-biased gene expression, which is likely to impact long-term IASC resolution.

4.2 - Introduction

The sexes have followed distinct evolutionary trajectories due to divergent selection regimes that have led to, and been exaggerated by, anisogamy (Parker 1979; Lande 1980, Hosken and Stockley 2004). This disparity has the potential

to ignite two forms of sexual conflict: interlocus and intralocus sexual conflict (IRSC and IASC, respectively). IRSC arises from a direct interaction between the sexes that increases the fitness of one sex at the expense of the opposite sex. On the other hand, IASC arises when males and females have different optimal trait values for a trait with a shared genetic basis. Here, alleles that have opposite fitness effects when expressed in each sex are maintained in a population. Both forms of conflict have been described as independent drivers of divergence and speciation (Parker and Partridge 1998; Rice and Holland 1997; Gavrilets 2000; Gavrilets 2014), and have important implications for the rate of trait evolution, the maintenance of genetic variation and sexual selection (Holland and Rice 1998; Gavrilets *et al.* 2001; Brommer *et al.* 2007; van Doorn 2009).

Rice and Holland (1997) integrated previous studies on various forms of intersexual conflict in their theory of interlocus contest evolution, which has since served as a basis for formal models of IRSC (Gavrilets 2000; Gavrilets *et al.* 2001; Rowe *et al.* 2005; Moore and Pizzari 2005). A key prediction of this framework is that IRSC will lead to coevolutionary arms races, similar to that seen between a parasite and its host. An analogy is drawn because in both scenarios one individual is gaining fitness at the detriment of another. Intersexual conflict, however, is centered on mating decisions and outcomes. Typically, males evolve adaptations for success in sperm competition and monopolisation of females (male offence traits), which often prevents females from obtaining fitness benefits through polyandry or sperm use (Arnqvist and Rowe 1995; Koene and Schulenberg 2005; Wigby and Chapman 2005). Subsequently, an arms race is initiated via the evolution of female counter-adaptations that reduce the fitness loss (female defence traits; Reinhardt *et al.* 2003; Koene and Schulenberg 2005). Repeated or even perpetual cycles of counter-adaptation in each sex are predicted to follow over evolutionary time, potentially leading to the rapid evolution of exaggerated trait values within populations (Gavrilets 2000). Rice and Holland (1997) propose that this coevolutionary process could affect the loci directly involved in intersexual interactions and other linked loci, thereby affecting a large proportion of the genome.

IRSC clearly manifests itself as a form of conflict in mating interactions, while IASC involves a more subtle type of sexual antagonism that operates at the level of phenotype expression. Here, conflict arises because the sexes share the same genome, but are nevertheless under selection to express different, sex-specific phenotypes (Rice 1984; Bonduriansky and Chenoweth 2009; Pennell and Morrow 2013). The resolution of IASC can be achieved via the evolution of sexual dimorphism (Lande 1980; Cox and Calsbeek 2009; Poissant *et al.* 2010; Wyman *et al.* 2013). Yet, the observation of negative intersexual correlations for fitness indicates that appreciable levels of IASC are maintained (Stewart *et al.* 2010; Gosden *et al.* 2012; Griffin *et al.* 2013; Ingleby *et al.* 2014), both in the wild (Brommer *et al.* 2007; Foerster *et al.* 2007; Mainguy *et al.* 2009) and in laboratory populations (Chippindale *et al.* 2001; Bedhomme *et al.* 2008; Innocenti and Morrow 2010; Hesketh *et al.* 2013). In fact, segregating sexually antagonistic alleles can be responsible for a substantial part of the standing genetic variation for fitness (Gibson *et al.* 2002) and are therefore likely to have an impact on adaptation and sexual selection (Brommer *et al.* 2007; van Doorn 2009). Moreover, the scope for sex-specific gene regulation and the rate at which sexual dimorphism will evolve in response to IASC, are likely to vary between populations, creating opportunities for trait divergence and the evolution of reproductive isolation, analogous to those arising from IRSC (Parker and Partridge 1998).

Given their different mode of operation, IASC and IRSC are traditionally considered as separate forces. In fact, in typical studies of IASC, fitness is frequency-independent and determined by a univariate trait, ruling out the possibility of coevolution between offence and defence traits characteristic of IRSC. Models of IRSC, on the other hand, consider interactions between at least two phenotypic characters expressed in a mating context, where the strategy of one sex is governed by a different set of loci than the trait(s) required by the other sex to counter-adapt (Rice and Holland 1997). This has commonly been interpreted to imply that loci involved in IRSC have sex-limited expression (Parker and Partridge 1998; Gavrilets *et al.* 2001; Rowe *et al.* 2005; Moore and

Pizzari 2005) and are, therefore, unaffected by IASC. Nevertheless, some authors have emphasized the role of pleiotropic side-effects, which may not be restricted to a single sex, in stabilising the dynamic of intersexual antagonistic coevolution (Gavrilets 2014; Rowe *et al.* 2005).

For instance, if the evolution of female indifference to a male mating signal is mediated by mutations in the female's sensory system (Holland and Rice 1998; Gavrilets *et al.* 2001), then those same mutations might be expressed in males as well. If so, potential negative side effects (like a reduced foraging efficiency) of female counter-adaptations to sexual conflict are subject to selection in both sexes. As in this example, many traits involved in sexual conflict have a complex genetic basis, providing ample opportunity for pleiotropic effects between male and female traits, by which the two processes of conflict can become linked. The potential that loci underlie both forms of conflict is further increased by the widespread occurrence of alleles associated with IASC or IRSC throughout the genome (Innocenti and Morrow 2010; Gibson *et al.* 2002; Andrés and Morrow 2003; Rice *et al.* 2005). Moreover, both IASC and IRSC are predicted to stem predominantly from reproductive traits, where the evolutionary interests of the sexes diverge the most (Stewart *et al.* 2010), although sexually dimorphic traits may, in fact, be subject to reduced IASC due to the prior evolution of sex-specific gene regulation (van Doorn 2009; Poissant *et al.* 2010). The potential for IRSC and IASC to interact has been highlighted recently (Pennell and Morrow 2013; and in **Chapter 1**), where it was noted that intersexual selection acting on a trait that is genetically correlated between the sexes, would often give rise to intralocus sexual conflict. It was also argued that the outcome of this interaction would depend on the opportunity for IASC resolution: IASC could persist and therefore prevent counter-adaptation of the trait in response to IRSC; or IASC could be resolved, resulting in the escalation of arms races stemming from IRSC. We here develop a formal, quantitative-genetic model of traits involved in inter- and intralocus sexual conflict, in order to verify these arguments and examine their implications for the evolution of sexual conflict. Our analysis supports the intuition that IASC can stabilise antagonistic male-female coevolution, but also indicates that the consequences of interaction between the two forms of sexual

conflict reach much further than anticipated. We finally discuss the implications of these results for the occurrence of perpetual arms races and the maintenance of sexually antagonistic variation in fitness.

4.3 - Methods

The Model

Biological Assumptions

Our analysis builds on a model of sexually antagonistic coevolution introduced by Rowe, Cameron and Day (2005), henceforth referred to as *"RCD05"*. The biological scenario considered in their study is that males and females are in conflict over the rate of mating, which is taken to be an increasing sigmoid function $\psi(s) = 1/(1 + \exp(-s))$ of the intensity of a mating stimulus, s. This particular formulation of the model captures the situation that mating is a contest between male offence and female defence traits, in which more extreme offence traits increase the rate of mating, whereas more extreme defence traits have the opposite effect. Biological examples of offence and defence traits include grasping and anti-grasping devices as seen in water striders (Arnqvist and Rowe 1995), or traumatic insemination and counter-adaptations to control its harmful effects, as found in bedbugs (Reinhardt *et al.* 2003) and hermaphroditic land snails (Koene and Schulenberg 2005).

In *RCD05*, the intensity of the mating stimulus perceived by a female is taken to be a function of three evolving phenotypic traits with sex-limited expression. Specifically, $s = z_{♀} \times (y_{♂} - x_{♀})$ depends on the difference between a persistence trait $y_{♂}$, expressed in males, and a female resistance trait, $x_{♀}$, reflecting the threshold amount of persistence required to induce mating. In addition, the perceived intensity of the mating stimulus depends on the sensitivity of the female, $z_{♀}$, which quantifies how strongly she discriminates between males that differ in their level of persistence. Male sexual fitness is modelled as an increasing function of the mating rate, such that sexual selection will invariably favour males who mate at a higher frequency. In contrast, females are assumed

to achieve maximal reproductive success at an intermediate mating rate θ_ψ. Selection may therefore act on females to reduce their rate of mating by increasing the mating threshold or evolving insensitivity to the mating stimulus. The latter response is likely when there are no pleiotropic constraints that prevent females from adjusting their sensitivity (Rowe *et al.* 2005). However, the sensory system underlying female mating behaviour is probably important in other contexts as well, such that the maximisation of female reproductive success may have negative consequences for fitness components unrelated to mating interactions. Similarly, evolving higher levels of persistence is presumably associated with increasing costs for males. In order to capture these effects, each of the mating traits is assumed to be subject to stabilising natural selection for an intermediate optimum.

In this paper, the analysis of *RCD05* is extended in two ways. First, if the mating characters have pleiotropic effects, then these need not necessarily be restricted to one sex. Therefore, we take into account that female resistance and sensitivity genes are expressed in males, denoting the corresponding phenotypic trait values as $x_♂$ and $z_♂$, respectively. Likewise, male persistence genes affect a correlated phenotypic character in females, of which the trait value is denoted as $y_♀$. Stabilising natural selection acts on x, y and z in both sexes in our model. The optimum trait values and the strength of stabilising selection are allowed to differ between males and females. Note that x, y and z still have sex-limited effects on the mating rate (as in *RCD05*), since their expression in the context of intersexual interactions is contingent on the asymmetry between male and female sex roles.

As a second extension, our model considers the dynamic of arms races also in cases where mating requires complementarity or matching of male and female mating characters. This alternative mating mechanism, which has frequently been considered in models of sexual conflict (Gavrilets 2014), is modelled by defining the mating rate as a unimodal function $\psi(s) = \exp(-s^2/2)$ of the mating stimulus $s = z_♀ \times (y_♂ - x_♀)$. In the same way as for sexual selection models, $x_♀$ can then be interpreted as a female mating preference, $y_♂$ as a male mating trait (e.g.,

an ornament) on which the preference acts, and $z_{♀}$ as a measure of female choosiness. For simplicity, we will continue to refer to the mating characters as threshold, persistence and sensitivity, as in *RCD05*, except when we are explicitly considering complementarity-based mating (in which case we will use preference, ornament and choosiness instead). Examples of sexually antagonistic mating systems that could be considered as complementarity-based include penis length-female reproductive tract coevolution in waterfowl (Hosken and Stockley 2004) and male seminal protein / female-receptor coevolution in fruit flies (Gioti *et al.* 2012).

A key feature of our model is that genes involved in IRSC are subject to distinct components of selection in males and females. As a result, selection is likely to favour different optimum trait values in the two sexes, setting the stage for IASC to occur. Prolonged IASC is expected when only a small fraction of the genes are regulated in a sex-specific manner, making it more difficult for males and females to diverge towards their sex-specific optima (Lande 1980; Cox and Calsbeek 2009; Gosden *et al.* 2012). The strength of the phenotypic correlation between brothers and sisters in their expression of a mating trait and the corresponding correlated character provides an observable measure of the degree of sex-differential expression. Additive-genetic intersexual correlation coefficients, which can be inferred from comparisons between opposite-sex relatives (Bonduriansky and Chenoweth 2009; Brommer *et al.* 2007), therefore play a prominent role as control parameters in our further analysis: their effect on the rate of IASC-resolution allows us to systematically vary the impact of IASC on antagonistic male-female coevolution.

Mathematical Representation

Based on fitness functions that capture the above biological assumptions, we calculated the strength of selection acting on each of the characters, and used this information to determine their rate of evolution (see Box 4.1). The evolutionary dynamic of the population average trait values is described by a multivariate breeder's equation (Lande and Arnold 1983), $d\mathbf{u}/dt = \mathbf{G}\beta(\mathbf{u})$, where $\mathbf{u}$ is a (column) vector $(\bar{x}_{♀}, \bar{z}_{♀}, \bar{y}_{♂}, \bar{x}_{♂}, \bar{z}_{♂}, \bar{y}_{♀})^{T}$ containing the average trait

values, and **G** is the additive genetic variance-covariance matrix. This matrix depends on the intersexual correlations r_x, r_y and r_z, as specified in Equation 4 of Box 4.1. The vector $\beta(\mathbf{u})$ is the selection gradient, given by

Equation 1:

$$\boldsymbol{\beta}(\mathbf{u}) = \begin{pmatrix} a\,\bar{z}_{\varphi}\,(\bar{\psi}-\theta_{\psi})\,\bar{\psi}' & -c_{x_{\varphi}}\,(\bar{x}_{\varphi}-\theta_{x_{\varphi}}) \\ a\,(\bar{x}_{\varphi}-\bar{y}_{\sigma})\,(\bar{\psi}-\theta_{\psi})\,\bar{\psi}' & -c_{z_{\varphi}}\,(\bar{z}_{\varphi}-\theta_{z_{\varphi}}) \\ b\,\bar{z}_{\varphi}\,\bar{\psi}' & -c_{y_{\sigma}}\,(\bar{y}_{\sigma}-\theta_{y_{\sigma}}) \\ & -c_{x_{\sigma}}\,(\bar{x}_{\sigma}-\theta_{x_{\sigma}}) \\ & -c_{z_{\sigma}}\,(\bar{z}_{\sigma}-\theta_{z_{\sigma}}) \\ & -c_{y_{\varphi}}\,(\bar{y}_{\varphi}-\theta_{y_{\varphi}}) \end{pmatrix}$$

Each element of the vector $\beta(\mathbf{u})$ quantifies the marginal fitness effect of varying one of the characters by one phenotypic unit, in the context of the current population with average trait values **u**. The upper three elements represent selection gradients acting on the mating traits $\bar{x}_{\varphi}$, $\bar{z}_{\varphi}$ and $\bar{y}_{\sigma}$, which depend on the fitness effects of mating interactions. The strength of sexual selection varies with ψ and $\bar{\psi}'$, the values of the mating rate function and its first derivative at $\bar{s} = \bar{z}_{\varphi} \times (\bar{y}_{\sigma} - \bar{x}_{\varphi})$. In addition, the impact of mating on female and male fitness is scaled by two parameters, *a* and *b*, that quantify, respectively, the cost to females of deviating from their optimum mating rate and the strength of the association between mating rate and male reproductive success. Direct selection on the correlated characters, reflected by the lower three elements of the selection gradient, occurs only in the form of stabilising natural selection, which also acts on the mating traits. Stabilising natural selection is parameterised for each trait by an optimum trait value θ and a selection intensity *c*, which determines how much fitness decreases when a phenotype is displaced by a given amount from its viability-selection optimum.

4.4 – Results

The coevolution of the mating characters $\bar{x}_{\varphi}$, $\bar{z}_{\varphi}$ and $\bar{y}_{\sigma}$ in the absence of between-sex pleiotropy has been analyzed by *RCD05*, and we briefly recapitulate

their results before examining the interaction between IRSC and IASC. A key finding is that, IRSC, acting by itself, has multiple potential evolutionary outcomes (Gavrilets 2014; Rowe *et al.* 2005). These include escalating arms races, the evolution of female indifference to the mating stimulus, and continual coevolution of threshold, persistence and sensitivity. Female indifference tends to evolve when females are able to adjust the shape of their preference function without major negative side effects, enabling them to avoid large fitness costs of IRSC at evolutionary equilibrium. By contrast, evolutionary arms races, which result in a significant reduction of female fitness, occur when adaptation of the female sensory system is constrained by a lack of genetic variation or strong stabilising selection on sensitivity in contexts other than mating. Hence, *RCD05* conclude that the outcome of IRSC depends critically on the constraints and selective forces that act on the female preference function.

Evolutionary Equilibria are Stabilised by IASC

Following Pennell and Morrow (2013; and **Chapter 1**), we hypothesised that IASC would restrain IRSC in cases with between-sex pleiotropic trait expression, potentially preventing the escalation of arms races. In particular, if the intersexual correlations are high and strong stabilising selection acts on the correlated characters $\bar{x}_{♂}$, $\bar{z}_{♂}$ and $\bar{y}_{♀}$, IASC is predicted to keep the mating traits fixed at an evolutionary equilibrium even if this would not be stable under the sole action of IRSC. To see why, suppose that one or several of the mating traits evolve away from the equilibrium under the influence of intersexual selection (i.e., sexual selection generated by variation in ψ). This change is associated with a correlated change of the homologous characters, causing those to deviate from their viability-selection optimum. As a result, the response to sexual selection is opposed by stabilising natural selection in the other sex, which pushes the traits back to their original values if the pleiotropic fitness effects outweigh the selective forces resulting from IRSC.

A formal equilibrium and stability analysis confirms this verbal argument (for detailed methods see **Appendix 1**), demonstrating that IASC can stabilise the dynamics of IRSC in the vicinity of evolutionary equilibria that would otherwise

be unstable. In such cases, IASC prevents the sexes from being engaged in an escalating arms race and allows them to sustain a stable 'truce'. The main prerequisite for stabilisation is that the intersexual genetic correlations and selection on the correlated characters must be sufficiently strong in the direction in phenotype space along which the arms race would have otherwise unfolded, so that stabilising natural selection is capable of overpowering the forces generated by IRSC.

The mathematical analysis leads to two additional insights. First, neither the location nor the number of fixed points depends on the values of the intersexual genetic correlations. As a result, the evolutionary equilibria of the mating characters are determined by sexual selection and within-sex stabilising natural selection, exactly as in *RCD05*, while the equilibrium values of the correlated characters are simply given by their respective optimum trait values $\theta\bar{x}_{♂}$, $\theta\bar{z}_{♂}$ and $\theta\bar{y}_{♀}$. Apart from being useful to characterise the evolutionary equilibria, this insight also restricts the range of phenomena that can be associated with qualitative changes in the dynamics of IRSC due to its interaction with IASC. Specifically, from the mathematical theory on qualitative changes in dynamical systems (bifurcation theory; Kuznetsov 2004), we infer that variation of the intersexual correlations can induce no other generic local bifurcation than a so-called Poincarè-Andronov-Hopf bifurcation, given that all other options require equilibria to move relative to one another. This bifurcation is associated with the emergence or disappearance of a cycle (i.e., periodic orbit), which can either act as an alternative evolutionary attractor or restrict the attainability of certain evolutionary outcomes. Hence, if IASC induces a qualitative change in the stability of an equilibrium, the associated appearance or loss of a cycle could dramatically alter the outcome of sexual conflict.

Dynamics of Sexual Conflict Away from Equilibria

To complement the insights offered by the local stability analysis, we studied the global evolutionary dynamic of the mating characters by means of numerical simulations, which were run separately for the two different mating scenarios. Figure 4.1 shows an illustrative outcome for the case that mating is a contest

between offence and defence traits. Here, IRSC leads to continuous fluctuations in the male persistence trait and the female sensitivity and mating threshold (cf. Figure 5b in *RCD05*). During these evolutionary cycles, the initiation of arms races between threshold and persistence is alternated by the evolution of female indifference to the mating stimulus, inducing the threshold and persistence trait to evolve back towards their optimal trait value under natural selection. The oscillations persist in the presence of low levels of pleiotropic expression between the sexes (Figure 4.1a), but their amplitude decreases if the intersexual genetic correlations are stronger. In that case, fluctuations of the mating traits induce a larger correlated selection response that is opposed more strongly by stabilising selection in the other sex. Modest-to-high values of the intersexual correlations (still below typical empirically observed values: Poissant *et al.* 2010) entirely prevent the initiation of arms races, causing the trait values to converge on a stable equilibrium (Figure 4.1b). These results are in line with the conclusions of the mathematical analysis.

More puzzling to explain are the simulation results obtained for the model variant with complementarity-based mating, illustrated in Figure 4.2. Here, again, panel 'a' shows the outcome of evolution when the intersexual genetic correlations are weak. In this case, females evolve costly choosiness, and we observe an arms race to exaggerated levels of ornamentation and corresponding costly preferences. Eventually, the sexes converge on a stable equilibrium, at which chase-away sexual selection (Holland and Rice 1998; Gavrilets *et al.* 2001) favouring further exaggeration is balanced by natural selection acting in the opposite direction. Antagonistic coevolution can escalate in two directions and thereby converge on two different fixed points (shown in the upper and lower part of panel a). When the intersexual correlations are high (Figure 4.2b), such that female choice is subject to strong pleiotropic constraints, arms races driven by chase-away sexual selection occur as well, but they are more effectively halted by natural selection, as a consequence of stronger pleiotropic side effects in the other sex. However, as the interlocus sexual conflict built up by the arms race is resolved, sexual selection on the female preference and male ornamentation changes direction, suddenly triggering an arms race in the

opposite direction. As a result, rather than stabilising the dynamics of IRSC, IASC prevents the coevolving sexes from reaching evolutionary equilibria, so that males and females are caught in a recurrent evolutionary cycle (thick lines in Figure 4.2b).

On first appearance, these results seem to contradict the conclusions from the mathematical analysis, which stated that stable equilibria of IRSC cannot be destabilized by IASC. However, the arms races observed in Figure 4.2b never get close to the endpoints of the coevolutionary chase in panel a, so we cannot yet infer the stability properties of the equilibria. We therefore ran simulations from other initial conditions, closer to the endpoints reached by evolution in panel a. The additional simulations (thin lines in the upper and lower part of Figure 4.2b) indicate that the sexes can still attain the same stable state characterised by exaggerated trait expression when the intersexual correlations are strong. In other words, no differences exist between panel a and b in the stability properties of the evolutionary equilibria, consistent with the analytical results. We are thus led to conclude that IASC has consequences for the dynamics of sexually antagonistic coevolution far away from equilibrium that contrast sharply with predictions derived from the local equilibrium stability analysis.

Figure 4.3 shows simulations for a different parameter set in which the evolutionary cycles observed in Figure 4.2b occur in a more basic form. These illustrate that the coevolutionary dynamic is dominated alternatingly by inter- and intralocus sexual conflict, with periods of arms races that displace the sexes from their optima, and phases of intralocus sexual conflict resolution that set the stage for the next arms race to occur. Note that the resolution of IASC in these simulations is only partly mediated by the evolution of sexual dimorphism. IASC is also resolved by dynamic changes in the direction of sex-specific selection and associated shifts in sex-specific fitness optima, which may temporarily align the selection gradients on correlated characters in males and females. At other times, the same process may cause sex-specific optima to diverge again, leading to the renewed build up of IASC. Furthermore, a comparison between the panels illustrates that the destabilising effect of between-sex pleiotropy is trait-specific:

in order for IASC resolution to reverse the direction of chase-away sexual selection (which requires that $x_{♀} - y_{♂}$ changes sign), the correlated selection response, which pulls the traits back to their viability selection optimum, must be larger in females than in males (see Figures S2-S4 in **Appendix 1**).

4.5 - Discussion

The potential for IASC to impact trait evolution and diversification caused by IRSC was emphasised in **Chapter 1** (reviewed in Pennell and Morrow 2013) but never before put to the test of formal analysis. Previous models of IRSC (Gavrilets 2000; Gavrilets *et al.* 2001; Moore and Pizzari 2005; Rowe *et al.* 2005; Gavrilets 2014), which have not included IASC, predict arms races of sexually antagonistic adaptation and counter-adaptation between the sexes, possibly leading to exaggerated traits and substantial fitness losses due to sexual conflict (Rice and Holland 1997). We included IASC in a quantitative-genetic model of IRSC trait evolution and found that, depending on the genetic architecture of traits (i.e., their degree of sex-limited expression) and the biological mechanism of mating interactions (i.e., whether compatibility is determined by a contest or trait complementarity), IASC can either restrain or induce male-female antagonistic coevolution.

The stabilising effect of IASC dominates near evolutionary equilibria. Here, selection is weak and trait values evolve slowly, so that there is ample time for the resolution of IASC. Since the correlated characters are close to their optimum, mutations expressed in mating interactions are also exposed to purifying selection in the other sex. A general mathematical argument confirms that this additional source of stabilising selection thwarts the initiation of arms races in populations at evolutionary equilibrium.

Far away from equilibrium, the mating traits evolve more rapidly, allowing unresolved IASC to build up. The pleiotropic effect of mating-trait mutations is then subject to directional selection in the other sex, which can slow down antagonistic coevolution, or even reverse its direction. The latter phenomenon occurs with complementarity-based mating, when the female preference, which

is ahead in the coevolutionary chase, is subject to stronger pleiotropic constraints than the male ornament, which is following behind (see Figures S2-S4 in **Appendix 1**). Furthermore, arms-race reversals occur above a critical level of the intersexual correlation, at which the correlated selection response to IASC resolution is sufficient to pull the mean female preference towards the other side of the male trait distribution, qualitatively changing the direction of chase-away sexual selection. In this way, rather than helping the sexes to maintain a truce, IASC fuels a never-ending cycle of IRSC arms races, interrupted by phases of IASC resolution, which set the stage for the next arms race to occur.

In conclusion, whether IASC stabilises or destabilises IRSC arms races is determined primarily by the balance between the rate of conflict resolution and the rate at which new sexually antagonistic variation is accumulated during episodes of rapid trait evolution. The time scale of both processes is affected by the genetic architecture of conflict traits, characterised in our analysis by the additive genetic intersexual correlation between mating characters and their pleiotropic homologues in the other sex. Research that has focused solely on IASC has shown that negative intersexual correlations for fitness are maintained in populations (Brommer *et al.* 2007; Cox and Calsbeek 2009; Foerster *et al.* 2007), suggesting that the resolution of IASC may be slow (Poissant *et al.* 2010; Stewart *et al.* 2010; but see Ranz *et al.* 2003). Yet, intersexual genetic correlations for individual traits are varied (Cox and Calsbeek 2009), with systematic differences existing among trait types (Poissant *et al.* 2010). Moreover, specific information on the extent of between-sex pleiotropy for traits involved in IRSC is scarce. Quantitative estimates of the strength of interaction between IASC and IRSC therefore require further developments in our understanding of the genetic basis of the two forms of sexual conflict.

The Genetic Architecture of Sexual Conflict

Currently, the most detailed studies of the genetics of IRSC come from seminal proteins. Sex peptide (SP), is one gene that has been recognised as a mediator of IRSC (Wigby and Chapman 2005), and other candidate genes have also been identified; including genes that are required by females to respond to SP post-

mating (Gioti *et al.* 2012). Furthermore, some candidate genes identified by Gioti *et al.* (2012) were not sex-limited in their expression, creating potential for them to also mediate IASC.

In addition to seminal proteins and their receptors, other traits that have direct roles in reproduction are plausible candidates to mediate both types of conflict. Given the complex genetic basis of many reproductive traits, pleiotropic effects in the other sex are not unlikely. Furthermore, reproductive traits are subject to strong selection, where the costs and benefits of mating are frequently different for males and females. Sex role asymmetries, fuelling conflicts of interest between the sexes, combined with divergent selection in males and females on traits with a shared genetic basis, thus set the stage for interactions between inter- and intralocus sexual conflict. In accordance, Innocenti and Morrow (2010) found that many of the transcripts associated with IASC were enriched in reproductive tissues, such as the male accessory gland and ejaculatory duct, and the female spermatheca. Altogether, this suggests that the same alleles could be involved in both IASC and IRSC.

Sexually antagonistic transcripts associated with IASC have also been identified in non-reproductive tissues (Innocenti and Morrow 2010), and several aspects of morphology and physiology that are exposed to sex-specific selection but not directly related to reproductive functions have strong genetic correlations between the sexes (Poissant *et al.* 2010). Non-reproductive traits may also be necessary in order to counter-adapt in an IRSC arms race. For example, in cockroaches and bed bugs, behavioural, morphological, and physiological adaptations are thought to be involved in adaptations to IRSC in both sexes (Moore and Pizzari 2005; Reinhardt *et al.* 2003). Non-reproductive traits may therefore participate in both IRSC and IASC, although reproductive traits appear more likely to mediate both types of sexual conflict and might generally be subject to stronger sex-specific selection.

Even when the molecular basis of conflict traits is known, estimating between-sex genetic correlations can be difficult due to the diffuse nature of pleiotropy.

Therefore, a promising complementary approach to functional-genomic analyses of the genetic architecture of intersexual conflict traits is to test for IASC effects using experimental manipulations of candidate genes previously identified for their role in sexually antagonistic interactions. It might also be insightful to conduct artificial selection experiments on traits involved in IRSC, to identify whether the traits have a shared genetic basis, where resulting phenotypes and their fitness effects could be quantified in each sex. A recent study by Fuchikawa and Okada (2013) used this method to study whether exaggeration of male mandibles in seed beetles affected female fitness via an intersexual genetic correlation. Although they found no evidence of IASC over this trait, it provides a framework for other studies of this kind.

Mechanisms of Mating Interactions

Apart from between-sex pleiotropy, a requirement for sustained arms-race reversals in our model is that mating compatibility is determined by trait complementarity rather than by a contest between offence and defence traits. IASC was observed to complicate the dynamics of arms races in the contest scenario as well (see Figure S1 in **Appendix 1**), but its destabilising effects never prevented evolution from ultimately reaching an equilibrium state. Both mating mechanisms have been considered in theoretical studies of IRSC and motivated by specific biological examples (Gavrilets 2014). *RCD05* already showed that evolutionary cycles, featuring fluctuations in sensitivity may occur when mating is determined by a contest. However, barring changes in sensitivity, changing the direction of chase-away sexual selection is precluded in the contest model, because male sexual fitness is an increasing function of the expression of the offence trait, irrespective of the level of female defence. A similar monotonic relationship holds for female sexual fitness as a function of the defence trait. These constraints, however, are an immediate consequence of the assumption that offence and defence are uni-dimensional traits. Male and female mating behaviours are frequently determined by many traits (including behavioural, morphological and physiological characteristics). Accordingly, intersexual arms races generally occur in multidimensional phenotype space. Therefore, male-female coevolution can unfold in many different directions, so that the resolution

of IASC may trigger sudden changes in the direction of arms races. Populations evolving in multidimensional phenotype spaces, in which mating interactions are governed by a contest between more than one offence and defence trait may therefore show similar complex dynamics of sexual conflict to what is observed in our model for complementarity-based mating. Consistent with this hypothesis, frequency-dependent selection operating on multivariate phenotypes is known to result in complex non-equilibrium dynamics or even evolutionary chaos (Doebeli and Ispolatov 2014).

Implications for Theory

The evolutionary cycling observed in our simulations of sexual conflict is a consequence of an interaction between processes on a fast and a slow timescale, typical of systems with delayed nonlinear feedback control (relaxation oscillators; Van der Pol 1940). Sexually antagonistic coevolution is the fast process (Gavrilets 2000), which drags along pleiotropically correlated characters. The accumulating displacement of these characters from their optimum acts as a control variable with a sudden, switch-like effect on the direction of intersexual selection, mediated by IASC resolution. The strength of the feedback and its timescale of operation are set by the additive genetic correlations. Broadening this analogy, we speculate that similar dynamical instabilities can occur in other coevolutionary processes that are subject to pleiotropic constraints, such as in the context of host-parasite coevolution (Rice and Holland 1997) or biological signalling (van Doorn and Weissing 2006). In fact, the mathematical argument presented in **Appendix 1** is not specific to sexually antagonistic selection, suggesting that pleiotropy may generally act to stabilise evolutionary equilibria if correlated characters are under stabilising selection. In systems with multiple equilibria, such stabilisation must necessarily lead to the emergence of alternative, nonstationary attractors, such as the evolutionary cycles observed in our simulations. These considerations warrant further theoretical investigation into the consequences of pleiotropy for the dynamics of evolution, given that pleiotropy has been seen predominantly as a source of evolutionary constraint so far.

Conclusion

Our findings inspire a systems perspective on the biology of sexual conflict that sheds new light on several issues debated in the field. First, we show that pleiotropic constraints, in the form of correlated-trait expression subject to stabilising selection, do not necessarily restrain arms races but may rather create conditions favourable to perpetual antagonistic coevolution. The reversal of arms races by IASC resolution provides a new mechanism for explaining the ongoing evolution of mating traits, despite the presence of stabilising natural selection preventing unlimited trait exaggeration. Second, our simulations, which show a recurrent build up of unresolved IASC during intersexual arms races, alternated by periods of conflict resolution, suggest that IASC may be more dynamic than has so far been recognized. This idea is consistent with the observation that closely related species show markedly different patterns of sex-biased gene expression (Ranz *et al.* 2003). It can also help to resolve the paradox that appreciable levels of sexually antagonistic genetic variation segregate in populations, whereas sexual dimorphism is known to evolve rapidly in many cases (van Doorn 2009; Stewart *et al.* 2010; Badyaev 2002). That is, even if the evolution of sexual dimorphism leads to a rapid loss of sexually antagonistic variation, new sexually antagonistic alleles that mediate IASC might be introduced continually as a pleiotropic side-effect of intersexual arms races.

Box 4.1 – Fitness Functions and Responses to Selection

Fitness Functions

Individual fitness is calculated as the product of survival and reproductive success. Reproductive success depends on the mating rate ψ in both sexes, but in qualitatively different ways: male fitness is an increasing function of ψ, whereas female fitness is maximised at an intermediate mating rate $\theta\psi$. Male and female survival are affected similarly by stabilising natural selection, which acts independently on each of the phenotypic characters expressed by the individual. Hence, the fitness of a female is a function of her own phenotype ($x_{♀}$, $y_{♀}$, $z_{♀}$) and of her mating rate, which also depends on the average persistence $y_{♂}$ of the resident males with whom she interacts

Equation 2:

$$W_{♀} = e^{-\frac{1}{2}a\left(\psi(z_{♀}(\bar{y}_{♂}-x_{♀}))-\theta_{\psi}\right)^2} \times e^{-\frac{1}{2}\left(c_{x_{♀}}(x_{♀}-\theta_{x_{♀}})^2+c_{y_{♀}}(y_{♀}-\theta_{y_{♀}})^2+c_{z_{♀}}(z_{♀}-\theta_{z_{♀}})^2\right)}$$

Likewise, the fitness of a male depends on his own traits ($x_{♂}$, $y_{♂}$, and $z_{♂}$), and on the average threshold $\bar{x}_{♀}$, and sensitivity $\bar{z}_{♀}$, of his mating partners.

Equation 3:

$$W_{♂} = e^{b\,\psi(\bar{z}_{♀}(y_{♂}-\bar{x}_{♀}))} \times e^{-\frac{1}{2}\left(c_{x_{♂}}(x_{♂}-\theta_{x_{♂}})^2+c_{y_{♂}}(y_{♂}-\theta_{y_{♂}})^2+c_{z_{♂}}(z_{♂}-\theta_{z_{♂}})^2\right)}$$

In these expressions, the parameters a and b scale the fitness consequences to females and males of IRSC. Moreover, θ_k and c_k (where k can stand for any of the phenotypic characters) specify the optimal value of character k under natural selection and the stabilising selection intensity, respectively.

Box 4.1 Continued

Calculating the Response to Selection

The strength and direction of selection on the phenotypic characters is quantified by the selection gradient, $\boldsymbol{\beta} = (\beta_{x♀}, \beta_{z♀}, \beta_{y♂}, \beta_{x♂}, \beta_{z♂}, \beta_{y♀})^{T}$. Its elements are calculated directly from the fitness functions (Equations 2 and 3), using standard methods from evolutionary quantitative genetics (Iwasa *et al.* 1991). Specifically, depending on whether k is expressed in females or males.

Equation 4:

$$\beta_k = \left.\frac{d \ln W_{♀}}{d\,k}\right|_{\substack{x_{♀}=\bar{x}_{♀} \\ y_{♀}=\bar{y}_{♀} \\ z_{♀}=\bar{z}_{♀}}} \text{ or } \left.\frac{d \ln W_{♂}}{d\,k}\right|_{\substack{x_{♂}=\bar{x}_{♂} \\ y_{♂}=\bar{y}_{♂} \\ z_{♂}=\bar{z}_{♂}}}$$

In the calculation of the selection gradients, we follow the common approach of assuming weak selection and limited phenotypic variation in the population. On the scale of a phenotypic standard deviation, the fitness function can then be approximated by a linear function, and the selection gradients become independent of the phenotypic variances.

The population average value of each character changes in response to selection acting on the character itself, and due to selection on correlated characters. The combined effect of the direct and indirect component of the selection response is found by multiplying the selection gradient with the genetic variance-covariance matrix **G** (Lande and Arnold 1983). Several of the off-diagonal elements of **G** represent additive genetic covariances between a mating character and its pleiotropic character in the other sex. These intersexual covariances are of prime interest, since they quantify to what extent the resolution of IASC is constrained

Box 4.1 Continued

by male and female traits sharing a common genetic basis. The other off-diagonal elements measure covariance between non-homologous characters due to pleiotropy or linkage disequilibrium. For simplicity, these elements of **G** are assumed to be negligibly small.

The number of parameters can be reduced further, if all traits are measured on a standardised scale. In that case, the additive genetic variances are equal to one and **G** takes the form of a correlation matrix

Equation 5:

$$\mathbf{G} = \begin{pmatrix} 1 & 0 & 0 & r_x & 0 & 0 \\ 0 & 1 & 0 & 0 & r_z & 0 \\ 0 & 0 & 1 & 0 & 0 & r_y \\ r_x & 0 & 0 & 1 & 0 & 0 \\ 0 & r_z & 0 & 0 & 1 & 0 \\ 0 & 0 & r_y & 0 & 0 & 1 \end{pmatrix}$$

Here, r_x, r_y and r_z denote the additive genetic intersexual correlations between the expression of a mating character in one sex and its homologous pleiotropic character in the other.

Quantifying IASC and IRSC

Following Cox and Calsbeek (2009), we consider IASC to arise when the selection gradients on genetically correlated characters in males and females point in opposite directions. The indices of IASC plotted in Figure 4.3 are therefore calculated as

$$I_x^{\mathrm{IASC}} = -\beta_{x♀}\,\beta_{x♂},\ I_y^{\mathrm{IASC}} = -\beta_{y♀}\,\beta_{y♂} \text{ and } I_z^{\mathrm{IASC}} = -\beta_{z♀}\,\beta_{z♂}$$

Box 4.1 Continued

Comparable indices for the strength of IRSC are calculated by multiplying the selection gradient of a mating trait with its effect on fitness in the other sex, i.e.:

Equation 6:

$$\begin{array}{lcccc} I_x^{\mathrm{IRSC}} & = & -\beta_{x_{\female}} \frac{d \ln W_{\male}}{d \bar{x}_{\female}} & = & b\, \beta_{x_{\female}}\, \bar{z}_{\female}\, \bar{\psi}', \\ I_y^{\mathrm{IRSC}} & = & -\beta_{y_{\male}} \frac{d \ln W_{\female}}{d \bar{y}_{\male}} & = & a\, \beta_{y_{\male}}\, \bar{z}_{\female}\, (\bar{\psi} - \theta_{\psi})\, \bar{\psi}', \\ I_z^{\mathrm{IRSC}} & = & -\beta_{z_{\female}} \frac{d \ln W_{\male}}{d \bar{z}_{\female}} & = & b\, \beta_{z_{\female}}\, (\bar{x}_{\female} - \bar{y}_{\male})\, \bar{\psi}' \end{array}$$

Figure 4.1 – Numerical Simulations Where Mating is a Contest: IASC stabilises sexually antagonistic coevolution of offence and defence traits. Male and female mating traits (solid lines) covary with their respective homologous character in the other sex (dashed lines) as a result of between-sex pleiotropic gene expression. The population-average trait values converge on an evolutionary cycle when the intersexual genetic correlations are low (a) but are driven towards a stable equilibrium (b) when sensitivity and threshold are subject to stronger IASC. Parameters are $a = 5$, $b = 0.5$, $\theta_{x♀} = \theta_{x♂} = 0.05$, $\theta_{y♀} = \theta_{y♂} = -0.05$, $\theta_{z♀} = \theta_{z♂} = 0.5$, $\theta_{\psi} = 0.2$, $c_{x♀} = c_{x♂} = 0.5$, $c_{y♀} = c_{y♂} = c_{z♀} = c_{z♂} = 0.1$. In panel (a), $r_x = r_z = 0.1$ and $r_y = 0.2$; in panel (b), $r_x = r_z = 0.5$ and $r_y = 0.2$.

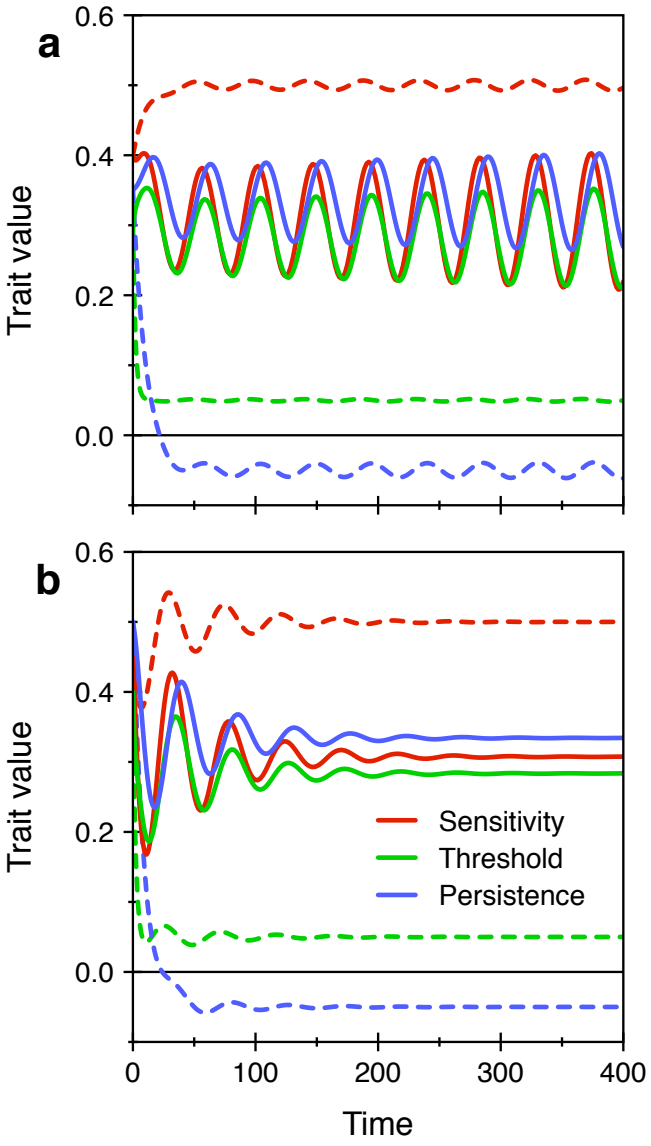


Figure 4.2 – Numerical Simulations Where Mating is Complementarity-Based: IASC gives rise to an alternative evolutionary attractor when mating is based on trait complementarity. (a) Chase-away sexual selection drives the population towards exaggerated trait values when the intersexual genetic correlations are relatively low (r_x = r_z = 0.5 and r_y = 0.2). The upper and lower part of panel a show simulations converging on two alternative equilibria. Two additional stable equilibria exist when choosiness is allowed to be negative at equilibrium. We do not show further results for this case, because the evolutionary trajectories at positive and negative values of choosiness are nearly symmetric. Thick lines in the upper and lower part of panel (b) show simulations from the same initial conditions as in (a), but at higher values of the intersexual additive genetic correlation (r_x = r_z = 0.95 and r_y = 0.2). Here, males and females engage in arms races, which are reversed halfway on their way towards attaining equilibrium. The cause of these sudden reversals is IASC resolution, which induces a correlated selection response in the mating traits, changing the relative positions of the sexes in their coevolutionary chase. The interaction of inter- and intralocus conflict thus keeps the sexes caught in a perpetual cycle of arms races, alternated by phases of conflict resolution. The equilibrium states attained in (a) are also potential endpoints of evolution in (b), but can only be reached from initial conditions close to the equilibria. This is shown by two example simulations (thin lines) that converge on the alternative equilibria in the upper and lower part of (b). Parameters (other than the intersexual correlations) are as in Figure 4.1.

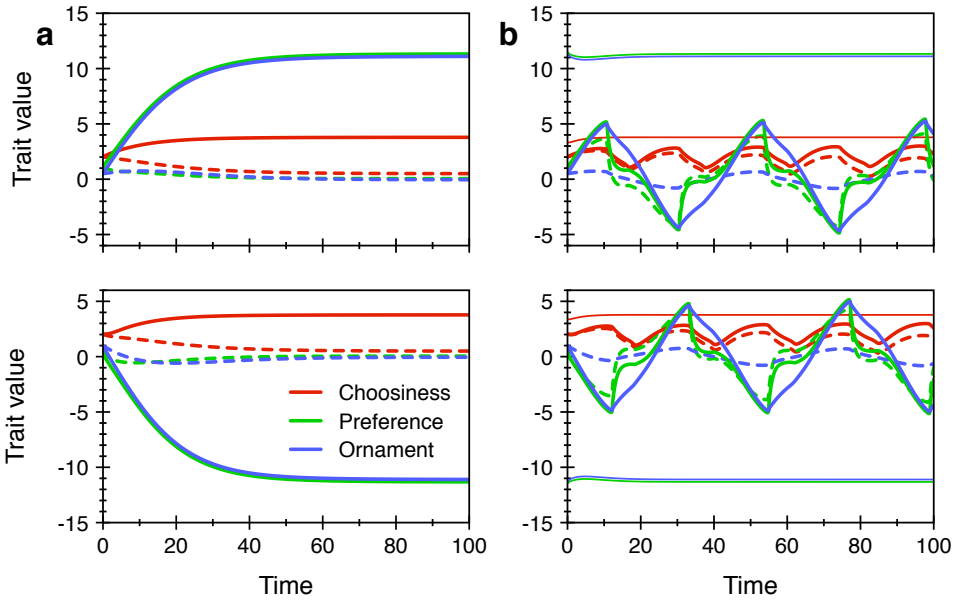
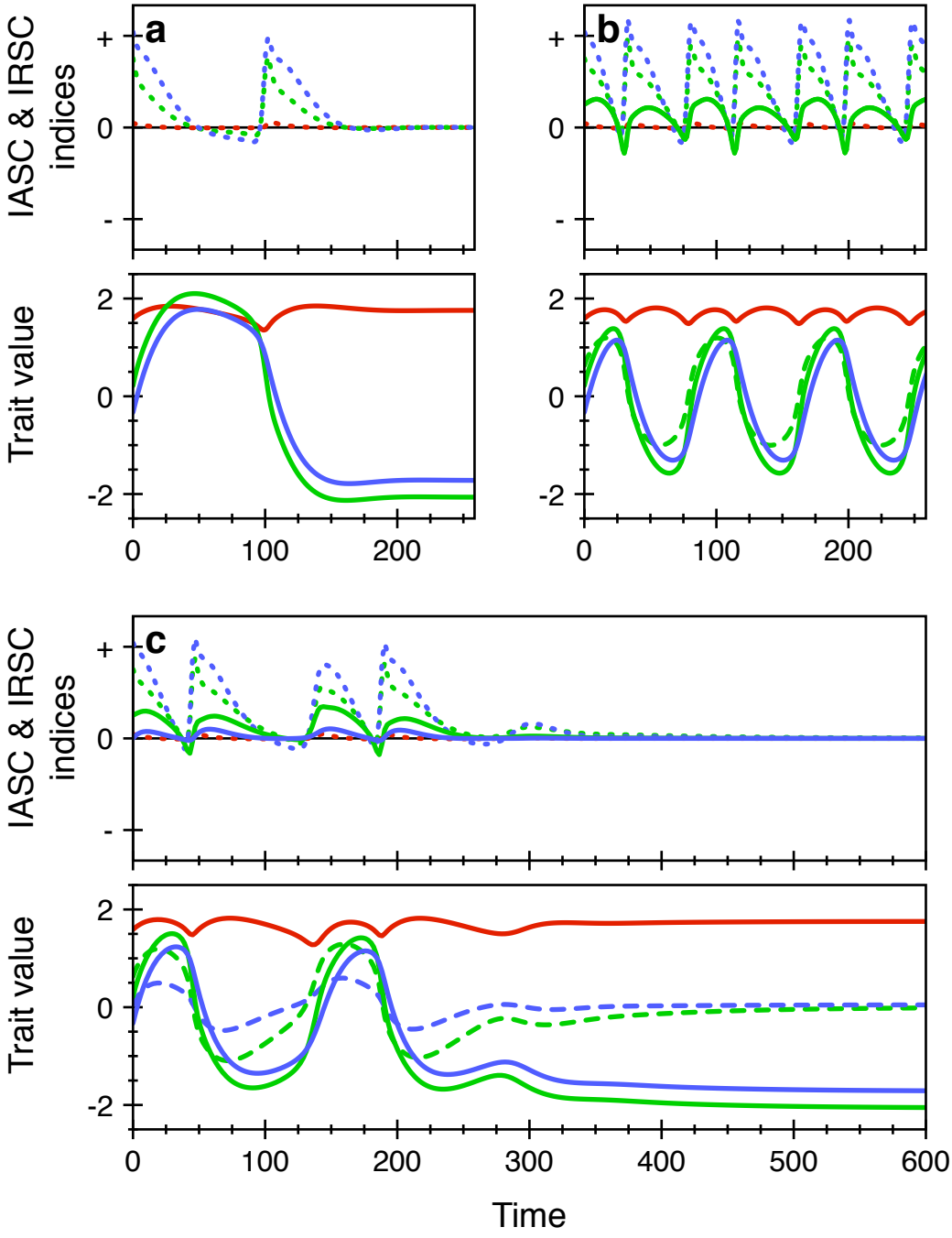


Figure 4.3 - Effect of Between-Sex Pleiotropy on the Dynamic of IASC and IRSC During Trait Evolution: each panel shows a simulation of evolving mean trait values (lower parts; line styles as in Figure 4.2) with a corresponding timeplot of trait-specific indices of sexually antagonistic selection (dashed: IRSC index; solid: IASC index). Positive values of IASC and IRSC indices are indicative of sexual antagonism, negative values indicate that fitness effects are concordant between the sexes (see Box 4.1) - (a) Complementarity-based mating without between-sex pleiotropy ($r_x = r_y = r_z = 0$). Preference and ornament evolve in an arms race driven by IRSC, first in one, then in the other direction, converging eventually on a stable equilibrium. In (b), the approach to the equilibrium is destabilized by IASC, which can be seen to build up during the period that IRSC is strong ($r_x = 0.8$; $r_y = r_z = 0$). IASC is resolved when $x_{♂}$ evolves back towards it sex-specific optimum, but this process induces a correlated change in $x_{♀}$ that causes the direction of sexual selection to reverse. As a result, a new IRSC arms race is triggered, initially accelerated by concordant selection on the preference and its pleiotropic character. (c) When also male ornamentation genes have pleiotropic effects in the other sex ($r_x = 0.8$; $r_y = 0.5$; $r_z = 0$) evolution can attain the equilibrium again. Though the overall level of between-sex pleiotropy and IASC have increased relative to (b), conflict resolution has become less effective in reversing the direction of sexual selection. This is because both $x_{♀}$ and $y_{♂}$ are pushed towards their viability-selection optimum by the correlated response to stabilising selection on, respectively, $x_{♂}$ and $y_{♀}$. The difference between the two traits, which determines the direction of chase-away sexual selection, is therefore less strongly affected by IASC resolution than in (b). Parameters are: $a = 0.4$, $b = 0.1$, $\theta_{x♀} = \theta_{x♂} = 0$, $\theta_{y♂} = \theta_{y♀} = 0.05$, $\theta_{z♀} = 0.95$, $\theta_{\psi} = 0.25$, $c_{x♀} = 0.1$, $c_{x♂} = c_{y♂} = c_{y♀} = c_{z♀} = 0.05$. For clarity, trait values for the correlated characters and IASC indices are not shown if the corresponding intersexual genetic correlation is equal to zero.

Figure 4.3 Continued



Chapter 5: Intralocus Caste Conflict: Building a New Research Framework Based on Sexual Conflict

5.1 - Introduction

Individuals within a population often have distinctive routes to maximise fitness, which they follow by having different morphological, behavioural and physiological trait values. As stated in previous chapters, the two sexes of sexually reproducing species are a prime example, where divergent reproductive roles have selected for sex-specific phenotypes (Trivers 1972; Parker 1979). However, despite being under divergent selection, the two sexes must largely share the same genome, which places limits on the degree of sexual dimorphism that can evolve (Lande 1980). This sets the stage for intralocus sexual conflict (IASC) and a gender load. IASC occurs whenever the fittest allele at a given locus is not the same in both sexes, leading to sexual antagonism. Gender load is defined as maladaptation resulting from intersexual genetic correlations (where selection in one sex causes a correlated response in the other sex), coupled with antagonistic selection on male and female traits (reviewed in Bonduriansky and Chenoweth 2009; van Doorn 2009; Pennell and Morrow 2013; **Chapter 1**). As well as being a key factor in the evolution of sexual dimorphism, IASC acts to maintain additive genetic variation for fitness within the sexes (Rice 1984), and has ramifications for adaptation, speciation and extinction (Bonduriansky and Chenoweth 2009; Connallon *et al.* 2010).

The core concept of IASC, namely that the shared genetics of different classes of individuals can constrain their independent adaptation, potentially applies to many other polymorphisms besides males and females, including different ploidy phases (Immler *et al.* 2011) or male fighter/sneaker dimorphisms (Buzzato *et al.* 2012). This chapter focuses on the breeding and non-breeding "castes" of eusocial and cooperatively breeding animals, which provide a particularly striking example of role-specific selection. In eusocial species for example, workers sacrifice their own reproduction to aid the reproduction of queens (Hamilton 1964). As with males and females, selection favours a different

phenotype in each caste, such that caste-specific adaptation could be constrained by the necessity of sharing a genome (Linksvayer and Wade 2005; Pennell and Morrow 2013; Holman *et al.* 2013; Holman 2014). We refer to this concept as intralocus caste conflict (IACC). IACC is expected to maintain additive genetic variation for fitness within castes, leading to maladaptation or 'caste load'.

IASC can be reduced through various mechanisms that permit the evolution of sexual dimorphism via sex-specific gene expression. However, multiple factors conspire to make the evolution of dimorphism incomplete, such that IASC is often strong in practice (van Doorn 2009; Pennell and Morrow 2013; Connallon and Clark 2014). For example, there are pleiotropic and epistatic constraints on the evolution of sex-specific gene regulation (Badyaev 2002; Ellegren and Parsch 2007; van Doorn 2009) and spatial/temporal variation in selection could help to maintain IASC by creating inconsistent selection for sex-biased gene expression, such that it does not evolve (Pennell and Morrow 2013). The same suite of mechanisms and barriers to conflict resolution probably also apply to IACC (Holman 2014), but this this has not been explored empirically or theoretically.

Because of the clear similarities between IASC and IACC, we believe that research relating to one conflict can aid our understanding of the other. The fact that IASC and IACC can arise in very different biological contexts also makes them interesting to compare; for example, how do genetic, ecological and social differences set these conflicts apart, and can the same mechanisms used to resolve one conflict be co-opted to resolve the other? Additionally, we suggest that IASC and IACC will act simultaneously to constrain trait evolution in social species, with interesting and unexplored consequences. Currently, our understanding of IASC is well developed, with both theoretical (Rice 1984) and empirical evidence suggesting its widespread occurrence in non-social sexual taxa (Bonduriansky and Chenoweth 2009; van Doorn 2009; Pennell and Morrow 2013). IACC is also predicted to be widespread in social systems (Holman 2014), but is much less well studied. The aim of this chapter is to compare and contrast predictions of IASC and IACC and highlight exciting new directions for research in species with a division of labour.

5.2 - Empirical Evidence for Sexual Conflict and Predictions for Caste Conflict

The hallmark of IASC is a negative intersexual genetic correlation for fitness – individuals of one sex with high fitness tend to have relatives of the opposite sex with low fitness. This occurs when a sufficiently high proportion of the genetic variance for fitness within a population is sexually antagonistic (Rice and Chippindale 2001). Negative intersexual genetic correlations for adult lifetime fitness and fitness correlates have been identified in a wide range of taxa, including: insects (Chippindale *et al.* 2001; Rice and Chippindale 2001; Gibson *et al.* 2002; Bonduriansky and Rowe 2005a; Bonduriansky and Rowe 2005b; Pischedda and Chippindale 2006; Long and Rice 2007; Bedhomme *et al.* 2008; Harano *et al.* 2010; Innocenti and Morrow 2010; Hesketh *et al.* 2013; Berger *et al.* 2014; **Chapters 2** and **3**), birds (Tarka *et al.* 2014), reptiles (Svensson *et al.* 2009), humans (Garver-Apgar *et al.* 2011; Stulp *et al.* 2012) and other mammals (Mainguy *et al.* 2009; Mills *et al.* 2012; Mokkonen *et al.* 2012).

The genetic architecture that is common to different social castes could have similar consequences: selection on one caste could cause a maladaptive correlated response in the other, potentially resulting in a negative correlation between the fitness effect of a gene when expressed in a breeder versus a non-breeder. Although intercaste genetic correlations for fitness have not yet been measured in any social system, evidence for a positive intercaste genetic correlation for ovarian development was found in the ant *Lasius niger*, such that especially fecund queens tended to produce more fecund workers (Holman *et al.* 2013). High fecundity is hypothesised to be beneficial for queens but detrimental in workers for colony productivity, since it might direct resources away from worker-specific tasks such as foraging. This finding could therefore contribute to a negative intercaste correlation for fitness. Holman (2014) also calculated that around 134 genes appeared to pleiotropically affect honeybee queen and worker fecundities in the same direction, based on microarray data from brain tissue (Grozinger *et al.* 2007). A later study of bumblebees found that reproductive workers and queens had a more similar gene expression profile than did reproductive and non-reproductive workers (Harrison *et al.* 2015), again

suggesting pleiotropy across castes for genes affecting fecundity. Whilst the correlations identified in these studies are consistent with IACC, a definitive demonstration would require the detection of a negative intercaste genetic correlation for total fitness, which although challenging, is possible to measure (see section 5.5).

5.3 - Comparing Mechanisms of Sexual and Caste Conflict Resolution

Sexual dimorphism provides evidence for past or ongoing IASC, since sex-specific selection selects for divergent trait values (Cox and Calsbeek 2009). Similarly, caste dimorphism suggests caste-specific selection and at least partially resolved IACC (Holman 2014). Castes differ in many aspects of their behaviour, morphology and physiology (particularly in "advanced" eusocial lineages, i.e. those with large, perennial colonies and many specialised social adaptations such as group foraging), and these phenotypic differences are accompanied by substantial intercaste differences in gene expression (e.g. Ferreira *et al.* 2013; Simola *et al.* 2013; Feldmeyer *et al.* 2014; Harrison *et al.* 2015; Morandin *et al.* 2015). Common functions associated with genes showing caste-specific expression include reproduction (egg production), metabolism, somatic maintenance and repair, digestion and feeding, pheromone recognition, cellular activity, protein structure and immunity, as well as many novel genes of unknown function (Ferreira *et al.* 2013). These extensive transcriptomic differences are consistent with caste-specific selection across much of the genome.

Two key mechanisms underlying sexual dimorphism in gene expression have also been linked to polyphenism in social insects: alternative splicing and gene duplication (see section 1.4). For example, much of the *Drosophila* genome shows sex-specific alternative splicing (Telonis-Scott *et al.* 2009), and evidence is accumulating for widespread caste-specific alternative splicing (Aamodt 2008; Jarosch *et al.* 2011; Bonasio *et al.* 2012; Foret *et al.* 2012; Terrapon *et al.* 2014). Additionally, gene duplication followed by subfunctionalisation has been hypothesised to play a role in both mitigating IASC (Gallach and Betran 2011; but

see Hosken 2011) and producing caste-specific gene expression (Claudianos *et al.* 2006; Xu *et al.* 2010, Terrapon *et al.* 2014).

DNA methylation, and other epigenetic mechanisms that affect gene expression, can also mediate polyphenism. Sex-specific methylation has been demonstrated in mammals, including humans (El-Maarri *et al.* 2007; Liu *et al.* 2010; Bermejo-Alvarez *et al.* 2010; Xu *et al.* 2013; Hall *et al.* 2014), and can arise early in the developing embryo. In comparison, no sex-specific methylation was identified in two species of non-social parasitic jewel wasp *Nasonia vitripennis* and *N. giraulti* (Wang *et al.* 2015), although it is present in other insects such as *Drosophila* (Avila *et al.* 2010). In humans, gene methylation levels are both sex-dependent and correlated with gene expression level, suggesting a role for methylation in sex-specific gene regulation (Xu *et al.* 2013). These epigenetic marks act to canalise sex-specific development, which is likely to alleviate IASC (but see Rice *et al.* 2012 on how epigenetic marks could also mediate IASC). In fact, sex-specific methylation was identified predominantly on the X chromosome in humans (Xu *et al.* 2013), which is predicted to be enriched for sexually antagonistic alleles (Gibson *et al.* 2002; Lindholm and Breden 2002; Fitzpatrick 2004; Tower 2006; Innocenti and Morrow 2010). Other evidence that methylation can resolve conflict between the sexes is shown in *Drosophila*, where Y-linked heterochromatin modulates autosomal gene expression (Lemos *et al.* 2010), which is a possible route through to sex-specific expression.

The social Hymenoptera also possess a full set of genes for applying, maintaining, and responding to DNA methylation (Wang *et al.* 2006). In adult honeybees, some studies suggest that the methylome is caste-specific (Lyko *et al.* 2010; Foret *et al.* 2012), though a better-replicated study found no caste-specificity (Herb *et al.* 2012). Nevertheless, knockout of a DNA methyltransferase gene (*dnmt3*) caused worker-destined larvae to develop queen-like traits (Kucharski *et al.* 2008) and significantly affected gene expression for 17% of the transcriptome (Li-Byarlay *et al.* 2013), consistent with a role for methylation in mediating polyphenism. In bumblebees (*Bombus terrestris*), workers treated with a DNA de-methylation agent developed queen-like traits, and there is

support for differential methylation between reproductive and non-reproductive workers (Amarasinghe *et al.* 2014). In ants, DNA methylation is again thought to be caste-specific (Bonasio *et al.* 2012). Termites, a lineage where sociality has evolved independently of Hymenoptera, also have DNA methylation, and there is some evidence that it may similarly encode differences between castes (Glastad *et al.* 2013). Furthermore, histone modifications, another important type of epigenetic modification, have been found to differ between castes in ants (Simola *et al.* 2013).

DNA methylation might also mediate patterns of genomic imprinting that differ between the sexes, such as expression of the maternally-derived allele in females and the paternally-derived allele in males (Day and Bonduriansky 2004), though evidence is currently limited (but see Hager *et al.* 2008). IACC might select for similar patterns, though because of complications resulting from the joint operation of IASC and IACC (see section 5.4), formal models are needed to confirm this. For example, workers could benefit from silencing the queen-derived allele, but only if the average male-derived allele is closer to the worker optimum than the average queen-derived allele. A fascinating recent study of methylomes found some evidence for allele-specific DNA methylation in ants, consistent with preferential methylation of one parent's allele (Bonasio *et al.* 2012). Additionally, the allele that was methylated for some loci was different in queens and workers, hinting at caste-specific genomic imprinting, in which offspring that are workers methylate one parent's allele and those that are queens methylate the other. For some loci, queens might maximize their fitness by expressing only the queen-derived allele and workers by expressing the male-derived allele, as proposed by Day and Bonduriansky (2004) for males and females. Additionally, imprinting could also work by mediating alternative splicing (Li-Byarlay *et al.* 2013) or other subtle forms of gene regulation.

IASC can also be mitigated through the movement of strongly sexually antagonistic genes from autosomes to sex chromosomes. For example, genes that benefit only the heterogametic sex might be selected to move to the Y (or W) chromosome, resulting in adaptive, sex-limited expression (Rice 1984). In

contrast, this route to conflict resolution is generally not available for IACC in Hymenoptera, as hymenopteran queens and workers share identical chromosomal complement. This could mean that they place more reliance on mechanisms such as methylation or alternative splicing to resolve conflict. For example, whilst methylation is often found in promoter regions in vertebrates, which could be important for between-tissue differences in gene expression that are unrelated to conflict resolution, in insects methylation is more often found in gene-body regions, which could be more important for alternative splicing (reviewed in Weiner and Toth 2012). The latter function could assist with caste-specific gene expression in social insects. Evidence for this is scarce however, and initial research in insects indicates that the level of sociality does not necessarily predict the extent of methylation (Weiner *et al.* 2013). It is therefore still unclear whether social insects are distinct from other insects with respect to methylation levels (Weiner and Toth 2012), and the picture may be obscured by both the extent of sexual dimorphism and environmental phenotypic plasticity that are likely to be mediated by the same mechanisms. Teasing apart these effects is therefore crucial for understanding IACC resolution.

Caste polymorphism has evolved multiple times within individual clades (e.g. 8 times within Hymenoptera; Hughes *et al.* 2008) and the same mechanisms, such as DNA methylation and alternative splicing, seem to have been implicated in mediating caste polyphenism across multiple independent evolutionary origins. This similarity could reflect convergent evolution, but it also seems likely that the evolution of sociality involves the repeated co-option of evolutionarily ancient mechanisms for regulating gene expression. The latter possibility is consistent with the "theory of facilitated variation", whereby ancient regulatory genes with a large, relatively conserved set of downstream targets are postulated to be the main sources of evolutionary novelty (Kirshner and Gerhart 1998). For example, castes might have arisen when a regulatory gene responsible for stimulating transcription of genes involved in oogenesis, perhaps in response to an individual's maturity, began instead to respond to the level of larval nutrition (Rehan and Toth 2015). This mode of evolution seems most plausible, because alternative scenarios that produce novel variants (e.g. sequential fixation of

novel mutations in downstream genes) are likely to be constrained by pleiotropy and correlated selection. Vitellogenin (*vg*), might be an example of a gene that has been co-opted to produce caste differentiation. For example *vg* is linked to reproduction and has recently been shown to play a role in sexual behaviour in a subsocial beetle *Nicrophorus vespilloides* (Roy-Zokan *et al.* 2015), but it has also been linked to behavioural changes in the reproductive division of labour between castes. Intriguingly, other examples of genes that are involved in sexual dimorphism and other polymorphisms exist, such as the ancient regulatory genes, *doublesex* and *transformer*. In insects *doublesex* is involved in sex-determination and also appears to have been co-opted to regulate polyphenisms in beetle mandible growth (Gotoh *et al.* 2014) and butterfly wing coloration (Kunte *et al.* 2014), and a recent paper showed that the sex differentiation gene *fem* is also differentially expressed between castes in a stingless bee (Brito *et al.* 2015).

The various pathways through which social castes become polymorphic could mitigate conflict over gene expression that arises as a result of IACC. However, the existence of caste dimorphism is not necessarily exclusively a reflection of complete conflict resolution (Cox and Calsbeek 2009): in IASC, sexually dimorphic genes have been associated with existing rather than fully resolved conflict (Innocenti and Morrow 2010). This means that despite sexual dimorphism, selection in one sex still acts to maintain genetic variation that has detrimental fitness consequences for the other sex. Sociogenomics research on the other hand has focused on mechanisms underlying phenotypic differences between castes, rather than on the role that these mechanisms may have in alleviating IACC. Genetic variation for gene expression within castes has tended to be overlooked, with studies typically utilising small, pooled samples of each caste to provide average phenotypic values. In the context of IACC, it is necessary to measure additive genetic variation for caste phenotype (e.g. for gene expression), as this could represent on-going conflict that is maintained because of selection acting on queens, despite maladaptive fitness consequences for workers. This could arise if intercaste genetic correlations for phenotype are not broken down fully (i.e., by sex-biased gene expression). To explore this

possibility would require disentangling environmental and genetic effects on caste phenotype, which could be achieved through cross-fostering experiments or by controlling for environmental effects in the laboratory. Indeed, genetically inherited components of caste phenotype have been identified in leaf-cutting ants (Hughes *et al.* 2003) and in strains of honey bees (Page and Fondrk 1995; Amdam *et al.* 2004). The link between IACC and genetic variation in caste phenotype could be explored by testing the correlation between genetic variation in caste phenotype and caste-specific fitness effects (but see section 5.5). This would provide an indication of how effective mechanisms that achieve caste-specific gene expression are in alleviating conflict in social species, thus permitting caste-specific adaptation.

5.4 - A Three- (or More) Way Conflict

Males are greatly understudied in species with reproductive castes, perhaps because social hymenopteran males are present for only part of the colony lifecycle, and because mating is often difficult to observe. There is also a tendency to regard sexual selection as comparatively weak and free of conflict in the social insects, with males typically thought to have "few if any sexually-selected traits" (Boomsma *et al.* 2005). However, a male's ability to reach maturity, fly, search, and mate is presumably highly polygenic, so that much of the genome may contribute to variance in reproductive success, and thus by definition be under sexual selection. Moreover, the sex ratio in social insect colonies is expected to be biased towards the cheaper sex (Grafen 1986; Boosma 1993), which means that males are produced in greater numbers due to their smaller size. This potentially creates large variance in male fitness, and hence strong selection on traits that affect mating success. Together, these factors suggest that selection on males might affect much of the genome, even in monogamous species lacking "active" sexual selection (choosy females, male fighting etc.) or classically sexually-selected adaptations such as ornaments and weapons.

Given that there is likely strong selection on males, and that males obtain fitness through routes that are very different from both queens and workers, we suggest

that IASC and IACC will act simultaneously in dioecious social species. This additional interaction is likely because the common genetic architecture of queens and workers is also shared with the opposite sex. The net evolution of a shared trait will therefore depend on its fitness consequences when expressed in different sexes as well as castes, and we predict that selection will frequently fail to optimise the fitness of all three phenotypes (Figure 5.1). Often, males, queens and workers will all have distinct mean values for a shared trait, implying that each has a different optimum, and that both IASC and IACC are only partially resolved (Cox and Calsbeek 2009). For example, males, queens and workers are commonly distinct in terms of body size, morphology and physiology (Stubblefield and Seger 1994; Hrassnigg and Crailsheim 2005; Zayed and Kent 2015). In other cases, queen and worker trait values are similar but differ greatly from those of males; for example, *Lasius niger* males have a very short lifespan and also short telomeres relative to queens and workers, while the female castes differ in lifespan but not telomere length (Jemility *et al.* 2007). For other traits, reproductives (queens and males) differ from workers, for example in wing phenotype (e.g. in ants: Abouheif and Wray 2002) and gamete production. In short, it seems certain that some loci are under both IASC and IACC, while selection at other loci may be concordant across some sexes/castes but not others.

A full theoretical treatment of the interaction between IASC and IACC is beyond the scope of this review, but we suspect it will be interesting for several reasons. Male Hymenoptera are haploid while queens and workers are diploid, and so selection on recessive alleles that affects fitness is more efficient in males, with recessive alleles in females experiencing little or no selection in heterozygotes. This ploidy difference might skew the phenotype towards the male optimum, and highlights that IASC and IACC likely act at once. Selection is also inefficient in workers relative to queens and males, since workers gain much of their fitness indirectly by increasing the productivity of related queens (Van Dyken *et al.* 2011). The relative efficacy of selection on workers correlates positively with the average relatedness between workers and the recipients of their help, as well as the frequency with which workers reproduce directly (Van Dyken *et al.* 2011).

We therefore expect the outcome of the IASC-IACC interaction to depend on colony relatedness structure (e.g. queen number and mating frequency), and the proportion of offspring that are produced by workers (which depends on the resolution of IACC; Holman 2014). The relatedness between workers and the queens and males that they rear creates an additional complication. A queen or male carrying perfectly queen- or male-adapted genes might nevertheless have low fitness, since many of the workers that raised it would often be carrying these same genes, which might be maladaptive when expressed in a worker due to IACC. Conversely, a queen or male with worker-adapted genes might have high fitness because it was raised by well-adapted workers. Thus, one needs to consider not only the direct genetic effects of an individual's own genotype on its fitness, but the indirect genetic effects on its fitness which depend on the genotypes of its social partners. Evolutionary predictions are complicated when the direct and indirect genetic effects covary (e.g. because social partners are kin), since indirect genetic effects and relatedness can together influence evolutionary trajectories in strong and unexpected directions (McGlothlin *et al.* 2010).

5.5 - Challenges for Caste Conflict Research

Measurement of IACC presents a number of obstacles that are not present when measuring IASC. Nonetheless, IACC is empirically tractable and social systems provide novel ways to quantify role-specific selection in social systems, which are not feasible in sexual systems.

In social systems, it can be difficult to measure intercaste genetic correlations, due to confounding effects of the common environment shared by queens and workers, and the necessity, for colony functioning, to keep the castes together (although workers can sometimes be kept separately). Maternal and sib-social effects can also strongly affect the phenotype, and must be considered when estimating the additive effects of genes (Linksvayer and Wade 2005). In order to separate these effects it may be possible to transfer individuals between social groups (or nests) in cross-fostering experiments (Holman *et al.* 2013), or use a multi-generational breeding design (Lynch and Walsh 1998). Another promising

option would be to use an 'inbred line' approach with a social insect species that can tolerate inbreeding (e.g. most supercolonial ants). By estimating the relative fitness of queens and workers derived from a number of genetically homogeneous lines, one could estimate the intercaste genetic correlation for fitness and other traits. This is a method that has been practised in IASC research, through the use of isofemale lines (Berger *et al.* 2014; Punzalan *et al.* 2014).

An alternative to using genetically inbred lines is hemiclonal analysis, where genes can be expressed as both males and females in a heterozygous state and the costs of homozygosity associated with inbred lines can be avoided (section 1.9; Rice 1996; Chippindale *et al.* 2001; Abbott and Morrow 2011). This technique has enhanced IASC research because it allows for the additive genetic sex-specific fitness effects of haplotypes to be directly quantified (Chippindale *et al.* 2001; Gibson *et al.* 2002; Pischedda and Chippindale 2006; Long and Rice 2007; Bedhomme *et al.* 2008; Innocenti and Morrow *et al.* 2010; Hesketh *et al.* 2013). Although this type of artificial genetic manipulation is unique to *Drosophila*, social insects have a distinctive mode of reproduction that will similarly help to disentangle the additive effects of genes on particular castes and sexes: clonal sperm. This is a feature of hymenopteran males, which means they are related to their daughters (future queens and workers) by 1, and their daughters are related to each other by 0.5. One way to explore the link between IASC and IACC would therefore be to partition variance in offspring phenotype within and between colonies of the same and different patrilines.

Finally, in a large enough population one could estimate genetic correlations between breeder and helper traits in a pedigreed, wild population using an animal model approach (Kruuk 2004). For example, in a pedigreed population of cooperatively breeding birds, one could measure the genetic correlation between breeder fitness and the effect that a helper has on the productivity of the breeding pair, though this may be constrained by the sample sizes required.

As well as measuring the extent to which caste-specific trait values can evolve independently, there is a need to quantify selection on a variety of caste-specific traits, and verify that it is indeed antagonistic. For example, it is likely that selection often favours larger body size in queens than workers, but it is not known whether queen and worker body sizes remain under directional selection in opposing directions (implying that the each caste has not reached its optimum; Figure 5.1). One approach to this question would be to ask whether the degree of caste dimorphism predicts fitness, across genetically divergent populations or colonies, after placing these in a common garden to minimize non-genetic effects (as has been done in the context of IASC; Rankin and Arnqvist 2008; Arnqvist and Tuda 2010).

Studying single traits and their involvement in IACC could help to answer general questions about social group dynamics. One example is exploring IACC over egg production and its effect on the partitioning of reproduction between the members of a social group (reproductive skew; Holman 2014). For example, there is a lack of between-colony variation in reproductive skew in primitively eusocial wasps, despite considerable variation in factors predicted to affect skew in strategic models (Field and Cant 2009). This might result from intercaste genetic correlations for traits affecting dominance and within-colony competition for reproduction, such that alleles resulting in more fecund queens also result in more fecund workers, with skew remaining unchanged.

In addition to phenotypic studies, modern molecular approaches could be useful in identifying traits that are under IACC. For instance, one could measure genome-wide patterns of gene expression (e.g. using RNA-seq) and search for transcripts associated with opposite caste-specific fitness effects. By using this approach it would be possible to generate lists of candidate processes or traits for further study at the whole organism level – a method that has been used successfully in the study of IASC in the fruit fly (Innocenti and Morrow 2010). A thorough understanding of the evolutionary dynamic of caste conflict ultimately requires identifying specific alleles that are maintained by caste-specific selection and that underlie maladaptive trait variation within each caste.

Although this has not been achieved at a whole-genome level in IASC research thus far, it could be achieved by correlating whole-genome sequence variation with caste-specific fitness effects. For both IASC and IACC, more comprehensive molecular datasets (that incorporate gene expression, sequence variation and functional annotations) could also answer fundamental questions regarding the genetic constraints on conflict resolution. For instance, are genes that are embroiled in conflict under pleiotropic or epistatic constraints that prevent complete caste specific expression? This has been explored in relation to IASC by forming links between sex-biased gene expression and proximate measures of pleiotropy, such as the tissue specificity and network connectivity of genes (Mank *et al.* 2008; Frings *et al.* 2012).

The goal of understanding both conflicts at the molecular level can be most easily achieved by studying organisms for which there is a history of genetic research. This is arguably true for IASC, where a rich source of genetic information and tools are available for the *Drosophila* model system (del Valle Rodríguez *et al.* 2012), which has aided IASC research (e.g. Rice and Chippindale 2001; Innocenti and Morrow 2010). The benefits of existing data from *Drosophila* extends further, as genetic homologs can also be scanned for in social insects to aid the functional annotation of genes under caste antagonism. For example, many genes have conserved functions between solitary insects such as *Drosophila* and social insects, such as those with reproductive and foraging functions (Toth and Robinson 2007), which might mediate traits that are involved in IACC. Outside of the *Drosophila* system, the honey bee *Apis* mellifera is fast becoming a model organism for social insect genetics since DNA sequence information was published (Honey Bee Genome Sequencing Consortium 2006). More recently, various social bee (Kocher *et al.* 2013), ant (Bonasio *et al.* 2010; Nygaard *et al.* 2011; Smith *et al.* 2011b; Smith *et al.* 2011c; Suen *et al.* 2011; Wurm *et al.* 2011; Gadau *et al.* 2012; Oxley *et al.* 2014), and termite (Terrapon *et al.* 2014) species also have sequence information available, as genome assembly methods are becoming efficient and accessible.

5.6 - Interesting Systems for Empirical Tests of Intralocus Caste Conflict

An extraordinarily diverse collection of social species exist that could provide different insights into genomic caste conflict (e.g. Figure 5.2). For example, species differ in their level of social complexity, from termites and ants (Figure 5.2) with worker castes that often completely forgo direct reproduction and have high functional specialisation (Eggleton 2011; Anderson and McShea 2001), to primitely eusocial bees and wasps that lack morphological castes (e.g. paper wasps: Sumner *et al.* 2010; Figure 5.2; and halictid bees: Danforth 2002). Some species also vary in their level of sociality at the population level (e.g. the sweat bees *Halictus rubicundus*: Soucy and Danforth 2002, Soro *et al.* 2010; and *Lasioglossum calceatum*: Sakagami and Munakata 1972). These differences lead to predictions that the most social species (or populations) require greater divergence between breeder and worker phenotypes, and are likely to show more extensive patterns of caste specific gene regulation and higher levels of existing IACC.

Termites stand out as a system for IACC research as they evolved sociality independently of Hymenoptera. It will be interesting to compare whether the same mechanisms to resolve IACC have arisen in these different lineages, representing convergent evolution. A factor that is likely to change the dynamic of caste conflict in termites is the presence of sex chromosomes as opposed to haplo-diploid sex determination. Levels of sexual dimorphism are also typically low in termites (Boomsma *et al.* 2005) and workers of both sexes exist (compared to exclusively female workers in Hymenoptera). Recent evidence suggests that termite species with greater levels of sexual size dimorphism tend to have workers of a single sex that are more specialised (Bourguignon *et al.* 2012). This suggests that sexual dimorphism might have enabled functional specialisation of worker castes, as predicted if mechanisms to resolve one conflict act to mitigate the other. Further research into this topic will benefit from data on Hymenoptera, where there is often greater divergence between male and female phenotypes (Beani *et al.* 2014).

Some social insects also have unusual genetic systems, in which we hypothesise that IACC should shape genomic architecture. In at least three ant species (and likely more), queens are produced asexually and workers sexually, while males are genetic clones of the queen's mate (Wenseleers and Van Oystaeyen 2011). Provided that workers are sterile and queens are never produced sexually, this means that the species is composed of two genetically isolated lineages: one that is present in queens and workers, and one in males and workers. We predict that worker-beneficial alleles should be more prevalent in the latter lineage and queen-beneficial ones in the former (if the male phenotype is closer to the worker optimum), though the evolutionary outcome will likely depend on the interplay between IASC and IACC. Additionally, strong genetic caste determination occurs in some species, such that crosses between genetically divergent lineages produce workers, while within-lineage crosses produce queens (Schwander and Keller 2008). We suspect that this mode of caste determinism may also interact with IACC, because workers will have greater genome-wide heterozygosity than queens in these species. Selection on recessive alleles with caste-specific fitness affects will therefore be more effective in queens, potentially causing shared phenotypes to be closer to the queen optimum relative to species without these unusual genetic systems.

5.7 - General Implications of Intralocus Caste Conflict

A defining feature of IACC is that it should act to maintain genetic variation within a caste that is maladaptive. The mechanism by which genetic variation is maintained within populations has long interested evolutionary biologists (Mather 1955; Charlesworth, 1987; Kingsolver *et al.* 2001; Rowe and Houle 1996; Andersson and Iwasa 1996; Haag-Liautard *et al.* 2007; Lynch and Walsh 1998; Trotter and Spencer 2007). The idea that correlated selection between the sexes could also maintain fitness variation was introduced by Rice (1984), but this could also arise as a consequence of correlated selection between any other individuals that have different phenotypic optima and a shared genetic architecture. Much like IASC, the maintenance of genetic variation that prevents caste-specific adaptation could impact trait evolution and influence a broad range of biological phenomena.

It is possible that IACC explains differences in caste ratio between colonies. This could arise if a queen can adjust offspring phenotype depending on whether the offspring genotype would best suit a queen or worker role. This is a similar concept to sex ratio adjustment in response to IASC, which has been suggested in non-social vertebrates and invertebrates (Blackburn *et al.* 2010). It would require some level of control by the queen through nutritional (Hunt 1991; Hunt 2007; Kucharski *et al.* 2008; Anderson *et al.* 2008), pheromonal (Vargo and Passera 1992; Matsuura *et al.* 2010) and hormonal (Schwander *et al.* 2008) regulation of caste fate. It would also require the presence of a cue through which queens could determine the genotype of offspring and therefore provision accordingly. An example of caste ratio adjustment that is dependent on a perceived cue is where shifts in caste occur in ants (due to nutritional provisioning) when they detect threat from competitors (Passera *et al.* 1996). As fitness is ultimately increased through the production of fertile future queens (as opposed to sterile workers), it is also possible that queens increase the production of worker offspring if their genotype is less suited to a worker role so as to increase colony productivity overall and aid the rearing of future queens. Alternatively, it could be that caste ratio differences arise because queens with a certain genotype produce more viable offspring of one caste than the other. For example, it is imaginable that high fitness queens produce more viable future queen offspring than worker offspring.

Another possibility is that IACC is responsible for maintaining some of the personality traits that are widely documented in social insects, which are defined as consistent behaviors across different contexts (e.g. when undertaking different tasks: Jandt *et al.* 2014). This includes differences in traits such as aggression and activity level between individuals of the same caste. As stated by Jandt *et al.* (2014), although differences in behaviours have been recognised between species (Davidson 1998; Holway and Saurez 1999), fewer studies have explored behavioural personalities among monomorphic individuals (e.g. a particular caste) within colonies. There is some evidence however, that behavioural traits in social insects have a heritable genetic basis (Penke *et al.*

2007; van Oers & Mueller 2010), which is one prerequisite for their involvement in IACC. To test whether they are truly involved in IACC, the behavioural traits should be shown to be repeatable in each caste, and associated with a negative correlation between caste-specific fitness. Other personality traits potentially maintained by IACC include learning differences and foraging preferences that are influenced by an individual's genetic background (see review: Jandt *et al.* 2014). This could not only impact colony productivity but also influence interactions between colony members (e.g. aggression and dominance hierarchy formation).

Caste conflict is also likely to maintain alleles that influence processes of ageing (Adler and Bonduriansky 2014). For example, both queens and workers (and males) are often highly dimorphic in lifespan (Carey 2001). An extreme case is provided by fire ants, where queens can live up to 30 times longer than their worker offspring (Holldobler and Wilson 1990). This divergent selection on lifespan is most likely mediated by extrinsic risk of mortality, such as predation, which is greater for workers than queens. Dimorphism in lifespan and senescence suggests either past IACC that has been resolved through caste-specific expression or partially resolved but on-going conflict. Evidence that IASC can occur over lifespan despite the evolution of sexual dimorphism is provided by two sources: Lewis *et al.* (2011) showed genetic constraints on longevity in a moth that displaced the sexes from their phenotypic optima; and Berg and Maklakov (2012) conducted artificial selection on longevity in a beetle, uncovering an intersexual genetic correlation coupled with a negative correlation for fitness between the sexes. With many social insects displaying extreme divergence in lifespan between castes, it is therefore a potentially widespread source of conflict, but one that currently lacks empirical support.

Finally, IACC could potentially contribute to the occurrence of disease, such as outbreaks that occur in commercial honey bee populations (Cox-Foster and van Engelsdorp 2009), which are of considerable public and scientific interest. In IASC research, immune function has been associated with conflict (Calsbeek and Bonneaud 2008; Mckean and Nunney 2005; Rolff *et al.* 2005; Svensson *et al.*

2009; Innocenti and Morrow 2010) and it is expected that disease or alleles influencing disease susceptibility are also maintained in human populations by sex-specific or sexually antagonistic selection (Gilks *et al.* 2014). For social insects, the immunity of queens and workers is under divergent selection because queens require higher immunity to increase lifespan and sustain fecundity for long periods, whereas workers do not. In line with this, caste dimorphism in immune genes has been demonstrated in social insects (e.g. Pereboom *et al.* 2005; Grozinger *et al.* 2007; Gräff *et al.* 2007). There is also heritable genetic variation within bee populations for susceptibility to parasites and infection (reviewed in Grozinger and Robinson 2015), but because intercaste genetic correlations have not yet been tested for, it is unclear whether this variation is maintained by IACC.

By exploring IACC in more detail, our understanding of adaptive processes of evolution, and the various phenomena described above, could be greatly improved. A diverse array of social systems can be exploited for future research, with populations and species that vary in their levels of sociality offering the perfect opportunity to explore IACC in greater depth. Sexual conflict research provides inspiration for questions that are outstanding in caste conflict research, while other questions that concern only species with division of labour could shed new light on the evolution of sociality. There is ample opportunity therefore for these two research streams to complement one another, as analogous conflicts in the two systems lend themselves to contrasting experimental approaches.

Figure 5.1 - Interactions Between IASC and IACC: IASC and IACC should act in concert to shape the phenotype of dioecious, social species. In panel A, there is sex-specific selection, shown the black lines representing selection pulling the sexually monomorphic phenotype (the red circle, which marks the male and female phenotypes z_M and z_Q) towards divergent optima (M_{opt} for males and Q_{opt} for females). In panel B, sexual dimorphism has evolved but genetic correlations between the sexes (shown by dashed grey line) prevent the male and female phenotypes from completely reaching their optima. In panel C, the species has evolved queen and worker castes, but there is not yet any phenotypic divergence between castes in Traits 1 and 2. The phenotype of both males and queens is deflected (grey arrows) by selection on workers; in this example, queens become more maladapted while males become better adapted compared to queens. In panel D, the species has evolved caste dimorphism, but maladaptation remains because of genetic correlations between sexes and castes. The evolutionary outcome can be thought of as a tug of war: the positions of the three phenotypes in multivariate space depend on the strength of selection pulling the shared phenotype towards three different optima, and on the extent of genetic constraints that prevent the phenotypes from complete diverging.

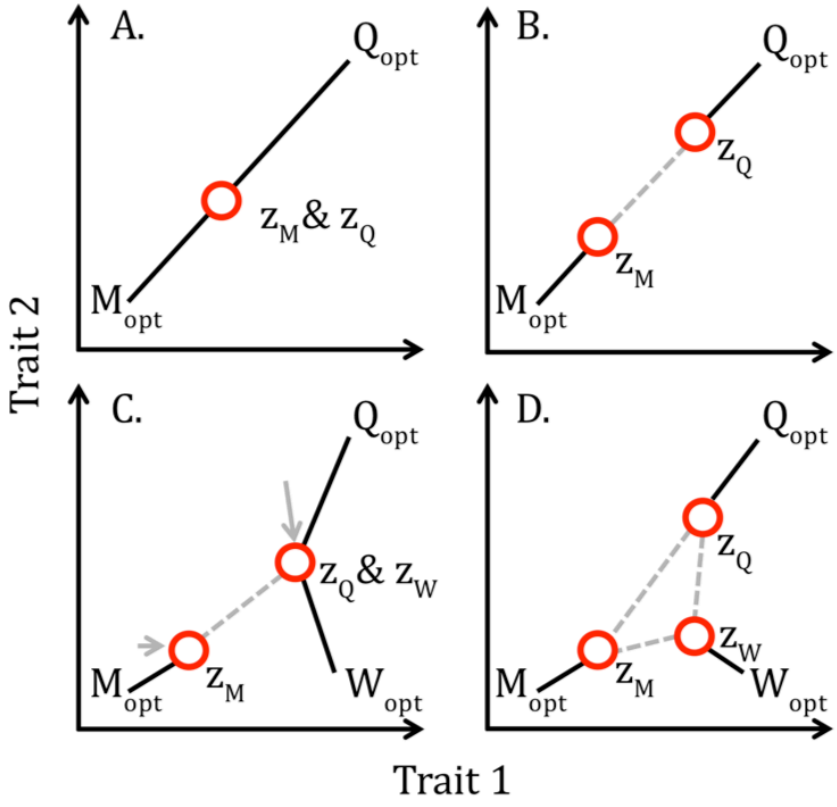


Figure 5.2 – Social Systems of Interest: a) primitively eusocial paper wasps *Polistes dominula*: co-foundresses fighting to attain dominance on the nest (taken by Tanya Pennell). Although they are morphologically similar, they will display different behavioural phenotypes, with the winning female dominating most of the reproduction and the loser foraging to feed her offspring. b) termites *Pterotermes Occidentis* evolved sociality independently of Hymenoptera: size dimorphism shown between worker (smaller and paler) and soldier (larger and darker) castes (courtesy of Feargus Cooney). c) and d) advanced social leaf-cutter ants: c) high reproductive skew and extreme queen and worker size dimorphism in *Atta colombica* (courtesy of Victoria Newman) and d) size distribution of castes in *Atta cephalotes*, from the smallest worker caste to the largest soldier caste, known to display distinct differences in behaviour as well as morphology (courtesy of Victoria Newman).

a)

b)

c)

d)

Chapter 6: General Discussion

There is mounting evidence for the existence of intralocus sexual conflict (IASC), which is also supported by the results presented in this thesis (**Chapters 2** and **3**); yet little is currently known about the evolutionary dynamic of this conflict, which presents difficulties for predicting trait evolution within populations. In this chapter, I discuss how the research in this thesis has contributed to an understanding of the timescale of resolution (**Chapter 2**), and of the physical (**Chapter 3**) and social (**Chapter 4**) environmental effects that are likely to inhibit long-term resolution. Furthermore, I explore fruitful avenues for future research that will shed light on IASC and its resolution. Given the persistent nature of IASC and its widespread occurrence, I also explore its influence on a broad range of other biological process that are likely to significantly affect animal behaviour and life history traits within populations. Finally, I discuss the complex dynamic when trait evolution is constrained by selection from additional polymorphisms besides males and females (**Chapter 5**).

6.1 - Factors Affecting Long Term Resolution of IASC

Rice (1984) suggested that IASC would act to maintain maladaptive genetic variation within a sex, and since this concept was introduced it has been unequivocally demonstrated that certain genotypes can in fact have opposite fitness consequences when expressed as either male or female (**Chapter 1**). In line with previous studies, **Chapter 2** demonstrates how IASC can still persist, even within a population that has experienced long-term adaptation to benign laboratory conditions. Such findings raise fundamental questions about when and how conflict will be resolved. As suggested previously (Stewart *et al.* 2010), even under constant conditions it might be difficult to resolve IASC because mechanisms to achieve sex-biased gene expression are likely to evolve over long timescales. Even then, factors such as pleiotropy and epistasis (Mank *et al.* 2008) could prevent these mechanisms from fixing. **Chapter 2** provides evidence that conflict can become partially resolved through long-term adaptation, but it supports the hypothesis that resolution is a slow process. The long-timescale for

resolution was evident even when the population had evolved under constant physical environmental conditions.

Slow-acting evolutionary processes are likely to be perturbed by fast-acting processes that can change the nature of natural or sexual selection. These quicker processes include environmental change, such as those brought about by stochastic physical conditions, or changes in the social environment. The inconsistency of sex-specific selection pressures that are likely to result from these processes might therefore prevent long-term IASC resolution through the evolution of sex-biased gene expression. Although there is some evidence that extreme changes in the environment can alter sex-specific selection in such a way, the effect of minor environmental changes was less clear. We addressed this gap in our understanding in **Chapter 3**, showing that very subtle changes in temperature can cause genotypes that were previously sexually antagonistic to become sexually concordant in their fitness effects. To increase our understanding of the environmental effects on IASC resolution, long-term evolution experiments to test the potential for resolution under static and fluctuating environments would be insightful. Additionally, changes in gene expression levels could be measured under different environmental conditions and combined with fitness data to quantify sex-specific selection at the molecular level. This will indicate the scale of environmental effects on IASC across the genome.

As well as physical variables such as temperature, the environment experienced by an individual also encompasses the social interactions that surround them. This includes interlocus sexual conflict (IRSC), involving interactions induced by one sex that increases their fitness at the detriment to the fitness of the opposite sex. This is another fast-acting evolutionary process, and one that is expected to lead to the rapid escalation and exaggeration of male and female traits through male-female coevolution (or arms races). The results presented from a mathematical model in **Chapter 4** suggest that the slower-acting resolution of IASC can act to slow down trait evolution resulting from IRSC. Another key finding was that the direction of arms races could shift due to constraints on trait

evolution imposed by IASC. This is interesting from the point of view of IASC resolution because it is another route through which sex-specific selection pressures can change suddenly, which will ultimately prevent sex-biased gene expression from evolving. This will allow perpetual cycles of IASC to arise in populations without IASC becoming fully resolved. Further insight into the potential interaction between these two conflicts could be gained empirically, by testing whether traits known to be involved in IRSC arms races share a genetic basis between the sexes. The strength of any existing correlation would indicate the evolvability of each trait under IRSC.

6.2 - Combining Phenotype and Genotype

The true sense of the term IASC is "conflict over genes that are shared by the sexes". Previous research, as well as the research presented in this thesis, has successfully shown that the additive effects of genes can have opposite effects on the phenotype (fitness) of each sex. Whilst this reflects IASC, there is still a large gap in our understanding of the genetic basis of this conflict, with questions regarding the number of genes involved and their chromosomal positions remaining largely unexplored. Whilst one study provided fruitful results on the extent of gene expression that was sexually antagonistic within a population (Innocenti and Morrow 2010), this is still the only study of its kind. Other research has identified specific alleles that are associated with IASC (Rostant *et al.* 2015; Morrow 2015), but not at the whole-genome level.

Information on the specific alleles mediating conflict will also aid our understanding of conflict resolution. For example, gene mapping combined with sex-specific fitness data will allow for the chromosomal positions, and extent of epistatic (Arnqvist *et al.* 2014) and pleiotropic interactions (Mank *et al.* 2008) to be deduced for genes that are mediating IASC. There are also other genetic constraints to consider, as gene expression needs to be optimised within an individual throughout their development. For example, there might be genetic constraints that prevent a larva from expressing genes at a higher level than is required for adult development. This is likely to affect the extent of IASC and the potential for resolution, particularly when considering the potential for between-

sex and between-stage genetic correlations to constrain the evolution of sexual dimorphism.

6.3 - The Broader Consequences of IASC

Much of IASC research is focused on its grave impact on population-level fitness, and it is clear that it can strongly impact genetic architecture by selecting for sexual dimorphism. Less apparent, however, are the broader consequences of IASC and its widespread evolutionary significance for animal behaviour and life history traits.

As mentioned in **Chapter 1**, one outcome of IASC is its potential to dramatically impact offspring sex ratio (Cox and Calsbeek 2010; Roulin *et al.* 2010; Katsuki *et al.* 2012). This is an important outcome in itself, but there are also broader scale implications within a population to consider. For example, changing sex ratios can profoundly affect mating behaviours and strategies (Weir *et al.* 2011). When the sex ratio of a population becomes female biased, male competition may be reduced and aggressive interactions between the sexes might become less frequent. In contrast, a male-biased sex ratio could increase male–male competition as females become limiting. This can consequently affect sexual selection on the sexes and significantly alter their evolutionary trajectories (and potentially the intensity of IRSC; **Chapter 4**).

van Doorn (2009) also explains how sex linkage of genes caused by sexual antagonism could have consequences for mate choice and sexual selection. Fisher's runaway hypothesis (Fisher 1958) for the exaggeration of male traits, and sexual selection based on "good genes" (Hamilton and Zuk 1982) are used as examples. These selection processes are facilitated by patterns of sex linkage (Kirkpatrick and Hall 2004) caused by IASC; however, for traits where conflict is still ongoing, runaway selection and sexual selection based on "good genes" may not work. For example, selection based on "good genes" will be less efficient because, while it allows males to be chosen on the basis of producing fit sons, any daughters produced may be of lower fitness (Pischedda and Chippindale 2006).

IASC has also been suggested to play an important role in speciation (Rice and Chippindale 2002). This may result if the gender load created by sexual antagonism causes coevolution between sexually antagonistic and gender-limited genes. It is then plausible that sexual coevolution within a population could subsequently cause allopatric populations to diverge, leading to hybrid infertility upon secondary contact. However, **Chapter 4** suggests that IASC could impact population divergence and speciation in different ways. The results from the mathematical model suggest that IASC could slow down IRSC trait evolution, which is traditionally linked to speciation due to its potential to cause rapid evolution of traits within populations and to create trait divergence between populations (Rice *et al.* 2005). The model in **Chapter 4** also demonstrates that IASC could alter the direction of arms races, causing them to chase-away in different directions. This could actually promote speciation, if different conflicts and patterns of trait divergence occur between allopatric populations.

Sexual antagonism can also have implications for modes of sex determination, leading to rapid evolutionary transitions in some species (van Doorn 2009). This encompasses both environmental sex determination (ESD), where the sex of an individual is determined by environmental cues, and genetic sex determination (GSD), where genes are exclusively responsible for determining sex. In a highly stochastic environment, ESD is likely to evolve if these fluctuations have dramatic sex-dependent fitness consequences (Charnov and Bull 1977). On the other hand, GSD may be favourable under circumstances where genetic variation has sex-dependent fitness effects (Rice 1986).

Abbott (2010) proposes a role for IASC in promoting shifts from hermaphroditism (one sex morph) to gonochorism (two sex morphs). This could occur if IASC leads to selection for linkage between sexually antagonistic alleles and loci for sex determination, consequently resulting in the evolution of proto sex chromosomes (Bedhomme *et al.* 2009). A focus on groups that make frequent transitions to and from gonochorism could be useful to study this concept further (Abbott 2010).

Finally, an interesting, and yet so far unexplored, consequence of IASC is its ability to maintain disease alleles within human populations (Gilks *et al.* 2014; Morrow 2015). In particular, this could apply to some early-onset diseases that are sex specific in their effect. A disease allele such as this would be beneficial to one sex, but increase disease susceptibility in the other. In this sense, it could be maintained within a population despite its negative effects on health. Further investigation into this is likely to have profound effects on the approaches taken to medical research and the design of personalised medicine in healthcare.

6.4 – The Potential for Multiple Sources of Conflict

A recent model showed that an analogous conflict to IASC could arise between queens and workers in eusocial and cooperatively breeding societies (Holman 2014), termed intralocus caste conflict (IACC; **Chapter 5**). Here, intercaste genetic correlations and caste-specific selection could displace castes from their respective fitness optima. **Chapter 5** draws upon this analogy and explores a research framework for caste conflict based on insights from sexual conflict. This chapter highlights the indirect evidence for IACC and shows that, despite its historical lack of attention, direct evidence is empirically tractable in numerous social species. It also raises the possibility that IASC and IACC could act in concert to shape genomic architecture and potentially constrain trait evolution, with greater implications than if IASC operates alone. In fact, this also applies to species where different morphs exist, such as male fighter/sneaker dimorphisms (Buzzato *et al.* 2012), where the expression of single genome needs to be optimised for individuals with different roles.

The dynamic of trait evolution is complicated even further when the results of **Chapter 4** are considered, where IASC and IRSC interact with dramatic effects on male and female trait evolution. In species where other forms of role specific selection operate (e.g. IACC), all of these forces of selection (e.g. IACC, IASC and IRSC) could shape the outcome for trait evolution. This is so far unexplored, and it is likely to be problematic to disentangle these interactions in empirical investigations. Nonetheless it should be considered when predicting the evolution of traits, due to its direct effects on adaptation, fitness variation and

the various other biological processes it influences, as emphasised in this general discussion.

6.5 – Conclusions

This thesis highlights the prevalence of IASC and the various obstacles presented by the physical and social environment that could prevent the evolution of sex-biased gene expression, which is fundamental for long-term resolution of the conflict. Changes in sex-specific selection could temporarily resolve conflict over certain traits, if the fitness effects of these traits become sexually concordant under different conditions. However, changing sex-specific selection likely sparks conflicts over new traits. Longer-term resolution on the other hand arises via sex-biased gene expression, and without it, perpetual cycles of IASC could arise over the same traits, acting to prevent the sexes from reaching their respective fitness optima. In the future, greater insight into the resolution of IASC could be gained by studying populations that have experienced long-term evolution under a range of environmental conditions.

This research is also highly multidisciplinary, as it builds a fundamental understanding of trait evolution and adaptation under various contexts, and stresses that IASC is likely to act in concert with other prominent forces of selection. The future for applying IASC research to other fields of biology is promising, particularly as intralocus genomic conflict between different morphs remains largely unexplored.

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Appendix 1: Supporting Material for Chapter 4

This appendix contains a step-by-step derivation of the general mathematical results (supported by Table S1), followed by supplementary Figure S1 (dynamic of IASC and IRSC indices in the contest mating scenario), Figures S2–S4 (detailing the mechanism of arms-race reversals), and Figures S5–S9 (individual-based simulation results).

Mathematical Analysis

Evolutionary Equilibria

The point of departure of the mathematical analysis is the multivariate breeder's equation (Lande and Arnold 1983)

Equation S1:

$$\frac{d\mathbf{u}}{dt} = \mathbf{G}\,\boldsymbol{\beta}(\mathbf{u}),$$

which describes the evolutionary dynamic of the average trait values. Except in degenerate cases ($r_k = 1$ or $r_k = -1$ for at least one of the traits), which we exclude in the further analysis, the genetic variance-covariance matrix $\mathbf{G}$ is non-singular. This means that $\mathbf{G}^{-1}$ exists, so that the equilibrium points of the system of ordinary differential equations (Equation S1) can be found by solving $\boldsymbol{\beta}(\mathbf{u}^*) = 0$ for the equilibrium trait values $\mathbf{u}^* = (x^*_{♀}, z^*_{♀}, y^*_{♂}, x^*_{♂}, z^*_{♂}, y^*_{♀})^{\mathrm{T}}$. As a further consequence, neither the number of equilibria nor their location are affected by the genetic variance-covariance matrix.

It follows straightforwardly from Equation S1 that $x^*_{♂}$, $z^*_{♂}$ and $y^*_{♀}$ are given by their respective optimal trait values $\theta_{x♂}$, $\theta_{z♂}$ and $\theta_{y♀}$. The equilibrium values of the three remaining characters can be expressed as functions of the mating stimulus $\bar{s}$:

Equation S2:

$$\begin{pmatrix} x^*_{\varphi}(\bar{s}) \\ z^*_{\varphi}(\bar{s}) \\ y^*_{\sigma}(\bar{s}) \end{pmatrix} = \begin{pmatrix} c_{x_{\varphi}} & -a\left(\psi(\bar{s})-\theta_{\psi}\right)\psi'(\bar{s}) & 0 \\ -a\left(\psi(\bar{s})-\theta_{\psi}\right)\psi'(\bar{s}) & c_{z_{\varphi}} & a\left(\psi(\bar{s})-\theta_{\psi}\right)\psi'(\bar{s}) \\ 0 & -b\,\psi'(\bar{s}) & c_{y_{\sigma}} \end{pmatrix}^{-1} \begin{pmatrix} c_{x_{\varphi}}\theta_{x_{\varphi}} \\ c_{z_{\varphi}}\theta_{z_{\varphi}} \\ c_{y_{\sigma}}\theta_{y_{\sigma}} \end{pmatrix}$$

Based on this result, the equilibria can be found by locating the roots of the function $f(\bar{s}) = z^*_{\varphi}(\bar{s})\,(y^*_{\sigma}(\bar{s}) - x^*_{\varphi}(\bar{s})) - \bar{s}$. The equilibrium condition $f(s^*) = 0$ cannot be solved analytically, except in a number of special cases discussed in Rowe *et al.* 2005. However, plotting the graph of f provides a straightforward graphical method to determine how many equilibria there are, while numerical root-finding methods can be applied to approximate the equilibrium values of the mating stimulus to arbitrary precision.

Stability Analysis

The stability of the equilibria is assessed by linearising Equation S1 around each of the equilibrium points,

Equation S3:

$$\frac{d(\mathbf{u}-\mathbf{u}^*)}{dt} \approx \underbrace{\mathbf{G}\left.\frac{\partial \boldsymbol{\beta}(\mathbf{u})}{\partial \mathbf{u}}\right|_{\mathbf{u}=\mathbf{u}^*}}_{\mathbf{M}}(\mathbf{u}-\mathbf{u}^*)$$

and evaluating the eigenvalues of the matrix **M**. This matrix, which is the product of the genetic variance-covariance matrix and the Jacobian of the selection gradient, can be written as a block matrix

Equation S4:

$$\mathbf{M} = \begin{pmatrix} \mathbf{I} & \mathbf{R} \\ \mathbf{R} & \mathbf{I} \end{pmatrix}\begin{pmatrix} \mathbf{J} & \mathbf{0} \\ \mathbf{0} & -\mathbf{C} \end{pmatrix} = \begin{pmatrix} \mathbf{J} & -\mathbf{RC} \\ \mathbf{RJ} & -\mathbf{C} \end{pmatrix}$$

where **I** is the 3 x 3 identity matrix, **0** is a 3 x 3 matrix of zeros, and **C** and **R** are diagonal matrices

Equation S5:

$$\mathbf{C} = \begin{pmatrix} c_{x_\sigma} & 0 & 0 \\ 0 & c_{z_\sigma} & 0 \\ 0 & 0 & c_{y_\varphi} \end{pmatrix} \text{ and } \mathbf{R} = \begin{pmatrix} r_x & 0 & 0 \\ 0 & r_z & 0 \\ 0 & 0 & r_y \end{pmatrix}$$

Throughout, we assume that $0 < r_k < 1$ and $c_k > 0$ for all characters, so that the eigenvalues of $\mathbf{R}^2$, $\mathbf{I}-\mathbf{R}^2$ and $\mathbf{C}$ are strictly positive. Finally, the matrix $\mathbf{J}$, given by

Equation S6:

$$\mathbf{J} = \begin{pmatrix} -a\, z_\varphi^{*2}\, \Psi'' - c_{x_\varphi} & a\, \Psi' + a\, s^* \Psi'' & a\, z_\varphi^{*2}\, \Psi'' \\ a\, \Psi' + a\, s^* \Psi'' & -a\, (y_\sigma^* - x_\varphi^*)^2\, \Psi'' - c_{z_\varphi} & -a\, \Psi' - a\, s^* \Psi'' \\ -b\, z_\varphi^{*2}\, \psi''(s^*) & b\, \psi'(s^*) + b\, s^* \psi''(s^*) & b\, z_\varphi^{*2}\, \psi''(s^*) - c_{y_\sigma} \end{pmatrix}$$

is a 3 x 3 submatrix of the Jacobian that specifies how small perturbations of $x_♀$, $z_♀$ or $y_♂$ away from their equilibrium value influence the strength and the direction of selection acting on each of the mating characters. Here, we used the shorthand notation $\Psi' = \psi'(s^*)(\psi(s^*) - \theta_\psi)$ and $\Psi'' = \psi''(s^*)(\psi(s^*) - \theta\psi) - \psi'(s^*)^2$. Furthermore, $s^* = z^*_♀ \times (y^*_♂ - x^*_♀)$ denotes the equilibrium value of the mating stimulus. Aside from the contributions of stabilising natural selection that appear on the diagonal, $\mathbf{J}$ captures the fitness consequences of IRSC, which are mediated by the effects of the mating characters on the value of the mating stimulus.

The equilibrium is stable if and only if all eigenvalues of $\mathbf{M}$ have negative real parts. Accordingly, if λ is the eigenvalue with the largest real part, a necessary and sufficient condition for stability is that $\Re(\lambda) < 0$ (a summary of our notation used for complex numbers is provided in Table S1). Let $\mathbf{w}$ be the eigenvector associated with eigenvalue λ. In accordance with the block structure of $\mathbf{M}$, $\mathbf{w}$ is split into two parts, which are written as linear combinations of two vectors $\mathbf{u}$, $\mathbf{v} \in \mathbb{C}^3$. We are primarily interested in the case that $\Re(\lambda) > 0$ for $\mathbf{R} \rightarrow \mathbf{I}$, implying that the equilibrium is not stable in the absence of intersexual genetic correlations (and aim to show that such an equilibrium can become stable for

some **R** ≠ **I**). In this case, **J** has at least one eigenvalue with positive real part. The further calculations simplify if we choose

Equation S7:

$$\mathbf{w} = \begin{pmatrix} \mathbf{u} + \mathbf{R}\mathbf{v} \\ \mathbf{v} \end{pmatrix}, \text{ such that } \mathbf{M}\mathbf{w} = \lambda\mathbf{w} \Leftrightarrow \begin{cases} \mathbf{J}\mathbf{u} + \mathbf{J}\mathbf{R}\mathbf{v} - \mathbf{R}\mathbf{C}\mathbf{v} = \lambda\mathbf{u} + \lambda\mathbf{R}\mathbf{v} \\ \mathbf{R}\mathbf{J}\mathbf{u} + \mathbf{R}\mathbf{J}\mathbf{R}\mathbf{v} - \mathbf{C}\mathbf{v} = \lambda\mathbf{v} \end{cases}$$

We note that **u** is a vector that tends to the dominant eigenvector of **J** as **R** → **I**. In that same limit, the vector **v** tends to a vector of zeros. Slightly rearranging the eigenvector equation in Equation S7 yields two other useful expressions

Equation S8:

$$\mathbf{G}^{-1}\mathbf{M}\mathbf{w} = \lambda\mathbf{G}^{-1}\mathbf{w} \Leftrightarrow \begin{cases} \mathbf{J}\mathbf{u} + \mathbf{J}\mathbf{R}\mathbf{v} = \lambda(\mathbf{I} - \mathbf{R}^2)^{-1}\mathbf{u} \\ \mathbf{C}\mathbf{v} = \lambda\mathbf{R}(\mathbf{I} - \mathbf{R}^2)^{-1}\mathbf{u} - \lambda\mathbf{v} \end{cases}$$

In order to calculate $\Re(\lambda)$, we make use of the properties of the inner product $\langle \mathbf{x}, \mathbf{y} \rangle = \mathbf{x}^\dagger \mathbf{y}$. In particular, for any real valued matrix $\mathbf{A}$, $\langle \mathbf{A}\mathbf{v}, \mathbf{v} \rangle = (\mathbf{A}\mathbf{v})^\intercal \mathbf{v} = \mathbf{v}^\intercal \mathbf{A}^\intercal \mathbf{v} = \langle \mathbf{v}, \mathbf{A}^\intercal \mathbf{v} \rangle$. In addition, $\langle \mathbf{v}, \lambda\mathbf{v} \rangle = \lambda \langle \mathbf{v}, \mathbf{v} \rangle$ and $\langle \lambda\mathbf{v}, \mathbf{v} \rangle = \bar{\lambda} \langle \mathbf{v}, \mathbf{v} \rangle$, such that

Equation :

$$\begin{aligned} \Re(\lambda) &= \frac{1}{2}(\lambda + \bar{\lambda}) \\ &= \frac{\langle \mathbf{v}, \lambda\mathbf{v} \rangle + \langle \lambda\mathbf{v}, \mathbf{v} \rangle}{2\langle \mathbf{v}, \mathbf{v} \rangle} \\ &= \frac{\langle \mathbf{v}, \mathbf{R}\mathbf{J}\mathbf{u} + \mathbf{R}\mathbf{J}\mathbf{R}\mathbf{v} - \mathbf{C}\mathbf{v} \rangle + \langle \mathbf{R}\mathbf{J}\mathbf{u} + \mathbf{R}\mathbf{J}\mathbf{R}\mathbf{v} - \mathbf{C}\mathbf{v}, \mathbf{v} \rangle}{2\langle \mathbf{v}, \mathbf{v} \rangle} \\ &= \frac{\langle \mathbf{R}\mathbf{v}, \mathbf{J}^{\mathrm{S}}\mathbf{R}\mathbf{v} \rangle - \langle \mathbf{v}, \mathbf{C}\mathbf{v} \rangle}{\langle \mathbf{v}, \mathbf{v} \rangle} + \frac{\langle \mathbf{R}\mathbf{v}, \mathbf{J}\mathbf{u} \rangle + \langle \mathbf{u}, \mathbf{J}^{\mathrm{T}}\mathbf{R}\mathbf{v} \rangle}{2\langle \mathbf{v}, \mathbf{v} \rangle}, \end{aligned}$$

where the long expression substituted for $\lambda\mathbf{v}$ in the third step of this calculation is taken from Equation S7. Appearing in the final step of this derivation is the symmetric part of the matrix **J**, defined as $\mathbf{J}^{\mathrm{S}} = \frac{1}{2}(\mathbf{J}+\mathbf{J}^{\mathrm{T}})$. A similar derivation, built on the result of Equation S8, gives rise to

Equation S10:

$$\begin{aligned}\Re(\lambda) &= \frac{\langle \mathbf{u},\, \lambda(\mathbf{I}-\mathbf{R}^2)^{-1}\mathbf{u}\rangle + \langle \lambda(\mathbf{I}-\mathbf{R}^2)^{-1}\mathbf{u},\, \mathbf{u}\rangle}{2\langle \mathbf{u},\, (\mathbf{I}-\mathbf{R}^2)^{-1}\mathbf{u}\rangle} \\ &= \frac{\langle \mathbf{u},\, \mathbf{J}\mathbf{u}+\mathbf{J}\mathbf{R}\mathbf{v}\rangle + \langle \mathbf{J}\mathbf{u}+\mathbf{J}\mathbf{R}\mathbf{v},\, \mathbf{u}\rangle}{2\langle \mathbf{u},\, (\mathbf{I}-\mathbf{R}^2)^{-1}\mathbf{u}\rangle} \\ &= \frac{\langle \mathbf{u},\, \mathbf{J}^{\mathrm{S}}\mathbf{u}\rangle}{\langle \mathbf{u},\, (\mathbf{I}-\mathbf{R}^2)^{-1}\mathbf{u}\rangle} + \frac{\langle \mathbf{R}\mathbf{v},\, \mathbf{J}^{\mathrm{T}}\mathbf{u}\rangle + \langle \mathbf{u},\, \mathbf{J}\mathbf{R}\mathbf{v}\rangle}{2\langle \mathbf{u},\, (\mathbf{I}-\mathbf{R}^2)^{-1}\mathbf{u}\rangle}\end{aligned}$$

We can now form a linear combination of Equations S9 and S10, and recognise that $\langle \mathbf{v},\, \mathbf{v}\rangle + \langle \mathbf{u},\, (\mathbf{I}-\mathbf{R}^2)^{-1}\mathbf{u}\rangle = \langle \mathbf{w},\, \mathbf{G}^{-1}\mathbf{w}\rangle$, yielding a result that only depends on the symmetric part of **J**:

Equation S11:

$$\Re(\lambda) = \frac{\langle \mathbf{u}+\mathbf{R}\mathbf{v},\, \mathbf{J}^{\mathrm{S}}(\mathbf{u}+\mathbf{R}\mathbf{v})\rangle - \langle \mathbf{v},\, \mathbf{C}\mathbf{v}\rangle}{\langle \mathbf{w},\, \mathbf{G}^{-1}\mathbf{w}\rangle}$$

Given that the matrices $\mathbf{J}^{\mathrm{S}}$ and **C** are both Hermitian $(\text{i.e., } \mathbf{J}^{\mathrm{S}} = (\mathbf{J}^{\mathrm{S}})^{\dagger} \text{ and } \mathbf{C} = \mathbf{C}^{\dagger})$ we next apply the following theorem from linear algebra to calculate an upper bound on $\Re(\lambda)$.

Theorem 1 (Rayleigh quotient theorem) For any n x n Hermitian matrix **H**, the Rayleigh quotient $Q(\mathbf{H},\mathbf{z}) = \langle \mathbf{H}\mathbf{z},\, \mathbf{z}\rangle / \langle \mathbf{z},\, \mathbf{z}\rangle$ cannot be larger than the largest eigenvalue of **H**, $\Lambda_{\max}(\mathbf{H})$. *Moreover*, $Q(\mathbf{H},\mathbf{z}) = \Lambda_{\max}(\mathbf{H})$, if and only if **z** is equal to the eigenvector associated with the largest eigenvalue. In the same way, Q(**H**, **z**) attains its minimum value when **z** is an eigenvector of **H** associated with the smallest eigenvalue $\Lambda_{\min}(\mathbf{H})$. Consequently, for any vector $\mathbf{z} \in \mathbb{C}^n$

Equation S12:

$$\Lambda_{\min}(\mathbf{H}) \leqslant Q(\mathbf{H}, \mathbf{z}) \leqslant \Lambda_{\max}(\mathbf{H})$$

The proof of this result builds on the fact that the eigenvectors of a Hermitian matrix form an orthonormal basis of $\mathbb{C}^n$ and that the associated eigenvalues are real, so that they can be ordered.

The application of the Rayleigh quotient theorem to Equation S11 yields

Equation S13:

$$\begin{aligned}\Re(\lambda) &= \frac{\langle \mathbf{u}+\mathbf{R}\mathbf{v},\, \mathbf{u}+\mathbf{R}\mathbf{v}\rangle Q(\mathbf{J}^{\mathrm{S}}, \mathbf{u}+\mathbf{R}\mathbf{v}) - \langle \mathbf{v},\, \mathbf{v}\rangle Q(\mathbf{C}, \mathbf{v})}{\langle \mathbf{w},\, \mathbf{G}^{-1}\mathbf{w}\rangle} \\ &\leqslant \frac{\langle \mathbf{u}+\mathbf{R}\mathbf{v},\, \mathbf{u}+\mathbf{R}\mathbf{v}\rangle \Lambda_{\max}(\mathbf{J}^{\mathrm{S}}) - \langle \mathbf{v},\, \mathbf{v}\rangle Q(\mathbf{C}, \mathbf{v})}{\langle \mathbf{w},\, \mathbf{G}^{-1}\mathbf{w}\rangle} \\ &= \frac{\langle \mathbf{w},\, \mathbf{w}\rangle}{\langle \mathbf{w},\, \mathbf{G}^{-1}\mathbf{w}\rangle}\left(\left(1 - \frac{\langle \mathbf{v},\, \mathbf{v}\rangle}{\langle \mathbf{w},\, \mathbf{w}\rangle}\right)\Lambda_{\max}(\mathbf{J}^{\mathrm{S}}) - \frac{\langle \mathbf{v},\, \mathbf{v}\rangle}{\langle \mathbf{w},\, \mathbf{w}\rangle} Q(\mathbf{C}, \mathbf{v})\right)\end{aligned}$$

which still depends on the relative magnitude of the two components **u** and **v** of the eigenvector **w**. However, we can already infer that the sign of $\Re(\lambda)$ is determined by the sign of the weighted mean of the dominant eigenvalue of $\mathbf{J}^{\mathrm{S}}$ and the eigenvalues of **C**, which are all negative. Accordingly, there is a range of values of $\Lambda_{\max}(\mathbf{J}^{\mathrm{S}})$ for which an unstable equilibrium can be stabilised, but equilibria for which $\Lambda_{\max}(\mathbf{J}^{\mathrm{S}}) < 0$ cannot become destabilised.

In order to obtain a result that explicitly depends on the intersexual correlations, we use the fact that **u** and **v** are related to each other by the second Equation on the right-hand side of Equation S8. As a consequence,

Equation 14:

$$\begin{aligned}\frac{\langle \mathbf{v},\, \mathbf{v}\rangle}{\langle \mathbf{w},\, \mathbf{w}\rangle} &= \frac{\langle \mathbf{v},\, \mathbf{v}\rangle}{\langle \mathbf{v},\, \mathbf{v}\rangle + \langle \mathbf{u}+\mathbf{R}\mathbf{v},\, \mathbf{u}+\mathbf{R}\mathbf{v}\rangle} \\ &= \frac{1}{1+\frac{\langle (\mathbf{P}_\lambda+\mathbf{R})\mathbf{v},\,(\mathbf{P}_\lambda+\mathbf{R})\mathbf{v}\rangle}{\langle \mathbf{v},\, \mathbf{v}\rangle}} \\ &= \frac{1}{1+Q((\mathbf{P}_\lambda^\dagger+\mathbf{R})(\mathbf{P}_\lambda+\mathbf{R}),\mathbf{v})}\end{aligned}$$

where $\mathbf{P}_\lambda = (\lambda\mathbf{R})^{-1}(\mathbf{C}+\lambda\mathbf{I})(\mathbf{I}-\mathbf{R}^2)$ is a complex-valued, 3 x 3 diagonal matrix that maps **v** to **u**. Substituting this result in Equation S13 and bounding the remaining Rayleigh quotients leads to the conclusion that

Equation S15:

$$\Re(\lambda) \leqslant \kappa\left(\Lambda_{\max}(\mathbf{J}^{\mathrm{S}}) - \left(\frac{r_{\min}|\lambda|}{|(1-r_{\min}^2)c_{\min}+\lambda|}\right)^2 c_{\min}\right)$$

where $c_{\min} = \min(c_{x♂}, c_{z♂}, c_{y♀})$ and $r_{\min}$ " $\min(r_x, r_z, r_y)$. Contained in the prefactor κ are several factors that are strictly positive and that, therefore, do not affect the sign of $\Re(\lambda)$, including a term that is bounded by the eigenvalues of the genetic variance-covariance matrix.

Varying one of the model's parameters in such a way that $\Re(\lambda)$ changes sign, causes a bifurcation event to occur, i.e., a qualitative change in the dynamical behavior of the model. Two different types of bifurcations can happen when $\Re(\lambda)= 0$, depending on whether the imaginary part of λ is zero at the bifurcation point or not. The first case, i.e., λ = 0, is accompanied by a change in the location (and sometimes the number) of equilibria, and requires that **M** is singular at the bifurcation point. Given that both **C** and **R** are positive definite, Equation S4 implies that **M** can only be singular if **J** is singular. This condition does not depend on the genetic variance-covariance matrix, so the corresponding bifurcations are independent of the intersexual genetic correlations. The reverse implication is that qualitative effects of intralocus conflict on the stability of intersexual selection equilibria, must involve bifurcations of the second type,

known as Poincaré-Andronov-Hopf (or, Hopf) bifurcations. A Hopf bifurcation is a local bifurcation at which a pair of two complex conjugate eigenvalues crosses the imaginary axis $(\text{i.e., } \Re(\lambda) \text{ and } \Re(\bar{\lambda})$ change sign while $\Im(\lambda) = -\Im(\bar{\lambda}) \neq 0)$. These events are associated with the birth of a limit cycle that branches from the equilibrium point.

Taking $r_{\min}$ as the bifurcation parameter of interest, we now return to inequality (Equation S15) and ask if a Hopf bifurcation can occur when the impact of IASC increases. For equilibria that go through a Hopf bifurcation, $|\lambda| \neq 0$, which implies that the right-hand side of inequality (Equation S15) is a strictly decreasing function of $r^2_{\min}$ in a neighbourhood of the bifurcation point. Therefore, the first conclusion we can draw is that IASC has in general a stabilising effect on the evolutionary dynamics of IRSC in the vicinity of equilibria. Furthermore, a qualitative change in the stability of an equilibrium can occur when an evolutionary fixed point is unstable under the sole action of IRSC (i.e., when $r^2_{\min} = 0$), but when stabilising natural selection on the homologous characters is sufficiently strong to overcome destabilising sexual selection. In particular, if $\Re(\lambda) > 0$ at $r_{\min} = \bar{0}$, such that $\Lambda_{\max}(\mathbf{J}^{\mathrm{S}}) > 0$, and if $\bar{c}_{\min} > \Lambda_{\max}(\mathbf{J}^{\mathrm{S}})$, then there is a critical value $r^*_{\min}$ such that the equilibrium is guaranteed to be stable for all $r^*_{\min} < r_{\min} \leq 1$.

Table S1 - Summary of Notation Used in the Mathematical Analysis

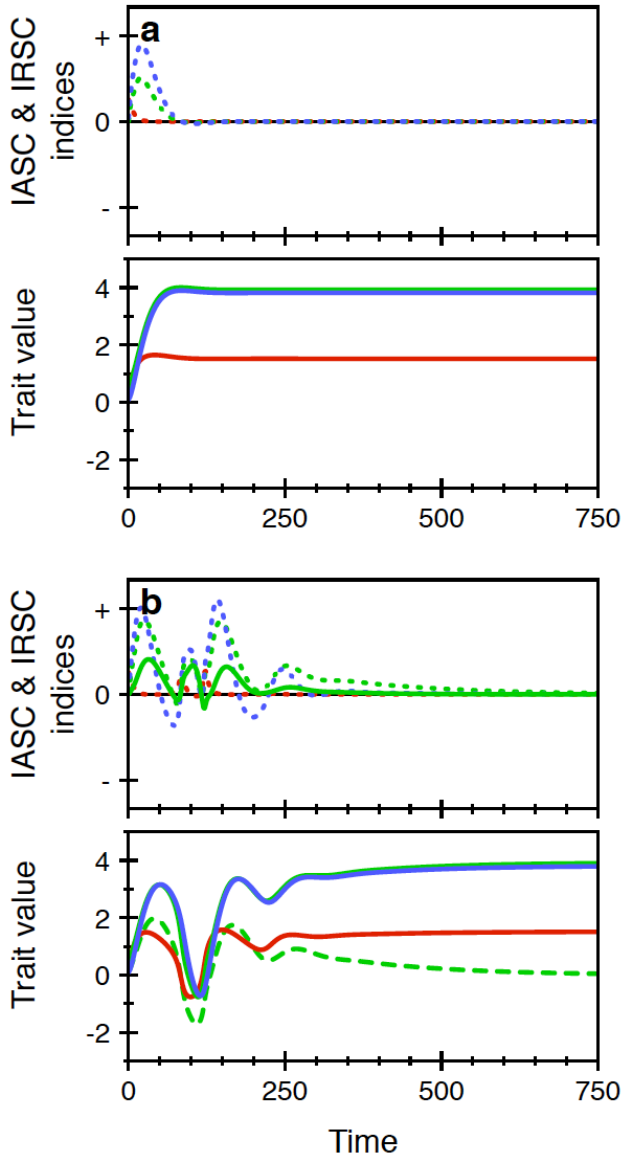
Table S1. Summary of notation used in the mathematical analysis

Notation	Definition
	Complex numbers[1]
$z \in \mathbb{C}$	Complex number $z = a + i\,b$, where i is the imaginary unit, defined by $i^2 = -1$
$\Re(z)$	Real part of z; $\Re(a + i\,b) = a$
$\Im(z)$	Imaginary part of z; $\Im(a + i\,b) = b$
$\bar{z}$	Complex conjugate of z, i.e., if $z = a + i\,b$ then $\bar{z} = a - i\,b$
$\lvert z \rvert$	Absolute value or magnitude of z, i.e., if $z = a + i\,b$ then $\lvert z \rvert = \sqrt{a^2 + b^2}$
	Vectors and matrices
$\mathbf{x}$	Lowercase boldface symbols represent (column) vectors
$\mathbf{x}_{[i]}$	The number at position i in vector $\mathbf{x}$
$\mathbf{x}^{\mathrm{T}}$	Transpose of a vector; transposition changes column vectors into row vectors and vice versa
$\mathbf{x}^{\dagger}$	Conjugate transpose of a vector; $\mathbf{x}^{\dagger} = \bar{\mathbf{x}}^{\mathrm{T}}$
$\langle \mathbf{x}, \mathbf{y} \rangle$	Inner product of $\mathbf{x}$ and $\mathbf{y}$; $\langle \mathbf{x}, \mathbf{y} \rangle = \mathbf{x}^{\dagger}\mathbf{y} = \sum_k \bar{\mathbf{x}}_{[k]}\mathbf{y}_{[k]}$
$\lVert \mathbf{x} \rVert$	Lenght of $\mathbf{x}$; $\lVert \mathbf{x} \rVert = \sqrt{\langle \mathbf{x}, \mathbf{x} \rangle}$
$\mathbf{A}$	Uppercase boldface symbols represent matrices
$\mathbf{A}_{[i,j]}$	The element at row i and column j of matrix $\mathbf{A}$
$\mathbf{A}^{\mathrm{T}}$	Transpose of a matrix; $\mathbf{A}^{\mathrm{T}}_{[i,j]} = \mathbf{A}_{[j,i]}$
$\mathbf{A}^{\dagger}$	Conjugate transpose of a matrix; $\mathbf{A}^{\dagger} = \bar{\mathbf{A}}^{\mathrm{T}}$
$\mathbf{A}^{\mathrm{S}}$	Symmetric part of matrix $\mathbf{A}$; $\mathbf{A}^{\mathrm{S}} = \frac{1}{2}(\mathbf{A} + \mathbf{A}^{\dagger})$
$\Lambda_{\max}(\mathbf{H})$, $\Lambda_{\min}(\mathbf{H})$	Largest and smallest eigenvalue of a Hermitian[2] matrix $\mathbf{H}$

[1] Definitions in this part of the table assume that $a, b \in \mathbb{R}$
[2] A matrix $\mathbf{H}$ is Hermitian if $\mathbf{H} = \mathbf{H}^{\dagger}$

Figure S1 - Dynamic of IASC and IRSC During the Evolution of Offence and Defence Traits: each panel shows a simulation of evolving mean trait values with a corresponding timeplot of trait-specific indices of sexually antagonistic selection (dashed lines: IRSC index; solid lines: IASC index; see Materials and Methods). Mating is modelled as a contest between offence and defence traits. (a) Evolution in the absence of between-sex pleiotropy ($r_x = r_y = r_z = 0$). Female threshold (green) and male persistence (blue) coevolve in an escalating arms race until eventually opposed by viability selection. In (b), the approach to the equilibrium is perturbed by IASC, which can be seen to build up during phases of rapid intersexual coevolution ($r_x = 0.9$; $r_y = r_z = 0$). Pleiotropic gene expression in males constrains the evolution of the female mating threshold, inducing females to reduce their mating rate by an alternative mechanism: lowering sensitivity (red) to the mating stimulus. When females become insensitive, threshold and persistence fall back towards lower levels, initially aided by the resolution of IASC (the IASC index is negative for a brief period). Viability selection then pushes female sensitivity up again, initiating a second arms race towards positive values of threshold and persistence. This time, a slightly lower level of IASC is built up, allowing the population to converge on the equilibrium. Parameters are: $a = 5.0$, $b = 0.5$, $\theta_{x♀} = \theta_{x♂} = 0$, $\theta_{y♂} = \theta_{y♀} = 0.05$, $\theta_{z♀} = 0.95$, $\theta_{\psi} = 0.25$, $c_{x♀} = 0.1$, $c_{x♂} = c_{y♂} = c_{y♀} = c_{z♀} = 0.05$.

Figure S1 Continued



Extra Supplementary Figures and Descriptions

Reversal of Arms Races

Here, we analyse the evolutionary trajectories of populations approaching equilibrium in order to clarify how IASC resolution reverses the direction of arms races when mating compatibility is determined by complementarity of mating traits. Simulation data are represented in two different ways in the following figures, one emphasising the coevolutionary chase between the sexes (left column in Figures S2 – S3), the other highlighting the build-up and resolution of IASC (right column in Figures S2 – S3). Figure S2 shows results for the simplified model also analysed in the main text of **Chapter 4**, for three different values of the additive genetic correlation r_x. At the lowest value of r_x (a, b; **Chapter 4**), the population can be seen to approach the green equilibrium, building up unresolved IASC on its way. The resolution of the conflict causes a temporary de-escalation of the arms race (Figure S2a), due to its pleiotropic effect on $x_{♀}$. However, the population never crosses the $x_{♀} = y_{♂}$ line (dashed diagonal in a), implying that the direction of IRSC does not change qualitatively. So, after IASC has been resolved, the population resumes the coevolutionary chase until it is halted at the green equilibrium by stabilising natural selection.

In Figure S2c-d, the intersexual genetic correlation is slightly stronger than in (a-b), such that higher levels of unresolved IASC build up during the arms race. By dragging down $x_{♀}$, which was ahead of $y_{♂}$ during the first phase of evolution, genetic conflict resolution switches the relative positions of the sexes in their coevolutionary chase, causing its direction to reverse. Initially aided by the natural selection gradients, this second arms race unfolds quickly, causing again high levels of IASC to build up. However, after a short phase of de-escalation, the correlated selection response is not strong enough to reverse the arms race once more, allowing the population to reach the red equilibrium.

The arms race towards the red equilibrium is more difficult to reverse, because a small asymmetry between the natural selection optima of $x_{♀}$ and $y_{♂}$ makes it slightly more difficult for the males to closely follow the females in that direction

of the coevolutionary chase. Hence, higher levels of unresolved IASC are required to switch the relative positions of the sexes, as, for example, shown in Figure S2e-f. Here, the population cycles several times, but note that the amount of IASC built up in the approach of the red equilibrium progressively decreases. Eventually, the population manages to resolve the genetic conflict and attain a truce with respect to intersexual conflict. At even higher levels of r_x, full resolution of IASC is no longer feasible without triggering a new arms race, leading to perpetual coevolution between the sexes.

The argument so far considers only a single character (the female preference) that is pleiotropically expressed in the other sex. Figure S3 illustrates what happens when another trait (i.e., the male ornament) is subject to IASC instead. In this case, the correlated selection response to IASC resolution holds back the males in their pursuit of the females, enlarging rather than reversing the difference between $x_{♀}$ and $y_{♂}$. As a result, IASC resolution for male mating traits tends to preserve the direction of intersexual selection. When acting simultaneously, IASC resolution for male and female mating traits have opposite effects on the stability of intersexual antagonistic coevolution (Figure S4). Arms race reversals, therefore, require stronger cross-sexual pleiotropic constraints on female mating traits (which are leading the coevolutionary chase) than on male traits (which are following behind). The scope for pleiotropy may frequently be asymmetric in this direction, as female preferences often rely on behavioural traits with a complex genetic architecture, whereas male ornamentation traits are usually highly sexually dimorphic already.

Figure S2 - Pleiotropic Expression of a Female Mating Trait in Males Reverses the Direction of Arms Race: large coloured dots denote the location of stable (red, green) and unstable (grey) evolutionary equilibria. Smaller open dots and coloured trajectories (red/green) indicate combinations of realised trait values at regular time points during three simulations, for different levels of the intersexual genetic correlation: r_x = 0.65 (a and b); r_x = 0.7 (c and d) and r_x = 0.75 (e and f). Data points in the left panels show the trait values ($x_{♀}$, $y_{♂}$); the corresponding right panels show the same simulations, summarised by two series of points, plotted at the coordinates ($x_{♀}$, $x_{♂}$) (in red/green) and ($y_{♂}$, 0) (white). Corresponding points in time of the two data series are connected by lines in (b, d, f), with different colours to indicate whether conflict resolution reverses the relative position of male and female mating traits (black → yes; white → no). This information is inferred from additional data contained in the plots: small grey dots in panel (a, c, e) indicate, at each point in time, the trait values that would result if IASC were to be fully resolved; grey lines (also present in b, d, f) trace the correlated selection response associated with such hypothetical, instantaneous IASC resolution. For completeness, dashed grey lines on the background of (b, d, f) also indicate the direction of trait evolution induced by IRSC and its correlated selection response. The red-green gradient used for trajectories, data points and the area of trait space that is traversed by conflict resolution provides a visual indication of the level of unresolved IASC.

Figure S2 Continued

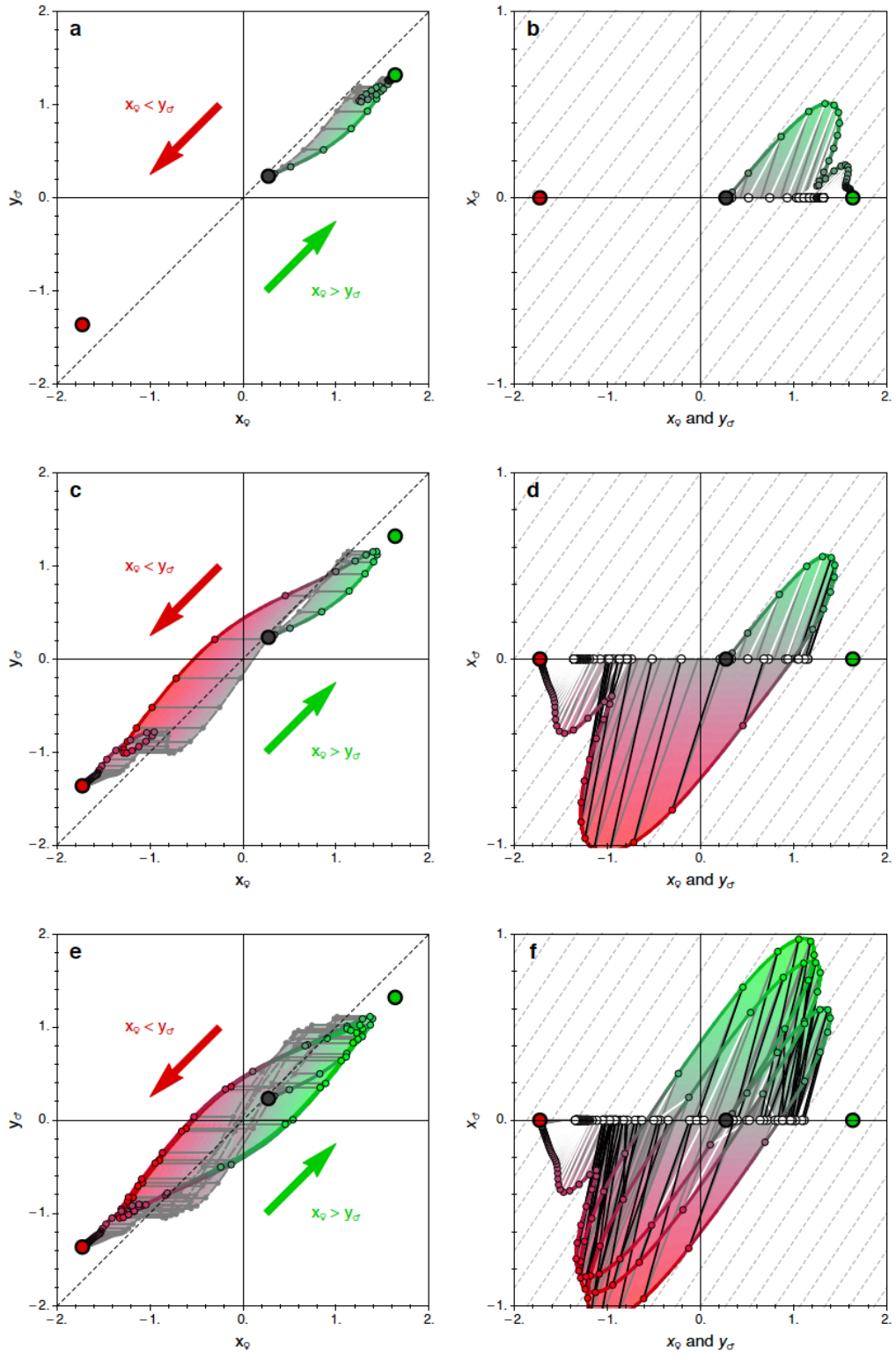


Figure S3 – Pleiotropic Expression of a Male Mating Trait in Females Does Not Destabilise the Approach to Evolutionary Equilibrium: conflict resolution plots (see the legend of S2 for details) of a reduced model (female choosiness is kept fixed at $z_{\varphi} = 1.5$), where only the male ornament genes are pleiotropically expressed in the other sex ($r_x = r_z = 0$; $r_y = 0.9$). Each panel shows data for two simulations, started from different initial conditions on the right and on the left of the interior fixed point.

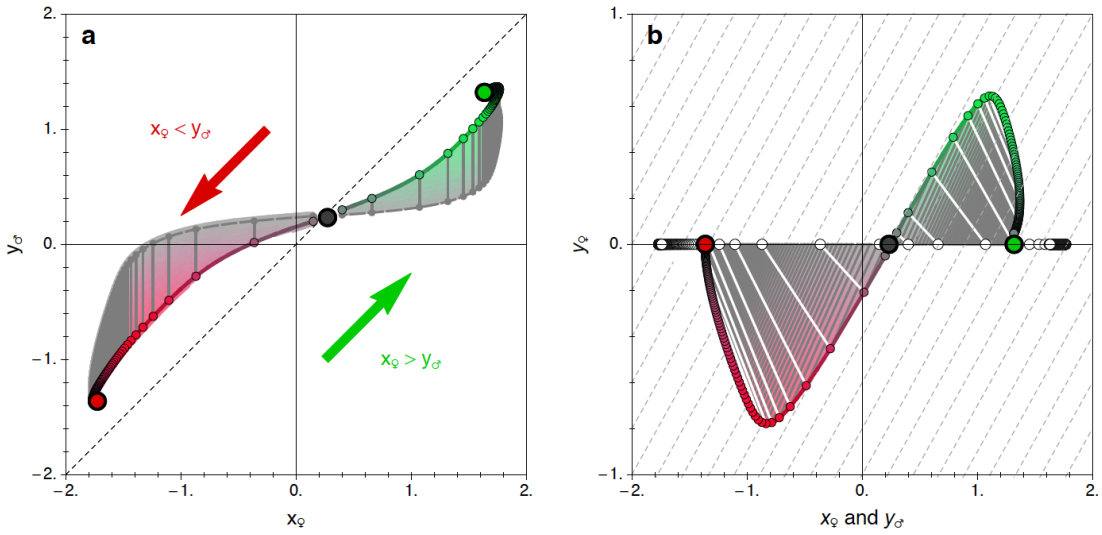
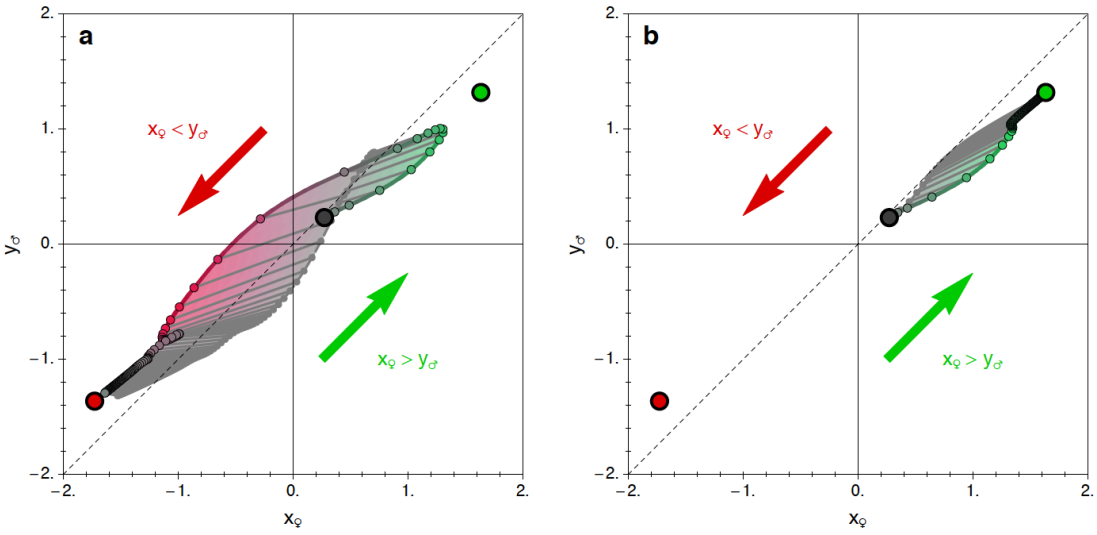


Figure S4 - Combined Effect of Between-Sex Pleiotropy for Male and Female Mating Traits: panel (a) and (b) show two simulations of a model variant with four evolving traits ($x_{♀}$, $y_{♂}$, $x_{♂}$ *and* $y_{♀}$; female choosiness is kept fixed at $z_{♀}$ = 1.5), i.e., both female preference and male ornamentation genes are expressed in both sexes. In (a) (r_x = 0.9; r_y = 0.7, r_z =0), the destabilising effect of IASC resolution for the preference genes (cf. Figure S2) dominates, so that the coevolutionary chase leading towards the green equilibrium is reversed. In (b) (r_x = 0.9; r_y = 0.8, r_z = 0), IASC resolution for the male ornamentation genes has a slightly larger impact, tipping the balance in favour of preserving the direction of intersexual selection (cf. Figure S3).



Individual-Based Simulations

Individual-based simulations were implemented in C++, closely following the assumptions of the quantitative-genetic model. The simulation program keeps track of a population of N individuals with equal proportions of males and females. Each individual carries separate sets of gene loci for x, y and z. Some of the loci are expressed in both sexes, others have sex-limited expression, so that the intersexual additive genetic correlation can be varied by modifying the proportion of sex-limited genes. We allowed for two alleles (denoted + and –) to segregate at a locus and included a low rate of mutation to introduce new genetic variation. Phenotypic trait values are calculated based on the assumption of additive gene action, i.e., each + allele increases the trait value by an amount $\delta/2$, whereas a – allele decreases the trait value by $\delta/2$.

Each generation in the simulation program proceeds in three steps. First, the phenotypes of individuals are determined from their genotype, depending on whether the individual is male or female. Second, the viability of each individual is calculated taking into account stabilising viability selection on the mating traits and the homologous characters. The last step in the life-cycle is the production of offspring. Here, in contrast to the quantitative genetic model, we did not evaluate reproductive success based on the population average trait values. Rather, the mating process was implemented in a more mechanistic fashion, allowing us to obtain a stronger validation of the quantitative genetic model: for every offspring, the simulation algorithm first randomly picks a female from the population of surviving females. This female is then assumed to encounter $n = 50$ different males sampled randomly from the surviving males. The mating rate of the focal female with each of the males (denoted by ψ_i for the i-th male) is evaluated. Next, a single mating partner is picked for the focal female from the sample of n males. This sampling step is implemented as a weighted lottery with weights given by male relative reproductive success $\exp(b\,\psi_i)$. The reproductive success of the female determines the probability that she will produce an offspring from the current mating attempt. Female reproductive success is calculated as $\exp\left(-a\sum_{1\leq i\leq n}(\psi_i-\theta_\psi)^2/(2n)\right)$, i.e., assuming multiplicative costs of interactions with all the n males encountered by the

female. The procedure is repeated until *N/2* male and *N/2* female offspring are produced. All surviving males and all surviving females are available to participate in each mating attempt, irrespective of how many mating attempts they have participated in already. After all offspring have been created, the parental generation is removed from the memory and replaced by their offspring. Inheritance was implemented assuming either haploid or diploid genetics and free recombination between loci.

To ensure correspondence between the generation time in the individual-based simulations and the time units of the quantitative-genetic model, we scaled the time variable of the breeder's Equation S1 by a factor 2 to take into account that each of the phenotypic characters is exposed to selection in only half of the individuals (i.e., either in males or in females). In addition, estimates for the elements of the **G**-matrix were derived from the parameters of the individual-based simulation, using approximations from the neutral theory of molecular evolution. In particular, under the assumptions of the infinite-alleles model (Kimura and Crow 1964), the probability F that a single locus is homozygous at mutation-drift equilibrium in a diploid population of size N is given by $F = 1/(1 + 4\mu N)$, where μ is the mutation rate. Given that the genetic variance at the locus is half of the heterozygosity, $1 - F$, we can now estimate V_L the additive genetic variance of a neutral phenotypic character that is encoded by L diploid loci:

Equation S16:

$$V_L = 2L \times \frac{1}{2}\left(1 - \frac{1}{1+4\mu N}\right) \times \delta^2 = L\delta^2 \frac{4\mu N}{1+4\mu N},$$

where δ is the phenotypic effect of a mutation. Similarly, the additive genetic covariance C_K between two neutral phenotypic characters that share a common genetic basis of K loci is given by

Equation S17:

$$C_K = K\delta^2 \frac{4\mu N}{1+4\mu N}.$$

The amount of additive genetic (co)variation that is present for phenotypic characters that are subject to selection is expected to converge to the neutral expectation (Equations S16 and S17) in the limit of weak selection. Hence, if selection is weak, we expect that the dynamic of the individual-based simulation is captured approximately by the following breeder's equation:

Equation S18:

$$\frac{d\mathbf{u}}{dt} = \frac{1}{2}\begin{pmatrix} V_L & 0 & 0 & C_{K_x} & 0 & 0 \\ 0 & V_L & 0 & 0 & C_{K_z} & 0 \\ 0 & 0 & V_L & 0 & 0 & C_{K_y} \\ C_{K_x} & 0 & 0 & V_L & 0 & 0 \\ 0 & C_{K_z} & 0 & 0 & V_L & 0 \\ 0 & 0 & C_{K_y} & 0 & 0 & V_L \end{pmatrix} \boldsymbol{\beta}(\mathbf{u}) = \frac{1}{2} L\delta^2 \frac{4\mu N}{1+4\mu N} \begin{pmatrix} 1 & 0 & 0 & K_x/L & 0 & 0 \\ 0 & 1 & 0 & 0 & K_z/L & 0 \\ 0 & 0 & 1 & 0 & 0 & K_y/L \\ K_x/L & 0 & 0 & 1 & 0 & 0 \\ 0 & K_z/L & 0 & 0 & 1 & 0 \\ 0 & 0 & K_y/L & 0 & 0 & 1 \end{pmatrix} \boldsymbol{\beta}(\mathbf{u})$$

Here, we have assumed (as in the individual-based simulations) that the number of loci coding for each phenotypic character (L) and the phenotypic effect of a mutation (δ) are identical for all characters. The number of loci that are shared between male and female characters however, are allowed to differ between traits, so that K_x, K_y and K_z can be varied to control the degree of between-sex pleiotropy for each character independently. As mentioned above, the factor 1/2 in front of the **G**-matrix appears because each phenotypic character is subject to selection in only one sex. Equation S18 applies to a diploid population. The analogous equation for a haploid population is given by

Equation S19:

$$\frac{d\mathbf{u}}{dt} = \frac{1}{4} L\delta^2 \frac{2\mu N}{1+2\mu N} \begin{pmatrix} 1 & 0 & 0 & K_x/L & 0 & 0 \\ 0 & 1 & 0 & 0 & K_z/L & 0 \\ 0 & 0 & 1 & 0 & 0 & K_y/L \\ K_x/L & 0 & 0 & 1 & 0 & 0 \\ 0 & K_z/L & 0 & 0 & 1 & 0 \\ 0 & 0 & K_y/L & 0 & 0 & 1 \end{pmatrix} \boldsymbol{\beta}(\mathbf{u})$$

Accordingly, for the same value of L and all other parameters, a haploid population evolves up to four times more slowly than a diploid population. The difference is due to two factors: first, relative to a diploid, a haploid individual carries only half the amount of gene copies, and therefore accumulates mutations at half the rate of a diploid individual; second, mutations are more rapidly lost from a haploid population, due to the smaller effective population size of its gene pool. As a result, the amount of genetic variation maintained at mutation-drift equilibrium in a haploid population is up to two times lower than in a diploid population.

Figures S5 and S6 compare the quantitative-genetic predictions based on Equation S19 with individual-based simulation results. The trajectories predicted by the two methods are, overall, in good agreement, both for a simulation that shows an arms race towards a stable equilibrium (Figure S5a, b) and for one that exhibits oscillations (Figure S6a, b). As expected, the additive genetic variances (panel S5c and S6c) are slightly lower in the individual-based simulation than predicted by Equation S16, since part of the variation is eroded by selection. However, given the observed time-scale correspondence between the two modelling methods, this discrepancy appears to have relatively minor consequences for the predicted rate of adaptive evolution.

Since we do not allow the allelic effect sizes or the number of loci to evolve, the phenotypic trait values in the individual-based simulations are restricted to a finite range (between $-L\delta/2$ and $+L\delta/2$ for haploid genetics). This constraint has three consequences that are ignored in the quantitative-genetic model. First, the maximal genetic variance decreases with the absolute mean trait value in the individual-based simulation, an effect that can clearly be observed in Figure S5c after generation 10000. Second, also the intersexual correlations are constrained by the finite genetic architecture of the traits when the mean trait values evolve towards the end points of the feasible phenotype range. This effect is only weak in Figure S6d, but appreciable in Figure S5d where systematic deviations of the intersexual correlations from their expected values are observed after generation 10000. Third, mutation can only act in one direction at the extreme

ends of the feasible phenotype range and generally has a tendency to bias evolution towards trait value 0. Consistent with these three effects, the individual-based simulation shows a retarded approach to equilibrium at lower escalated trait values (Figure S5a).

Mutation bias may also partially explain why the amplitude of the oscillations in the individual-based simulations is less than predicted by the quantitative-genetic model (Figure S6a, b). However, additional simulations show that the discrepancy in amplitude also depends on the population size (Figure S7) and other factors that influence the amount of standing genetic variation (mutation rate, number of loci and allelic effect size). Therefore, we hypothesise that the presence of genetic variation, which is not taken into account in the derivation of the selection gradients, causes the arms race to reverse prematurely, reducing the amplitude of the evolutionary oscillations. Data recorded from simulations across a range of population sizes are consistent with this hypothesis (Figures S7 and S8). The same simulations also validate the quantitative genetic model (Equation S18) for diploid populations.

Surprisingly, the predicted dynamics of the quantitative-genetic model is mirrored most accurately in relatively small populations, even though the impact of genetic drift on evolution is generally inversely related to population size. The pattern suggested by Figure S7 is confirmed by a more careful quantification of the period and amplitude of the evolutionary oscillations observed in the individual-based simulations (Figure S8). Close correspondence between the two modelling methods is found for populations smaller than 20000 individuals, whereas substantial deviations in amplitude and period occur in populations larger than that size. The two outcomes coincide with two distinct population-genetic regimes: if $4\mu N << 1$, evolution is mutation-limited, the amount of genetic variation present in the population is low and adaptation proceeds as a sequence of discrete mutation and trait-substitution events; by contrast $4\mu N > 0.1$ when $N > 20000$ (given that $\mu = 1.28 \times 10^{-6}$), implying that an appreciable level of standing genetic variation is present in the largest simulated populations.

To complete the analysis, we ran individual-based simulations of the mating-contest scenario. Unlike the other simulations, which were first run for a while to allow genetic variation to build up, these simulations were started with a population of genetically identical individuals from which data were recorded immediately. This was necessary to enable the visualisation of the transient dynamics. The low initial genetic variation in the simulation caused the initial dynamic to slow down, but otherwise the match between simulation data and the quantitative-genetic model was satisfactory (Figure S9): we recovered the expected qualitative contrast between sustained oscillations and convergence to a stable equilibrium at low and high intersexual correlations, respectively. However, the oscillations in the individual-based simulations were slower and had a lower amplitude. These effects appear to be due mainly to a reduction of the genetic variance for the female mating threshold.

In summary, we conclude that the individual-based simulations (figures S5-S9) altogether confirm the robustness of our main results.

Figure S5 - Comparison Between Individual-Based Simulation and Quantitative-Genetic Predictions: for the same parameters as in Figure 2a, the evolution of the mating traits (solid lines) and correlated characters (dashed lines) was modelled using a stochastic individual-based simulation. The trajectory of the mean trait values in a simulated population of N = 10000 individuals (a) matches in detail with the corresponding prediction from the quantitative genetic model (Equation S19), (b) except for minor differences in the rate of convergence to the equilibrium and the equilibrium trait values. These qualitative differences relate to discrepancies between the observed (coloured lines) and predicted (black lines) genetic variances (c) and intersexual genetic correlations (d), which are a consequence of the genetic architecture of the traits in the individual-based simulation (see Discussion in **Chapter 4**). Each phenotypic character was determined by L = 600 haploid, bi-allelic loci. Some of these were expressed in both sexes and therefore affected a mating character and a correlated character in the other sex: the phenotypic characters $x_{♀}$ and $x_{♂}$ were assumed to share a common genetic basis of K_x = 300 loci (so that 300 loci exhibited sex-specific expression; r_x = 300 / 600 = 0.5), $y_{♂}$ and $y_{♀}$ shared K_y = 120 loci (implying that each was also affected by 480 sex-specific loci; r_y = 120 / 600 = 0.2) and $z_{♀}$ and $z_{♂}$ shared again K_z = 300 loci (r_z = 300 / 600 = 0.5). Mutations occurred at a rate of 0.001 per genome per generation (corresponding to a rate of $\mu = 3.47 \times 10^{-7}$ per gene copy). The phenotypic effect size of mutations was set to δ = 1/15, allowing all trait values to range from -20 to 20. Panel (e) shows the values of the average additive genetic correlations between traits (orange: choosiness x preference; purple: choosiness x ornament; blue-green: preference x ornament). Between-trait correlations are ignored in the quantitative genetic model, but may evolve in the individual-based simulation due to non-random mating and genetic drift, potentially affecting the evolutionary trajectory. Lines in (c-e) represent smoothed data (low-pass filter; data-reduction factor 4); raw data are indicated by dots.

Figure S5 Continued

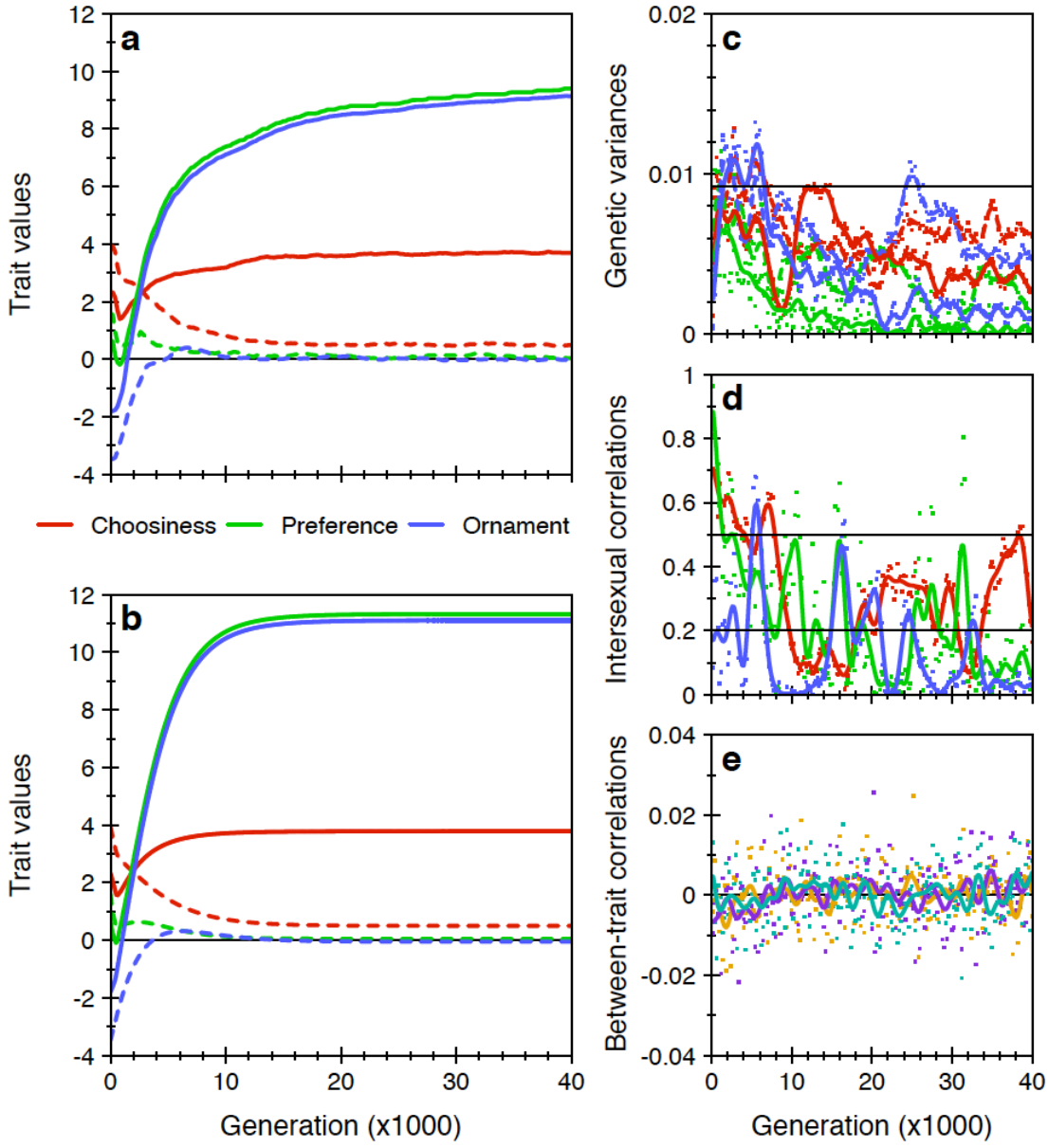


Figure S6 - Occurrence of Oscillations in an Individual-Based Simulation: the correspondence between individual-based simulation results (a) and the quantitative-genetic model (b) extends to the parameter regime where oscillations occur (parameters are as in Figure 2b). In this simulation, the evolving trait values remain far from the edges of the feasible phenotype range (from -20 to +20) and selection is weak, such that the between-trait correlations (e) remain negligible and the observed genetic variances (c) and intersexual correlations (d) match well with their expected values (black lines) based on Equation S19. Population size and genetic parameters were as in Figure S5 (see the legend of that figure for further details), except that K_x and K_z were increased to 570 (r_x = r_z = 570 / 600 = 0.95). This also had an effect on the per-locus mutation rate, which increased to μ = 4.27 x 10^{-7}, still corresponding to a genomic mutation rate of 0.001 per generation.

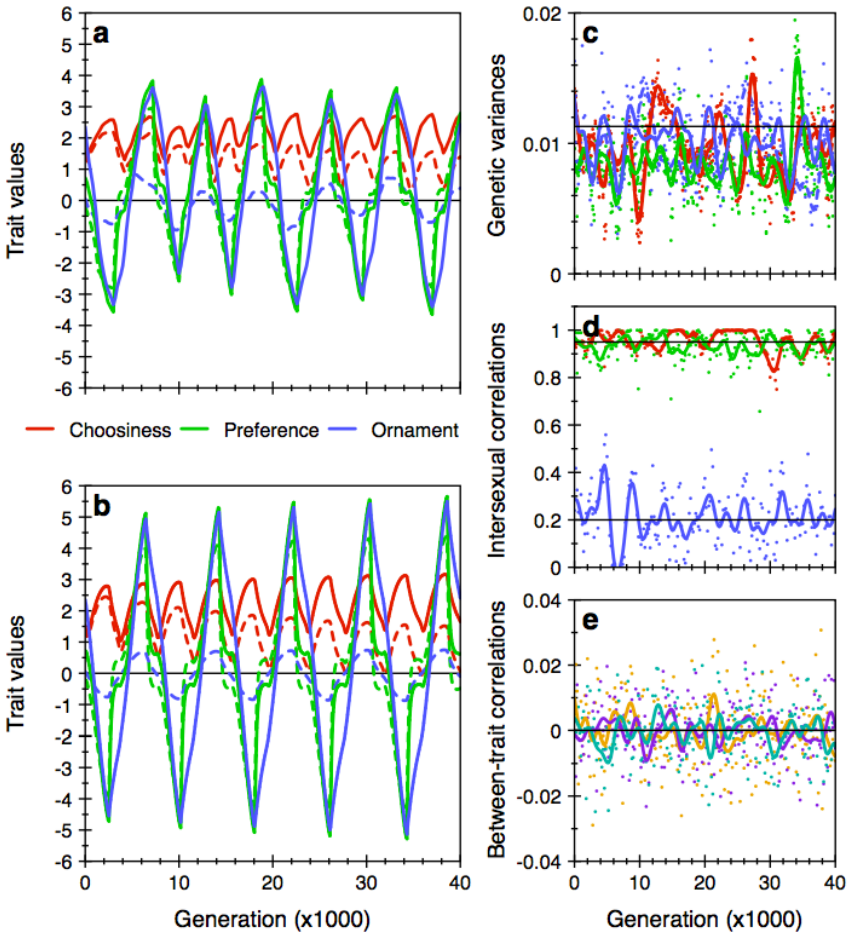


Figure S7 - Individual-Based Simulations of Diploid Populations Across a Range of Population Sizes: also for diploid populations, individual-based simulations show evolutionary oscillations, in agreement with the predictions of the quantitative-genetic model (parameters are as in Figure 2b). Across a range of population sizes (row (a): N = 1000; (b): N = 2000; (c): N = 5000; (d): N = 10000; (e): N = 20000; (f): N = 50000; (g): N = 100000) the three columns show, respectively, the dynamic of average trait values for the mating characters, their additive genetic variances and the intersexual genetic correlations r_x, r_z and r_y. Throughout, individual-based simulation results are shown in colour, whereas the corresponding quantitative genetic predictions are shown in black. Quantitatively, the agreement between simulation results and quantitative genetic predictions is better at the lower population sizes, despite the dynamics of the genetic variances (middle column) and the larger effect of genetic drift, which are ignored by the breeder's equation (Equation S18). As population size increases, the amplitude of the oscillations in the individual-based simulation decreases, presumably due to the presence of higher levels of standing genetic variation in large populations. A systematic change in the dynamics occurs at population size 20000 and above (panel e-g): here the genetic variances and intersexual correlations start to exhibit regular oscillations, and the dynamic of the average trait values slows down. Genetic parameters are: L = 100 diploid loci, $K_x = K_z$ = 95, K_y = 20, δ = 0.06, μ = 1.28 x 10^{-6} corresponding to a genomic mutation rate of 0.001. As before, raw data (dots) are presented along with smoothed data (lines) in the middle and right column of panels.

Figure S7 Continued

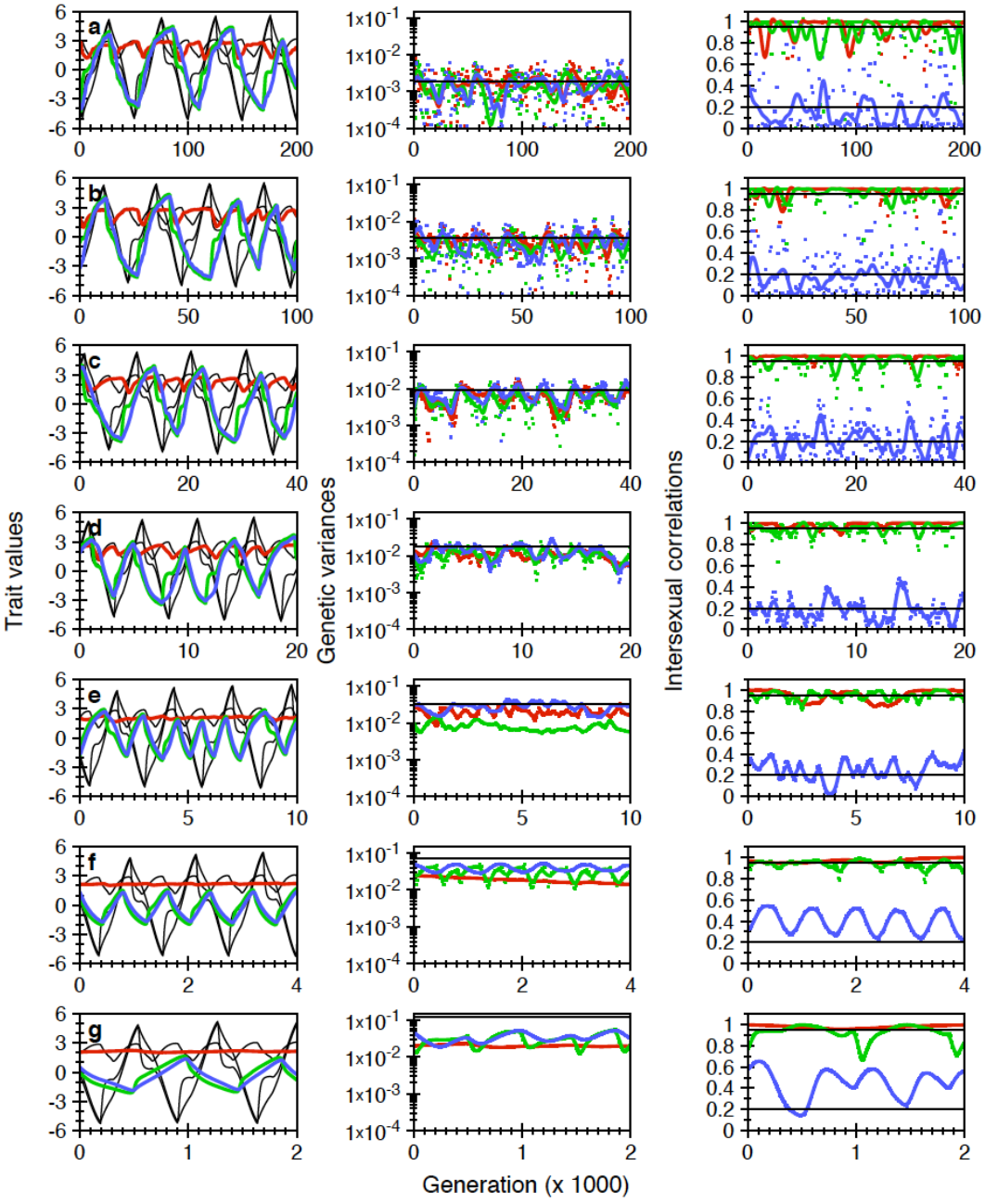


Figure S8 - Period and Amplitude of the Oscillations in the Individual-Based Simulation Model: the simulations shown in figure S7 were extended to include 10 full evolutionary cycles, from which we estimated the average period (a; orange filled squares) and amplitude (b) of the oscillations as a function of the population size. Lines show corresponding predictions from the quantitative genetic model (Equation S18). Also shown in (a) are the average additive genetic variances observed in the simulations, for the mating characters (red, blue and green filled circles for choosiness, preference and ornament, respectively) and their correlated characters (corresponding open circles). The same symbols are used in (b) to indicate the amplitude of the oscillations for each trait (line styles for the predicted values follow the convention used in earlier figures). Throughout, error bars indicate the standard deviation of the estimates obtained from the individual-based simulations. Data points have been slightly displaced in the horizontal direction to improve clarity.

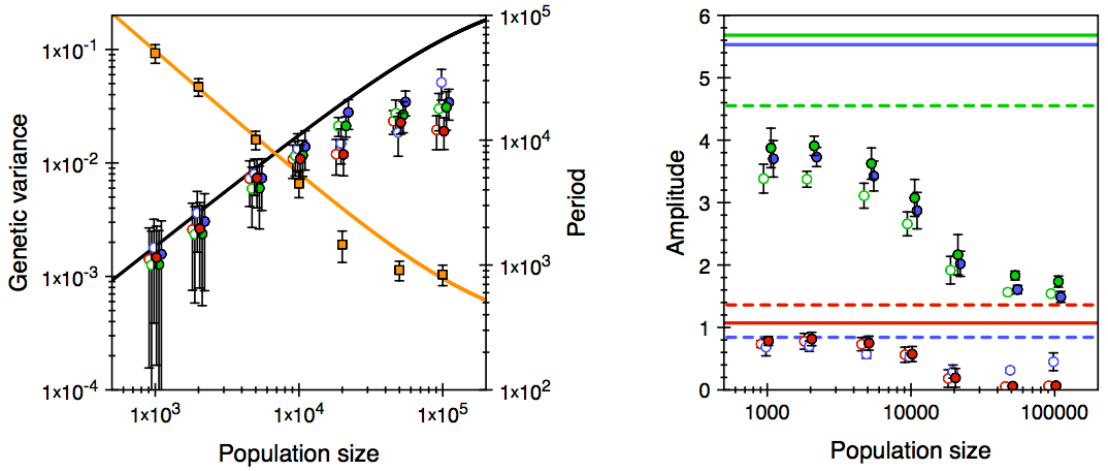
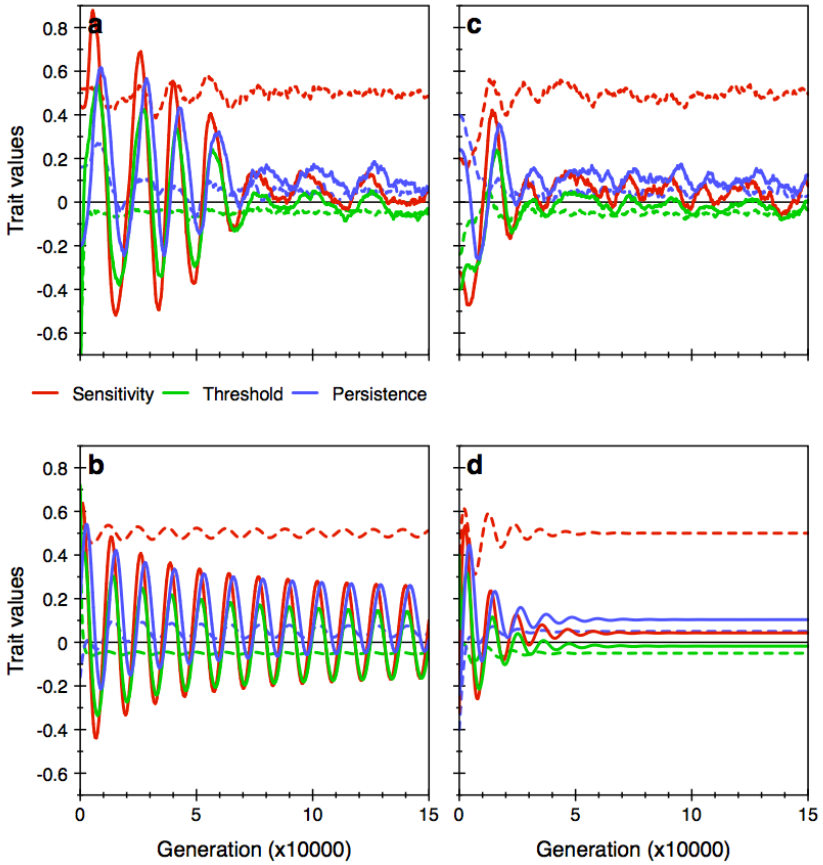


Figure S9 - Individual-Based Simulations of the Contest Mating Scenario: evolutionary oscillations of male offence and female defence traits are stabilised by between-sex pleiotropic gene expression in individual-based simulations (upper panels), in agreement with the corresponding runs of the quantitative-genetic model (lower panels). (a) At low values of the intersexual genetic correlations (r_x = r_z = 0.1; r_y = 0.2), the mating traits show regular oscillations, but with a smaller amplitude and longer period than in the quantitative-genetic model (b). These quantitative differences are the result of a reduced genetic variance in the female mating threshold, which causes this trait to lag behind in the oscillations during the second half of the simulation. (c) The regular oscillations are lost at higher values of the intersexual genetic correlations (r_x = r_z = 0.5; r_y = 0.2), in line with the results of the quantitative genetic model (d). However, the individual-based simulation continues to show irregular, damped oscillations around the equilibrium point, as a result of genetic drift. Parameters: a = 5, b = 0.5, $\theta_{x♀}$ = $\theta_{x♂}$ = -0.05, $\theta_{y♀}$ = $\theta_{y♂}$ = 0.05, $\theta_{z♀}$ = $\theta_{z♂}$ = 0.5, θ_{ψ} = 0.2, $c_{x♀}$ = $c_{x♂}$ = 0.5, $c_{y♀}$ = $c_{y♂}$ = $c_{z♀}$ = $c_{z♂}$ = 0.1, N = 25000, L = 500 haploid loci, K_x = K_z = 50 in (a) and K_x = K_z = 250 in (b), K_y = 100, δ = 0.04, μ = 3.57 x 10^{-7} in (a) and μ = 4.17 x 10^{-7} in (b), both corresponding to a genomic mutation rate of 0.001.



Appendix 1 References

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