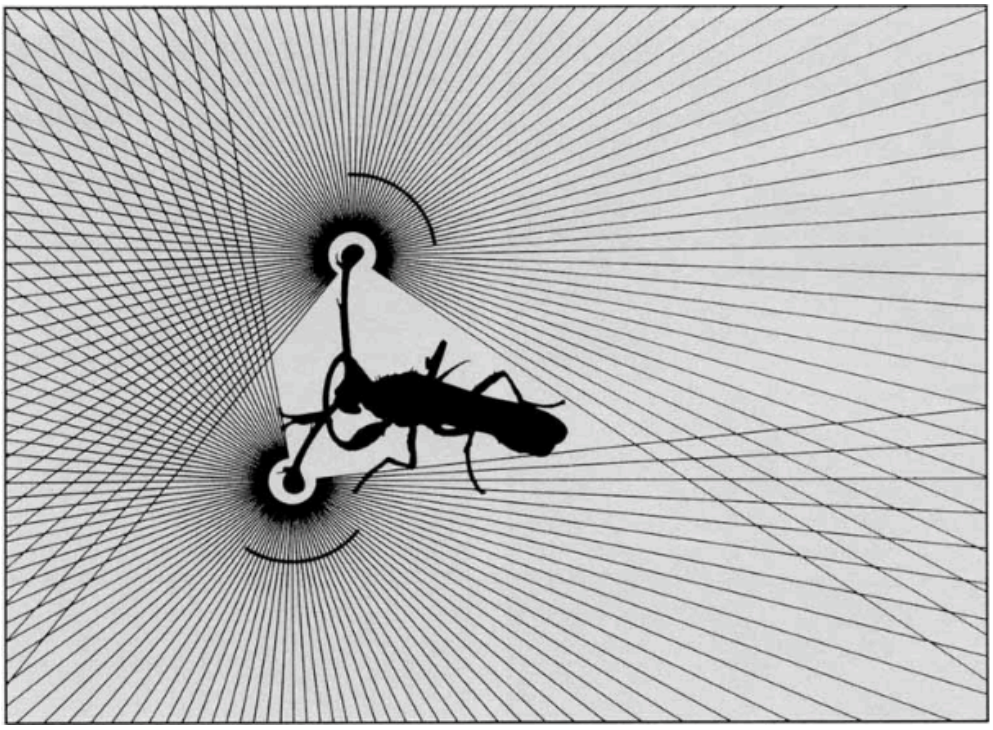

de la Motte & Burkhardt 1983

Female Mate Choice and Male Ornamentation in the Stalk-Eyed Fly, *Diasemopsis meigenii*

James Malcolm Howie

Submitted for Ph.D.

University College London

I, James Malcolm Howie, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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A B S T R A C T

Female mate choice is a crucial driver of sexual selection, leading to the evolution of the diverse male sexual ornaments seen in nature. Yet little is known about the factors that cause variation in different components of female preference, or how these components interact to influence the series of choices that exert selection on male ornaments. Likewise, it is not known how the signals of genetic condition revealed by male ornaments, or reproductive capacity, vary across environments. But this variation is important for both the direct and indirect genetic benefits of female mate choice, as well as for the male-driven effects of reproductive investment on ejaculate allocation and sexual selection. Here, I use the sexually dimorphic African stalk-eyed fly species *Diasemopsis meigenii* to conduct empirical studies to address these issues. First, I manipulate female mating status (virgin versus mated) and use mathematical and statistical techniques to decompose mate choices made in a repeated sequential no-choice design into estimates of preference, and selection on the male ornament. I show that choosiness and selection, but not the preference function, are elevated in mated females. Second, I use larval diet manipulation and a series of crosses within and between a suite of inbred lines to investigate the across-environmental genetic condition dependence of male sexual ornaments relative to nonsexual traits. I find evidence for the heightened condition dependence of the male sexual trait (male eyespan), and for a novel gene-by-environment interaction in which the effects of genetic stress on sexual trait expression are masked in both high and low but not intermediate environments. Finally, I measure the effects of environmental (E) and genetic (G) stress on reproductive, fertility and attractiveness traits, and find evidence for a qualitative alignment of trait responses (all tend to be positive), but a negative integration across traits (traits that respond most to E respond least to G). The results have important implications for the operation of sexual selection in nature.

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CHAPTER 1

GENERAL INTRODUCTION

1.1 OVERVIEW

In this introduction, I firstly outline the classical models of sexual selection and discuss the mechanisms by which female preference for elaborate male secondary sexual ornamental traits can evolve. I then describe the lek paradox, introduce the concept of condition and discuss how condition dependence and gene-environment (G x E) interactions can interact to increase additive genetic variation (V_A) in sexual traits, resolve the lek paradox, or undermine ornament reliability and the indirect benefits of choice. I explain that condition can also influence reproductive investment, and that it can impact on male fertility, ejaculate allocation and the direct female benefits associated with choice. I also discuss the consequences of the above for female preference, the role of female preference variation in the maintenance of V_A in sexual traits, and its wider role as a driver of sexual selection. I then review the literature on stalk-eyed flies and discuss their advantages as a valuable model for studies that seek to test the predictions of sexual selection theories related to mate choice variation, condition-dependent trait expression, reproductive investment, ejaculate allocation and male attractiveness. Finally, I provide a brief outline of the aims and content of each subsequent chapter, and set the scene of the rest of this thesis.

1.2 SEXUAL SELECTION: AN HISTORIC OVERVIEW

A limitation to classical natural selection is that it is unable to explain the evolution of a special class of elaborate traits – the colours, sounds, behaviours and characters – which, present in one sex, flamboyant and detrimental to survival, can be observed to adorn the natural world. It was this limitation that led Darwin, first in 1859, and in detail in 1871, to conceive of the battle between individuals for mates and for fertilisations as analogous to the direct contest for resources and survival that is typified by natural selection. He realised that this was a process capable both of driving and explaining the evolution of a special class of 'secondary sexual' traits. And it was a process to which he gave the name:

– *sexual selection*.

As first conceived, sexual selection was considered to operate by two main mechanisms. The first of these was the direct battle between members of the same sex – most often males (Darwin 1859, 1871; Bateman 1948) – to win mates. This was thought to explain the evolution of traits such as antlers, tusks and spurs, and to contribute to the evolution of sexual dimorphism. But the idea that Darwin's "weapons of offence", "courage" and "pugnacity" could be used in fights to win females was an old one. It had been proposed by his grandfather, Erasmus

Darwin, in 'Zoonomia' (1794) and by a host of other naturalists of the time (Aiken 1982). The extension of this idea into sexual selection, therefore, received little criticism in Darwin's time, and was accepted by Alfred Russell Wallace (Wallace 1889). In part, this was because, as a competition of contests, endurance and scrambles (Andersson 1994), such 'intra-sexual selection' (Huxley 1938a,b) could be seen as a simple extension of natural selection: in the most literal case, the death of an individual coming not from the predator's claw, but from the rival's tusk (Wallace 1889); in a less direct case, physical strength wining possession not of food, but of mates and of sex. A now classic illustration of this comes from the study of the elephant seal, where a male's size, strength and tusks contribute to the death and defeat of his rivals, as well as to his success in holding possession of a vast harem of females (Batholomew 1952, 1970; Ghiselin 1974; Stirling 1975).

A second, less intuitive, mechanism of sexual selection that was proposed by Darwin was that of the indirect contest between members of one sex – usually males – to be chosen by members of the other sex – usually females – as mates (Darwin 1859, 1871). This 'inter-sexual selection' (Huxley 1938a,b) was proposed, not as an explanation for the evolution of horns and tusks and weapons of power, but for that of the bright coloured plumes and tufts, exotic feathers and crests, dances and melodies – for that of the male ornaments that, "serve[d] … *only* to … excite or allure the female[s]" (Darwin 1871 II, pg 332). However, in contrast to the male driven intra-sexual selection, this female choice driven inter-sexual selection (Huxley 1938a,b) was controversial, and received serious criticism at the time. For instance, while Wallace accepted that sex-limited male weapons could evolve due to inter-male combat, he vehemently opposed the idea

that female choice could directly drive the evolution of male ornaments, citing instead selection based on mimicry, warning colouration or recognition (Wallace 1889). A part of the reason for this was that Darwin provided no evolutionary explanation for the origin of female mate choice, or the female's "standard of beauty" (Darwin 1859, pg 89). A consequence of this was that these criticisms persisted across the decades (reviewed in Pomiankowski 1988; Andersson 1994), and declined only after the inception of two crucial theoretical developments in inter-sexual selection: the 'Fisher Process', conceived by Ronald A. Fisher (1915, 1930, 1958), in which exaggerated ornaments were thought to evolve *despite* their costs, due to a female mate choice based on the production of 'sexy sons'; and the 'Handicap Principle', developed by Amotz Zahavi (1975, 1977a), in which such ornaments were, in contrast, thought to evolve explicitly *because* of their costs, and to thus act as indicators of a male's genetic quality, condition, or 'good genes'.

Another mechanism of sexual selection, with the potential to explain the evolution both of the tusks and weapons of power *and* the tufts and ornamental traits – also conceived by Fisher (1930), was an extension of intra-sexual selection based on his theoretical developments in inter-sexual selection. It consisted, not of a direct contest, but of an indirect, intra-sexual contest to win mates. Here, the weapons were not physical, but "psychological": "war paint[s]" used to dazzle, and "gaudy uniform[s] used for battle" (Fisher 1930, 1958; Hingston 1993, pg 114). As was the case for the male ornaments thought to elicit female choice, these "weapons" could be ritualised colours or displays. Likewise, such weapon-ornaments (Maynard Smith & Harper 2004) could be used as indicators (Zahavi 1977b; Baker & Parker 1979) of rank, or power (Huxley 1938c; Peek 1972; Smith

1972; Borgia 1979; Rohwer 1975, 1982) or condition (Parker 1982a; Andersson 1982). The role of such traits was, therefore, thought to be similar to that of the standard ornaments, but with their use centred in an *intra*-sexual rather than an inter-sexual context. However, in contrast to the female choice driven evolution of standard ornaments, the evolution of such emblematic traits was realised to be free from a range of difficult restraints that limited the former – for instance, such "weapon-ornaments" did not need to be heritable (Andersson 1994). Hence, while the traits were similar, indirect intra-sexual selection was less controversial.

Across the years, both theoretical (Parker 1983; Maynard Smith & Brown 1986) and empirical evidence (reviewed in: Andersson 1994) has piled up in favour of a role for *direct* intra-sexual competition as a driver of both sexual selection and the evolution of male weapons. Likewise, the role of *indirect* intra-sexual competition in the evolution of emblematic traits and indicators has remained uncontroversial. Yet neither is able to explain in full the evolution of female mate choice, or the evolution of extravagant ornaments that it drives. Despite a flourish of work in recent decades, this indirect contest is still not understood. Hence, the focus of the rest of this introduction, and of this thesis, shall be

– inter-sexual selection.

1.3 THEORIES OF INTER-SEXUAL SELECTION

1.3.1 *An overview of intersexual selection*

As noted above, all theories of inter-sexual selection must seek to understand the evolution of female mate preferences and the male ornaments for which these preferences exist. In addition, each must do so in a manner that accounts for the

costs both of mate choice, in terms of search time or effort, and of ornamentation, in terms of resource use or survival. A number of explanations and models have thus been proposed that fall into a range of categories based on the type of benefits that a female can obtain via her preference for a more ornamented male. These include models based on 'no-benefits', 'direct benefits', 'indirect genetic benefits' or even 'inverse benefits'. A brief discussion of each is provided below:

1.3.2 *No Benefits – or 'Sensory Bias'*

A simple explanation for the evolution of indirect inter-sexual selection – thought to be the likely origin of female preference – is that it arises, in the absence of benefits, as a "pleiotropic by-product of natural selection on the sensory system" (Kirkpatrick 1987; Kirkpatrick & Ryan 1991): that is, via 'sensory bias'. Here, the male ornament exploits the neural network or sensory system of the female. For instance, a male insect produces a pheromone with similarities to that of a fruit in order to elicit the response of a female (Baker & Cardé 1979; Löfstedt *et al.* 1989). To this extent, the male ornament can be viewed as a tool for the manipulation of female behaviour – as a tool of "sensory exploitation" (Ryan 1990), or as a "sensory trap" (West-Eberhard 1984). However, such sense-biased male traits can also reduce female search costs. Louder or brighter males will be easier to find in species that use sound or light for hearing or sight. Hence, in concert with the increase in reproductive output for louder or brighter males, this reduction in mate search costs can be seen – theoretically – to overcome the costs of both mate choice and of ornamentation, and to lead to a process of 'sensory drive', in which the male ornament is exaggerated even where there is little genetic variation or change in female preference. Models based on neural networks imply

that signal recognition mechanisms will lead to inevitable biases of this type (reviewed in Enquist & Ghirlanda 2005; Phelps 2007; and see also Fuller 2009).

1.3.3 *Direct Benefits*

An even simpler explanation for the evolution of female mate preference is that based on 'direct benefits'. In this case, a male's ornament is thought to reveal important information about his individual attributes – for instance, about his parental ability, the size of the nuptial gift that he will be able to provide to the female, or the quality of the territory that he will be able to defend (see Williams 1975; Kirkpatrick & Ryan 1991; Andersson 1994, for a list of further direct benefits). A female that exhibits mate preference for such ornamented males will, as a consequence, obtain direct benefits that can offset the resource or reproductive costs associated with mate choice (for instance, mate choice requires both time and energy and a cost of remaining un-mated, Kokko & Mappes 2005). As in the 'no benefits' scenario, the more ornamented males will thus be chosen more often, will have increased reproductive output, and will – theoretically – be able to compensate for the costs associated with ornamentation. Hence, on the assumption that a male ornament provides honest information about a male's quality, or parental ability, or fertility, both female mate preference and the male ornamental trait will be expected to coevolve, leading to the exaggeration of the male ornament as well as the female preference until equilibrium is reached (at which point the benefits of ornaments and preference will be offset by the costs).

A consequence of this is that such male ornaments are often expected to be particularly costly, so as to be unfakeable. An advantage of this is that such traits

– or handicaps (Zahavi 1975) – will reveal honest information about the quality of the male, which is in turn likely to be associated with parental ability, or territory, or the fertility of the male. Indeed, a number of studies have shown that non-heritable benefits associated with condition (such as parental care) can provide a basis for the evolution of female preference and male ornamentation (see Grafen 1990; Price *et al.* 1993; Iwasa & Pomiankowski 1999). A caveat, here, is that while these (and other: Heywood 1989; Hoelzer 1989) 'direct benefits' models do not require heritable indirect genetic benefits in order to work, 'condition' is, nonetheless, expected to pool environmental and genetic variation – so, in nature, environmental and genetic effects will often be confounded, or will arise in unison or interact: and models should be specified to consider both (Kuijper *et al.* 2012). Likewise, it is notable that condition need not relate to high parental care if a male mates with multiple females, as in such cases a male could instead split care between females or focus on a 'favourite' female (Cotar *et al.* 2008; Tazzyman *et al.* 2012). Each of these points is discussed in sections 1.3.5 and 1.4.

1.3.4 *Indirect Genetic Benefits – The Fisher Process*

An alternate explanation for the (co)evolution of male ornaments and female mate preference is that based on indirect genetic benefits. In this case, the male ornament is expected to convey information about heritable benefits to the female's offspring, which can then increase the female's overall fitness. The need for such ideas was first realised in relation to cases where a male provides no obvious direct benefit to a female, and leaves her with only his sperm or ejaculate. A number of such benefits are possible, and will be discussed in detail below. But, I will start at the beginning, with Ronald A. Fisher, and his "Fisher Process".

As touched on in the 'Historic Overview', the first coherent explanation for the evolution of female mate preference and for the operation of inter-sexual selection in the absence of direct benefits was provided by R. A. Fisher (1915, 1930, 1958) as the 'Fisher Process' and it's associated 'Fisherian Runaway'. Here, Fisher conceived of a two-step process. First, a heritable male trait – for instance, tail length – would exist in a population, with heritable genetic variation associated with both the size of the trait and with male survival. Second, female mate preferences for this trait would also exist – for instance, due to the increased fitness of the female due to the increased survival of her sons (or due to sensory bias, a direct benefit, or any other process). Heritable variation in this female preference would also be associated with the strength of the preference. Then – these priors in place – the heritable basis for both the female mate preference and the male ornament would start to become coupled. The sons and daughters of choosy females would also be those of ornamented males. After a number of generations this coupling would lead to an ever greater genetic correlation between the male trait and the female preference, in a process now referred to as, "The Fisher Process". The alleles for each trait would also start to increase in frequency in the population; due to the direct benefits to the choosy female, the indirect benefits related to her male offspring's survival, and the increased reproductive success of the chosen males. Then, at a certain point, the benefit to a female due to her offspring's survival would be reinforced by the dual facts that her male offspring were sexier (more ornamented), and her female offspring choosier. At this point, a self re-inforcing feedback loop, "Fisherian Runaway", would cause both the female mate preference and the male ornament to "run

away", and would lead to dramatic trait exaggeration – with trait size increased at an ever faster rate until, after a rapid sweep, checked at last by natural selection; for instance, due to the higher mortality costs associated with male ornamentation.

Yet – while Fisher found this idea self-evident, indeed, "easy to see" (Fisher 1930, pg 137) – it was a verbal model with far reaching consequences and counterintuitive predictions. As such, it was treated with scepticism, until the 1980s, when the first formal mathematical models of this process were created.

Despite earlier work in this direction (O' Donald 1962, 1967), the full first models of the Fisher process were those created by Lande (1981) and Kirkpatrick (1982). Although the two models were set in different frameworks – the quantitative genetic and population genetic frameworks, respectively – the models were quite similar. Each was a two-locus (or two trait) model, with terms for the female preference trait, and the male ornament trait (either its phenotype, in Lande 1981, or its presence or absence, in Kirkpatrick 1982). Each also contained a term, or a set of terms, to describe the statistical association or correlation between the traits (based on the additive genetic variance within and covariance between the traits, in Lande 1981, and based on the level of linkage disequilibrium between the 'trait-present' alleles, for the two traits, in Kirkpatrick 1982). Each model showed that, based on the direct selection on each trait in tandem with the indirect correlated selection due to the statistical association or linkage, the system would (Lande 1980) or could (Kirkpatrick 1982) evolve to a semi-stable equilibrium.

A point of particular interest, was that in each case, the equilibrium was not a point, but a line; that is, for each value of the preference trait there was a stable level of male ornament exaggeration, at which point the mating advantage of ornamentation would be balanced by the reduced survival associated with the over-sized trait. The level of each trait was also able to slide over time, and move up or down the line of equilibrium – returning at a different point on the line each time the balance was upset, for instance due to a stochastic fluctuation, dependent on the direction and force of the initial disruption. It was also of interest that in Kirkpatrick's (1982) population genetic model, the frequency of the preference allele would increase in the population only when the linkage disequilibrium parameter was not equal to zero; while, in Lande's (1981) quantitative genetic model, it was also shown that, if the statistical association between preference and ornamental trait was strong enough, then the line of equilibrium could become unstable, such that a disruption of sufficient size (where females became sufficiently critical or where the costs of ornamentation were reduced) could lead to an evolutionary trajectory in excess of the line of equilibria, which would then lead to trait evolution away from this line at ever faster speeds – and that this "run away" could, indeed, lead on to the ever increased trait values predicted by Fisher.

An issue with these initial models of sexual selection, via the Fisher process, was that female choice was considered to be 'free', in that it had no cost. In real life, female choice is expected to have a cost – either in time, or effort, or due to a loss of reproductive output whilst "on the lookout" (Kokko & Mappes 2005). But when costs were added to these models, the lines of equilibria were lost. The populations evolved to single points, where female fitness was maximized – at

zero preference, and zero ornamentation (Pomiankowski *et al.* 1991 and see also Kirkpatrick 1982; Pomiankowski 1987; Bulmer 1989). However, the Fisher process was also shown to be rescuable via the addition of additional mechanisms, such as mutation bias (mutations that have mainly negative effects on male ornaments, Pomiankowski *et al.* 1991) or migration bias (an influx of migrant males with smaller ornaments, Day 2000), into the models; or via use of very small costs to *both* female preference and male ornamentation (Hall *et al.* 2000). In this latter case, the male trait values did not converge at an equilibrium, but oscillated forever on a 'limit cycle' (Kuijper *et al.* under revision), similar to the cyclic evolution described in an earlier model of the Fisher process (where selection on the male trait was made weak around the natural selection optimum) that ignored the costs of choice (Iwasa & Pomiankowski 1995). Here, a stable equilibrium was achievable, but only when there was extremely high mutation bias.

In summary then, the Fisher process has now been well documented theoretically, and the idea that females can increase their fitness via preference for male ornamentation, even in the face of costly choice, due to the increases in the reproductive success of their sons, has been confirmed as a force that will almost certainly operate in nature (although direct empirical demonstrations remain rare).

1.3.5 *Indirect Genetic Benefits – Good Genes*

Another indirect process with the potential to explain the evolution of female choice and male ornamentation – also realised by Fisher (1930), though less often attributed to him – is that based on heritable genetic viability; the so called, 'good genes models'. Here, rather than heritable sexiness, the benefit to female mate

choice (or preference) is heritable genetic quality (often interpreted as increased offspring survival, but more correctly considered as increased offspring reproductive value, Kokko 2001; Kokko *et al.* 2002). In fact, the models of such 'good genes' processes are very similar to those used to model the Fisher process. Where the basic Fisher process model has two loci – one for the male trait, one for the female preference (and a term for the correlation between them) – the standard base of the 'good genes' model includes one additional locus: a viability locus (or set of loci). As a consequence of the similarities between the models, because all models based on 'good genes' contain some aspects of the Fisher process, and based on this new focus on reproductive values, a number of researchers have even come to consider the differences between these two forms of model and perspective as both small, and superficial (Kokko 2001, *et al*. 2002).

However, there are some important differences to consider. First, all good genes models contain at least this one additional viability parameter, and thus exhibit different system dynamics (Kuijper *et al.* 2012). Second, the Fisher process requires a genetic correlation between the female preference and the male trait (recall that the frequency of the preference allele would not increase unless the linkage disequilibrium between the preference and trait alleles was not equal to zero in Kirkpatrick 1982). Yet, the good-genes models can both work and lead to runaway dynamics in the absence of genetic correlations between ornamental and preference traits, for instance through genetic correlations between alleles linked to the preference traits and to heritable quality. Finally, in the Fisher process, the ornament and preference genes are directly coupled, such that the question of ornament reliability is mute – the preference is directed at the ornament, so larger

ornament males will be preferred, and preferring females will have more ornamented (and thus sexier) sons. As will be seen below, such concerns are, in contrast, of crucial importance for good genes models. Hence, while the similarities are striking, the differences between these mechanisms should not be ignored.

To build on this point, an important debate within the field of good-genes based models is that based on ornament reliability. How can a female determine whether a male ornament is an honest or dishonest source of information about his heritable genetic quality? As has been touched on in both the 'Historic Overview' and the '*Direct Benefits*' sections, a potential resolution to this issue was provided by (Zahavi 1975, 1977a) in the form of his 'Handicap Principle'. Here, rather than evolve in spite of the costs, as is the case in the Fisher process, Zahavi proposed that ornaments could evolve specifically because of the costs. The idea was that a costly trait would be an honest trait. Just as this idea could work for direct benefits, it could potentially also work for indirect genetic benefits; if with several caveats. The first of these caveats is that not all costly signals are reliable indicators of quality (Getty 2006: a tortoise shell is costly to produce, but does not in itself reflect the condition of the tortoise). Another, is that honest traits do not always require extra costs (Számadó 2011; Higham 2013: the size of an animal, or its height, has no 'additional' cost, but is honest). Nonetheless, in principle, this Handicap Principle works as follows: an ornamental trait has a cost associated with it, so males with better viability (that is, all components of fitness other than mating success, Maynard Smith 1987) are better able to pay, survive longer, and so have a higher reproductive value than males with lower viability. Given this, a

female can choose a more ornamented male and obtain heritable fitness benefits for her offspring; and she can rest assured that the information provided was honest.

In fact, a range of different types of handicaps have now been proposed and tested in mathematical models. These include, Zahavi's handicap, revealing handicaps, condition-dependent handicaps and epistatic signals (reviewed in Pomiankowski 1988; Andersson 1994; also see Maynard Smith 1985; Maynard Smith & Harper 2004; for formal definitions see Van Doorn & Weissing 2006). In brief, the 'Zahavi handicap' is based on the idea that all males express an ornamental trait, while those in lower condition suffer the costs (this is similar to the epistatic indicator, in that trait expression does not depend on viability). The revealing handicap, or 'index' (Maynard Smith 1985) is not in fact a handicap at all (Maynard Smith & Harper 1995), but a trait that reveals the quality of the male directly; for instance, the size of an animals head could reveal information about the size and thus quality of the animal (a stag's bellow is another example). Here, a set level of investment results in a better trait, in the better quality individuals (all individuals develop the trait, but lower quality individuals develop a smaller or a less well maintained form of the trait). A similar idea lies behind the condition-dependent handicap. However, the condition-dependent handicap is a real "handicap". In this case, the expression of an ornamental trait relates to the condition of the individual, so that a lower condition individual will pay a higher relative cost to express a trait. Traits are thus expressed in proportion to the condition of their bearers (Zahavi 1977a; Kodric-Brown & Brown 1984; Andersson 1986; Zeh & Zeh 1988; Pomiankowski & Møller 1995; Rowe & Houle 1996).

In broad terms, the balance of theoretical findings is that female preference for ornaments evolves when the handicap in place is based on a revealing handicap or a condition dependent handicap, while the role of Zahavi handicaps and epistatic indicators has been found to be less important. Nonetheless, the evolution to an equilibrium cycle for pure epistatic indictors has been shown to be possible (Van Doorn & Weissing 2006; and also see Kirkpatrick & Barton 1997).

1.3.6 *Additional Benefits – Conflict, Cost Avoidance, and Multiple Benefits*

As a final note in this section, it is also important to briefly mention a few new developments in the field. First, there is a new wave of models based on 'compatible genes'. Whereas the standard 'good genes' model assumes that there is an overall directional genetic quality intrinsic to the genetic makeup of the organism, compatibility models 'realise' that life is not so simple (Hunt *et al.* 2004b; Puurtinen *et al.* 2009). Male ornaments could instead reflect information about their local adaptation (Proulx 2001; Reinhold 2004), or relate information about genes that will be better or worse – and more or less desirable – dependent on the frequencies of other genes in the population (Van Doorn *et al.* 2009). Likewise, females could prefer not an ideal, 'best' genetic quality male, but a male that carries a set of alleles that are compatible with the set that she carries. The general expectation is that effects of this type will weaken selection on female mate preference. But, recent models show that such sexual selection can arise under certain conditions: i.e. in the presence of biased mutations (Lehmann *et al.* 2007).

In addition to these compatible-genes models, another set of recent models includes those based on 'inverse benefits', or 'cost avoidance'. A large number of

studies have shown that females experience (sometimes severe) mating costs – such as those related to seminal fluids (that evolve under male sperm competition), or the physical damage that can be inflicted via male genital spines (Rice 1996; Arnqvist & Rowe 2005). Hence, in addition to the direct benefits of mate choice discussed above, there can be further benefits to the prudent – or resistant (Kokko *et al.* 2006) – female, in the form of a reduction in mating associated costs. Here, a female that mates with too large a number of males will suffer a reduction in reproductive value, for instance due to early death; a female that mates with too few males may also fail due to a lack of sperm, or a low genetic diversity of sperm (unless she has chosen a high quality male). The (co)evolution of female resistance and male harm is the common outcome in such a case. However, the conclusions change if females become insensitive rather than resistant (Rowe *et al.* 2005). Here, evolution can come to a standstill. But it is notable that females have to 'consider' another factor: persistent males could provide indirect benefits, so females might prefer to mate with persistent males so as to obtain persistent sons.

As has been hinted at throughout this section, these different classes of model and of benefits are not as separate as could be imagined. The Fisher process is a crucial part of most 'good-genes models'. The condition-dependent basis utilised in multiple 'good-genes' models will also capture environmental variation; and could thus lead to covariation between indirect and direct benefits; or even to trade-offs between such classes of benefit. An example of this would be where unattractive males provide increased parental support as a result of their low expectation (or reality) of mating with multiple females. In contrast, the attractive low parental input males could well provide better indirect benefits – a potential

cause of female extra pair copulations; in birds, for instance (Tazzyman *et al.* 2012). And, in addition to all this, there is the potential for multivariate ornamentation and preferences, each of which could lead to correlated selection across traits in relation to the genetic correlations (positive, neutral, negative) between these traits. So, while a lot has been learned, there is still much to resolve.

1.4 MODELS OF CONDITION-DEPENDENT SEXUAL SELECTION

An important subset of models for this thesis are those based on condition-dependent handicaps (one of the three types of handicap noted in the previous section). Under this form of handicap, the level of trait exaggeration is expected to be proportional to the overall condition of the male. Hence, males in better condition will have ever higher ornamentation and viability (Zahavi 1977a; Kodric-Brown & Brown 1984; Zeh & Zeh 1988; Rowe & Houle 1996; Cotton *et al.* 2004a; Getty 2006). As has been outlined above, the condition dependent handicap hypothesis provides the basis for a wide range of models of sexual selection based on 'good genes'. But, as an extension of Zahavi's (1975) standard 'Handicap Hypothesis', this idea can also be applied in situations where both male quality and ornamental traits are non-heritable (see Grafen 1990 for details). In fact, both forms of model produce similar output as long as two crucial assumptions are met. The first of these is that higher values of condition confer higher values of fitness (Maynard Smith 1977; Cotton *et al.* 2004a). The second is that condition must have an inexhaustible source of variance, be that genetic, environmental, or both (Iwasa & Pomiankowski 1994; Rowe & Houle 1996; Iwasa & Pomiankowski 1999; Cotton *et al.* 2004a). As an illustration of this, I provide – below – a brief overview of three important models of sexual selection,

each of which operates via the use of condition-dependent traits and benefits.

First, I provide an outline of Iwasa and Pomiankowski's (1994) quantitative genetic model so as to describe the main requirements for the evolution of condition-dependent handicaps. Here, Iwasa and Pomiankowski examined the evolution of male ornaments and female mate choice, each associated with a cost, in a model with four traits, t, t', p and v. All traits were polygenic, and, for simplicity, only additive genetic effects were modelled. To turn first to the males, the size of the realised male ornaments, s, was based on the linear model

$$s = t + t'v \quad , \qquad [1]$$

where t represents the value of genes for the trait per se, v is viability, and t' is a term for condition dependence. The value t' reflects the relationship between ornament size and viability. When $t' = 0$, the ornaments will evolve as Fisherian traits. When $t' > 0$, ornament size, s, will increase as a function of viability, v. t and t' are sex-limited male traits, while v is expressed in both males and females.

Male fitness is determined by the interaction between natural and sexual selection. For sexual selection, it relates to female preference, p. As the average female preference, $\bar{p}$, increases, so the mating success of more ornamented males increases in exponential proportion. Here, the expression of the p term is sex limited to females. A mate preference for males with larger than average ornaments (s-$\bar{s}$>0) is expressed where $p > 0$, while females mate at random where $p = 0$. Hence, the more ornamented males are selected more often when $\bar{p} > 0$ (and have a higher reproductive fitness due to this sexual selection). In contrast, the natural selection part of male fitness comprises of two elements. The first is

the direct effect of male viability, where (survival) fitness increases as a function of v. The second arises due to the costs associated with male ornament size, as

$$cost = \frac{c}{1+kv} s^2 \quad , \qquad [2]$$

where c and k are constants, and the cost to survival increases as the male ornament size increases away from the natural selection optimum; that is, for simplicity, where $s_{opt} = 0$. Around either side of this point the chances of survival decrease in symmetrical fashion. A crucial assumption for the handicap principle is that the chances of survival with an ornament of a set size will vary with viability. Hence, alterations to the cost differential, k, that mediates the relation between viability and the effects of ornamentation on survival, allow for tests of this hypothesis. Where $k = 0$, viability has no effect on survival. Where $k > 0$, low viability males (those of low intrinsic quality) pay a higher cost for a set ornament than do males with a higher viability (that is, with a higher value for the term, v).

As for male fitness, female fitness also increases as a function of the natural selection viability term, v. However, like ornamentation, there is a cost to mate choice. At $p = 0$, females mate at random, with no cost. At $p > 1$, females mate with males that have larger than average ornaments, and the cost, for female fitness, of this increased discernment increases in exponential proportion with the increase in preference, p. Unlike male ornamentation, it is not assumed that the viability term has an affect on female mate choice, so the cost is less variable (although, in reality, such a viability- or condition-dependent cost could well exist).

Based on this model, Iwasa and Pomiankowski (1994; and also see Pomiankowski, Iwasa & Nee 1991 for a similar example) showed that two

conditions are essential for the evolution of mate choice, in the face of associated costs, under the handicap principle. The first, as touched upon in the previous section, is that viability must be subject to deterioration – for instance, that induced via biased deleterious mutations; to maintain variation in fitness. The second is that ornaments must be expressed in a condition-dependent fashion. That is, for costly mate choice to evolve, t' must be > 0; and further, as the condition dependence of the trait, t', was shown to be proportional to cost-differential of trait expression for a set viability, k, (i.e. where $t' \propto k$), condition dependence was found to evolve only when the cost of ornamental expression caused lower viability males to pay higher survival costs for a set larger ornament size (i.e. where k>0). Hence, as predicted by the handicap hypothesis, higher values of condition related to a higher fitness, ornaments became condition dependent only when associated with an asymmetric survival cost, and females were able to obtain honest information about the genetic quality of the male, and were thus able to obtain heritable fitness benefits for their offspring even in the face of the costs to their survival incurred via the execution of mate choice. At equilibrium (when $\Delta p = 0$) the costs of mate preference were then balanced by the benefits accrued through increased offspring viability. And the whole system persisted as such, so long as there was an infinite level of genetic variation in viability.

As an extension to this model, Iwasa and Pomiankowski (1999) were also able to show that – in addition to these indirect benefits to mate choice that arise due to heritable viability – such handicap models could also work when males provide direct benefits. Here, the 'good-parent' handicap, was based on a direct benefit to females, in which condition-dependent male ornaments revealed male viability, which was related to a direct benefit to female reproductive success. For female

preference to evolve, ornaments once again needed to be condition dependent. Here, at equilibrium, the strength of female preference was controlled by the product of the signal efficiency, phenotypic variance in male quality, and the effectiveness of male quality in increasing female reproductive success. However, the equilibrium that arose in this environmental 'good-parent handicap' was exactly the same as that seen in the previous 'good-gene handicap'. Both showed cyclic evolution, as seen in a pure Fisherian model; but with the addition that handicaps could lead also to stable equilibria. Hence, Iwasa and Pomiankowski were able to show that both environmental and genetic sources of variation can allow condition-dependent handicap models of sexual selection to work/operate.

All initial models of the handicap hypothesis (such as those above) were dependent on the Fisher process, even if the results went beyond those of purely Fisherian models. These models were also based on heritable traits – male ornaments, and female preference; and, in some cases, heritable benefits. To test whether such traits can evolve in the absence of the Fisher process, and in the absence of direct trait heritability, Grafen (1990) utilised another type of model based on the application of biological game theory (Maynard Smith 1982). Here, Grafen constructed a simple model based on a haploid population in which variation at a single locus controlled both sex-limited ornamental expression (in males) and preference rules (in females). As the Fisher process requires a correlation between the genes of independent traits it was thus precluded from this model (although it could be argued that the correlation in this model was in fact equal to 1). After the model was run, Grafen found that, in concordance with the results of Iwasa and Pomiankowski (1994, 1999), an equilibrium of female preference and male ornamentation could evolve, provided that male ornaments

were costly and condition dependent; so that the cost of 'advertisement' varied with the quality of the male, and was relatively increased for lower quality males.

A key to the understanding of this model is that while neither the male nor female traits were heritable, the rule that described the level of advertisement (the size of the ornament) in relation to the quality of the individual (i.e. the level of condition dependence) was. Male fitness related to his actual quality, his advertisement, and the female perception of his advertisement. Female fitness related to her ability to discern a male's real quality. A caveat to this model (and more recent related models: Gintis *et al.* 2001; Seymour & Sozou 2009), though, is that it was prone to cheats – males that have lower qualities, but exhibit larger ornaments. Hence the model required that the costs of ornamentation were relatively higher for low condition males, and relatively lower for high condition males. Where this was not the case, the ornaments would become dishonest, and the benefits of female preference would shrink relative to the costs of choice (as all males would become well ornamented, so the value of ornaments would be lost). However, while real life is expected to include incomplete honesty and non-perfect perception, Johnstone and Grafen (1992) were able to build on this first model to show that the handicap equilibrium can be maintained in the face of this variation, so long as the inferred quality of a male covaries with his true value.

So, across a range of models, based on either direct or indirect benefits to female choice, the use of heightened condition dependence as an honest handicap has been shown to allow for the evolution of female mating preference and male ornamentation, both with and without the Fisher process, as long as the two crucial assumptions are met: 1] high values of condition confer higher fitness, due

to the role of condition in the maintenance of reliable advertisement; and 2] that there is an infinite source of environmental and/or genetic variation in condition.

1.5 THE MAINTENANCE OF ADDITIVE GENETIC VARIANCE

As well as a requirement for honest handicaps based on the condition dependence of sexual trait expression, another essential factor in all indirect genetic models of the handicap principle (and all models based on 'good genes') is the presence of additive genetic variance (V_A) in both the trait and the fitness that it relates to (and more broadly, a source of inexhaustible variation). An issue with this requirement is that additive genetic variance is expected to be depleted in the face of the persistent directional selection arising from female mate choice and viability selection (Kirkpatrick & Ryan 1991; Rowe & Houle 1996; Hine *et al.* 2004). Hence, the benefits to female choice should be expected to decrease in each generation, as beneficial alleles become fixed, until the benefit of choice is outweighed by the cost of exerting choice; with selection on both choice and the male ornament consequently relaxed, and then lost altogether (Taylor & Williams 1982; Tomkins *et al.* 2004). It is this that has been termed, the "lek paradox" (Borgia 1979). However, in reality, sexual ornaments exhibit high levels of V_A (Pomiankowski & Møller 1995; Prokuda & Roff 2014), so this concern to solve the lek paradox is more one of seeking explanations for why suitable variation persists to favour continued mate preference, when such depletive forces are expected to exist.

The most widely discussed resolution to this 'paradox' concerns the condition-dependent expression of sexual ornamental traits based on genic capture

(Radwan 2008; Kotiaho *et al.* 2001; Kotiaho *et al.* 2008). Here, condition – considered as the "pool of resources" to be allocated by (Rowe & Houle 1996) or the residual reproductive value of (Williams 1996) an individual, and related to a wide range of morphological, physiological and life history traits (Houle 1992) – is expected to sum variation across numerous genetic loci and exhibit high persistent V_A due to genic capture (as loci across the genome contribute to condition, a mutation at almost any point in the genome can lead to variation in the expression of a condition-dependant trait: Houle 1992; Pomiankowski & Møller 1995; Rowe & Houle 1996; Tomkins *et al.* 2004). A trait that evolves, or is predisposed towards, condition dependence is therefore expected to provide honest non-depletable information about the quality of the individual (Zahavi 1975; Pomiankowski 1987; Iwasa & Pomiankowski 1994; Tomkins *et al.* 2004; Johnstone *et al.* 2009). Hence sexual ornaments are predicted to exhibit heightened condition-dependent responses to environmental and genetic variation relative to nonsexual traits (de Visser *et al.* 2003; Cotton *et al.* 2004a, b, c; Tomkins *et al.* 2004a; Bonduriansky & Rowe 2005); the latter expected to relate to fewer loci and to have evolved buffers to environmental variation (Cotton *et al.* 2004a, b, c; de Visser *et al.* 2003; Tomkins *et al.* 2004; Bonduriansky & Rowe 2005).

In line with expectations, a plethora of studies have shown heightened environmental condition dependence in sexual ornamental traits (e.g. Zuk *et al.* 1990; David *et al.* 1998; Kotiaho 2000; Holzer *et al.* 2003; Cotton *et al.* 2004a; Punzalan *et al.* 2008; McGuigan 2009; Rashed & Polak 2010). These insights are useful because environmental variation is an important source of variation in natural populations, and because it seems plausible that the mechanisms of

condition dependence are similar for environmental and genetic variation. But this can't be assumed; it needs to be demonstrated; especially as environmental responses and genetic responses are not always coordinated (Bonduriansky *et al.* 2015). In contrast to this environmental insight, though, little is known about the genetics of condition dependence (Cotton *et al.* 2004b; Bellamy *et al.* 2013). That is, little is known about the extent to which secondary sexual trait responses to variation in genetic condition, or stress, are heightened, or not. What is known is often flawed (Bellamy *et al.* 2014). The reason for this is that most studies have used covariation in the V_A of ornaments and condition as a test for genetic condition dependence (Bellamy *et al*. 2014). But, while the level of V_A in a trait is theoretically simple to measure (i.e. via the use of half-sib mating designs, Green 2001), reliable estimates of condition are difficult to obtain. A common approach, for instance, is to use the residuals of a body mass on body size regression as an estimate of fat reserves, or the "pool of resources" (Jakob *et al.* 1996). But this is a proxy for condition, not condition. Body-mass residuals do not always relate to fitness (Witter & Cuthill 1993; Cuthill *et al.* 2000). Furthermore, their use requires that a range of oft-violated assumptions be met (e.g. that the relationship between mass and body size is linear, that body size and mass are independent of each other and of condition, that the body size indicator trait is accurate, Green 2001).

A powerful tool for the direct manipulation of genetic condition is inbreeding (Rowe & Houle 1996; Cotton *et al*. 2004b; Tomkins *et al*. 2004; Bellamy *et al*. 2013, 2014). Inbreeding increases homozygosity (Wright 1977, Chapter 2). It exposes recessive deleterious alleles (i.e. the dominance hypothesis: Roff 1997, 2002; Charlesworth & Charlesworth 1999; Charlesworth & Willis 2009). And it

causes further reduction-of-fitness effects due to overdominance (or 'heterozygote advantage': Wright 1977; Bulmer 1980; Charlesworth & Charlesworth 1987). Irrespective of mechanism, inbreeding leads to inbreeding depression (Darwin 1876; Roff 2002), which increases as a function of the inbreeding coefficient, and leads ultimately to extinction (Frankam 1995; likey due to the accumulated effects recessive alleles on organismal function/reproduction). As such, the inbreeding coefficient is a useful and easily manipulated measure of genetic quality, or 'condition' (Bellamy *et al*. 2013, 2014). And it is one that links directly to the condition dependence theories of sexual selection, as both ornamentation (Bellamy *et al*. 2013) and sexual selection (Lumley *et al.* 2015) are known to protect against extinction in repeatedly inbred lines: more ornamented lines (Bellamy *et al*. 2013), or lines exposed to sexual selection (Lumley *et al.* 2015), take longer to become extinct under genetic stress; likely because these lines have been selected for increased genetic condition, so that these lines have better dominant alleles across the genome, and degrade less quickly. Inbreeding thus allows for a manipulation of genetic condition relevant to female choice, as females will prefer to mate with males that have fewer bad recessive genes. A further advantage to the use of inbreeding and inbreeding coefficients to apply genetic stress is that neither require an over-specific *a priori* definition of condition, nor the use of indices, as critiqued above (Bellamy *et al*. 2014). But this possibility has rarely been explored.

A small number of studies have used inbreeding to apply direct genetic stress to test for the heightened condition dependence of sexual ornamental traits (reviewed in Bellamy *et al*. 2014; and discussed further in Chapter 3). But the results have been mixed. For instance, heightened inbreeding depression in sexual

traits has been found in guppies (Sheridan & Pomiankowski 1997; van Oosterhout *et al.* 2003; Zajitschek & Brooks 2010), killifish (Ala-Honkola *et al.* 2009), *Drosophila montana* (Aspi 2000) and the stalk-eyed fly, *Diasemopsis meigenii* (Bellamy *et al*. 2013). But there have been mixed result in crickets (Drayton *et al.* 2007). And heightened inbreeding depression was not found in either the zebra finch (Bolund *et al.* 2010) or in another stalk-eyed fly, *Teleopsis dalmanni* (Prokop *et al.* 2010). In addition, the validity of the studies may be limited: either due to a lack of control for body size, or a failure to contrast sexual ornamental with nonsexual control traits; or due to inbreeding specific issues such as low line replication, low numbers of flies per line, low inbreeding coefficients, or a lack of outbred control (Lynch 1988; Bellamy *et al.* 2014). However, it is notable that a number of well-conducted studies on the responses of sexual ornamental traits to genetic variation do, nonetheless, exist (e.g. Sheridan & Pomiankowski, 1997; Bellamy *et al*. 2013). Nevertheless, even these are usually carried out in relatively benign environments that can mask the effects of genetic variation on trait expression (Bellamy *et al*. 2014). Moreover, it is not possible to understand the genetics of condition dependence without observation across a full range of natural environments (Cotton *et al.* 2004a; Armbruster & Reed 2005; Fox & Reed 2011). Yet this has been done in very few cases (reviewed in Bussière *et al.* 2008).

Indeed, an associated, and also important, resolution to the lek paradox is that related to gene-by-environment (G x E) interactions. Due to variation in the performance of different genotypes across environments (Jia *et al.* 2000a; Rodríguez & Greenfield 2003; Danielson-François *et al.* 2006), or due to life-cycle environmental-cycle interactions (where the life cycle of the organism is shorter

or longer than the fluctuation of the environment, so that ornaments become more or less reliable signals of genetic quality, Rodríguez 2013), or even where dispersal and migration are slow or fast relative to the life-cycle (Greenfield & Rodríguez 2004; Kokko & Heubel 2008), G x E interactions can theoretically increase V_A in sexual traits (Gillespie & Turelli 1989; Ellner & Hairston 1994; Danielson-François *et al.* 2006; and see Via & Lande 1985, 1987 for the standard evolutionary genetic basis). For instance, the effect of genetics (specific alleles or overall genetic condition) on trait expression can be lost in high quality environments, so that selection becomes weaker, which can allow mutations to accumulate. However, the increase in V_A arises due to blurring of the genetic signal in the ornament due to environmental variation (Higginson & Reader 2009). That is, environmental variation, and G x E can reduce the rate or loss of V_A, but to do so must blur or mask the genetic signal in ornaments and undermine the reliably of such traits (Kokko & Heubel 2008); even while reliability and honesty are crucial requirements of the condition-dependent handicap hypothesis.

In other contexts, however, a G x E could instead exaggerate or enhance the genetic signal (for instance, if environmental stress precipitates a difference in performance of ranked genotypes that would be hidden under more benign conditions, David *et al.* 2000), and could thus lead to a more rapid loss of V_A as well as a more honest signal. Hence, both condition dependence and G x E are in fact two sides of the same coin (Kokko & Heubel 2008): individual alleles contribute to ornamental trait expression, while condition dependence means that the number of such alleles is large and that the rate of loss of V_A is low – because the initial levels of V_A, and the subsequent mutation rates, will be high; likewise,

G x E reduces the loss of V_A where environmental variation masks the signal of specific alleles or overall genetic condition in trait expression – but also reduces the information content of the ornaments, and thus undermines the value of the signal in terms of sexual selection; in contrast, a G x E that leads to an enhanced role of genetics in the expression of ornaments, increases the information content of the ornaments, but also increases the rate of loss of V_A. As an extension to the point made above, it is, then – from the perspective of the maintenance of V_A in sexual traits, and of the evolution of costly female preferences for indirect genetic benefits – not possible to understand condition dependence in the absence of environmental variation (Cotton *et al.* 2004b; Armbuster & Reed 2005; Fox & Reed 2011), nor G x E in the absence of condition dependence, and both should, thus, be treated as such (Jia *et al.* 2000a; Hunt *et al.* 2004a; Kokko & Heubel 2008).

A number of studies have, therefore, looked at the genetic variance in environmental condition dependence. For instance, David *et al.* (2000) used a full-sib design to test for genetic variation in ornamental trait expression across a range of three food stress environments and found that some families developed large ornaments under all conditions, while others produced smaller ornaments as conditions deteriorated. However, despite a recent spate of such studies on the presence and scale of G x Es in sexual traits (reviewed in Greenfield & Rodríguez 2004; Bussière *et al.* 2008; Ingleby *et al.* 2010; and see also Ahuja *et al.* 2011; Weddle *et al.* 2012; Ingleby *et al.* 2013; Evans *et al.* 2015), which have demonstrated both 'ecological crossover' (where the order or rank of the different genotypes switches, crosses or is reversed across environments and can thus blur the signal of genetics in ornamental traits) and classic 'variance G x E s' (which

can enhance the signal, and increase the loss of V_A) none, aside Bonduriansky *et al.* (2015), have used explicit, controlled manipulations of both environmental and genetic condition to look at cross-environmental sexual trait expression for genotypes of explicitly higher or lower quality. Moreover, *no* studies have done so across multiple levels of environment for sexual versus nonsexual traits – in part because the controlled manipulation of genetic condition is extremely difficult.

It is not possible to understand the operation of sexual selection in nature without an understanding of the interactions between the effects of heightened genetic condition dependence and environmental variation on the expression of male sexual ornaments (G x E masking or enhancing the signal of genetics in male sexual ornamental traits). This is true both in terms of and understanding of the evolution (and maintenance) of female preferences, and in terms of understanding the basis of the traits at which these preferences are directed. As such, there remains a need for well conducted, controlled studies of the interaction between explicit environmental and genetic condition on the expression of sexual traits.

1.6 REPRODUCTIVE INVESTMENT

1.6.1 *The Role of Condition in Reproduction*

Another set of traits that could also be dependent on condition are reproductive and fertility traits. Due to the competitive nature of male reproductive success in species in which females mate polyandrously (Darwin 1871; Parker 1970; Andersson 1994; Birkhead & Møller 1998; Parker & Ball 2005; Pizzari & Parker 2009; as is the case in the majority of taxa: Jennions & Petrie 2000; Simmons 2001), the reproductive traits involved in achieving copulation, ejaculate delivery

and sperm competitive success could be under similar selective pressures as sexual ornaments (Eberhard & Cordero 1995; Arnqvist 1998; Griffith 2000; Dixson & Anderson 2004; Ramm *et al.* 2005; Ramm *et al.* 2007; Martin-Coello *et al.* 2009; Wigby *et al.* 2009; Perry & Rowe 2010). These classes of trait are also closely related to condition-dependent life history traits. Hence, they could often evolve to be condition-dependent (Alatalo *et al.* 1988; Houle 1992; Rowe & Houle 1996; Cotton *et al.* 2004b; Bonduriansky & Rowe 2005), and to integrate both environmental and genetic condition in their development (Pizzari & Birkhead 2002).

A consequence of this is that we can potentially expect the expression of such traits to mirror that of exaggerated sexual ornaments. So reproductive trait size should relate to a wide range of loci (Pomiankowski & Møller 1995; Rowe & Houle 1996; Tomkins *et al.* 2004) and exhibit heightened environmental and genetic condition dependence (de Visser *et al.* 2003; Cotton *et al.* 2004a, c, b; Bonduriansky & Rowe 2005; Tomkins *et al.* 2004). This leads to several potential outcomes for male reproductive success and female benefits. The first possibility is that the dependence of both sexual ornaments *and* reproductive traits on condition will lead to a positive covariance between these traits, such that males with larger ornamentation will also have increased reproductive investment, in part to account for the increased number of female partners that they are able to attract. Male sexual ornaments could, therefore, reflect fertility and the genetics of fertility (Trivers 1972; Birkhead & Pizzari 2002; Pizzari *et al.* 2004), as predicted by the 'phenotype-linked fertility hypothesis' (Sheldon 1994; Pizzari *et al.* 2004).

An alternate expectation, however, arises from a life history, resource allocation or ejaculate allocation perspective (Williams *et al.* 2005; Tazzyman *et al.* 2009; Engqvist 2012). Here, one could instead expect a trade-off between ornaments and reproductive or ejaculate traits (Parker 1998; Simmons & Emelen 2006), which could lead to negative covariation between these traits (Evans 2010; Simmons *et al.* 2010; Engqvist 2011; Evans *et al.* 2015). The direct resource-mediated trade-off between ornamental and reproductive traits is simple to understand: an individual male of a given condition has a set level of energetic resources, and can invest these in either ornamentation (to increase attractiveness) or in reproductive traits and fertility traits (to increase per mating reproductive success). In contrast, to this direct ornament-reproductive trait trade-off, the present-future trade-off in ejaculate investment that is predicted by sperm competition theory is more complex and so requires a more detailed explanation.

1.6.2 *An Overview of Limited Ejaculates*

As a result of his classic experiment on fruit flies, Bateman (1948) was able to demonstrate a 'fundamental' difference between males and females. He showed that male reproductive success was dependent on – and limited by – access to female partners. In contrast, female reproductive success was constrained by the female capacity to produce offspring; to lay eggs. As a consequence, males have been considered capable of producing near *unlimited* quantities both of the small, cheap gamete that is their sperm, and of offspring. To summarise this, Dawkins wrote in 'The Selfish Gene' (1976, p.164) that, "excess has no meaning for a male".

One reason why males typically produce such vast numbers of individual sperm is that males often compete post-copulation for fertilisation success, in a process known as 'sperm competition' (Parker 1970; Parker 1982b; Birkhead & Pizzari 2002). Ejaculates that contain a large number of sperm are more competitive than those that do not. However, despite this traditional idea of sperm as infinite or unlimited, recent evidence has shown that spermatogenesis has clear limits (such as energetic limits, Nakatsuru & Kramer 1982), and that males have evolved various mechanisms to control sperm transfer so as to allocate their reserves in a strategic manner and maximise their reproductive returns (Tazzyman *et al.* 2009).

A reason for this limitation on sperm production is that, while individual sperm do not cost a lot to produce, sperm are not transferred in ones, but in hundreds, or thousands or millions (Dewsbury 1982). That is, the costs are not trivial. A demonstration of this has been provided by Nakatusuru & Kramer (1982), who showed that male lemon tetras were able to produce only four times as many offspring as females, even when access to females was unlimited. And further examples can be provided both by Vanvoorhies (1992), who showed that sex was responsible for a dramatic reduction in the lifespan of *Caenorhabditis elegans* as a result of increased sperm production (rather than physical activity), and by Gage and Cook (1994), who showed that reductions in diet quality resulted in the constraint of sperm production in the Indian meal moth *Plodia interpunctella*. Hence, the production of sperm has been shown to require heavy investment and to be a limited, condition-dependent resource that must often be allocated with care.

Where individuals mate multiple times, selection will favour those males that can control their release of mature sperm rather than use it all at once. In line with

this, males from numerous species have evolved mechanisms that allow partitioning of sperm over a series of matings. In many taxa, males store mature sperm in specialised regions of the reproductive tract that are adjacent to but distinct from the testes (such as the epididymis and vas deferens in mammals, and the seminal vesicle in insects). For example, males of the blue head wrasse *Thalassoma bifasciatum* regulate their sperm release across successive spawnings in relation to female fecundity – with the controlled ejaculate allocation facilitated via the use of a specialised multi-chambered sperm duct (Rasotto & Shapiro 1998).

As noted, ejaculate allocation is predicted to be governed by the trade-off between current and future reproduction (Parker 1982b), and to depend upon multiple factors. For instance, in male-biased populations males are expected to favour current reproduction, while the opposite is true in female-biased populations, where a male can find multiple partners, and should thus consider future reproduction as well as his present reproductive opportunities. Males are expected to partition ejaculate resources with care across the available partners (Pitnick 1993). So, male ejaculate allocation will depend not just on population level parameters, but also on individual level factors such as a male's attractiveness (i.e. the ease with which he can obtain partners) and the size of a male's trade-off between survival and total investment in ejaculates – which will often be condition dependent (i.e. the relative costs will increase as condition is reduced). Attractive males may invest more in absolute terms in ejaculates, but are expected to attract more potential mates, and are thus expected to be more conservative with allocation than non-attractive males (Tazzyman *et al.* 2009). Hence, increased condition could potentially lead to increased ornamentation, but decreased investment in ejaculate products (such as sperm and accessory fluids)

in a single mating context (that is, a lower fractional investment of total reserves). This could, in turn, lead both to a negative correlation between ornaments and ejaculates, or related fertility traits, and the observation of negative condition dependence in such ejaculate/fertility traits (although it could lead to the opposite if the more attractive males run out of reserves less quickly over multiple matings).

1.6.3 *A Brief Overview of Empirical Evidence*

Evidence for a positive response of reproductive and fertility traits to variation in environmental condition is abundant, with positive responses found for reproductive traits (testes size: Droney 1998; Jensen *et al.* 2004; Rogers *et al*. 2008; Vasudeva *et al*. 2014; accessory gland size: Fedina & Lewis 2006; Rehm *et al.* 2008; Rogers *et al.* 2008), as well as for functional correlates of ejaculate quality (spermatophore or ejaculate size: Gwynne 1990; Cerolini *et al.* 1995; Delisle & Bouchard 1995; Kast *et al.* 1998; Watanabe & Hirota 1999; Jia *et al.* 2000b; Ferkau & Fisher 2006; Lewis & Wedell 2007; Blanco *et al*. 2009; sperm size, number, velocity or viability: Fedina & Lewis 2006; McGraw *et al.* 2007; Perez-Staples *et al.* 2008; Simmons 2011; Gasparini *et al.* 2013; Rahman *et al.* 2013, 2014 ; O'Dea *et al.* 2014; Cordes *et al.* 2015; Kahrl & Cox 2015; ejaculate composition: Perry & Row 2010; seminal proteins: Wigby *et al.* 2016) and resulting fertility (Vasudeva *et al.* 2014; Kahrl & Cox 2015). A small number of studies have also provided evidence for similar responses to variation in genetic condition, such as that based on increased versus decreased homozygosity (inbreeding). For instance, Wildt *et al.* (1982) found that the number of sperm per ejaculate was lower in inbred than in outbred foxhounds. Roldan *et al.* (1998) and Gomendio *et al.* (2000) found an inverse relationship between inbreeding coefficient and various ejaculate traits in

the endangered gazelle species *Gazella cuvier*. Fitzpatrick and Evans (2009) demonstrated an impairment of ejaculate quality with increased homozygosity across 20 mammalian species. Although some studies have found no responses in certain ejaculate traits (e.g. Michalczyk *et al.* 2010; Gasparini *et al.* 2013), taken as one, these studies have demonstrated a clear pattern of increased trait size with environmental quality, and decreased trait size in relation to environmental stress.

At face value, then, there is a lot of support for the idea that reproductive traits, like ornaments, respond to condition. However, studies on environmental and especially genetic correlations between these traits have been rarer, and have provided more mixed results. For instance, Hosken *et al.* (2008) found a positive genetic correlation between male attractiveness and siring success in *Drosophila simulans*, while in the dung beetle *Onthophagus taurus*, soma weight (a proxy for 'condition') was found to be positively genetically correlated with both attractiveness (Kotiaho *et al.* 2001) and testes weight (Simmons & Kotiaho 2002). In contrast, a number of recent studies of the relationship between ornaments, or attractiveness, and ejaculates have found evidence of negative genetic correlations (e.g. in *P. reticulata*: Evans 2010; in the Australian cricket *Teleogryllus oceanicus*: Simmons *et al.* 2010; and in *P. cognata*: Engqvist 2011) and have thus provided support for the economic trade-off hypothesis (see Chapter 4 for further details). So, the type of relationship between other traits and the male ornament appears to vary from trait to trait and species to species. But it is at present difficult to come to any clear conclusions because the evidence is sparse. As such, there remains a need to conduct further studies of the responses of reproductive traits to environmental and genetic condition, and to test the coordination of these responses with male ornaments. For further details on these points, see Chapter 4.

1.7 FEMALE MATE CHOICE

1.7.1 *An overview of female mate choice*

As seen above, a large number of theoretical and empirical studies have been conducted on male ornamentation and the information that these ornaments reveal (Pomiankowski & Moller 1995; Rowe & Houle 1996; Cotton *et al.* 2004a; Tomkins *et al.* 2004). In contrast, while the series of mate choices that females make – for instance, due to female mate preference – are the force that drives sexual selection and the evolution of male ornamentation (Poulin & Vickery 1996; Rolff 1998; Cotton *et al.* 2006a), far less is known about female mate choice or the factors that drive female mate choice (Jennions & Petrie 1997). For this reason, I will now focus on female mate choice, mate preference, and its variation.

Female mate choice is complex, and is influenced by a range of factors. These include, the range of potential mates from which a choice can be made (Andersson 1994), the capacity of the female to discriminate between these potential mates (Burkhardt & de la Motte 1983; Buschbeck & Hoy 1998; Hingle *et al.* 2001b; Secondi *et al.* 2015), and the extent to which female choices are free from or forced by male dominance (Cordero 1999; Dukas & Jongsma 2012) or skewed in relation to factors such as male mate choice (Amundsen & Forsgren 2001; Chenoweth *et al.* 2007; Myhre *et al.* 2012; Cotton *et al.* 2015). But, while such factors can limit or alter the array of possible choices or actual choices that a female makes (or appears to make), the crucial driver of mate choice is female mate preference (Jennions & Petrie 1997). In contrast to a mate choice, which is the outcome of multiple factors (including the both 'internal' and 'external' factors listed above, *in addition to* the underlying mating preferences of the female, (Jennions & Petrie 1997; Widemo & Sæther 1999; Cotton *et al.* 2006b), mate

preference can be considered as the behavioural and sensory capacity of females that leads to the differential mating of males based on their phenotype (Heisler *et al.* 1987). This distinction between the factors that influence choice and preference is an important distinction to make, and it is one that is often neglected.

As an important driver of variation in female mate choice, female mate *preference* is also complex. The precise nature and definition of its constituent parts are debated (Heisler *et al.* 1987). A range of terms – such as 'tolerance', 'discrimination', 'permissiveness', 'receptivity', 'selectiveness', 'sensitivity' and 'tightness' – exist in the literature, and overlap, both with each other, and with terms used in other related fields (Figures 1, 2; reviewed in Edward 2015). Yet, despite this profusion of terminology, female mate preference can in principle be broken down into, and understood as a composite of, two main components: the preference function, and the level of female choosiness (Jennions & Petrie 1997).

The first component – the preference function – is equivalent to the order or rank of mate phenotypes (Jennions & Petrie 1997), or the ideal "standard of beauty" (Darwin 1859). Aside potential neurological costs, there is no clear cost to the existence of an idealised preference function. Hence, it is expected to vary, in the main, with the genetics of the female (modelled in Tomlinson & O'Donald 1996; reviewed in Cotton *et al.* 2006b). In contrast, the second component – female choosiness – relates to the effort "put in to" the preference function: for instance, the effort used to assess mates prior to acceptance. It includes factors such as 'sampling effort' and 'motivation' (Jennions & Petrie 1997). The efforts put in to express this ideal preference function in a mate choice or series of mate choices have clear costs (Pomiankowski 1987; Reynolds & Gross 1990). As such, choosiness is expected to vary with the various costs of mate choice (see below).

Figure 1

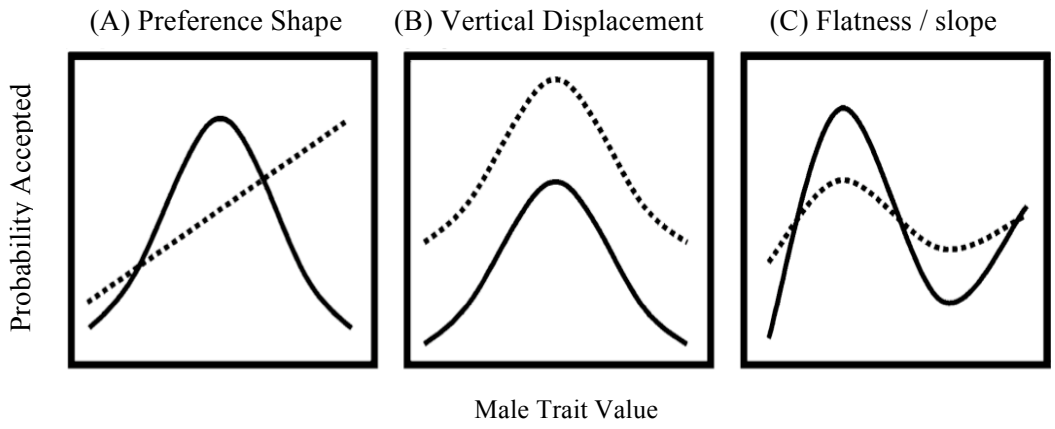


Figure 1. Attributes of female preference function (adapted from Bailey 2008). Hypothetical examples of independent variation in three aspects of female mate preference. The *x* axis represents a continuous male ornamental trait. The *y* axis represents the probability that a female will accept a male, or the likelihood that she will mate with a male, or even the effort she will put in to find a male with a given ornamental trait value. (A) Variation in the shape of the preference function. The solid line shows a stabilising preference function, the dashed line an open-ended linear preference function. Each has a similar vertical displacement and flatness, or slope. Females discriminate at an equal level between males that are the 'most' and 'least' attractive, but the shape of the preference function differs. (B) Variation in the vertical displacement of the preference function. In Bailey (2008) this is referred to as 'responsiveness'. The shape of the two functions is constant, but the female with the dashed preference function will accept all males with a higher probability. A consequence of this is that that proportional difference between the most and least accepted male will decline; so selection will weaken, even though the difference

between the most and least attractive male remains the same for both the dashed and solid line females. A similar plot could be drawn for an open-ended linear function. (C) Variation in the flatness of the curve, or the slope of a linear function. This is the vertical displacement of parts, rather than, all of the preference function. In Bailey (2008) this is referred to as 'discrimination'. Here, the shape and vertical displacement of the two preference functions is the same. But the dashed line represents the preference function of a female that shows less variation in her acceptance or rejection of males based on the male ornament phenotype. In the context of an open-ended linear preference function, this would result in a shallower slope. A further representation could also be drawn to show horizontal displacement.

Figure 2

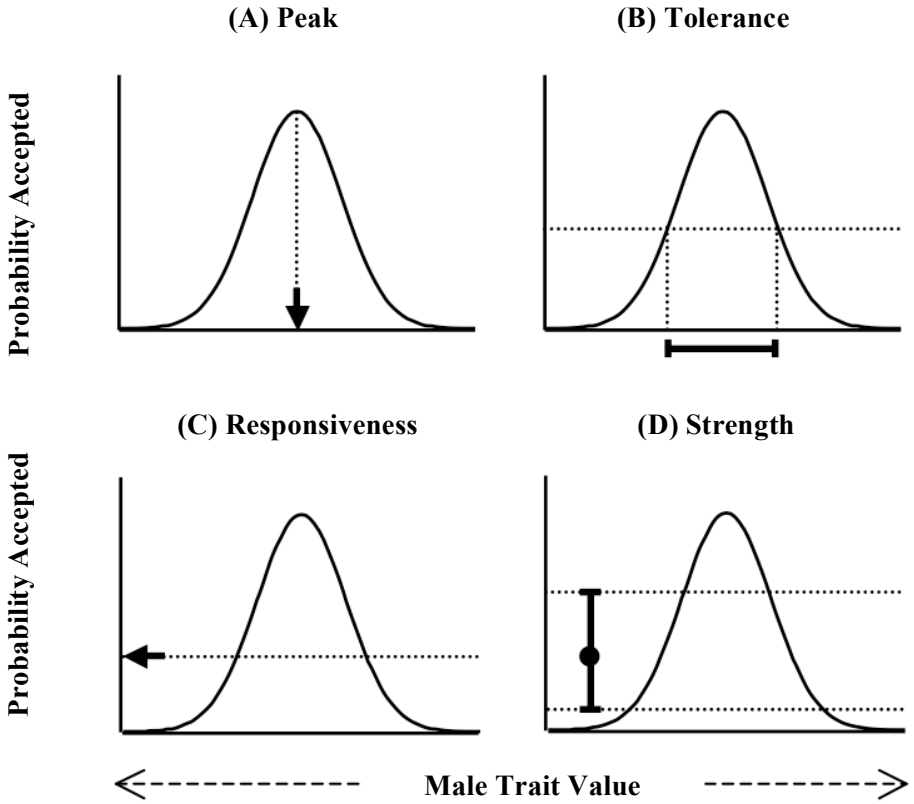


Figure 2. Attributes of female preference function (adapted from Fowler-Finn and Rodríguez 2011). Hypothetical examples of variation in four aspects of the female mate preference function. The axes are as described for figure 1. (A) The 'peak' of the preference function relates to the point on the *x* axis at which the female is most likely to accept a male: that is, it relates to the most preferred ornament size. The peak can exist for any 'shape' of preference function (see figure 1). It can move on the *x* axis, for instance, due to the condition dependent costs of male damage on females, or in relation to (genetic) compatibility, or overall male (genetic) quality. It can also move on the *y* axis. That is, the peak can stretch, or become flatter (see figure 1). (B) The 'tolerance' of the preference function is the width of the preference function at a 50%

drop from the highest response. It is indicated by the black horizontal bar. This measure also relates to the flatness of the preference function. It is equivalent to 'tightness'. It can also be applied to an open-ended linear slope, but a gradient for the slope is more useful in this case. (C) The 'responsiveness' of a preference function relates to the mean response across a range of stimuli. It is similar to vertical displacement, and to Bailey's (2008) 'responsiveness'. However, in this case, the value relates not to the overall vertical displacement of the whole curve, but to the mean response that arises irrespective of the parts of the curve that vary in vertical displacement. (D) The 'strength' of the preference function is the square of the coefficient of variation across the range of stimuli or ornaments (based on Schulter 1988). It relates to the 'tallness' (which is the inverse of 'flatness'), and thus to the vertical displacement of all, or in most cases parts, of the female preference function.

A note on the figures: In all, each female will have an ideal preference function based on genetics or condition that can vary between females in all aspects described in figures 1 and 2. However, variation in the cost of choice will affect the vertical displacement of all or part of the curve. Hence, variation in the cost of choice will alter vertical displacement, 'flatness', 'tolerance', 'responsiveness' and the 'strength' of preference curve. All can thus be considered under the umbrella term – 'choosiness'.

Another way to conceive of the preference function is as a literal function, as a 'function valued trait' (Lande 1981, Ritchie 1996, McGuigan *et al*. 2008; Fowler-Finn & Rodríguez 2011; Rodríguez *et al*. 2013). In this sense, variation in the male ornament phenotype is represented on the x axis, while variation in the female's likelihood of accepting a given mate (or the effort put into finding that mate) is represented on the y axis (Edward 2015). Here, the preference function captures variation associated with both 'preference' and 'choosiness'. It is a useful form of measurement, but can be confounded. Nonetheless, it is possible to separate out the effects of 'preference' and 'choosiness' on the shape of the preference function so as to better explain this concept, and so as to provide a basis for theoretical expectations for the type of variation that should be seen in each component of the preference function, and the factors that could cause this variation to arise.

In an idealised scenario, in which the costs of choice (all costs of the expression of the internal female preference function) are removed, it should be possible to record a univariate preference function on an $x - y$ plane as noted above. Here, the preference function may take several forms. For instance, it could be a simple threshold. Likewise, it could be a directional slope, open-ended, or otherwise. A slope or threshold could also have various forms of curve, such as those based on exponential or sigmoidal functions. There could also be humped curves based on normal or 'Gaussian' bell shapes, or even spikes, and inverted forms of each. The mathematical description, categorisation, quantification and empirical investigation of such curves is difficult. But various sub-terms have been defined.

A simple way to look at the preference function, or curve, is to consider it based on three aspects: the shape (which captures all the above), the displacement of the

shape (on a vertical, *y*, or horizontal, *x*, plane), and the shallowness of the curve.

With the exception of the horizontal displacement, this set of descriptions was used by Bailey (2008); and can been seen in figure 1. He referred to these attributes as 'shape', 'responsiveness' and 'discrimination'; he noted that each was or could be independent of the other, and he assumed that the latter two – responsiveness, and discrimination – would be reflective of the costs of choice as well as of the underlying 'ideal' preference. To further categorise the shape of the preference function, Fowler-Finn and Rodríguez (2011) have added further terms such as the 'peak' of the preference function, as well as the 'tolerance' (similar to others' 'tightness'), 'strength' and 'responsiveness' of the curve (Fowler-Finn & Rodríguez 2011; see Figure 2). The first of these terms relates to the horizontal displacement of the peak (but not of all) the preference function on the *x* plane, while the other terms capture aspects of both Bailey's (2008) 'responsiveness' and 'discrimination'. The 'shape' is not captured in the Fowler-Finn and Rodríguez's (2011) definitions, as these are applicable to forms of bell curve or spike, and can not easily be applied to slopes (although a crossover in terms could be achieved, the attributes would be more easily captured by a slope for a linear curve). This brief snapshot captures some of the variability and overlap that was noted before.

The main point though, is that the preference function can take various shapes, which may vary between individuals, for instance due to their genetic condition. Likewise the shapes will vary between populations. A population that uses multiple types of syllable in male call songs could lead to the evolution of less 'tight' preference functions, while one with fewer call syllables could lead to a 'tighter' preference function (as was observed in Ritchie 1996). A population with

assortative mating, or compatible gene effects, could lead to variation in 'peak' preference between individuals, while one with a clear 'best' male could lead to a specific peak, or an open-ended preference. (With the addition of mating associated male damage, the peak of the preference function could even shift on the *x* axis in relation to female condition). Additionally the form of the preference function, in itself, could also lead to concordant variation in male ornaments – and could have effects for evolution and even speciation, due to the potential effects of linear directional, stablising, or disruptive preference functions, as a driver of mate choice, and sexual selection (Gomulkiewicz 1991; Judge 2010; and see O'Donald 1980; Maynard Smith 1991; Hoikkala & Aspi 1993; Ritche 1996).

It is for the various reasons described above that researchers are interested in the ideal preference function. However, each part of the shape of the recorded 'ideal' preference function will vary not only with underlying 'ideal' preference, but also with the effects of choosiness. As noted before, choosiness will respond to variation in the costs of choice. For instance, if it takes more effort to search for a mate, or to resist a mate, then the overall probability that a particular mate is accepted may increase, irrespective of his ornamentation. Here, the point is that variation in the costs of choice will alter the level of choosiness, and this will alter, in turn, the expression of the ideal preference function in choices (which can then be recorded, and used to construct a recorded preference function). An important point is that the variation in choices due to variation in choosiness will affect the vertical displacement, on the *y* axis, for all, or part of the curve of the preference function. It is plausible that *x* axis variation could arise, for instance in a threshold model. But here, the preference function and choosiness are fused and cannot be separated. In fact, in a more complex threshold (such as a tapered, diagonal

threshold), as with an open-ended slope, the variation on the *x* axis can even be explained in terms of variation in the *y* axis (a long ruler, held at an angle, and raised vertically past the edge of a table on one plane, will produce lateral movement in the point at which the ruler bisects the table, while the slope is stable – as each point in the ruler becomes, in turn, the new 'viewable' preference slope).

Hence, variation in the costs of choice can alter choosiness and in turn alter aspects of the expressed preference function that relate to the *y* axis. An implication of this is that both aspects of female mate preference – preference function *and* choosiness – affect sexual selection. Further, it is important to realise that, to measure the preference function, the effects of the cost of choice must be removed or controlled; or the two parts of mate preference must be otherwise separated. Where this is not the case, aspects of Bailey's (2008) 'responsiveness' and 'discrimination', Fowler-Finn and Rodríguez's (2011) 'tolerance', 'responsiveness' and 'strength', and Ritchie's (1996) 'tightness' will reflect, not variation in actual, ideal (or underlying) preference functions, but instead aspects of choosiness. Hence, it is critical that such aspects are separated, and controlled.

To summarise, then, female mate preferences are complex, but consist of two main sub-components – the preference function, and choosiness (Jennions & Petrie 1997). The first, relates to the shape of the curve of mate responses, the second to the overlaid vertical displacement of all or part of this shape of the preference function (Lande 1980, Ritchie 1996, Bailey 2008, Edward 2015). The preference function is expected to vary with the 'internal' properties of the female, such as her environmental (Gray 1999; Hingle *et al.* 2001a; Hunt *et al.* 2005; Cotton *et al.* 2006a; Holveck & Riebel 2010; Holveck *et al.* 2011) or genetic

condition (modelled in Tomlinson & O'Donald 1996; reviewed in Cotton *et al.* 2006b) that alter the relative costs of preference for higher quality, optimal or compatible mates (Tregenza & Wedell 2000; Qvarnström 2001; Badyaev & Qvarnström 2002) (or that alter the costs of resistance, and thus of the height or horizontal displacement of the 'peak' of the preference function). In contrast, choosiness is expected to vary with external factors that alter the relative costs of mating (Kokko & Monaghan 2001; Bleu *et al.* 2012) or not mating (De Jong & Sabelis 1991; Kokko & Mappes 2005; Lynch *et al.* 2005), such as environmental variables – density (Arnqvist 1992), sex ratio (Berglund 1994; Holveck *et al.* 2015), or social structure (Fowler-Finn & Rodriguez 2012; Bailey & Macleod 2014) – that influence mate encounter rates and the costs of forgoing a mate. Finally, the output of this dualistic, female 'mate preference' in terms of choice and selection is then expected to be further limited in relation to limits on discrimination (Burkhardt & de la Motte 1983; Buschbeck & Hoy 1998; Hingle *et al.* 2001b; Secondi *et al.* 2015) and the range of available mates (Andersson 1994).

Hence, the variation in, and shape of, each component of preference, as well as the interactions between these components, and the factors that cause variation in these interactions and affect the expression of 'ideal' preferences in female mate choices, and in sexual selection, need to be studied – and at the individual level.

1.7.2 *The measurement of female mate preference*

Due to this complexity, both choosiness and preference are difficult to measure. The data available to measure each are often not the preferences themselves, but series' of choices, or even indirect measures of association times. Each form of data (direct or indirect) presents it's own challenges. Nonetheless, a relatively

simple list can be provided for the considerations that need to be taken account of in order to obtain reliable estimates of the different components of preference.

First, it is important to realise that choices (or association time) are the end products, not the preference *per se* (the choices that an experimenter records will have been filtered in relation to the number and type of mates available, and in relation to factors such as the presence or absence of competition, either between females, or between the males that are chosen). Then, in the construction of an assay it is preferable, where possible, to use direct rather than indirect measures of choice (for example, to observe a choice rather than to measure association time), as these minimize inference and assumption (direct measures record the actual choices females make; indirect measures assume that association times or other behaviours reveal the choices that females will make, Shackleton *et al.* 2005; Cotton *et al.* 2006a; Reinhold & Schielzeth 2015), and as a clear relation between the two types of measure cannot always be found (Gabor 1999). (It is, of course, acceptable to use such indirect measures in as far is these measures can be shown to correlate with eventual choices made by the females (acceptance or rejection)).

Next, in the construction of the assay of female mate choices, it is often better to use a 'no-choice' test rather than a 'choice' test (that is, to provide sequential rather than simultaneous choices). This is because choice tests can inflate estimates of preference and increase the risk of overestimation or type I errors (Dougherty & Shuker 2015): both due to a reduction in the costs of rejection and the increased ease of discrimination in simultaneous choice tests; where females can pick up subtle differences that would not be observed in natural situations, where direct comparisons would less often be experienced (look at two shades of

yellow in sequence, or at the same time – can you tell the difference?). This last point is especially relevant in cases where a direct comparison of potential mates is not possible, or is rare, in nature. As before though, the use of simultaneous choices will be appropriate in so far as it reflects a natural behaviour (for instance, in a classical lek species, such as the sage grouse, such tests could be appropriate).

Another factor to consider is the level of the measurement. Where possible, it is better to measure preference at an individual level (indeed, it is not possible to deconstruct preference into choosiness and the preference function with population level preference measures), as such measures allow for the more accurate characterisation of preference components, and provide a basis to examine the levels of variation between and within individuals (Cotton *et al.* 2006a). To do so, females should be presented with a full range of ornament phenotypes – either via the sequential no-choice presentation of individual males, or via the linear modulation of stimuli (e.g. mate calls or colours) – as studies that use only two stimulus males are unable to accurately measure the strength of directional selection (Cotton *et al.* 2006a), detect stabilising (e.g. Sappington & Taylor 1990; Greene *et al.* 2000) or disruptive preference functions (e.g. Gerhardt 1991; Ritchie 1996; Hunt *et al.* 2005), and have low power to resolve individual differences in preference (Wagner 1998) (this is because, with binary choices, preferences can either be positive, or negative, or neutral, while no information about their shape can be extracted). Furthermore, at each 'presentation' it is crucial to isolate female mate decisions from the influence of male effects, such as male choice (e.g. Amundsen & Forsgren 2001; Chenoweth *et al.* 2007; Myhre *et al.* 2012; Cotton *et al.* 2015), mating ability (e.g. Rogers *et al.* 2005a) or forced copulation (e.g. Cordero 1999; Dukas & Jongsma 2012) as these can alter the

outcomes of choice and can invalidate measures of preference (Cotton *et al.* 2006a) (these factors can be biologically relevant, in terms of the expression of preference, but will blur the measurement underlying preference functions, and make it hard to determine the factors that cause variation in these preference functions). Likewise, other factors that can alter the outcomes of female choice – such as light levels, or encounter rates – should also be controlled for this reason.

As can be seen, mate choice and female mate preference are complex, and difficult to measure and to disentangle. Nonetheless, the past 10 – 20 years have seen a flourish in research on this topic, and a lot is now known about shape of preference functions (Ritchie 1996; Ritchie *et al.* 2001; McGuigan *et al.* 2008), their variation between individuals (Bakker & Pomiankowski 1995; Wagner *et al.* 1995; Ritchie *et al.* 2005; Cotton *et al.* 2006a), their plasticity within individuals (Qvarnström *et al.* 2000; Coleman *et al.* 2004; Fowler-Finn & Rodriguez 2012; Rodríguez *et al.* 2013; Tinghitella 2014), and the delineation of preference into different components (reviewed in Edward 2015). The consequences of the dual condition and context dependence of female preference and male ornamentation for the process of sexual selection have thus started to come into focus. However, it remains true that most studies have been conducted under non-natural conditions: for instance, via the use of virgins (to control mate status, and to increase the accuracy of preference measures, but with the potential to alter the costs of choice and to alter the estimation of choosiness, the preference function and resultant selection) or via the use of laboratory conditions. Hence, there is a need for studies to investigate the effect of variation in condition or context on the individual components of preference and on the series of choices and selection that this leads to, under more realistic conditions in the laboratory, and in nature.

1.71.8 A REVIEW OF THE LITERATURE ON STALK-EYED FLIES

1.8.1 *An Introduction to Stalk-Eyed Flies*

Across the last 30 years, stalk-eyed flies (Diopsidae; Diptera) have become an established model used for the study of sexual selection (Andersson 1994; Wilkinson 2001; Maynard Smith & Harper 2004; Chapman *et al.* 2005). In this section, I review our current knowledge on the evolution and natural history of stalk-eyed flies, with a special focus on the aspects that are relevant to this thesis.

Approximately 150 species of stalk-eyed flies (Diopsids) have been characterised (Feijen 1989) since they were first described by Linnæus in 1775 (reviewed in Shillito 1974), although some estimates are closer to 300 (Wilkinson & Dodson 1997). The distribution of these flies is focused around the tropics, in South East Asia and Africa. Nonetheless, some species can be found in both North America and Europe (for instance, those of the genus *Sphyracephala*, Feijen 1989; Papp *et al.* 1997; Wilkinson & Dodson 1997), where the prehistoric *Prosphyracephala* genus has also been documented (in fossil amber dated 22 Ma., Schumann 1994). Each Diopsid species is characterised as such, in part, due to a form of hypercephaly in which the eyes and antennae of each sex are located on the end of lateral projections from the head capsule – otherwise known as eyestalks (Shillito 1940).

Although hypercephaly has also been documented in other Diptera (Grimaldi & Fenster 1989; Wilkinson & Dodson 1997), the Diopsids are unique in that both males and females of all species exhibit this trait (Baker *et al.* 2001b). In each sex, the length of these stalks is variable, and can exceed the length of the body in certain species (Baker & Wilkinson 2001). In addition to hypercephaly, eyestalk sexual dimorphism is also a common feature of Diopsids: it can be observed in

multiple species, and is thought to have evolved at least four times (Baker & Wilkinson 2001). In these instances, male eyespan has evolved to be far greater than female eyespan (Baker & Wilkinson 2001). However, this is not a ubiquitous feature of the stalk-eyed flies, and the monomorphic state is thought to be plesiomorphic (Wilkinson & Dodson, 1997, Baker *et al*. 2001b, Baker & Wilkinson 2001), with a number of extant monomorphic species also documented (see, for instance, in the South African *Sphyracephala beccarri,* Cotton *et al*. 2004b).

The natural or sexual selective force that first drove the evolution of the eyestalks is not known. However, it is likely that this, "first push" arose due to natural selection based on visual acuity. The number of ommatidia (the visual units that make up the surface of the insect compound eye) on each eye increases with the width of the eyespan (up to around 2600 in males and 2500 in females in some species, Burkhardt & de la Motte 1983; de la Motte & Burkhardt 1983), and is claimed to be associated with increased binocular field vision (over 135°, Burkhardt & de la Motte 1983) – though this has not been established. The near field (the distance from the animal at which at least one ommatidium in each eye is able to see a specific point) is also thought to be ~ 400-800x higher in flies with eyestalks than in flies without (Burkhardt & de la Motte 1987). Hence, the large eyespan of stalk-eyed flies potentially allows for increased visual resolution, and range. It would be a major advance for this to be established experimentally.

However, it is also likely that a number of costs are imposed by wider eyespans, such as an increase in damage, reduced aerial agility (Swallow *et al.* 2000; but see below), and increased predation risk (Worthington & Swallow 2011). Further, while stalk-eyed flies may have increased visual acuity (similar to that of far larger

insects, such as Dragonflies, Buschbeck & Hoy 1998), empirical studies have shown that the requisite neural networks are associated with further costs, and that increased axon lengths could lead to a reduction in a fly's ability to resolve images quickly, and in turn limit their ability to move at speed (Buschbeck & Hoy 1998). (It is notable that condition dependence itself implies a cost, and that a number of studies – discussed below – have also shown that eyespan is condition dependent).

An important potential cost is that of the reduced aerial turning performance that could arise due to the increased moment inertia of the head in more ornamented males (Swallow *et al*. 2000; for context, the eyes and eyestalks of a stalk-eyed fly can account for around 13% of its total weight, de la Motte & Burkhardt 1983). However, recent empirical experiments have failed to detect any such effect (Swallow *et al.* 2000; Ribak & Swallow 2007). In fact, male *T. dalmanni* were able to turn in the air as well, or better than, female conspecifics in a trial by Ribak and Swallow (2007). This appeared to be due to the increase in thoracic mass (Swallow *et al*. 2000; Ribak & Swallow 2007) and larger wing length and body size (Ribak *et al.* 2009; Husak *et al.* 2011) of male stalk-eyed flies. The implication is that the costs are real, non-trivial and have led to the evolution of male adaptations.

A potential outlook, then, could be that more ornamented males must pay greater energetic costs to exist with these ornaments (the eyespan, and all other external morphological traits are fixed at eclosion, de la Motte & Burkhardt 1983). However, it is notable that – across multiple species of stalk-eyed flies, which include the oft-studied *T. dalmanni*, *Teleopsis whitei*, and *D. meigenii* – (relative) male eyespan has been shown to be negatively evolutionarily correlated with (body size corrected) wing beat frequency (Hussak *et al.* 2011). That is, species

with larger males have fewer wing beats per second, as do larger relative to smaller eyespan males within species, as do males, relative to females, in line with the level of sexual dimorphism in the species (Hussak *et al.* 2011). Hence, certain costs could be real, but could have become less important due to evolutionary compensations that reduce energetic costs and increase aerial agility.

Irrespective, while vision based natural selective advantages are able to provide potential explanations for the origin of hypercephaly, they cannot readily explain the evolution of eyespan sexual dimorphism. It *is* plausible that this sexual dimorphism could have evolved due to selection for niche differentiation between the sexes (as seen in the example of the beak size differentiation in huia, Darwin 1871; Doflein 1914; Lande 1980; Andersson 1994). However, there is no evidence for such niche specialism as a driver of eyespan differentiation between the sexes in stalk-eyed flies, and there is also some evidence against it, in the sexes' shared behaviours. For instance, a typical feature of stalk-eyed flies is that the adults of both sexes spend the day foraging in the forest, and feed on mould, fungi, and rotten leaf litter (de la Motte & Burkhardt 1983; Feijen 1989; Wilkinson & Dodson 1997). Likewise, adults of both sexes also spend a large proportion of the day "grooming". For instance, in African (Seibt 1972; Wickler & Seibt 1972) and Asian (de la Motte & Burkhardt 1983) species of stalk-eyed fly. And adults of both sexes also react with aggression to encounters with conspecifics of either sex during the day, in both African and Asian species (Lorch *et al.* 1993).

It is also unlikely that natural selection can account for the extreme exaggeration of the male eyespan that is observed in some species. In part, this is because of aforementioned costs, which could potentially limit extreme exaggeration. In part

it is because monomorphic species have relatively similar life histories to dimorphic species, with the apparent exception of their mating behaviours (adults in four out of five dimorphic Malaysian species were found to roost in clusters at night, Burkhardt & de la Motte 1987; Wilkinson & Reillo 1994; as was a dimorphic Kenyan species, *Diasemopsis fasciata*, Wilkinson & Dodson 1997; while no such nocturnal clusters have been observed in monomorphic species, Wilkinson & Dodson 1997, Kotrba 1996); so that the non-mating associated selection on eyespan should be similar between these classes of species. Finally, it is also, crucially, notable that, across species, the level of eyespan exaggeration (the slope of its allometric relationship to body size) is positively associated with the level of sexual dimorphism (Baker & Wilkinson 2001) – the wider the dimorphism, the greater the exaggeration, and vice versa. In all then, natural selection is unlikely to account for the extreme eyespan exaggeration and sexual dimorphism that is observed across multiple species of stalk-eyed flies (or, Diopsids).

In contrast, a likely explanation for this sexually dimorphic hypercephaly can be provided by sexual selection. In nature the flies (that is, the dimorphic Diopsids) often live in forests or near low level vegetation, and aggregate at dawn and dusk to roost and mate (Burkhardt & Delamotte 1985; Burkhardt & de la Motte 1988; Lorch *et al.* 1993; Wilkinson & Reillo 1994; Wilkinson & Dodson 1997, pg 320). The 'lek' sites can be root hairs that overhang the tangled banks of streambeds (as is well documented in a range of Asian species: Burkhardt & de la Motte 1987; Wilkinson & Reilo 1994) or can be the leaves of broadleaved plants (as has been observed in at least one African species, *D. fasciata*, Wilkinson & Dobson 1997), often near streams or in primary or secondary forest (Feijen 1989). Due to the location and time, the light levels tend to be low, and this may favour increased

visual capacity. This effect would operate on both males and females and would allow for an increased capacity to locate food sources, roost and lek sites or oviposition (egg-lay) sites. But there could also be an advantage to sexual selection.

A simple advantage to wider eyespans would be in the sense that wider eyespan males are more attractive. Indeed, numerous studies have shown that females prefer to roost with wider eyespan males (Wilkinson & Reillo 1994), and that such males mate more often (Lorch *et al.* 1993), and achieve higher reproductive success (Burkhardt *et al.* 1994). There is also evidence that that there is a role for male eyespan – ritual and physical – in male-male contests (this is well documented in Asian species, Burkhardt & de la Motte 1983; Lorch *et al*. 1993; Panhuis & Wilkinson 1999; Small *et al*. 2009, but has also been observed under laboratory conditions in the African species, *Diasemopsis meigenii*, J.H. pers. obs.), at lek sites, and at ovipositon/resource sites (de la Motte & Burkhardt 1983; Burkhardt & de la Motte 1983). And there could be further advantages to wider eyespans if wider eyespan females are better able to assess males (as implied in Hingle *et al*. 2001b, discussed in 1.8.3). Nonetheless, it is the relationship between the size of a male's eyespan and female mating choice that is most striking, and which has drawn the most attention (as discussed in the next two sections below).

As in other species, it is of interest to know what it is about the male eyespan that the female prefers. Does it relate to a direct benefit? Or to the possibility of so called "sexy sons"? Or does it reveal information about the genetic quality of the male: overall, or in terms of compatibly. A number of studies have been conducted in relation to each of these points. But I shall focus here on those studies that have investigated the prediction that male eyespan be condition

dependent, and in a heightened manner relative to nonsexual traits; as is expected under Zahavi's (1977) condition-dependent handicap hypothesis of sexual selection.

1.8.2 *Male Eyespan: A Condition-Dependent Trait*

As noted above, a potential resolution to the question, 'why is there sexual dimorphism in the level of hypercephaly in stalk-eyed flies', is that the dimorphism is driven by female mate choice/preference for larger eyespan males.

A key hypothesis of the handicap principle is that sexual traits should exhibit heightened condition-dependent expression (Zahavi 1975; Cotton *et al.* 2004b). Given this, a number of studies have been conducted in various species of stalk-eyed fly to investigate the condition dependence of male eyespan relative to female eyespan and other non-sexual traits. This has been done in relation to variation in both environmental factors (e.g. larval food stress), and genetic factors (see below); and the results have started to shed light on the answers to this question.

A number of studies have shown that male eyespan exhibits heightened condition-dependent responses to variation in levels of environmental quality or stress relative to non-sexual traits. This is the case in both sexually dimorphic (David *et al.* 1998; Cotton *et al.* 2004a) and monomorphic species (Cotton *et al.* 2004c). For instance, after the manipulation of larval diets (in terms of food quantity and quality) in the sexually dimorphic Malaysian *T. dalmanni*, male eyespan was found to decrease more in response to increased stress (low food levels or low diet quality) than was either female eyespan, male wing length or female wing length (David *et al.* 1998; Cotton *et al.* 2004a). Likewise, a heightened response was also observed under similar conditions in the dimorphic African stalk-eyed fly species, *D. meigenii* (a result which persisted after control for body

size, Bellamy *et al.* 2013) . In addition to this, a similar response to larval environmental stress was observed even in the monomorphic *Sphyracephala beccarii*, where the eyespan trait shows minimal exaggeration in either sex (Cotton *et al.* 2004c), suggesting that the condition dependence of eyespan is ancestral. As in the case of *D. meigenii*, these results also held after control for body size. Hence, the heightened condition dependence observed cannot be accounted for by body size scaling – a point to which weight is added by a further study in the African species *Diasemopsis aethiopica,* in which males reared under low dietary stress invested their additional resources into increased eyespan rather than body size (Knell *et al.* 1999). Here, female larvae raised on high quality diets had larger adult eyespans and body lengths than their low quality counterparts, while low stress males had larger eyespans but similar body lengths compared to high stress males. That is, males fed on a higher quality diet were able to increase their fitness by investing their extra resources in larger eyespans rather than larger body sizes.

In addition to environmental variation, further studies have revealed high levels of genetic variation in the response of male eyespan to larval food stress. David *et al.* (2000) used full- and half-sib families of the Malaysian *T. dalmanni* to demonstrate the presence of a genotype-by-environment (G x E) interaction for male eyespan. Male eyespan was large across all three levels of environmental stress for some genotypes, and decreased as stress increased in others. While female eyespan, male wing length and female wing length also showed genetic variation in condition-dependent expression, their genetic responses were entirely explained by body size or 'allometric' scaling. *However*, David *et al.* (2000) attempted to remove the effect of body size scaling using a ratio method – that is, they divided eyespan by thorax length. But this ratio method is flawed. It cannot

remove the effect of a variable (in this case body size) on another variable (in this case 'absolute' male eyespan) unless the trait allometries pass directly through the origin (Packard & Boardman 1999). To address this, Cotton (2004) repeated the experiment using inbred lines of *T. dalmanni*. He included thorax as a covariate and thus overcame this issue, and came to similar conclusions to those of David *et al*. (2000). Male relative eyespan was more sensitive to changes in larval diet than were the equivalent measures of female eyespan, male wing length and female wing length. Taken together, these studies show that male eyespan exhibits higher genetic variance under high stress levels than it does under low levels of stress (David *et al*. 2000; Cotton 2004) and that the genetic component of environmental variation in male eyespan is far larger than that in the equivalent nonsexual traits.

Yet, while these studies look at the genetic variance in environmental condition dependence, they do not test for heightened condition dependence in relation to explicit variation in genetic condition or stress. A recent development in this line has come as the result of two further studies: that by Prokop *et al.* (2010) and that by Bellamy *et al.* (2013). The handicap hypothesis, coupled with expectations based on genic capture, predicts that ornamental traits will exhibit both heightened *reductions* in size in response to inbreeding (inbreeding depression), and heightened *increases* in response to outcrossing (heterosis), as such ornaments should depend on a larger number of loci than non-sexual traits (Rowe & Houle 1996; Cotton *et al*. 2004b; Tomkins *et al*. 2004; Bellamy *et al*. 2014). To test this, Prokop *et al*. (2010) used one generation of full-sib inbreeding in the Malaysian *T. dalmanni* to induce genetic stress. Male eyespan and all nonsexual traits exhibited inbreeding depression, and male eyespan also decreased significantly more than female eyespan. However, the decline in male eyespan and female

eyespan were both fully explained by the decline in body length. In contrast, Bellamy *et al*. (2013) applied repeated genetic stress through eleven generations of inbreeding, which led to a significant decrease in male eyespan relative to other non-sexual traits (including female eyespan) that could not be explained by changes in body size. Bellamy *et al*. (2013) also used a cross protocol to produce outbred flies, and observed distinct heterosis in male eyespan relative to non-sexual traits and the female homologs of these non-sexual traits (but not relative to the female homolog of male eyespan). This provides clear evidence of heightened genetic condition-dependence of male eyespan. However, it is notable that both studies were conducted in benign laboratory conditions. No studies have looked at trait responses to explicit genetic stress in stressful environments, or at the shape of across-environmental genetic condition dependence in Diopsids.

Nonetheless, consistent with the theoretical predictions of genetic condition-dependent sexual selection, male eyespan has been found to be characterised by high levels of additive genetic variance. In both *T. dalmanni* and *Teleopsis whitei* the additive genetic variance in male eyespan is over twenty times greater than that associated with the nonsexual trait, thorax width (Meier & Baker 2002). Additionally, the additive genetic variance observed in a monomorphic *Teleopsis quinqueguttata* is three times less than that of its sexually dimorphic relatives (Meier and Baker 2002). When compared to female eyespan, the eyespan of male *T. dalmanni* and *T. whitei* has treble the additive genetic variance (Wilkinson & Taper 1999). While the genes that contribute to the genetic variance in eyespan have not been fully identified, there is evidence to suggest that the X chromosome has significant influence upon male relative eyespan. In *T. dalmanni* artificial selection for large and small male eyespan-to-thorax ratios resulted in strong

bidirectional trait changes (Wilkinson 1993). And, a large proportion (~ 25%) of the variation between these selected lines has since been shown to be accounted for by the X chromosome (Wolfenbarger & Wilkinson 2001; Johns *et al.* 2005).

All these studies, taken as one, show that hypercephaly is a complex polygenic trait with a strong association with condition that may have arisen prior to the evolution of female preference for hypercephaly; as demonstrated by the presence of a heightened condition-dependence of eyespan relative to wing length in the monomorphic species *S. beccari* (Cotton *et al*. 2004c). The observation raises the intriguing possibility that the reason female mate preference for hypercephalic males has evolved so often is that hypercephaly itself reflects male quality: that is 'pre-adapted' to heightened condition dependence, and thus to a role as an honest indicator. Irrespective, the studies show that in dimorphic species the male eyespan and relative (thorax controlled) eyespan reveal information about environmental and genetic condition in a heightened manner relative to analogous nonsexual traits: with clear consequences for the benefits of female mate choice.

1.8.3. *Female Mate Preference for Male Eyespan*

An initial selective advantage associated with hypercephaly would provide a basis for female choice, leading to the eventual exaggeration of male eyespan beyond that which is naturally selected for. Thus far the majority of the experimental work in stalk-eyed flies has used the Asian species *T. dalmanni* and *T. whitei.* As touched on earlier, in natural populations the males and females form nocturnal aggregations on exposed root hairs overhanging the tangled banks of rainforest streams (Burkhardt and de la Motte, 1985, Wilkinson and Reillo, 1994, Wilkinson and Dodson, 1997). Arriving shortly before dusk, males compete to

control the best sites, the contests frequently won by the male with the largest eyespan (Burkhardt & de la Motte 1983; Panhuis & Wilkinson 1999; Small *et al.* 2009). Females arrive shortly afterwards, and preferentially roost and mate with the largest eyespan males (Wilkinson and Reillo, 1994; Wilkinson and Dodson, 1997 Hingle *et al*. 2001b, Cotton *et al*. 2010). After the females land, the males continue to patrol their leks both to mate with the newly arrived females, and to fight off rival males (J. Howie, *pers. obs*.). At this point, some smaller males will avoid detection and thus 'sneak' mate. The females will also move between lek sites to obtain mates, and larger females will be able to mate with larger males (J. Howie, unpublished data; the larger males often ignore the solicitations of smaller females). Male mate choice for females is also known to exist (Cotton *et al.* 2014). Per lek, the mixed-sex aggregations can contain 1 - 4 males and have been observed to contain up to 24 females (although this is likely not the biological maximum). Irrespective, males will typically mate with all the females on their lek, so the number of females present is a good indicator of male reproductive success (Lorch *et al.* 1993; Burkhardt *et al.* 1994). More than 90% of copulations occur at dawn (with a number more completed at dusk), and individual males have been reported to mate up to 40 times per day (Burkhardt *et al*. 1994). In such root hair scenarios, females experience males in a sequential manner, although simultaneous situations will occur. Likewise, however, in the African *D. fasciata*, in which 'leks' occur on leaves, males have also been shown to be overdispersed, while females are distributed randomly between leaves, as males also fight off other males in this species (Wilkinson & Dobson 2007). Hence, sequential mate encounter seems to be a common feature across multiple species of stalk-eyed fly.

In the laboratory, female preference for large eyespan males is well documented

(Wilkinson & Reillo 1994, Wilkinson *et al.* 1998, Hingle *et al.* 2001, Cotton *et al.* 2006). The majority of early studies used a choice-based experimental design, where females are given two different male options to choose between. For instance, female *T. whitei* that were presented with 'dummy' males (dead males with artificially lengthened eyestalks) tended to roost with larger males (Burkhardt & de la Motte 1988). Wilkinson and Reillo (1994) also demonstrated a genetic association between female choice and male eyespan by subjecting flies to thirteen generations of selection for large or small eyespan to body length ratios. When presented with a choice between either a large or small male, females from the large lines and a non-selected control population chose to roost with large eyespan males. In contrast, females from small eyespan lines chose to roost with small males more often than females from the unselected population. As selection was restricted to male eyespan, the observed change in female mate preference was attributed to a genetic correlation between male eyespan and female mate preference (Wilkinson & Reillo 1994). And, as female mate choice (a binary measure of preference) was measured on non-selected (wild type) males, the result is unlikely to have arisen as a response to kin or 'same-group' recognition (that is, the females in large eyespan lines are unlikely to have recognised large males as 'kin' or 'same group', and vice versa, unless this was based on eyespan).

The studies described above demonstrate female mate preference for male eyespan based on the female choice to roost with larger eyespan males. Further to these studies, simultaneous choice tests (where females were presented with two males) have then shown that larger eyespan males also obtain a higher proportion of copulations than do smaller eyespan males in *T. dalmanni* and *T. whitei,* but not in the related monomorphic species *T. quinqueguttata* (Wilkinson *et al.* 1998). The

results also hold after controlling for body size. However, female ability to accurately discriminate between similarly sized males is limited (Hingle *et al.* 2001b). Based on the proportion of copulations received by large and small eyespan males, Hingle *et al*. (2001b) showed that *T. dalmanni* females were able to distinguish between males when the difference in male eyespan was large (mean difference = 3.17mm), but not when the difference was intermediate (2.40mm) or small (1.45mm). However, in the intermediate category, the ability to distinguish different eyespan males covaried with female eyespan. That is, the proportion of copulations with large eyespan males was significantly greater for large eyespan females (> 6.00mm) than for females with smaller eyespans (< 5.75mm). This implies that female mate preference could vary with condition (which is associated with size body size); potentially, as noted before, due to increased visual acuity. In line with this, Hingle *et al*. (2001b) went on to show that female preference in *T. dalmanni* does indeed vary with condition. Adult females maintained on a high quality diet (sweet corn) mated more frequently with large eyespan males, but mated at random when the diet was switched to a low quality food type (sucrose). This pattern was shown to be reversible, and adds weight to the idea that female mate preference, like male eyespan, is condition-dependent.

An issue with the use of species such as *T. dalmanni* and *T. whitei is* that the females do not exhibit a clear rejection response to unwanted suitors. Hence it is difficult to discern male versus female effects in the recorded mate choices (or, to do so requires the use of less direct associated measures). Given this, recent studies of female mate preference have utilised the African species of stalk-eyed fly, *Diasemopsis meigenii*. While little is known of the natural history of this species – but see the information in "Box 1 – *Diasemopsis meigenii*", below – an advantage

to the use of this species is that the females exhibit a clear rejection response towards unwanted suitors through the vigorous shaking of their abdomens and through the extension of their ovipositors (Kotrba 1996). Hence, it is possible to present males sequentially (as is the likely experience of females that arrive on a lek site of individual, male-controlled leaves, or of those that encounter individual males), to record clear-cut responses, and to assay the mating responses of a single female to a full range of male ornament phenotypes, rather than to use binary comparisons based on roost behaviour (Cotton *et al.* 2006a; Small 2009).

Box 1: The Natural History of, *Diasemopsis meigenii* [Westwood, 1837]

As a brief aside, it is worth noting what is known about *D. meigenii*. *D. meigenii* is part of one of four genera in the genera-group "*Diasemopsis"*. However, it is an "aberrant" [that is, 'unusual'] *Diasemopsis*, which has led some to prefer the older "*Chaetodiopsis*" clade name; with this *Chaetodiopsis* type included as a novel genera, embedded within the wider *Diasemopsis* genera-group (Feijen in prep.). Little is known of the natural behaviour of this species. However, it is known to be one of the commonest Diopsids in sub-Saharan Africa, with a range that spans at least North and South Africa, and extends as far as the Arabian Peninsula (Feijen, in prep.). The vast scale of this area means that it has been sighted near towns, rice fields, streams, rivers, lakes, in mountainous areas (Descamps 1957) and in secondary rainforests (Feijen, in prep.). Most observations are of individuals or small clusters of individuals, often on rocks or on the forest floor

(Descamps 1957; Feijen, in prep.). Small clusters have been observed in the wild on the leaves of plants (similar to the leks that have been observed in the related *D. fasciata*, Wilkinson & Dodson 1997), as has a larger cluster of 20-50 individuals (in 2011, Uganda, in secondary rain forest, H. Feijen, pers. comm.); as well as several mid-size clusters, in the botanic gardens and fruit markets of Maputo, as well as at the waterfall near Namaacha (H. Feijen, pers. comm.). It is also known that small clusters can be induced in a laboratory, on the leaves of plants; and that males and females will fight with each other upon encountering one another on these leaves (J. Howie, pers. obs.). This species is also known to feed off rotten fruit and vegetation, and is more attracted to fruit than are related species (H. Feijen, pers. comm). Mating behaviour has not, to the author's knowledge, been observed in the wild. However, under laboratory conditions, mating takes place at dawn and dusk (artificial light) as a 'scramble': individual males attempt to mate with individual females, and are either accepted, or rejected by the female. In these situations, it is common for both males and females to mate multiple times, often with multiple individuals of the opposite sex (J. Howie, pers. obs).

Given this, Cotton *et al*. (2006) were able to use a repeated sequential no-choice assay of female mate responses to a full range of male phenotypes to show that the strength of female preference is related to female eyespan; a trait that is in part dependent on larval stress (Bellamy *et al*. 2013; and see Chapter 3 of this thesis). Large eyespan females tended to mate more frequently with larger eyespan males and tended to reject smaller eyespan males. The smaller eyespan females rejected

males at random. The results show that there is a potential role for condition (or body size) on overall female choice outcomes, either due to a direct effect on female choosiness or female preference functions, or due to an effect on discrimination (via increased visual acuity). A further experiment has shown that (as in *T. dalmanni*) female preference is also condition-dependent in response to adult nutritional stress (Small 2009). Females maintained on a full diet of sweet corn accepted large eyespan males and rejected small eyespan males more often than did females reared on a low quality, sucrose, diet. These experiments confirm that the strength of sexual selection on male eyespan due to female mate choice in both *T. dalmanni* and *D. meigenii* depends upon the condition of females. Nonetheless, it is notable that in each case, the males and females were virgin. So it is unclear whether similar results would hold in a natural, mated context. And there remain further open questions about the effects of interactions between female reproductive context and condition on components of female preference (choosiness, preference functions), about the interactions between different types of environmental and genetic condition on these, and about the broad stroke and finer resolution details of the genetical basis of these aspects of female preference.

1.8.4. *Sperm Limitation and Reproductive Investment in Stalk-Eyed Flies*

Relatively little is known about the benefits that female stalk-eyed flies gain from mate preference. As expounded above, a potential range of benefits are those related to genetic quality (higher quality outcross males have larger eyespans, Bellamy *et al*. 2013). As male eyespan is at least in part heritable, there are also potential indirect benefits for female choice based on the Fisherian idea of 'sexy sons'. However, it is also possible that the benefits of mate choice are more direct. In stalk-eyed flies, males provide only sperm, accessory products and a

spermatophore when they mate (Kotrba 1996). They do not provide nuptial gifts or parental support. The spermatophore itself is also ejected after copulation (within a few hours, Kotrba 1996). But, while sperm is often viewed to be an unlimited resource, this is not always the case. If males are limited in their sperm reserves, this can potentially lead to a situation in which females are sperm limited (a common feature of stalk-eyed flies; for references see below). Where sperm is limited it then has the potential to influence female mate choice, because both the direct fertility benefits to mate choice and the related costs (to females) of mate rejection will be increased (especially in the virgin state or when not recently mated, after the number of sperm in storage has declined, Kokko & Mappes 2005).

A potential scenario then is that male eyespan reveals information about reproductive quality or fertility. Variation in fertility is an important component of fitness in females. In the field, only ~ 55% of female *T. dalmanni* eggs are fertilised (Cotton *et al.* 2010) and laboratory studies have shown that females also need to mate multiply to achieve and maintain high fertility in both *T. dalmanni* (Baker *et al.* 2001a) and *D. meigenii* (Small 2009). This is probably in part because of the small size of the male spermatophore (Kotrba 1996; Rogers *et al.* 2006) and the small number of sperm stored after a single copulation in *T. dalmanni* (about 35, Wilkinson *et al.* 2005; Rogers *et al.* 2006). It is a problem that is compounded in field populations due to X-linked meiotic drive. Up to 25% of *T. dalmanni* males carry a 'drive' allele on the X-chromosome that kills rival sperm bearing the Y-chromosome (Presgraves *et al.* 1997). The X chromosome is now also known to be highly masculinised (Baker *et al.* 2016). Females mated with meiotic drive carrying males suffer from reduced fertility (Wilkinson & Fry 2001; Wilkinson & Sanchez 2001; Wilkinson *et al.* 2006). However, this is less likely the case in *D.*

meigenii, as drive has not been observed, as the spermatophores are larger, and as a far larger number of sperm are stored post copula (~150 - 300, J. Howie, *pers. obs*.).

When isolated from mating opportunities, female fertility declines significantly over time in both *T. dalmanni* (Cotton *et al*. 2010) and *D. meigenii* (Small, 2009) as females use up their limited supplies of stored sperm (in *D. meigenii* this occurs across a 2-3 week phase, with rapid loss across the first 2-5 days J. Howie, unpublished data). Hence, the extent to which male eyespan relates to reproductive output could be important for females. Male reproductive quality is positively associated with the size of accessory glands and testes. The size of male accessory glands in *T. dalmanni* is genetically (Rogers *et al.* 2005a) and phenotypically associated with male mating frequency (Baker *et al.* 2003; Rogers *et al.* 2005b). Testes size is also positively associated with the number of sperm stored in a female's spermathecae following copulation (Fry 2006) and is therefore likely to affect female fertility. As male eyespan is a key predictor of male reproductive organ length (Rogers *et al.* 2008), sperm-limited females may well select large eyespan males based upon their fertilisation potential. In support of this hypothesis is the finding that female *T. dalmanni* housed with large eyespan males have higher fertility than those housed with small eyespan males (Rogers *et al.* 2008). In *D. meigenii*, testes size and accessory gland size are also related to the size of a male's eyespan (Harley *et al.* 2013). However, as a result of male ejaculate allocation strategies, the benefits for females are not necessarily straightforward.

Recent studies conducted by Harley *et al*. (2013) in *D. meigenii* showed that males allocate ejaculate (spermatophore size and area of sperm per spermatophore) based on female size. But smaller males provide as much sperm in a single mating

as do larger males. This implies either that larger males reduce their allocation in expectation of a greater number of mating opportunities (as they are more attractive) or that smaller males are able to increase their allocation in a single mating as they expect fewer future mating opportunities (and thus provide a similar total output, but a larger output relative to their reserves). In this case, a female cannot obtain information about sperm loads based on male eyespan size. However, in an unpublished follow up study, Harley (2013) then looked at male ejaculate allocation across multiple (three) consecutive matings. The result of this study was that, on the first mating, both large and small males allocated a similar size spermatophore and a similar number of sperm (per spermatophore) to a large eyespan female. However, after the second and third mating, the size of the spermatophore and amount of sperm provided by the smaller males had dropped significantly below that of the larger eyespan males. The result provides insight into male ejaculate strategies. It shows that females could become sperm limited due to a limited male resource (as the amount of sperm provided to a female declines across multiple matings), but it also shows that in a multiple male-female, multiple mating scenario, a female is likely able to obtain information about direct reproductive and fertility benefits in relation to the size of a male's eyespan – as larger eyespan males provide more sperm across a larger number of matings (this backs up, in part, the results of a theoretical model by Tazzyman *et al*. 2009).

In summary, stalk-eyed flies are a useful model for the study of sexual selection. As such, their use in empirical experiments allows for some of the many issues raised throughout this introduction to be addressed in the three empirical chapters that constitute the rest of this thesis. An overview of this thesis is now provided:

1.9 OVERVIEW OF THESIS CHAPTERS

The rest of this thesis comprises of three 'results' chapters, followed by a 'General Discussion' of the main findings. In each chapter, the work is conducted using the African stalk-eyed fly species, *Diasemopsis meigenii*. I focus first on the variation in female mate choice that is associated with female mating status. I then move on to look at the across-environmental pattern of genetic condition dependence in the male sexual ornament (male eyespan). Finally, I take a similar look at the condition dependence of male reproductive and fertility traits, and I also test the integration of trait responses to variation in environmental and genetic condition for ornamental, and reproductive, and fertility classes of traits. The work here presented was carried out under the supervision of Professor Kevin Fowler and Professor Andrew Pomiankowski. The author designed, performed, analysed and interpreted all of the experiments henceforth presented.

1.9.1 Chapter 2. **Mating status affects components of female mating behaviour and sexual selection in the stalk-eyed fly, *Diasemopsis meigenii*.**

Female preference is a crucial driver of intersexual selection, and causes the evolution of the diverse ornamental traits seen in nature. The last two decades have seen an explosion in the literature on the shape of preference, its variation between individuals, and its delineation into different components. Yet little is known about the way that the different components such as choosiness and the preference function respond to different factors, and interact to influence the series' of mate choices and that drive sexual selection. Both the reliability – and applicability – of what *is* known is also questionable. In part this is because

preference is difficult to measure. In part it is because studies have tended to use mating status controls to increase measurement accuracy – for instance, via the use of virgins. However, virgins are rare in nature, and are likely to behave differently to mated females due to variation in the reproductive costs of mate choice (that is, it costs more for a virgin to say, "no"). So, it is important to look at the effect of female mating status on individual components of preference as well as selection. To address this, I conduct two related studies on the stalk-eyed fly, *Diasemopsis meigenii*. First, I manipulate female mating status (virgin/mated). I then assay female mating responses to sequentially presented males of two or five ornament size classes. I use mathematical and statistical techniques to decompose these responses into estimates of choosiness, the preference function and selection. I show that virgin females are less choosy, have similar preference functions, and exert less selection than mated females. The results have clear implications for our understanding of sexual selection and its operation in nature.

1.9.2 Chapter 3. **Environmental variation can amplify or mask the signal of genetic condition in sexual ornaments, in stalk-eyed flies.**

Next I move on to the male ornament. An important area of sexual selection that is unresolved is the nature of the indirect genetic benefits that females obtain via mate choice. Male sexual ornaments can reveal information about both environmental and genetic condition in a heightened manner relative to nonsexual traits. But how does environmental variation alter the genetic condition dependence of these ornaments; does it enhance or mask the genetic signal? No studies have been conducted that can answer this question. To address this, I use three levels of larval diet and exploit a series of crosses within and

between a set of 17 inbred lines to directly investigate the environmental, genetic and gene-by-environmental (G x E) condition dependence of an exaggerated sexual ornament, male eyespan, relative to nonsexual traits, in *D. meigenii*. To do so, I measure a set of traits: male and female thorax length, eyespan, and wing length, as well as male testes length and accessory gland length. I then contrast the environmental, genetic and G x E responses of these traits. I find evidence for heightened condition dependence of the sexual trait (male eyespan), for a shared genetic basis with the analogous female trait (female eyespan), and for a novel G x E interaction on sexual ornament expression, in which the effects of genetic stress on relative trait size are masked in both high and low, but not intermediate, quality environments. I also find evidence for the integration of environmental, genetic and gene-by-environmental condition dependence of the sexual and nonsexual traits. As in Chapter 2, the results have consequences for our understanding of sexual selection under the variable conditions that exist in nature.

1.9.3 Chapter 4. **Environmental and genetic condition dependence of pre- and post-copulatory reproductive traits in the stalk-eyed fly, *Diasemopsis meigenii*.**

Finally, I investigate these environmental, genetic (and G x E) condition responses in a set of important reproductive and fertility traits. These traits are often under direct selection, similar to that experienced by secondary sexual traits. Due to this, such pre- and post-copulatory traits can be expected to covary with either environmental or genetic condition, and to integrate their responses to these factors. In contrast, life-history resource allocation trade-offs can also be expected to lead to a lack of integration, or to negative covariation – as could also arise due

to the present-future ejaculate allocation trade-offs that are predicted by sperm competition theory. The extent to which trait responses to environmental and genetic condition are integrated, and the extent to which trait responses do or do not covary has important consequences for sexual selection: both due to the direct selection on males and to the indirect and direct benefits that females can obtain via mate choice. Yet – despite recent work on the pairwise phenotypic and genetic correlations between pre- and post-copulatory (ornamental and reproductive) traits – the full coordination of pre- and post copulatory trait responses to variation in environmental and genetic condition has not been studied. To address this, I use a set of crosses within and between 17 inbred lines to generate explicitly low (incross) and high (outcross) condition individual *D. meigenii*, as in Chapter 3. I use larval diet manipulation to exert three levels of explicit environmental stress, also as in Chapter 3. I then measure the responses of a suit of set of pre- and post-copulatory male traits (related to attractiveness and fertility): that is, male thorax length, eyespan, wing length; male testes length and accessory gland length; male spermatophore size and the area of sperm within it; the number of sperm a male transfers to a female over three days, and the resultant number of offspring; male latency to mate, and mating length; and the number of mating attempts that a male made prior to acceptance by a female (inverse 'attractiveness'). I look at the overall responses, at the relationships between each trait and the ornamental trait (male eyespan), and at the across-trait integration of responses to variation in environmental and genetic condition, or stress. I find evidence of a broad qualitative alignment in trait responses, but a negative integration across traits (most traits increase in with condition, but those that respond most to genetic condition respond least to environmental condition, and vice versa). I also find

evidence for variation in the relationship between ornamental and reproductive traits across environments. As in each prior chapter, the results have important consequences for our understanding of the operation of sexual selection in nature.

1.9.4 Chapter 5. **General Discussion**

Here, I draw out the key results of chapters 2, 3 and 4, tie these together, and place them in a wider historic and theoretical context. I also provide direction for several lines of future experimentation that will likely produce profitable insights.

1.9.5 Appendix 1 a – c: Supplementary Information

Finally, I attach the Supplementary Information for chapters 2, 3 and 4 in turn. In each, I present the model output tables for GLM, GLMM and GLME models. I also provide extended methods, results and discussion where this is relevant.

1.9.6 Appendix 2 – Female Sneak Copulation

This is the text for a book chapter that I wrote with A. Pomiankowski as I corrected this PhD. It is in press at The Encyclopedia of Evolutionary Psychology.

1.9.7 Appendix 3 – Evolution: Sex or Survival

This is a re-print of an article that I wrote during my PhD, published in Current Biology (2013), with A. Cotton and A. Pomiankowski.

CHAPTER 2

Mating status affects components of female mating behaviour and sexual selection in the stalk-eyed fly, *Diasemopsis meigenii*

A B S T R A C T

Female preference is a crucial driver of intersexual selection, and causes the evolution of the diverse ornamental traits seen in nature. Yet little is known about the way that its different components, such as choosiness and the preference function, respond to different factors, and interact to influence sexual selection. In part this is because preference is difficult to measure. Also, studies have tended to standardise female mating statuses to increase the accuracy of female choice measurements – for instance, using virgins. Yet, virgins are rare in nature, and are likely to behave differently to mated females due to their greater reproductive cost of mate choice. Indeed, it is possible that virgin studies will underestimate choosiness and selection, lower the resolution of estimates of the preference function, and increase the variation in preference function estimates within and between females. To test these hypotheses, I conduct a pair of studies on the stalk-eyed fly, *Diasemopsis meigenii*. I manipulate female mating status (virgin/mated) and assay female mating responses to sequentially presented males drawn from two or five ornament size classes. I use mathematical and statistical techniques to deconstruct these responses into individual level estimates of choosiness, the preference function and selection. I show that, when virgins are assayed, choosiness is underestimated, variation in the preference slope is overestimated, and sexual selection is underestimated. I show that simple 2-size and complex 5-size techniques can provide this insight. I also provide the first description of a full preference function in a stalk-eyed fly and show that, while robust to variation in female mating status, its estimation is less precise in virgin females. I conclude that the use of virgins in the study of female mating preferences should be treated with caution, and I provide directions for future studies in this and related species.

2.1 INTRODUCTION

Female preference is a crucial driver of intersexual selection, and causes the evolution of the diverse ornamental traits seen in nature (Andersson 1994). The last two decades have seen an explosion of literature attempting to better define the intricacies of preference, including the shape of the preference function (Ritchie 1996; Ritchie *et al.* 2001; McGuigan *et al.* 2008), its variation between individuals (Bakker & Pomiankowski 1995; Wagner *et al.* 1995; Ritchie *et al.* 2005; Cotton *et al.* 2006a), its plasticity within individuals (Qvarnström *et al.* 2000; Coleman *et al.* 2004; Fowler-Finn & Rodríguez 2012; Rodríguez *et al.* 2013; Tinghitella 2014), and its delineation into different components that contribute to female mate choice (reviewed in Edward 2015). Yet little is known about the way that these elements of preference respond to different factors, or how they interact to influence sexual selection (Judge *et al.* 2014). In part, this is because preference is difficult to measure. However, the current understanding of preference is also limited because, in an effort to standardise the measurement of female mating responses, the mating status of females is often controlled via the use of virgin or singly mated focal females. This fails to take account of the fact that variation in female mating status is likely to have a large effect on the relative costs of mate choice and the expression of underlying preferences in the mate choices recorded.

Female preference is complex (Heisler *et al.* 1987). The precise nature and definition of its constituent parts are much debated (Edward 2015). A range of terms – such as 'tolerance', 'discrimination', 'permissiveness', 'receptivity', and 'selectiveness' (reviewed in Edward 2015) – exist in the literature, and overlap, both with each other, and with terms used in other related fields. But, despite this

profusion of terminology, the basis of a female mate preference can be broken down into two main components: choosiness, and the preference function (Jennions & Petrie 1997; Widemo & Sæther 1999; Cotton *et al.* 2006b). The former relates to the effort used to assess mates prior to acceptance, the latter to the order or rank of mate phenotypes (Jennions & Petrie 1997). Both factors can be complex, multivariate traits. But the concept can be explained via a simple univariate analogy. Here choosiness can be considered as the vertical displacement and/or 'flatness' of a curve of mating responses to different male phenotypes (are males accepted more, or less, overall, irrespective of their ornamentation), while the preference function can be considered as the slope, shape and horizontal displacement of the same. These key attributes of the curve are sometimes termed the 'peak', 'tolerance', 'responsiveness', and 'strength' of the preference function (see Figure 2,in the *General Introduction* for further details).

As mate choice is costly (Pomiankowski 1987; Reynolds & Gross 1990), choosiness and preference function are expected to vary with both context and condition (Jennions & Petrie 1997; Widemo & Sæther 1999). In the main, choosiness is expected to vary with external factors that alter the relative costs of mating (Kokko & Monaghan 2001; Bleu *et al.* 2012) or not mating (De Jong & Sabelis 1991; Kokko & Mappes 2005; Lynch *et al.* 2005) – including variables such as density (Arnqvist 1992), sex ratio (Berglund 1994; Holveck *et al.* 2015), or social structure (Fowler-Finn & Rodriguez 2012; Bailey & Macleod 2014) – that can influence mate encounter rates and the costs of forgoing a mate (Kokko & Mappes 2005). In contrast, the preference function is expected to vary with 'internal' qualities such as environmental (Gray 1999; Hingle *et al.* 2001a; Hunt *et*

al. 2005a; Cotton *et al.* 2006a; Holveck & Riebel 2010; Holveck *et al.* 2011) or genetic condition (modelled in Tomlinson & O'Donald 1996; reviewed in Cotton *et al.* 2006b) that alter the relative costs of preference for higher quality, optimal or compatible mates (Tregenza & Wedell 2000; Qvarnström 2001; Badyaev & Qvarnström 2002); but see Syriatowicz and Brooks (2004) for a rare example of the effects of condition on choosiness. The two components are then expected to interact – after limitation by the range of mates available to choose between (Andersson 1994), and by the physiological (Burkhardt & de la Motte 1983; Hingle *et al.* 2001b; Secondi *et al.* 2015) and neurological (Buschbeck & Hoy 1998) limits to discrimination between these mates – to lead to the series of mate choices that exert selection on the male secondary sexual trait (Heisler *et al.* 1987). Hence the inherent difficulties in the delimitation and measurement of female mate preference, its components, and the resultant selection, are compounded by this variability (Wagner 1998; Gibson & Langen 1996; Chenoweth & Blows 2006).

To enable accurate measurement and quantification of the different components of female mate preference, empiricists have tried to control variation in context and condition. A common approach has been to use virgin females, despite their rarity in natural populations (Bateman 1948; Burns 1968; Trivers 1972; Burkhardt & de la Motte 1988), to control for variation in mating status (e.g.Ritchie 1996; Rosenqvist & Houde 1997; Cotton *et al.* 2006a; Fowler-Finn & Rodriguez 2012; Judge *et al.* 2014; Tinghitella 2014; critiqued in: Peretti & Carrera 2005). However, the use of virgins rather than mated females could also have important implications for the measurement of preference. For instance, as the reproductive costs of the choice to forgo a mate are higher for virgins (Jennions & Petrie 2000;

Kokko & Mappes 2005), choosiness could be underestimated if virgin females are used in preference assays, as a virgin female may be less likely to reject a suitor. Furthermore, while individual preference functions may not be affected by mating status, if choosiness is low *and* experimental resolution limited, then estimation of the slopes of preference functions could be compromised in relation to stochastic measurement error (as most females will accept most males, and differentiation will be near random). The use of virgins could thus have an important impact on our inferences about the strength (Gomulkiewicz 1991; Judge 2010), direction (Turner & Burrows 1995) and type (Ritchie 2007) of sexual selection that we expect to see in nature. This has knock-on consequences for the estimation of the heritability and within individual variation of preference, and so on the inferences to be drawn about the mechanisms underpinning sexual selection.

Various studies have been conducted on the effects of mating experience on choosiness (Peretti & Carrera 2005; Bailey & Zuk 2008; Rebar *et al.* 2011; Bailey & Macleod 2014; Stoffer & Uetz 2015), the direction of preference (Hebets 2003; Hebets & Vink 2007), choosiness and the direction of preference (Bateman *et al.* 2001; Lynch *et al.* 2005; Uetz & Norton 2007), choosiness and the preference function (Fowler-Finn & Rodriguez 2012), complex, multiple stage preference (Qvarnström *et al.* 2000), multivariate preference (Qvarnström *et al.* 2000; Gershman *et al.* 2014) and resulting sexual selection on males (Judge 2010; Gershman *et al.* 2014). These studies show the expected increase in choosiness with experience, as well as a relatively limited effect on preference. But, only six involve direct contrasts of virgins and non-virgins (Bateman *et al.* 2001; Lynch *et al.* 2005; Peretti & Carrera 2005; Uetz & Norton 2007; Judge 2010; Gershman *et*

al. 2014). Of these, only one considers the slope of the preference function (Gershman *et al.* 2014), and none consider the shape of the preference function. Furthermore, only two consider selection: the first without consideration of either choosiness or the preference function (Judge 2010), and the second based on a design where male and female effects cannot be separated (Gershman *et al.* 2014). (Yet an understanding of the responses of each component is required to understand in full the effects of female mating status variation on sexual selection).

These six studies can also be criticised in relation either to sample size or weak experimental assay design. For reliable estimates of preference variation, several important factors must be taken into account. First, it is better to use direct rather than indirect measures of choice, for example, to directly observe choice rather than infer it from measurements of the association time between males and females (Shackleton *et al.* 2005; Cotton *et al.* 2006a; Reinhold & Schielzeth 2015). A clear relation from indirect measures to choice cannot always be found (Gabor 1999). Second, it is preferable to use a 'no-choice' rather than a 'choice' test (that is, to provide sequential rather than simultaneous choices). 'Choice' tests can inflate estimates of preference and increase the risk of overestimation or type I errors, because of the lower costs of rejecting worse males (Dougherty & Shuker 2015). Finally, the level of the assay is critical. It is best to use individual level estimates of preference where possible – such as those that require repeated sequential sampling of individuals – rather than population level estimates (Wagner 1998). The former may be more difficult to obtain, but permit a more accurate characterisation of preference components, and provide a basis to examine both the levels of inter- and intra- female variation (Cotton *et al.* 2006a).

In order to be able to evaluate the shape of the preference function, assays must also include two further design elements. First, they must record female mating responses to a wide range of male phenotypes, because simple studies that use only two stimulus males have a low power to resolve individual differences in preference (Wagner 1998) and are unable to accurately measure the strength or shape of selection; for example, that based on directional (Cotton *et al.* 2006a), stabilising (e.g. Sappington & Taylor 1990; Greene *et al.* 2000) or disruptive preference functions (e.g. Gerhardt 1991; Ritchie 1996; Hunt *et al.* 2005b). Second, they must also isolate female mating decisions from the influence of male effects, such as those related to male mate choice (e.g. Amundsen & Forsgren 2001; Chenoweth *et al.* 2007; Myhre *et al.* 2012; Cotton *et al.* 2015), male mating ability (e.g. Rogers *et al.* 2005) and factors such as male competition, domination, or forced copulation (e.g. Cordero 1999; Dukas & Jongsma 2012). If such factors cannot be removed, then male effects may alter the outcomes of female choices, and could thus, in turn, invalidate measures of preference (Cotton *et al.* 2006a).

To test how female mating status effects components of preference and selection, and to explore the benefits and limitations of a simpler two-male-size and more complex five-male-size preference test, I conduct here a pair of studies using the sexually dimorphic African stalk-eyed fly species, *Diasemopsis meigenii* (previously, *Chaetodiopsis meigenii* Baker *et al*. 2001). Stalk-eyed flies (Diptera: Diopsidae) are characterised by the lateral displacement of their eyes on elongated stalks in both sexes (Wilkinson & Dodson 1997), and are a valuable model for the study of sexual selection (Wilkinson & Dodson 1997; Wilkinson 2001). Females exhibit

preferences for males with larger relative eyespans (Burkhardt & de la Motte 1988; Wilkinson & Reillo 1994; Wilkinson *et al.* 1998a; Hingle *et al.* 2001a, b; Cotton *et al.* 2006a) associated with direct benefits (David *et al.* 1998; Cotton *et al.* 2004a, b; Rogers *et al.* 2008) and indirect genetic benefits (Wilkinson *et al.* 1998b; Knell *et al.* 1999; David *et al.* 2000; Cotton *et al.* 2010; Bellamy *et al.* 2013; Cotton *et al.* 2014). Female preference varies with condition (Hingle *et al.* 2001a) and size (Cotton *et al.* 2006a). The capacity of females to discriminate between males also varies with eyespan, itself dependent in part upon condition (Burkhardt & de la Motte 1983; Hingle *et al.* 2001b). Moreover, the species here used, *D. meigenii,* is especially suited to studies of mate choice, as females exhibit an unequivocal rejection response to the mating attempts of undesired suitors (Cotton *et al.* 2006a).

The first explicit investigation of individual level female preference in a stalk-eyed fly was conducted using this species, and reported covariation in preference with female size (Cotton *et al.* 2006a). This study used virgin females to control for variation in female mating status (mating interrupted prior to male ejaculation). Here, I build on this work, and examine the effects of differences in female mating status, virgin versus mated, on the mating preference of individual female *D. meigenii*. I record female mating responses to the sequential presentation of a variety of male eyespan phenotypes in a pair of related experiments that assayed two and five male phenotypes, respectively. I then derive estimates of choosiness, the slope of the preference function, and the selection exerted by choice. I also use statistical models to visualise preference functions and investigate their shapes and peaks at low resolution via the use of *post-hoc* interrogative tests. Finally, I contrast the levels of variation in each component of preference and selection, and ask how each aspect of preference varies between individuals and across time.

2.2 MATERIALS AND METHODS

2.2.1 *Production of experimental flies*

The laboratory-adapted stock population of *D. meigenii* used in this study was collected in West Africa in 2000 by Sabine Hilger. A large population (> 200 individuals) was subsequently maintained in cage culture at 25°C on a 12 : 12 hour light : dark cycle, with flies fed twice weekly on puréed sweet corn mixed with a low dose of antifungal Nipagin. Artificial dawn and dusk periods were created via the phased illumination of two 58W, 'cool white', 1-10 V, dimming tube lights over a period of 15 minutes at the start and end of each light cycle.

Eggs were collected from stock cages on petri dishes lined with moist cotton pads containing 0.3 – 3.0 grams of puréed sweet corn. Variation in larval diet was used to produce a wide range in the size of eclosing flies (Cotton *et al.* 2004a). Two weeks after eclosion the flies were separated by sex and raised until sexual maturity (~ 8 weeks after pupation). All flies were maintained until this point (eclosion) and afterwards as described above for the stock population. All experimental flies were between 8 – 16 weeks old at the start of the experiment.

The eyespan (the distance between the outermost tips of the eyes; David *et al.* 1998) and thorax (the distance between the centre of the most posterior point of the head to the joint between the meta-thoracic legs and the thorax) of each fly was measured, to a tolerance of 0.01mm, using a video camera mounted on a monocular microscope and ImageJ image capture software (v. 1.46, Rasband 1997-2012; Abramoff *et al.* 2004; Schneider *et al.* 2012). Flies were anaesthetised on ice before measurement.

2.2.2 *Measurement of female preference*

2.2.2.1 *Two studies – but why?*

I conducted two related studies: each used the direct observation of female mating responses (accept versus reject) to sequentially presented males of different phenotypes, in a 'no-choice' design, to obtain individual level estimates of female preference. The studies differed in that the first used two male sizes across three days, while the second used five male sizes across three weeks. The reasons for the use of a two- and five-size test were two-fold. First, experimental studies are often proven incorrect over time (or have a 'half life', Ioannidis 2005). However, this is less often the case where a result is found in repeated tests that use different methods. Second, it is not always possible to use a complex multi-level preference assay – for instance, where a larger number of experimental blocks are required experimenter time can become a limit. Hence, it can be useful to test the relative limitations, overlap between, and benefits of simpler versus more complex studies.

2.2.2.2 *Two-size assay of female mate choice*

Individual virgin females (n = 196) and males (n = 302) were isolated in 500ml containers (with a moist cotton base and containing a single plastic food tray) at least two weeks prior to the preference observations. A dark blue paper lining was added to female pots so that eggs laid during the experiment could be easily counted. As female preference is positively associated with female eyespan (Cotton *et al.* 2006a), I used only large females (≥ 5.90 mm eyespan). Males were allocated to one of three eyespan categories: 6.40 - 7.20 (n = 58), 7.20 – 8.00 (n = 153) and 8.00 – 8.60 mm (n = 91) (the 'small' and 'large' males were used in the female preference assays while the 'intermediate' males were mated to the non-

virgin females). Females were allocated at random to one of two mating status categories (virgin, n = 81 or mated, n = 64). Virgin females were stored individually for two weeks prior to preference observations. Mated females were housed with two intermediate males (7.20 – 8.00 mm) over this period, with dead males replaced daily. Males were removed from the pots that housed the mated females at least 18 hours prior to the preference assays of those females.

To assay female mating preference, each female was then presented with two males per day, one large and one small, in random order, for three successive days; with males drawn randomly from within their eyespan categories. Males were used for one or two matings per day (this number depended on the availability of males). Acceptance and rejection were recorded, and mating was interrupted prior to the transfer of ejaculate by aspiration using a pooter. Acceptance was defined as the engagement of genitalia after a male mounted a female (Cotton *et al.* 2006a). Rejection was defined as the unambiguous and vigorous shaking of the female body, often accompanied by a copulation prohibiting extension of the ovipositor (Cotton *et al.* 2006a). As soon as the female's response was determined the male was removed. If no mating attempt was made after 15 minutes, a replacement male from the same size category was provided. All observations were conducted over the first three hours of each day.

After the assay phase was complete, males were re-added to the containers with the mated females and all females were maintained for a further week under similar conditions to those used during the pre-assay phase. Food and water were replenished twice weekly throughout the experiment, as were the blue paper

linings in the female containers. The number of eggs laid by each female was counted twice during the second and fourth weeks and once during the assay week to provide a measure of fecundity. The counts were derived from a minimum of three counts over 11 days, and a maximum of five counts over 18 days. Fecundity measures served to verify that the mated females had in fact mated.

2.2.2.3 *Five-size assay of female mate choice*

To build on the above a five-size test was conducted. Individual females (≥ 5.90 mm eyespans, n = 418) were isolated, assigned to mating categories (virgin, n = 161 or mated, n = 239), and housed during the first two weeks of the experiment as described above for the two-size assays. Males were allocated to one of five eyespan categories reflecting a full range of natural variation: 6.4 – 6.8 (n = 67), 6.8 – 7.2 (n = 101), 7.2 – 7.6 (n = 70), 7.6 – 8.0 (n = 66) and 8.0 – 8.4 (n = 164).

Female preference was then assayed over a three week period, with a single assay completed per week. In each assay, individual females were presented with a single male from each size category, one male per day, over five days. The order of presentation of size categories was randomised (for each set of five males), and males were drawn randomly from within their categories (individual males typically used for 1 – 3 matings per day). In order to maintain the integrity of the treatment categories, each of the mated females were housed with two intermediate males (7.20 – 8.00 mm) for a period of 48 hours between each weekly assay. During the assays, rejection and acceptance were recorded, and successful copulations were interrupted as described previously, for the two-size assays.

After the assay phase, females were housed as in the pre-assay phase for a further week. Food, water and blue paper linings were replaced twice weekly throughout the experiment. The number of eggs laid by each female was counted twice per week to provide a measure of fecundity. The counts were derived from a minimum of six counts over 11 days and a maximum of nine counts over 31 days.

2.2.3 ***Estimation of components of preference and selection***

The individual mating response data collected in the two- and the five-size assays was then used to derive per female estimates of choosiness, of the direction and slope of each preference function, and of the strength of selection exerted on the male ornament via each female's mating decisions (all individual level estimates).

2.2.3.1 *Two-size assay of female mate choices*

Rejection, R

An estimate for the level of choosiness was calculated per female as '*rejection*', R; that is, the proportion of males rejected by a female, regardless of male size. The values were calculated as

$$R = a/b \quad , \qquad [1]$$

where a represents the total number of males rejected by, and b the total number of males presented to a female.

Preference slope, P_1

An estimate of the slope and direction of each preference function was then calculated per female as the differential rejection of small versus large males, scaled via the number of males seen in total. The estimates of this '*preference slope*', P_1, were calculated as

$$P_1 = \frac{2(c-d)}{b} \quad , \qquad [2]$$

where c is the total number of small, and d the total number of large males rejected by a female. $P_1>0$ indicates preference for large male eyespan, and $P_1<0$ indicates preference for smaller male eyespan, with bounds $-1 \leq P_1 \leq 1$.

Selection, S_1

Finally, the strength and direction of the selection exerted on the male ornament by the female mating decisions was calculated per female as '*selection*', S_1, the proportion of accepted males that was large (as opposed to small), as

$$S_1 = e/f \quad , \qquad [3]$$

where e is the number of large males accepted, and f is the number of males accepted in total, large or small.

The expectation is that *rejection* will reveal a female's level of choosiness, while *preference slope* will reveal the strength or slope of an individual female's preference function (but will reveal no information about the shape). Given this, rejection

and preference slope were set up to be unrelated, except in cases where rejection is very low or very high, which will force neutral level preference slopes. In contrast, *selection* was set up to respond to both rejection and preference slope, so as to reflect variation in selection on the male ornament due both to the effects of female choosiness and the female preference function on a female's mate choices (for instance, the same *preference slope* with a larger *rejection* will lead to stronger *selection*, as the proportion of accepted males that are *large* will now be increased).

2.2.3.2 *Five-size assay of female mate choices*

The level of choosiness was estimated per female as '*rejection*', *R*, using equation 1 above, as for the two-size assay.

Preference slope, P_2

An estimate of the slope and direction of the preference function was calculated per female. In order for this to be broadly equivalent to P_1, it was calculated as

$$P_2 = \frac{\bar{X}_A - \bar{X}_P}{SD_{\max,\min}} \quad , \qquad [5]$$

where $\bar{X}_A$ is the mean eyespan of males accepted by a female, and $\bar{X}_P$ is the mean eyespan of all males presented to a female. $SD_{\max,\min}$ is the maximum or minimum value difference between $\bar{X}_A$ and $\bar{X}_P$ given the number of males accepted by a female and the distribution of eyespans amongst the males presented to the female (a female that sees several males with a narrower range or smaller mean will have a different maximum or minimum *SD* for a given rejection rate than will

a female that sees a wider range of males). The denominator forces bounds of -1 $\leq P_2 \leq 1$, with $P_2 > 0$ meaning female preference for larger male eyespan, and $P_2 <$ 0 meaning female preference for smaller male eyespan, equivalent to that of P_1.

To calculate $SD_{\max,\min}$, the males presented to a female were ranked by eyespan in descending (large to small, $X_{P[DS]}$) and ascending (small to large, $X_{P[AS]}$) order, and the theoretical maximum or minimum average eyespan that could have been accepted by the female, given the range of males actually presented, and the number accepted, was calculated as

$$T_{\max,\min} = \frac{\sum_{i=1}^{j} x_i X_{P[DS,AS]}}{j} \quad , \qquad [6]$$

where $T_{\max}$ was calculated using $\sum_{i=1}^{j} x_i X_{P[DS]}$, and $T_{\min}$ using $\sum_{i=1}^{j} x_i X_{P[AS]}$, where $\sum_{i=1}^{j} x_i X_{P[DS]}$ and $\sum_{i=1}^{j} x_i X_{P[AS]}$ refer to the sum of ranked male eyespans ($x_i X_P$) up to the j^{th} rank in descending ($x_i X_{P[DS]}$) and ascending ($x_i X_{P[AS]}$) orders, respectively (j equal to $b - a$; or the number of males accepted).

A theoretical maximum or minimum *SD* was then calculated for each female as

$$SD_{\max,\min} = [-]\bar{X}_A - T_{\max,[\min]} \quad , \qquad [7]$$

with SD_{max} calculated using T_{max} where $\bar{X}_A - \bar{X}_P$ was greater than zero, and SD_{min} calculated using T_{min} where $\bar{X}_A - \bar{X}_P$ was smaller than zero.

Finally, a '*preference slope*', $P_{2,}$ was calculated for each female by expressing the observed $\bar{X}_A - \bar{X}_P$ as a proportion of the maximum or minimum possible $\bar{X}_A - \bar{X}_P$, given the males presented, and the number accepted using equation 5; SD_{max} used where $\bar{X}_A - \bar{X}_P$ was larger than zero, SD_{min} where $\bar{X}_A - \bar{X}_P$ was smaller than zero.

P_2 provides a measure for the selection on the male ornament due to female preferences after the effects of rejection are removed. P_2 is superior to the simple difference $\bar{X}_A - \bar{X}_P$. This is because $\bar{X}_A - \bar{X}_P$ scales with rejection. As the number of males rejected increases, so the theoretical maximum and minimum possible values of $\bar{X}_A - \bar{X}_P$ increase (that is, a female that rejects 14 out of 15 males will have accepted one male, which could be the largest or smallest of all males; in contrast, a female that rejects one out of 15 males will have accepted 14 males, which must include both large and small males. Hence, the maximum and minimum values of $\bar{X}_A - \bar{X}_P$ must scale with *R*). Hence, the expression of $\bar{X}_A - \bar{X}_P$ as a proportion of the maximum or minimum value (i.e. $SD_{max,min}$), controls for this and allows for an estimate of the slope of an individual female's preference function. By definition, P_2 varies between ±1 making it equivalent in scale to P_1.

Selection , *SD*

The strength and direction of the selection exerted on the male ornament via female choice was calculated per female as a '*selection differential*', *SD*, based on (Lande & Arnold 1983; Falconer 1989) as

$$SD = \bar{X}_A - \bar{X}_P \quad , \qquad [9]$$

where $\bar{X}_A$ and $\bar{X}_P$ are defined as in equation 5. Note therefore the similarity of the preference slope (P_2) and selection (*SD*) measures. But recall that P_2 gives a measure of preference that is independent of a female's rejection rate and the distribution of males presented to that female, whereas *SD* incorporates the effects of both the preference function and rejection on the set of decisions that the female makes in relation to the particular set of males that she has encountered.

As in the two-size case, the values *rejection* and *preference slope* reflect choosiness and the slope of the preference function; and should be unrelated, even at quite extreme (but not at maximal or minimal) levels of rejection, as the effects of rejection are controlled for P_2. As before, *selection* is set up to relate to an interaction between rejection and preference slope: both in the literal sense, and also in a mathematical sense. The measure focuses on the acceptance responses to males, and integrates both variation in rejection level (and so choosiness) and in preference slope (and so preference functions). Hence, the set of values used in the five-size test is different to, but *analogous to*, that used in the two-size test. The measures permit a test of method corroboration, and tests of the main hypotheses.

2.2.4 *Statistical analysis*

Only females that had completed at least two complete assays – four decisions in the two-size assay (virgin, $n = 58$; mated, $n = 37$), and a minimum of 10 decisions in the five-size assay (virgin, $n = 102$; mated, $n = 103$) – were included for analysis. As further prior, I tested for an effect of mating status on female fecundity and found that, as expected, fecundity was lower in virgin than in mated females in the two-size ($p < 0.001$) and five-size experiment ($p < 0.001$, SI.D1, 2).

2.2.4.1 *Two-size assay of female mate choices*

As the relationships between the components of preference and selection are important for the interpretation of the mating status results, I examined the relationships between the components of preference and selection via the use of a set of linear regressions, fitted to test for effects of rejection on preference slope, and, in turn, for potential effects of both rejection and preference slope on selection.

To test for the effect of female mating status on the components of preference and selection, I compared mating status means in a series of general linear models (GLMs). As female relative eyespan and fecundity are known to influence preference (Cotton *et al.* 2006a; Small 2009), each model included, as fixed effects, female eyespan, female thorax, the eyespan x thorax interaction, fecundity, and female mating status. To contrast means with null expectations, I then split the data via female mating status and used Wilcoxon signed-rank tests. For rejection, no clear null could be specified. For preference slope, the mating status means were contrasted with 0, while for selection the contrast was with 0.5.

To test if the relationship between rejection and preference slope varied across mating status, I then fitted a GLM for preference slope that included as fixed effects, rejection, mating status and the interaction. To test for variation in the relation between rejection and preference slope with selection across mating status, and to further test for interactions between rejection and preference slope on selection, I then fitted a GLM for selection, with rejection, preference slope, mating status, and all two- and three-way interactions included as fixed effects.

To test for effects on female mating responses of experimental day, and the order in which males were presented to females, as well as to contrast preference functions by mating status groups, I then fitted a set of generalized linear mixed effects models (GLMEs) with binomial error structures and logit link functions to the raw mating response data (models fitted in lme4, Bates *et al.* 2015). First, I fitted three models in turn to test for effects of female eyespan, thorax and fecundity on rejection responses. A fourth model was fitted to test for effects of the order of male presentation, experimental day and the order x day interaction. A full model was then constructed to test for effects of female mating status and male size. It included female mating status, male size and the mating status x male size interaction as fixed effects. In each model, female ID was included as a random effect. As I used a single random effect, parameters were estimated via Gauss-Hermite quadrature. A minimal adequate model was selected, and all effects were tested via likelihood ratio tests (Crawley 2009). As the random effect levels (Agresti 2002; Demidenko 2004) and the ratio of the level of fixed effects to the total sample size were >40 (Bates & Pinheiro 2000) the *p*-values can be

considered reliable. However, to be conservative (Bolker *et al*. 2009), I also tested the interaction via bootstrap at 10,000 repetitions to obtain empirical *p*-values. I obtained confidence intervals via bootstrap at 1000 repetitions and estimated marginal and conditional R^2_{GLMM} in MuMIn (Nakagawa & Schielzeth 2013; Johnson 2014; Bartón 2015); a package in 'R'. Each model was tested for overdispersion via the comparison to the square root of the penalised residual sum of errors divided by the number of observations (Bolker *et al.* 2009).

2.2.4.2 *Five-size assay of female mate choices*

The statistical analyses used for the five-size assay were similar to those used for the two-size assay, but with several important differences. First, to test for effects of mating status on selection I fitted a generalized linear model (GLMz) with an exponential error structure and reciprocal link function rather than a GLM, so as to obtain a normal residual error. Second, I contrasted mean virgin and mated selection differential values with a null of zero (rather than to a null of 0.5, as was done in the two size test). Third, I fitted a GLME for experimental day, week and the day x week interaction, (rather than one for order and day, as was used in the two size test) as a single male was presented to each female per day, with one assay completed per week (rather than two males per day over three days, as in the two-size assay). This was done to determine whether day, week or the interaction had an affect on female mating responses; the results were used to decide whether these variables were included as random effects in the 'full' model. I then fitted a 'full' GLME which included female thorax length, female mating status, male size and the interaction of female mating status and male size as fixed effects, with experimental day nested within experimental week and

female ID included as random effects (due to the significant effects of each on the female response data, and to account for both days and weeks). Due to the use of multiple random effects, model parameters were estimated using Laplacian approximation rather than the more powerful Gauss-Hermite quadrature. Finally, to investigate the shape of the preference function, I used Tukey contrasts to compare the female mating responses to males in the different size classes, both overall, and after the mating response data were split via female mating status.

As mating response variation between and within individuals is important for sexual selection (Forstmeier & Birkhead 2004; Kuijper *et al.* 2012) I also ran additional analyses on the effects of mating status on both intra-female mating response stability, over time, and inter-female mating response variation. First, to test for effects of mating status on the variation in mating responses across time, I fitted a binomial-logit GLME to the raw mating response data. It included as fixed effects, female mating status, male size, experimental week, and all of their interactions. Female ID was included as a random effect. Parameters were estimated via Gauss-Hermite quadrature. This allowed for a test for variation in female rejection/acceptance responses across weeks (mating status x week interaction), male size specific rejection/acceptance responses across weeks (male size x week interaction), and the effect of female mating status on each in turn (week x mating status interaction; week x size x mating status interaction). I then tested for effects of female mating status on the level of inter-female variation in rejection, preference slope and selection using Brown-Forsythe variance tests.

To visualise individual female preference functions, and to aid the understanding of the levels of variation in the preference functions between females and mating status categories, I then examined individual preference functions, for each of the individual virgin and mated females. This was done using nominal logistic models and non-parametric smoothers (see Figure 10): an individual regression fitted per female to her rejection/acceptance responses, per male size class, at $\lambda = 10$.

All statistical analyses were conducted in JMP v. 11. 2 and R v. 3. 1. 3. All GLM(z), and GLME output tables provided in the Supplementary Information.

2.3 RESULTS

2.3.1 ***Two-size assay of female mate choices***

2.3.1.1 *Female mating status, components of preference and selection*

Female mating status had a large effect on rejection. Virgin females rejected fewer males than mated females (GLM: $F_{1,85} = 25.84$, $p < 0.001$; Figure 1). In contrast, preference slope, P_1, did not vary with mating status (GLM: $F_{1,85} = 0.672$, $p = 0.415$; Figure 2); although virgin females had neutral (Wilcoxon Signed-Rank test: $sr = 124$, $df = 57$, $p = 0.100$) and mated females positive preference slopes (Wilcoxon Signed-Rank test: $sr = 67$, $df = 34$, $p = 0.035$; neutral = 0). In line with the pattern seen for rejection, selection, S_1, was lower in virgin females than in mated females (GLM: $F_{1,75} = 9.48$, $p = 0.003$; Figure 3). But, in line with preference slopes, virgin females had neutral levels of selection (Wilcoxon Signed-Rank test: $sr = 78$, $df = 55$, $p = 0.278$) while mated females had positive levels of selection (Wilcoxon Signed-Rank test: $sr = 100$, $df = 26$, $p < 0.001$; neutral = 0.5).

Figure 1:

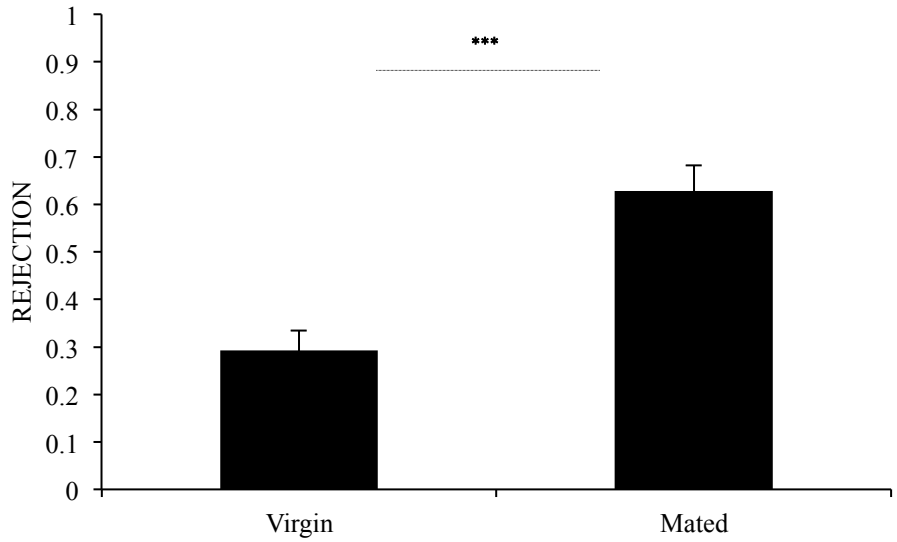


Figure 1. Two-size assay of female mate choices. Effect of female mating status on rejection, *R*. Error bars show ± SEM. The difference between mating status categories is shown on the horizontal line, denoted by asterisks (*** $p < 0.001$).

Figure 2:

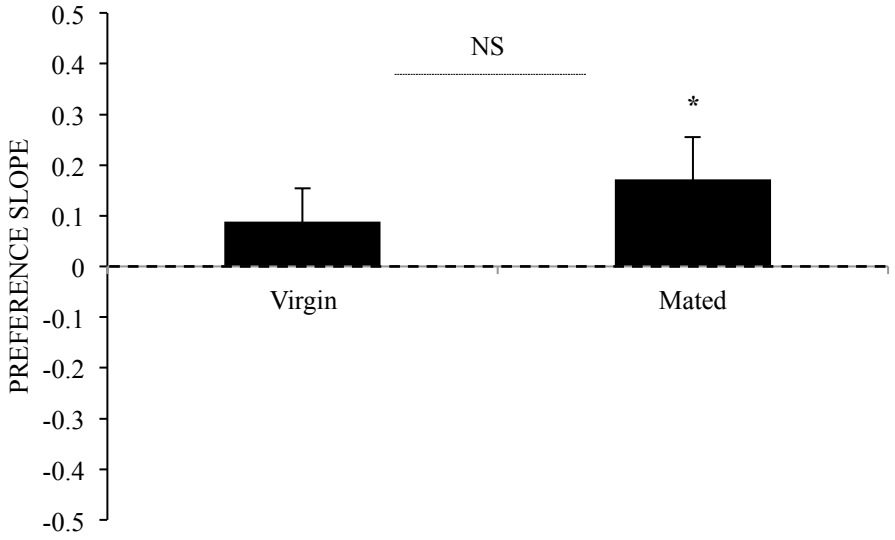


Figure 2. Two-size assay of female mate choices. Effect of female mating status on preference slope, P_1. Error bars show ± SEM. The lack of difference between mating status categories is shown on the horizontal line (NS). Each mating status category was also contrasted with the null expectation of $P_1 = 0$ (thick dashed line at Y = 0). A significant difference to null is denoted by an asterisk (* $p < 0.05$) above a mating status bar; an absence of asterisks denotes a lack of significant difference.

Figure 3:

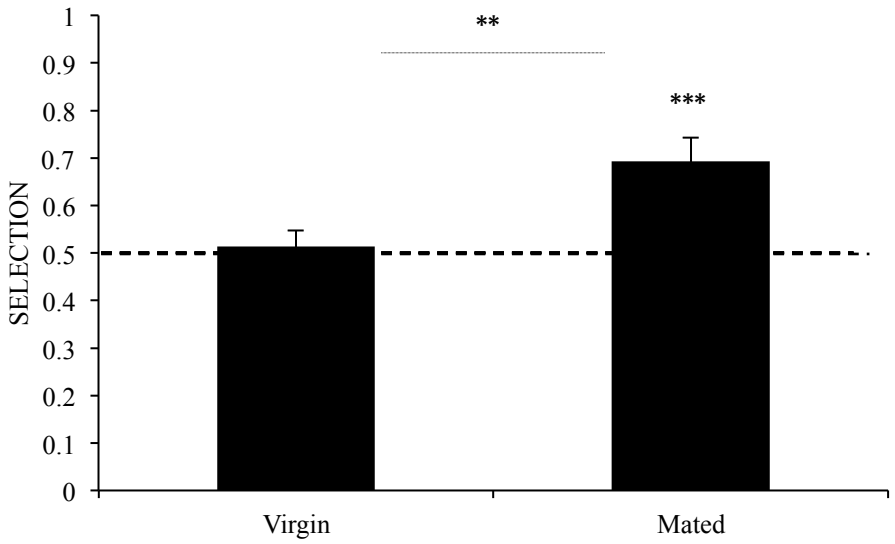


Figure 3. Two-size assay of female mate choices. Effect of female mating status on selection, S_1. Error bars show ± SEM. The difference between mating status categories is shown on the horizontal line, denoted by asterisks (** $p < 0.01$). Each mating status category was also contrasted with the null expectation of $S_1 = 0.5$ (dashed line). A significant difference to null is denoted by asterisks (*** $p < 0.001$) above a mating status bar; an absence of asterisks denotes a lack of significant difference.

2.3.1.2 *Interrelation of rejection, preference slope and selection*

Rejection and preference slope were not related (linear regression: $R^2 < 0.01$, $df = 91$, $t = 0.42$, $p = 0.678$). There was also no effect of mating status on the relation between rejection and preference slope (GLM: $F_{1,75} = 0.59$, $p = 0.443$). Further, both rejection (linear regression: $R^2 = 0.22$, $df = 81$, $t = 4.71$, $p < 0.001$) and preference slope (linear regression: $R^2 = 0.55$, $df = 81$, $t = 9.96$, $p < 0.001$) were positively associated with selection. In the full GLM for selection, the rejection x mating status interaction was significant (GLM: $F_{1,,75} = 9.91$, $p = 0.002$): the relation between rejection and selection was weaker in virgins. In contrast, the preference slope x mating status interaction was not significant (GLM: $F_{1,,75} = 3.73$, $p = 0.058$), although a trend existed for a weaker P_1-S_1 relation in the virgin state.

2.3.1.3 *Preference slope analysis*

Neither female eyespan (GLME: $\chi^2_1 < 0.01$, $p = 0.988$), female thorax length (GLME: $\chi^2_1 = 0.06$, $p = 0.804$) nor fecundity (GLME: $\chi^2_1 = 0.176$, $p = 0.675$), had an effect on female mating responses. This was also true of the order of male presentation (GLME: $\chi^2_1 = 1.62$, $p = 0.203$), experimental day (GLME: $\chi^2_2 = 3.74$, $p = 0.154$), and the male order x day interaction (GLME: $\chi^2_2 = 2.18$, $p = 0.337$). In contrast, female mating status and male size had large effects. Virgin females were less likely to reject males (GLME: $\chi^2_1 = 15.74$, $p < 0.001$) and large males were rejected less often than small males (GLME: $\chi^2_1 = 10.21$, $p = 0.001$). However, the differential rejection by females of smaller relative to larger males did not vary with mating status (GLME: $\chi^2_1 = 0.112$, $p = 0.738$; Figure 4). That is, mean preference slopes were positive and similar across mating status categories.

Figure 4:

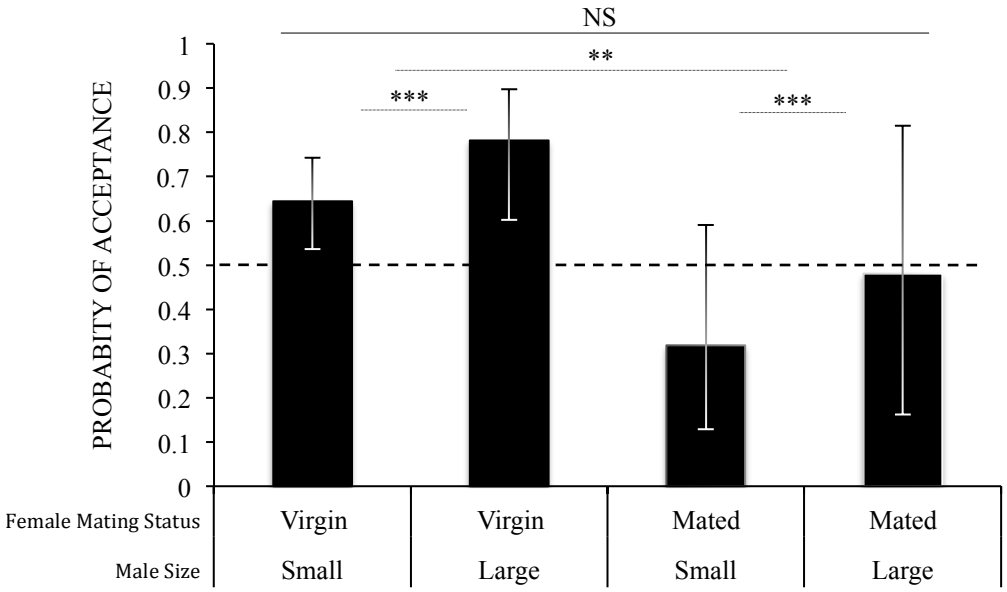


Figure 4. Two-size assay of female mate choices. Effect of female mating status, male size and the mate status x size interaction on the probability that a female accepts a male (the inverse of the probability that a female rejects a male, shown for clarity). Error bars show converted log-odds (mating probabilities) ± 95% C.I. The significance of the female mating status effect is shown on the dotted line, denoted by asterisks (** $p < 0.01$), The significance of the male size effect is shown on the lower pair of dotted lines, denoted by asterisks (*** $p < 0.001$). The female mating status x male size interaction was not significant (shown on the solid line).

2.3.2 *Five-size assay of female mate choices*

2.3.2.1 *Female mating status, components of preference and selection*

As in the two-size assay, virgin females rejected fewer males than did mated females (GLM: $F_{1,199} = 50.62$, $p < 0.001$; Figure 5). Again, the mean preference slope, P_1, did not differ between virgin and mated females (GLM: $F_{1,174} = 0.876$, $p = 0.351$; Figure 6). However, in contrast to the two-size test pattern, both the virgin and mated females now had positive mean preference slopes (*t*-test: virgin: $t = 4.52$, $df = 91$, $p < 0.001$; mated: $t = 7.06$, $df = 87$, $p < 0.001$; neutral = 0). Again, as in the two-size test, selection, *SD*, was weaker in virgin females (GLMz: $\chi^2_{1,121} = 7.16$, $p = 0.008$; Figure 7). However, in line with the five-size test preference slope pattern, selection was positive in both virgin (*t*-test: $t = 2.55$, $df = 101$, $p = 0.012$) and mated (Wilcoxon Signed-Rank test: $sr = 1338.5$, $df = 101$, $p < 0.001$) females (neutral = 0). There were subtle differences between the test types.

2.3.2.2 *Interrelation of rejection, preference slope and selection*

As in the two-size test, rejection and preference slope were not related (linear regression: $R^2 < 0.01$, $df = 177$, $t = -1.02$, $p = 0.307$), and there was no effect of mating status on the relation between rejection and preference slope (GLM: $F_{1,170} = 1.21$, $p = 0.272$). Likewise, both rejection (linear regression: $R^2 = 0.05$, $df = 201$, $t = 3.33$, $p = 0.001$) and preference slope (linear regression: $R^2 = 0.51$, $df = 177$, $t = 13.65$, $p < 0.001$) were positively associated with selection. However, unlike the two-size test, the rejection x mating status (GLM: $F_{1,,170} = 10.34$, $p = 0.002$), preference slope x mating status (GLM: $F_{1,,170} = 115.03$, $p = < 0.001$), rejection x preference slope (GLM: $F_{1,170,} = 227.77$ $p = < 0.001$), and the three-way rejection x preference slope x mating status (GLM: $F_{1,170,} = 5.99$, $p = 0.015$) interactions all

Figure 5:

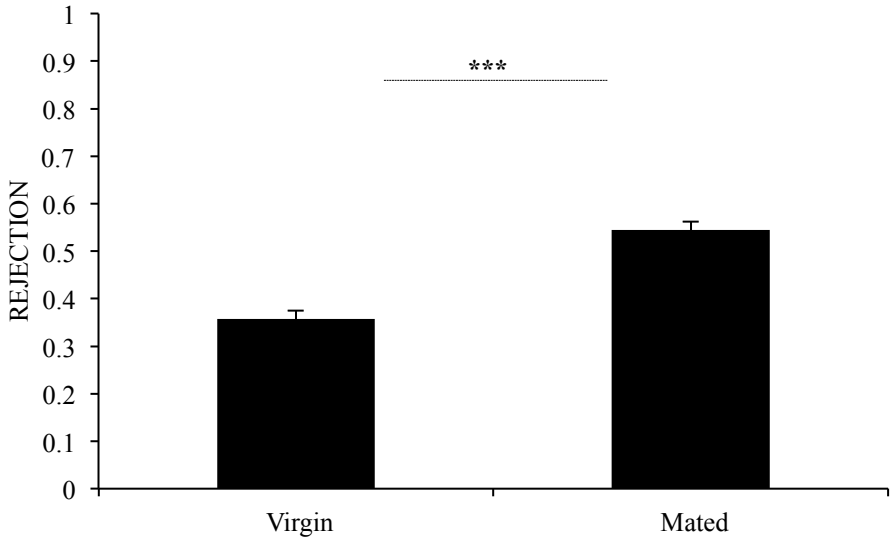


Figure 5. Five-size assay of female mate choices. Effect of female mating status on rejection, *R*. Error bars show ± SEM. The difference between mating status categories is shown on the horizontal line, denoted by asterisks (*** $p < 0.001$).

Figure 6:

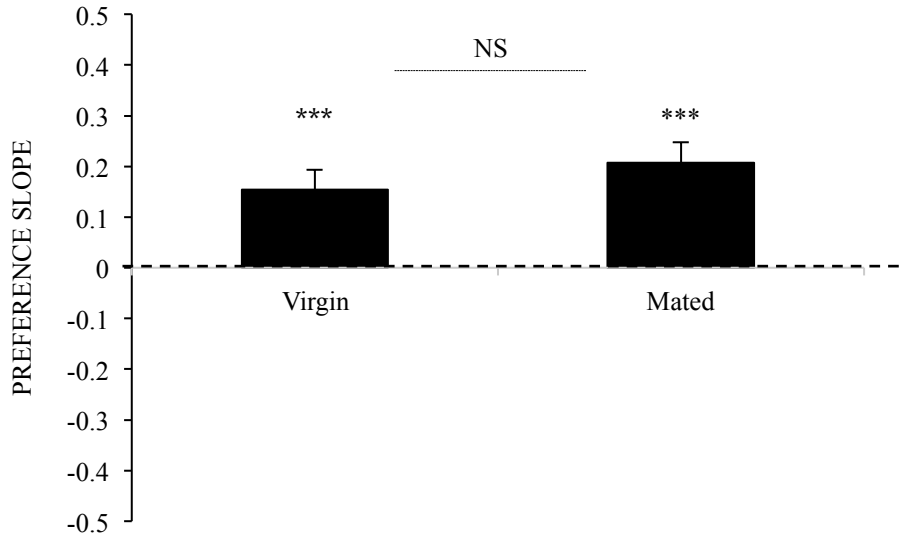


Figure 6. Five-size assay of female mate choices. Effect of female mating status on preference slope, P_1. Error bars show ± SEM. The lack of difference between mating status categories is shown on the horizontal line (NS). Each mating status category was contrasted with the null expectation of $P_2 = 0$ (thick dashed line at Y = 0). A significant difference to null is denoted by an asterisk (*** $p < 0.001$) above a mating status bar; an absence of asterisks denotes a lack of significant difference.

Figure 7:

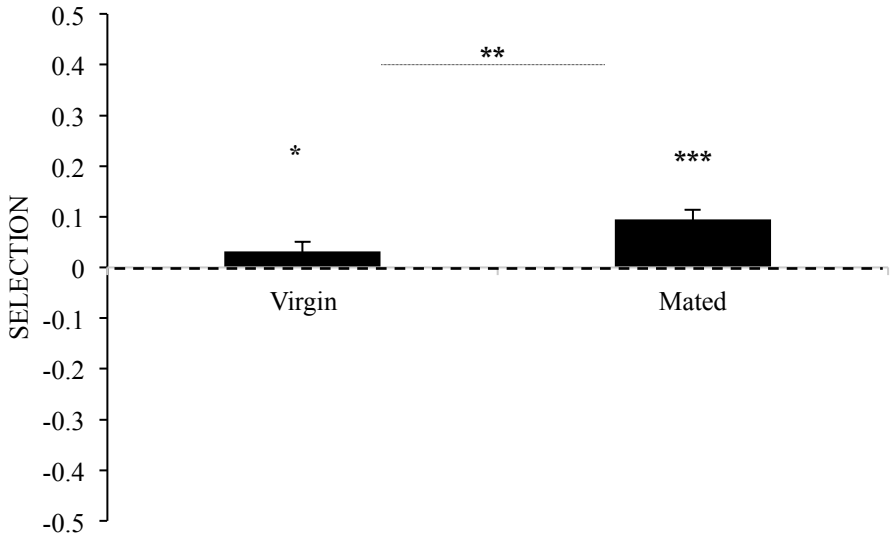


Figure 7. Five-size assay of female mate choices. Effect of female mating status on selection, *SD*. Error bars show ± SEM. The difference between mating status categories is shown on the horizontal line, denoted by asterisks (** $p < 0.01$). Each mating status category was also contrasted with the null expectation of $SD = 0$ (thick dashed line at Y = 0). A significant difference to null is denoted by an asterisk (* $p < 0.05$, *** $p < 0.001$) above a mating status bar.

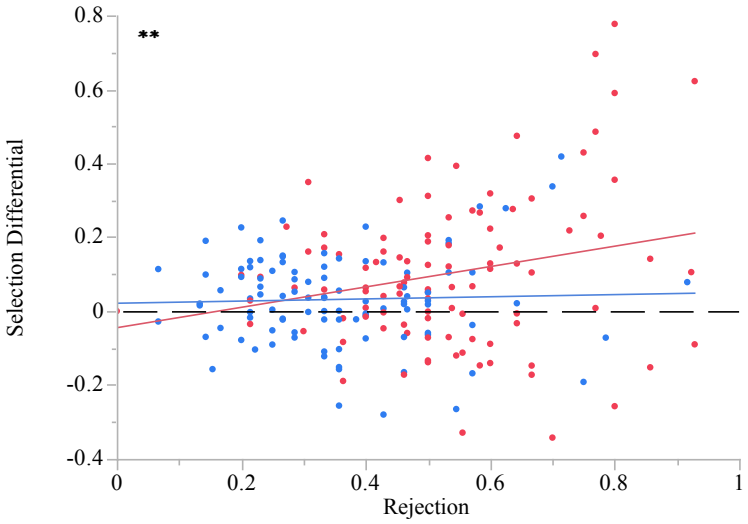
explained variation in selection. The relationship between each component of preference and selection was weaker in virgins (Figures 8 a, b). While the interrelation between components of preference and selection were similar to those in the two-size tests, mating status was found to have a still stronger effect on the interrelation of rejection, preference slope and selection in the five-size tests.

2.3.2.3 *Preference function analysis*

Female rejection/acceptance of males was not significantly affected by female eyespan (GLME: $\chi^2_1 = 0.44$, $p = 0.507$), the eyespan x thorax interaction (GLME: $\chi^2_1 = 0.32$, $p = 0.574$) or female fecundity (GLME: $\chi^2_1 = 1.16$, $p = 0.281$). However, females with larger thoraxes were more likely to reject males (GLME: $\chi^2_1 = 7.26$, $p = 0.007$), and there were also effects of experimental day (GLME: $\chi^2_4 = 23.57$, $p < 0.001$), week (GLME: $\chi^2_2 = 6.67$, $p = 0.036$), and the day x week interaction (GLME: $\chi^2_8 = 56.74$, $p < 0.001$). Rejection was higher on the first day of the week (Tukey Contrasts: all $z > 3.84$, $p < 0.002$) but did not change directionally across weeks (Tukey Contrasts: all $z < 1.16$, $p > 0.478$). The 'first-day effect' appeared to be lost in week three (see SI.C2.S1). As in the two-size test, there was also a large effect of both mating status and male size on female responses. Virgin females were less likely to reject males overall (GLME: $\chi^2_1 = 89.34$, $p < 0.001$). Larger males were less likely to be rejected (GLME: $\chi^2_4 = 25.83$, $p < 0.001$). As in the two-size assay, there was no effect of the interaction mating status x male size interaction on female mating response (GLME: $\chi^2_4 = 2.93$, $p = 0.569$): preference slopes were positive in both virgin and mated females.

Figure 8:

a)



b)

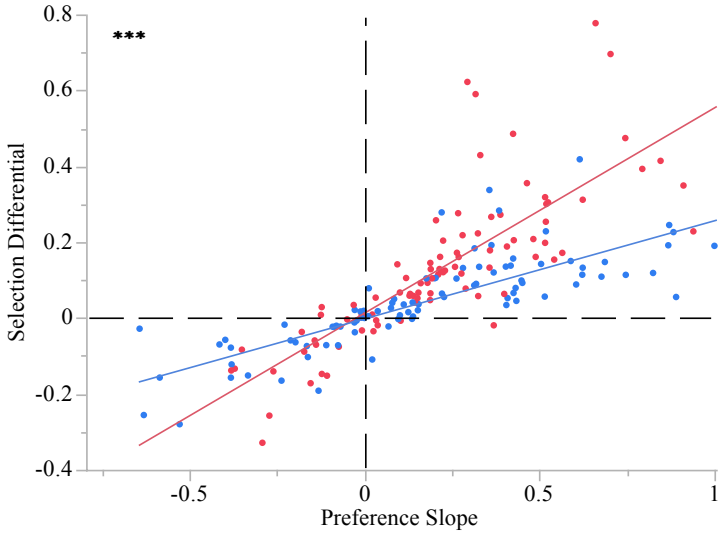


Figure 8 a, b. GLM model output for effects of interactions between rejection, *R*, and preference slope, P_2, with female mating status on selection differentials, *SD*, for the five-size test. Individual virgin females are represented by blue dots, mated females by red dots. The blue fit is for virgin, the red for mated females. The significance of each interaction is denoted by asterisks (** $p < 0.01$, *** < 0.001).

Given this, I analysed the shape of the preference function as a trait pooled across mating status (Figure 9). Males in the 'D' size class (7.6 – 8.0 mm eyespans) were less likely to be rejected than those in the 'A' size class (Tukey Contrast: $z = 3.39$, $p = 0.006$), 'B' size class (Tukey Contrast: $z = 4.79$, $p < 0.001$) or 'C' size class (Tukey Contrast: $z = 3.28$, $p = 0.009$) (size classes: 6.4 – 6.8, 6.8 – 7.2, ≥ 7.2 – 7.6 mm eyespans). Males in the 'E' size class (8.0 – 8.4 mm eyespan) were less likely to be rejected than those in the 'B' size class (Tukey Contrast: $z = 2.89$, $p = 0.031$), but not those in the 'A' size class (Tukey Contrast: $z = 1.45$, $p = 0.596$).

In a further analysis, I split the data into virgin and mated female status, to test for subtle variations in the preference function. In mated females 'D' size males were less likely to be rejected than either 'A' (Tukey Contrast: $z = 3.07$, $p = 0.018$) or 'B' (Tukey Contrast: $z = 3.01$, $p = 0.022$) size males. In virgins females 'D' size males were less likely to be rejected than 'B' sized males (Tukey Contrast: $z = 3.69$, $p = 0.002$), but not 'A' sized males (Tukey Contrast: $z = 1.68$, $p = 0.446$). Hence, while the ability to measure the preference function varied to a small extent with mating status, its slope and shape were stable in relation to mating status variation, with a potential peak preference at male eyespan ≥ 7.6 – 8.0 mm.

2.3.2.4 *Intra-female variation*

In the GLME, there was no effect of female mating status on the amount of change in overall female rejection/acceptance response across the experimental weeks (mating status x week: GLME: $\chi^2_2 = 0.28$, $p = 0.867$). However, female rejection/acceptance responses to male size classes did vary across weeks (male size x week: GLME: $\chi^2_8 = 32.09$, $p < 0.001$) and mating status had a positive

Figure 9:

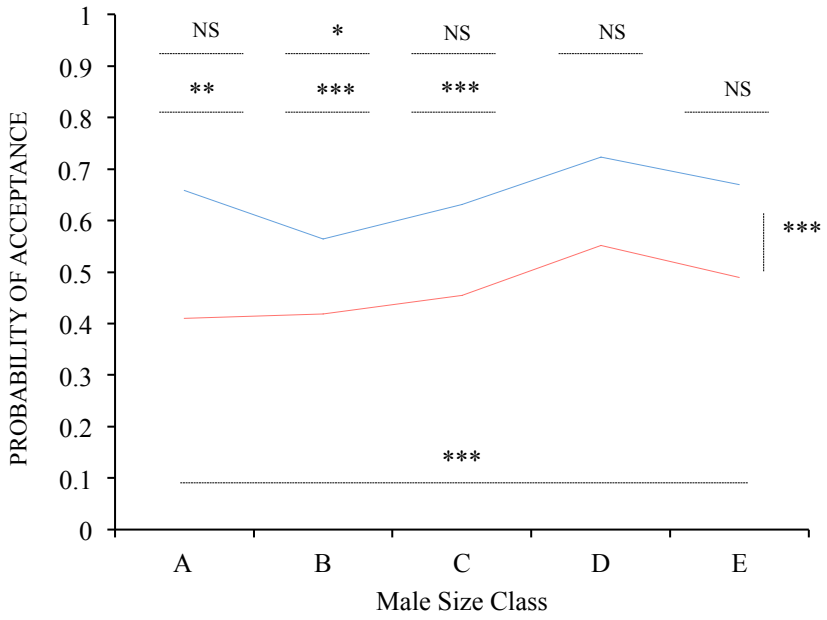


Figure 9. Five-size assay of female mate choices. Effect of female mating status, male size and the interaction on the probability that a female accepts a male. Error bars are omitted for clarity. Asterisks show significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Virgin females are represented by the blue line, mated females by the red line. Each line connects the peaks of 5 bars (not shown). Lines are used, rather than bars, to enhance visualisation of the preference function, and to make it easier to compare between virgin and mated responses. However, the lines are illustrative, not quantitative. The lack of significance of the 2-way interaction (female mating status x male size) is not shown, for clarity. The significance of the female mating status effect is shown on the vertical (dotted) line (at *** $p < 0.001$). The significance of the male size effect is shown on the wide horizontal (dotted) line (at *** $p < 0.001$). Individual male size contrasts are shown on the narrow horizontal (dotted lines). The top row shows contrasts between class E males and those of class A, B, C, and D. The second row shows contrasts between class D males and those of class A, B, C, and E.

effect on the amount of change in these male size specific, across week female responses (mating status x male size x week: GLME: $\chi^2_8 = 16.28$, $p = 0.039$). Both virgin and mated females increased in peak preference for 'C' and 'D' class males across weeks 2 and 3. But the increase in 'D' class male acceptance (in week 3) was larger for virgin females (see SI for model output). Hence, overall the mean variation female in preference functions across time was higher in virgins.

2.3.2.5 *Inter-female variation*

There was no effect of female mating status on the level of inter-female variance in rejection (Brown-Forsythe test: $F_{1,201} = 1.079$, $p = 0.300$). In contrast, inter-female variance in preference slope was far higher in virgins (Brown-Forsythe test: $F_{1,176} = 5.382$, $p = 0.022$), while that in selection was far lower in virgins (Brown-Forsythe test: $F_{1,200} = 13.34$, $p < 0.001$). (See Figure 10 for a visualisation of the individual level female preference functions split by female mating status).

2.4 DISCUSSION

In this chapter, I investigate the effects of female mating status variation on individual level female preference, and selection, in the sexually dimorphic African species of stalk-eyed fly, *Diasemopsis meigenii*. To do so, I manipulate female mating status (virgin and repeatedly mated), and conduct a pair of experiments that assay female mate choice via the repeated sequential presentation of males from either two or five ornament size classes. I use mathematical and statistical techniques to deconstruct the responses into two crucial components of preference – choosiness, and the slope of the preference function – as well as measuring sexual selection derived from the choice

Figure 10:

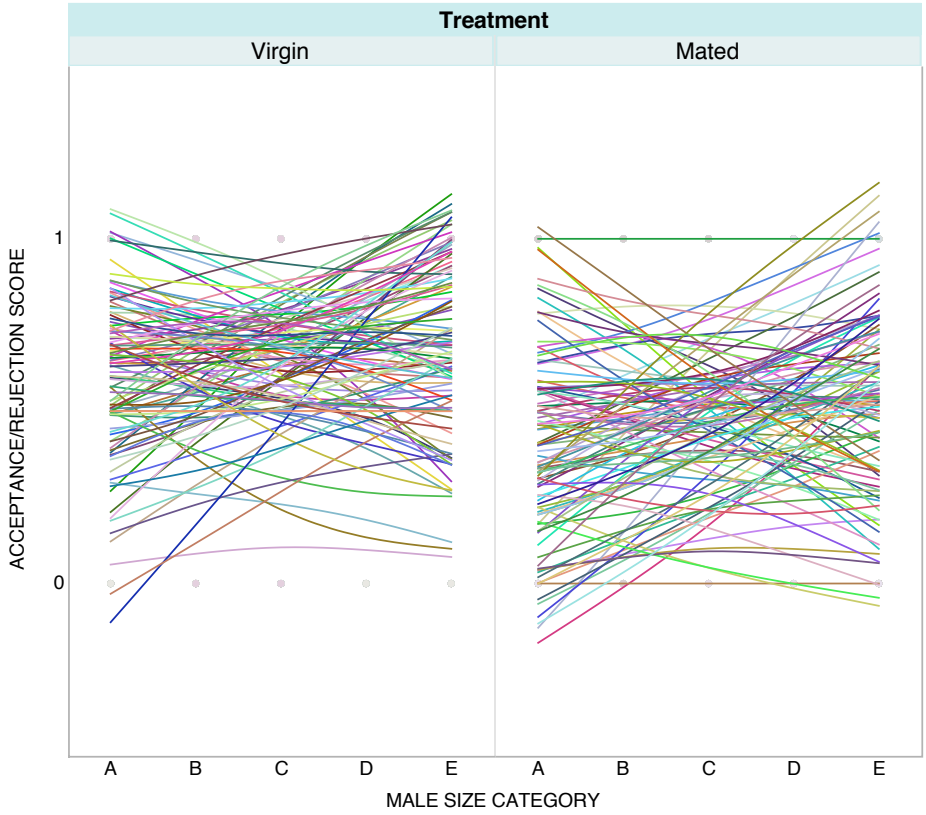


Figure 10. Five-size assay of female mate choices. Nominal logistic plot with smoothers for female mating response to different sized males, per female; a visualization of variation in choosiness and the shape and slope of the preference function between individuals across mating status categories, and between females.

outcomes. I also estimate aspects of the shape of the preference function, and test the level of variation in each component of preference within females (over time), as well as between females in relation to mating status categories. As such, I am able to test three hypotheses about the use of virgin rather than mated females to measure mating responses: first, that it will lead to reduced estimates of choosiness and selection; second, that it will result in a loss of resolution in estimates of the preference function; and third, that it will inflate (or deflate) estimates of variation in the slope of the preference function within females across time, and between females. Due to the use of two experiments I am able to answer these questions clearly, and I am also able to reveal some of the benefits and limitations of simpler and more complex experimental techniques. My findings are relevant to current ideas on the operation of sexual selection in nature.

As expected, both 'rejection' (the overall propensity of females to reject males, and a measure of 'choosiness') and 'selection' (the proportional relationship between the number of large and small males accepted by a female, and a measure of the selection exerted on the male ornament by a female's mating decisions) were lower in virgin females, while 'preference slopes' (the relative number of large versus small males rejected by a female, and a measure of the slope of the preference function after most of the effects of the female's rejection level have been removed) were robust to mating status variation in the two-size test, as was also the case in the more complex five-size equivalent (see section 2.2.3.2 for definitions). These patterns can be explained in relation to the costs of choice. As the reproductive cost of forgoing a mating is higher in virgins (Kokko & Mappes 2005), the choosiness of virgins should be reduced relative to that of

mated females. In line with this, the virgin female rejection levels were lower than those of the mated females in both the two and five size assays (Figures 1 and 5).

In contrast, variation in the cost of mate choice was not expected to alter preference slopes. In stalk-eyed flies, female preferences are expected to be positive and in the direction of larger males (Hingle *et al.* 2001a, b; Cotton *et al.* 2006a). I found that mean female preference slopes did not differ via mating status in either study; and that both virgin and mated female mean preference slopes were qualitatively positive (Figures 2 and 6), even if the direction could not be detected statistically in the virgins in the two-size test. There was also a trend for lower preference values in virgin females, especially in the two-size test (Figure 2). It is possible that this trend reflects that a real difference was not 'detected' due to a small sample size. However, in the five-size test the trend was far weaker. Alternatively then, the trend could reflect an imperfect mathematical decoupling of rejection and preference slope. That is, as virgin females rejected fewer males, some smaller males will have been accepted, even for the same 'underlying' preference functions. If true, then the difference between virgin and mated female preference slopes should have declined as the number of per female observations increased, as was observed for the five-way versus two-way contrast. A formal comparison is not possible, as these were two different experiments. Nonetheless, the outcome adds some weight to the importance of repeated, per female samples.

Finally, selection was also expected to vary with the costs of choice (like rejection). This is because selection is predicted to emerge from an interaction between a female's choosiness (rejection rate) and preference function (preference

slope). Hence, even while a female's preference function remains stable its outcome in terms of mate choices should be suppressed in virgins, where choosiness is lower, due to the increased costs of choice in this state. As expected then, the selection exerted on the male ornament in virgin females was lower than in mated females. And, while the direction of selection reflected that of the preference function (all mean selection values were qualitatively positive), the strength of the observed selection was affected not just by this function, but was, it seems, modulated by choosiness (that is, it was lower in virgins; Figures 3 and 7).

The interpretation that female choosiness (rejection) modulates the expression of the preference function (preference slopes) as the series of choices that lead to sexual selection (selection) was backed up by an examination of the interrelations between the preference components and selection, and of the effects of mating status on these. As expected, 'rejection' and 'preference slope' did not covary in either the two- or the five-size tests, while rejection *and* preference slope positively covaried with selection in both cases (that is, the measures worked as designed). The relationship between preference slope and selection was also stronger than that between rejection and selection. Hence, as would be expected, the preference function was shown to have the lead role in influencing the effect of mate choices on selection. However, while the relationship between rejection and preference slope did not vary with female mating status in either the two or five size test (and was thus robust to the cost of choice context as well as to the experimental set up), the relationship between rejection and selection was found to be weaker in virgins in both tests (see Figure 8 a for the five-size test). Likewise, the effect of the preference slope, and of the interaction between rejection and the preference

slope, on selection was also weaker in virgins, in the five-size test (see Figure 8 b for five size test for preference slope). Hence, consistent with the interpretation of the pattern of means (above), selection can be seen to have covaried with both rejection and preference slope. In each case, the direction of the preference slope was determined by the direction of the sexual selection exerted. But the strength of this selection was modulated also via 'rejection', which itself varied in relation to the costs of mate choice, or mating status, so that the influence of 'rejection' (choosiness) and 'preference slope' (preference function) on selection was weaker in the virgin state. It is, of course, notable, that while these relationships must reflect biological variation (virgin and mated females differed), they will also have arisen to some extent due to mathematical constraints – for instance, rejection limits the values of preference slope that can be expressed when it is very low or high (especially in the two size test), while positive values of preference must mathematically lead to positive selection, and must be curbed by rejection levels.

A mechanistic explanation for this modulation is that, while the differential rejection of smaller versus larger males (i.e. 'preference slope') was not affected by variation in the cost of choice, the reduced overall rejection (i.e. 'rejection') in the higher cost virgin state resulted in an increase in the proportion of smaller relative to larger males that was accepted for a given preference slope. Hence, the relationships between rejection and selection, preference slope and selection, and rejection x preference slope and selection became less important in virgins: in part, because virgin rejection responses became more random and homogenous, in part because the expression of preference functions in their decisions was suppressed by the lowered rejection rates (which may also have increased the

importance of stochastic variation). Taking into account, then, the overall responses of each component of preference and selection, as well as the effects of variation in female mating status on the interrelations of these components, it is clear, that both choosiness and the preference function contributed to mate choices and selection. It is also clear that variation in the costs of choice associated with variation in female mating status led to variation in choosiness and thus to variation in the expression of preference functions as the series of choices that exerted selection. And it is clear that while this could be in part a product of mathematical constraint, there is real biological effect, and real biological relevance – as the mating status categories differed (effect), and as in the wild, low choosiness will lead to increased acceptance, and lower efforts to find alternate mates, and so to a less direct expression of the preference function in mate choice (relevance). As such, I was able to add to the small number of studies that have previously shown that choosiness can be reduced in the virgin state due to the increased reproductive costs of mate choice in that state (other examples are the field cricket *G. bimaculatus*, Bateman *et al*. 2001; the túngara frog, *Physalaemus pustulosus*, Lynch *et al*. 2005; and the wolf spider, *Schizocosa ocreata*, Uetz & Norman 2007; but see Gershman *et al*. 2014 for an example in which choosiness does not vary, in *D. serrata*) and that a reduction in choosiness can lead to a concomitant reduction in selection. An important consequence of this is that the use of virgin rather than mated females could lead to the underestimation of both choosiness and selection and could limit the extent to which simple (i.e. two size) studies can determine the scale or direction of preference functions, so that future studies should treat of the use of virgins in this context with caution.

What can we conclude about the shape of the preference function and the effects of mating status variation on this shape? No previous studies have extracted full population level or individual level preference functions in any species of stalk-eyed fly. A direction and 'scale' for the preference function has been calculated by Hingle *et al.* (2001b) in *T. dalmanni*, while Cotton *et al.* (2006a) was able to build on this to fit the first individual level logistic curves to female mate responses, based on a threshold model of preference. Likewise, Wilkinson *et al.* (1998) were able to fit a form of 'discrimination' based preference function in T. *dalmanni*, but this was based on the differences in eyespan between pairs of males, rather than the size of male eyespans *per se*. In each case, females expressed preferences for larger males. In Cotton *et al.* (2006a) and this current study, the overall curve was relatively linear. So, the first implication is that the use of a simple linear index, such as the 'preference slope', is valid in *D. meigenii*, because such linear slopes will capture most of the relevant information about the preference function.

Here, I utilised recent advances in statistics to build on this further by estimating aspects of the shape of the overall preference function as well as testing for variation in this shape associated with variation in the female mating status (based on methods similar to those used in studies by Fowler-Finn & Rodríguez 2011; Gershman *et al.* 2014). I found that the shape was that of a plateauing curve, rather than either a threshold, or an open-ended slope (Figure 9). Females preferred larger males, but only up to a point (with a potential dip down at the top end – though there is no statistical evidence for this). Why female preference saturated at the higher level is not clear. It is possible that females are simply unable to tell the difference between the eyespan of males at the top end. In the

related *T. dalmanni*, Hingle *et al.* (2001b) showed that females were unable to tell the difference between males where differences were small. Furthermore, the extent to which females could tell was related positively to female eyespan. So, this discrimination-limit idea is plausible. More perplexing though is the possibility that there really is a peak preference, because the extra large males have lower reproductive value; for instance, because on average large males suffer too many costs associated with such excessive eyespans. This should be investigated further.

An alternative interpretation of the overall shape of this preference function, with curved ends and stable peak (the peak did not vary with female mating status, and was thus stable to fluctuations in the costs of choice; Figure 9), is that it could be a 'stabilising' preference function, with an 'optimal' male size class. Such functions have been found in a number of other species. Examples include: the female response to male call frequency in the *Enchenopa binotata* treehopper complex (described in Rodríguez *et al.* 2006; Fowler-Finn & Rodríguez 2011, 2012; Rodríguez *et al.* 2013) and the black cricket, *Teleogryllus commodus* (Hunt *et al.* 2005b), as well as responses to the percentage of long-chirps in the field cricket, *Teleogryllus oceanicus* (Bailey 2008), to intra-pulse carrier frequency in *Drosophila montana* (Ritchie *et al.* 2001) and to call syllable number in both monosyllabic and polysyllabic populations of the bush cricket *Ephippiger ephippiger* (Ritchie 1996). Stabilising preference functions are often thought to imply stabilising selection (O'Donald 1980; Maynard Smith 1991; Hoikkala & Aspi 1993). However, this depends on whether the peak of selection overlies the peak of the frequency distribution of male size. My results show that the *D. meigenii* preference function was displaced well beyond the population mean, towards large eyespan.

Furthermore, this *D. meigenii* preference function also resembles, to a large extent, the linear directional functions that have been described in related species such as *D. montana* in relation to the pulse length (Ritchie *et al.* 2001) or carrier frequency (Ritchie *et al.* 2005) of male songs. It is still such open-ended directional preference functions that are expected to lead to the most trait exaggeration. Hence, the extreme male ornament exaggeration seen in *D. meigenii* could well be tied to the equally exaggerated, near open-ended, displaced-peaked female preference function trait. Note, though, that it is also plausible that the peak would shift if females with a different range of sizes were tested. Female eyespan covaried with the strength of preference in a previous study (Cotton *et al.* 2006a). In the current study, females were intermediate to large in size. So, the use of even larger females, for example, could potentially lead to an increase in the peak preferred male eyespan. Hence it would, in the future, be interesting to examine the consequences of varying female eyespan in further assays of female preference.

A final result of interest related to the levels of variation within and between individual females in each component of preference, and in the resultant selection. For both intra- and inter-female measures, there was no effect of female mating status on the level of variation in 'rejection' (choosiness). In contrast, the preference function was less stable over time *within* artificial virgins relative to mated females, and also varied more *between* such artificial virgins relative to mated females. A potential reason for this is that the [mathematical] decoupling of 'rejection' and 'preference slope' was not perfect. As virgin females were less choosy, and accepted more males with less discretion, the importance of stochastic effects as an influence on preference slopes could have been larger in

virgins. The random acceptance of a smaller male could have abolished or inverted preference functions. This could explain the increase in variance in the virgin state. In terms of temporal variation, there is also the possibility that some accessory products were able to enter the females during the preference assays before 'accepted' males were removed, which could have caused low level fluctuations in female 'mating status' over time in the 'virgins'. However, this is unlikely. Firstly, in this species sperm and accessory fluids are delivered in a single spermatophore (Kotrba 1996), which is not delivered before 150 seconds of mating (Harley *et al.* 2013). In both of our experiments, the males were removed from the females within a maximum of 20 seconds. So, the chance that either accessory fluids or sperm could have entered the females is very low. Moreover, it is notable that if either class of female was to vary in the level of 'mating status' over time it would more likely be the mated females, if sperm stores declined between the remating opportunities provided at the end of each assay week. Yet, this was not the case. Finally, the inter-female variation in selection was lower in virgins. This is likely because, while the variance in preference function was higher in virgins (or equivalent, in the case that this was an estimation resolution effect) the expression of the preference functions in choices was lower in virgins due to their lower choosiness. The variance in selection in virgins would have been reduced relative to that in mated females as the variable preference functions led to more random choices due to the effects of the virgin status on the costs of choice and on choosiness, as well as on choices recorded (and thus on 'selection').

2.4.1 *Conclusions and further research*

To summarise, I found and have presented evidence in favour of each of the three main hypotheses tested in this chapter. First, I have shown that estimates of female choosiness (rejection) and sexual selection (selection), but not preference function (preference slope), are lower or suppressed in virgin females. Second, I have shown that preference functions are estimated at a lower resolution in virgins. Finally, I have shown that both intra-female and inter-female variations in the estimation of the preference function are inflated in virgins females, in *D. meigenii*.

In addition to this, I have shed some light on the limitations and benefits of simpler (i.e. the two size test) and more complex (i.e. the five size test) assays of female mate choice. Both tests revealed near identical patterns for the responses of each component or preference, and selection, to variation in female mating status. Further, the interrelations of the analogous measures to one another were near identical in each case. In the first instance, this shows that a biological signal was detected – as the two tests utilised different mathematical frameworks methodologies. In the second instance, this shows that a simple test will be sufficient for most purposes. This insight is valuable, as experiments that use multiple levels or complex nested or blocked designs could limit the feasibility of full-scale multi-level preference tests: for instance, due to the levels of experimenter time required. Nonetheless, there are clear limits to the simpler tests. As has been implied in the introduction, such tests are not able to look at aspects of the shape of the preference function. Then, in addition to this, the two size test seems liable to detect artifactual variation in female preference functions.

Finally, as such tests cannot be used to look at variation in the shape of preference functions, they can only be used effectively if such functions are linear.

These conclusions lead to various implications. For instance, in the narrow sense, the effects of female mating status on the level of (and variation in) preference components and selection (within between females) mean that virgin estimates cannot be easily generalised to the non-virgin state. As polyandry is the normal state in stalk-eyed flies (Wilkinson & Dodson 1997; Chapman *et al.* 2005) and many other species (across animals, Jennions & Petrie 2000; Simmons 2001; and in insects, Arnqvist & Nilsson 2000), this suggests that such parameters need to be estimated in mated females, even if this complicates experimental design.

There are also wider implications. The leading sexual selection theories make predictions about the level of variation in preference. For instance, good genes theories predict low preference variation between or within individuals due to selection for 'best' combinations of genes (Forstmeier & Birkhead 2004). In contrast, the Fisher process relies on preferences with high levels of heritable genetic variation within populations (Lande 1981). The compatible-genes process predicts weak preference with variation between but not within individuals (Tregenza & Wedell 2000), the chase-away process, variation within the individuals as well (Holland & Rice 1998), while the sensory bias theories require stable population level choosiness and preference functions (Kuijper *et al.* 2012). These predictions could be contested. But the general point is that estimates of genetic variation are key to assessing a variety of plausible mechanisms supporting the evolution of mate preferences. Hence, the use of virgins could lead

to a systematic bias. For example, in the present study, inter-female variation in preference functions was higher in virgin females, which could lean towards a Fisherian interpretation. In actual fact, the level of variation was high in both the virgin and mated females (Figure 10). However the peak of the preference function and prior evidence about the nature of the male ornament (as a signal of genetic condition, Bellamy *et al*. 2013; Howie *et al*. Chapter 3; and of reproductive investment, Howie *et al*. Chapter 4) leaves room for a role for both indirect genetic 'good genes' benefits and direct reproductive or fertility benefits in the evolution of female preference functions in stalk-eyed flies. In all, this further highlights the need for future studies to consider the consequences of using virgin females to study female mate choice to use virgin or mated females. As a final point, it could be worth the inclusion of mating status in meta analyses that report results such as low or standard hertiabilities for preference (Prokuda & Roff 2014).

And, finally, it is possible to use simple experimental tests, so long as the shape of the preference function can be captured in a simple manner, to investigate variation in the components of preference and selection with context or condition. However, fine detail studies will require more complex designs that are able to look at variation in the shape of preference functions in response to such factors.

CHAPTER 3

Environmental variation can amplify or mask the signal of genetic condition in sexual ornaments, in stalk-eyed flies.

A B S T R A C T

An area of sexual selection that remains unresolved is the nature of the indirect genetic benefits that females obtain via mate choice. Male sexual ornaments reveal information about both environmental and genetic condition in a heightened manner relative to nonsexual traits. But how does environmental variation alter the genetic condition dependence of these ornaments; does it enhance or mask the genetic signal? Here, I test the hypothesis that environmental stress precipitates the signal of genetic condition in male sexual ornaments. To do so, I manipulate larval diets and exploit a series of crosses within and between a suite of 17 inbred lines in the sexually dimorphic African stalk-eyed fly, *Diasemopsis meigenii*. I find evidence for the heightened condition dependence of the sexual trait (male eyespan), for a shared genetic basis with the analogous female trait (female eyespan), and for a novel gene-by-environment interaction on sexual ornamental trait expression in which the effects of genetic stress on relative trait size are masked in both high and low, but not intermediate, stress environments (food levels). I also find evidence for an alignment between environmental and gene-by-environmental condition dependence in sexual and nonsexual traits. These results have important implications for the indirect benefits of female mate choice and the operation of sexual selection in nature.

3.1 INTRODUCTION

An area of sexual selection research that remains unresolved is the nature of the indirect genetic benefits gained through mate choice. Male sexual ornaments reveal information about condition (Houle 1992; Pomiankowski & Møller 1995; Rowe & Houle 1996; Tomkins *et al.* 2004; Prokuda & Roff 2014), and respond in a heightened manner relative to nonsexual traits to variation in environmental (Zuk *et al.* 1990; David *et al.* 1998; Kotiaho 2000; Holzer *et al.* 2003; Cotton *et al.* 2004a; Punzalan *et al.* 2008; McGuigan 2009; Rashed & Polak 2010) and genetic condition (Sheridan & Pomiankowski 1997; Aspi 2000; van Oosterhout *et al.* 2003; Drayton *et al.* 2007; Ala-Honkola *et al.* 2009; Bellamy *et al.* 2013). But despite a recent spate of studies on the extent of gene-by-environment interactions (G x Es) in sexual traits (Greenfield & Rodríguez 2004; Bussière *et al.* 2008; Ingleby *et al.* 2010; Ahuja *et al.* 2011; Weddle *et al.* 2012; Ingleby *et al.* 2013; Evans *et al.* 2015), little is known about the effects of environmental variation on the genetic condition dependence of sexual versus nonsexual traits (Cotton *et al.* 2004b). It is not known whether increased environmental stress precipitates or masks the signal of explicit genetic condition in exaggerated male sexual ornamental traits.

In part, this is because genetic condition is difficult to manipulate. A potentially powerful tool to achieve this is inbreeding (Rowe & Houle 1996; Cotton *et al.* 2004a; Bellamy *et al.* 2013; Tomkins *et al.* 2004); although this possibility has barely been investigated (Bellamy *et al.* 2014). Inbreeding increases homozygosity (Wright 1977). It exposes recessive deleterious alleles (i.e. the dominance hypothesis, Roff 1997; Charlesworth & Charlesworth 1999; Roff 2002; Charlesworth & Willis 2009) and further reduces fitness at loci subject to

heterozygote advantage (Wright 1977; Bulmer 1980; Charlesworth & Charlesworth 1987). This inbreeding depression (Darwin 1876; Roff 2002) increases as a function of the inbreeding coefficient, and leads ultimately to extinction (Frankham 1995; Liao & Reed 2009). As such, the inbreeding coefficient is a useful and easily manipulated measure of genetic stress, or 'condition' (Rowe & Houle 1996; Tomkins *et al.* 2004). It is one that links directly to the condition dependence theories of sexual selection, as both ornamentation and sexual selection are known to protect against inbreeding; with larger-ornament lines (Bellamy *et al.* 2013), and lines exposed to sexual selection (Lumley *et al.* 2015) less likely to become extinct as a result of serial inbreeding. A further advantage of the use of inbreeding to apply genetic stress is that it requires neither an overly-specific *a priori* definition of condition, nor the *post hoc* quantification of condition via the use of indices such as body-mass residuals, which require that a range of often-violated assumptions be met (Green 2001). Inbred individuals simply have a lower genetic condition than non-inbred ones.

A small number of studies have, in consequence, used inbreeding to apply direct genetic stress to test for heightened genetic condition dependence of sexual ornaments (reviewed in Bellamy *et al.* 2014). The results have been mixed. For instance, strong inbreeding depression in sexual traits (compared to non-sexually selected control traits) has been found in guppies (Sheridan & Pomiankowski 1997; van Oosterhout *et al.* 2003; Zajitschek & Brooks 2010), killifish (Ala-Honkola *et al.* 2009), *Drosophila montana* (Aspi 2000) and the stalk-eyed fly, *Diasemopsis meigenii* (Bellamy *et al.* 2013). But there have been mixed results in crickets (Drayton *et al.* 2007). And weak inbreeding depression has also been

reported in the zebra finch (Bolund *et al.* 2010) and in another stalk-eyed fly, *Teleopsis dalmanni* (Prokop *et al.* 2010). In addition, the validity of many studies is limited. This is due to a number of experimental design flaws, including: a lack of control for body size, a failure to contrast the sexual ornament with nonsexual control traits and a variety of issues specific to each study such as low replication of the number of inbred lines, low numbers of individuals per line, low inbreeding coefficients, or a lack of an outbred control (Lynch 1988; Bellamy *et al.* 2014). Most studies have also been conducted in single, often benign, environments.

To date, only one study has included inbreeding-by-environment interactions (Zajitschek & Brooks 2010). Whilst this study of guppies stands out for originality, it suffers from weak experimental design. It used only two generations of full-sib inbreeding and two levels of environmental stress. The semi-natural 'high stress' environment was not a controlled treatment, so individuals may have experienced quite different levels of environmental stress. There was a lack of control for body size and no non-sexual trait comparison, which disqualifies any inferences about heightened condition dependence. An interesting contrast is provided by a different approach that used *Drosophila melanogaster* mutation accumulation (MA) lines instead of inbred lines to generate controlled genetic variation (Bonduriansky *et al.* 2015). The MA lines were subjected to dietary manipulations to test for an alignment between the effects of genetic and environmental condition on trait expression. The study was robustly designed, with 19 haploid genomes and 50 generations of MA. It was also novel, adding to our knowledge with a direct, affirmative test of the hypothesis that condition integrates environmental and genetic variation (in which a correlated across-trait response to E and G was observed). But it lacked a clear, simple, condition-

dependent sexual trait (the traits used were CHC complexes and male sex combs: the former is complex, that later cannot be thought of as a condition-dependent sexual ornament). Further, the use of two levels of environmental stress limits interpretation about directionality and the across-environmental shape of genetic condition dependence (Cotton *et al.* 2004a). So, there remains a need for further well-conducted G x E studies using inbreeding to investigate the genetic basis of condition dependence in male sexual ornaments (Cotton *et al.* 2004b; Tomkins *et al.* 2004) and to provide insights into the across-environmental shape of genetic condition dependence in sexual ornamental versus nonsexual traits: to ask, "does environmental stress amplify the signal of genetic condition in male ornaments?"

To address this, I investigate the effects of interactions between the inbred and outbred status of individual flies and a range of environments, decreasing in condition, on the expression of the sexual ornamental trait in the African stalk-eyed fly species, *D. meigenii*. As in many stalk-eyed fly species (Wilkinson 2001; Chapman *et al.* 2005), male *D. meigenii* have larger eyespans than females, even after controlling for allometric scaling (Baker & Wilkinson 2001). Females exhibit strong mate preferences for males with larger eyespan (Cotton *et al.* 2006). Male eyespan has been shown previously to have heightened condition dependence to both environmental and genetic stress (Bellamy *et al.* 2013). I use 17 highly inbred lines ($f \sim 0.908$; Falconer & Mackay 1996) produced by Bellamy *et al*. (2013) to induce two distinct levels of genetic stress by crossing between lines (outcross) or within lines (incross). I raise the offspring on three distinct larval diets that likely span the range of natural variation. I assess the consequences for the sexual ornament (male eyespan) compared to other non-sexually selected traits. I test the

integration of trait responses to variation in environmental, genetic and gene-environmental condition for sexual and nonsexual traits in both sexes. As such, I am able to ask, firstly, whether the response to environmental and genetic variation is heightened for the sexual trait (male eyespan) relative to a suite of non-sexual traits; then, whether the level of genetic information in the sexual trait increases as environmental stress increases; whether a suite of condition dependent traits integrate environmental and genetic variation, and whether the male sexual trait does so most of all, as anticipated by the handicap hypothesis of sexual selection. Ultimately, I ask: "why do females prefer more ornamented males" and, "how does this impact on the operation of sexual selection in nature".

3.2 MATERIAL AND METHODS

3.2.1 *Production of experimental flies*

The laboratory-adapted stock population of *Diasemopsis meigenii* was collected in West Africa in 2000 by Sabine Hilger. A large population (> 200 individuals) was subsequently maintained in cage culture at 25°C on a 12 : 12 hour light : dark cycle, with artificial dawn and dusk periods created via the phased illumination of two 58W, 'cool white', 1-10 V tube lights over 15 minutes at the start and end of each light cycle. Each cage was lined with moist cotton. The flies were fed twice per week with puréed sweet corn mixed with a low dose of antifungal Nipagin.

A suite of inbred lines was founded in 2008 (Bellamy *et al.* 2013). In brief, 105 F_0 male-female virgin pairs were selected at random from the stock population. From the progeny, 5 F_1 brother-sister virgin pairs were set up per line. Each of

these sub-lines was maintained until at least one had produced 10 male and 10 female F_2 offspring. This sub-line was chosen to generate the next F_3 generation. This procedure was repeated until F_{11}. At that point there were 26 extant lines with expected inbreeding coefficients of $f \sim 0.908$ (Falconer & Mackay 1996). The inbred lines were then kept in population cage culture under the same conditions as the outbred stock population. At the start of this experiment, 17 lines were still extant and varied between $F_{24} - F_{31}$.

Experimental flies for this study were obtained from cage populations of inbred lines. Eggs were collected on petri dishes containing excess puréed sweet corn (3 grams) to ensure that the maximum possible number of viable adult flies were obtained. At eclosion, all flies were placed in large cages (15 litre). Two weeks after eclosion the flies were separated by sex. The flies were then raised until maturity (that is, until ~ 8 weeks of age) before being used in experimental crosses.

3.2.2 *Variation in environmental and genetic effects*

Variation in genetic state was created by comparing incross flies from the inbred lines to outcross flies created by crossing between lines. The crossing protocol was a modified version of that used in Prokop *et al.* (2010) and Bellamy *et al.* (2013) (see Figure 1). Such crosses balance allelic richness between flies of the two incrosses and the outcross, and permit a comparison of homozygotic (incross) and heterozygotic (outcross) states. For each cross, 4 males and 4 females were placed in a 1.5l pot with moist cotton, a blue paper liner to visualise eggs, and *ad libitum* corn. Eggs were collected twice a week over the following 23 days (and food replaced). Groups of 3-5 eggs from a single cross were transferred to petri

Figure 1

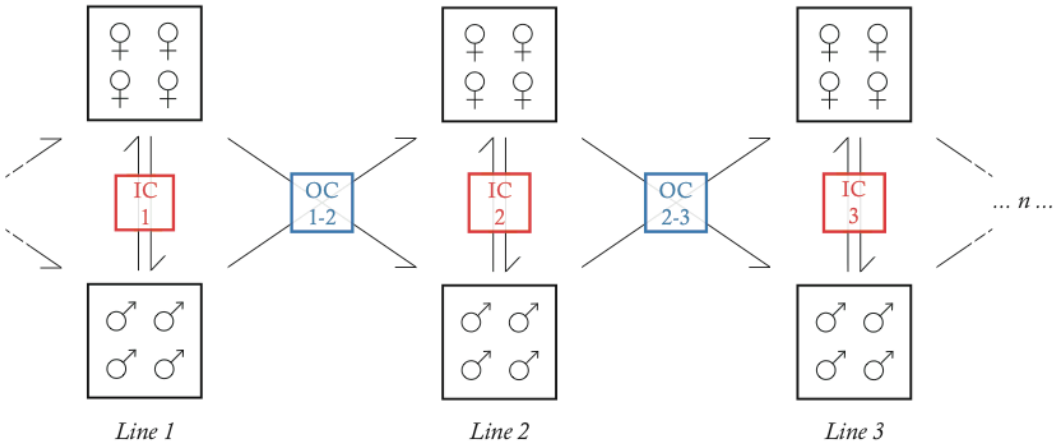


Figure 1. The cross-protocol used is represented above. Each initial inbred line was crossed with itself to create incross flies (red), or with another line to create outcross flies (blue). Each cross used 4 males and 4 females. Each cross was repeated. Full details of the scheme are provided in the Materials and Methods, section 3.2.2, above.

dishes containing two small cotton pads, 15 ml water and 5 ml of different quality food media to induce controlled variation in environmental state (see below).

For incrosses (n = 67, between 15 inbred lines), males from one inbred line were crossed with females from the same inbred line. For outcrosses (n = 50, between 16 inbred lines), males from one inbred line were crossed with females from another inbred line. Variability in the number of inbred lines used reflected the availability of suitable numbers of mature flies at the time the experiment was set-up. Each of the incrosses was replicated 1 – 8 times, each of the outcrosses 1 – 4 times. This variation reflected expectations of survival (inbred flies generally have low fecundity and viability) and the unknown nature of outcrosses (which could fail due to incompatibility between different lines). The replicate crosses were reciprocal, to balance sex chromosomal, cytoplasmic and other male/female effects. The number of times that a line was used in an incross was also balanced with the number of times that it was used in an outcross, to balance line (including autosomal) effects between genetic treatments. Finally, the number of times a line was used overall (in incrosses and outcrosses) was also balanced.

Variation in environmental state was created using three levels of quality in the food media. The levels varied in accordance with the composition of mixtures of "pure" corn diluted with water at ratios of 1:1, 1:10, and 1:20. The "pure" corn was created by forcing puréed sweet corn through a fine sieve to remove husks and non-blended corn that could otherwise form nutrient low or hotspots. Variation in larval diet was used to generate variation in environmental stress because variation in larval stress causes variation in adult size (Cotton *et al.*

2004a) and because the external morphology of stalk-eyed flies is known to be fixed at eclosion (de la Motte & Burkhardt 1983). The specific levels were chosen based on a pilot experiment (so as to capture a wide range of variation, without large mortality effects). As lower eclosion was expected in incross and outcross flies at low food levels, approximately double the sample size of these crosses was set up. All larvae were maintained in constant temperature rooms until eclosion.

3.2.3 *Adult morphology*

At eclosion, flies were transferred to cross-specific 1.5l pots, with 8 – 10 flies per pot. After two weeks, flies were separated by sex. Females were frozen at - 20°C. Males were housed at the same density in cross-specific pots and maintained until sexual maturity when they were used in experimental work. After this, all flies were measured for eyespan (the distance between the outermost tips of the eyes; David *et al.* 1998), thorax (the distance between the centre of the most posterior point of the head to the joint between the meta-thoracic legs and the thorax) and wing length (branch point of the MA and r-m veins to the terminus of the RP4 vein; Gullan & Cranston 1994, p 45; measurement *x* in David *et al.* 1998) to a tolerance of 0.01mm, using a video camera mounted on a monocular microscope and ImageJ image capture software v. 1.46 (Rasband 1997 - 2012).

In addition, the male reproductive organs were measured. This was done to allow me to test the integration of environmental (E), genetic (G) and gene-by-environmental (G x E) variation in condition. In the case that condition integrates E, G and G x E, then condition-dependent traits should respond to each – either on a similar scale, but to different extents, as some traits are more

condition-dependent than others; or on different scales, with some trait responses of the type, "more to E, less to G", and vice versa (i.e. if there are different types of condition dependence, as implied by Bonduriansky *et al.* 2015). To allow for a quantitative test, several condition-dependent traits are needed (rather than just eyespan, Bonduriansky *et al.* 2015). Both testes and accessory glands are related to life history traits, and can be expected to be condition dependent (Rowe & Houle 1992). As such, both organs were dissected from each male, and placed on a slide with a drop of phosphate-buffered saline solution (Baker *et al.* 2003) and photographed at x 50 with a Leica DFC295 digital camera mounted on a compound microscope. The length of each organ was estimated using a trace along the midline in ImageJ (Rogers *et al.* 2005). A mean testes length and mean accessory gland length was then taken for each male (Baker *et al.* 2003), with all measurements taken when the males were mature, at 10 – 14 weeks. These measurements were taken either at natural death, or after the completion of ejaculate output and male attractiveness assays (see Chapter 4). For the latter, males were left for ~ 12 hours prior to dissection, to allow post-mating recovery time (Rogers *et al.* 2005). The expectation was that all traits would integrate E and G (and G x E), and that there would be positive correlation across traits for trait responses, with male eyespan integrating the most E and G (and G x E), as the most condition-dependent trait. A concomitant expectation was that thorax would fall well off the correlation as it is expected to be a relatively canalised trait.

3.2.4 ***Statistical analysis***

3.2.4.1 *Mean effects of environment, genetics and their interaction*

To test for effects of food level, genetic status and G x E on morphological trait variation, several sets of general linear mixed effects models (GLMMs) were fitted via REML to the trait data. In each model, food level, genetic status (incross/outcross) and their interactions were included as fixed effects. The number of eggs on the petri dish that the flies were reared on was included as a covariate to control for variation in size due to larval competition. Each cross was included in the models as a random effect. GLMMs were fitted for absolute thorax length, eyespan and wing length, relative eyespan and relative wing length (adding thorax as a covariate to control for body size for relative traits), separately for males and females. To contrast eyespan and wing length, the models also included trait type and its interactions with food level and genetic status (including the three-way interaction, trait type x food level x genetic status), with male ID used as a random effect. Likewise, to contrast male and female trait responses, the analyses were repeated with sex and its interactions instead (including the four-way sex x trait type x food level x genetic status interaction).

3.2.4.2 *Integration of environmental and genetic effects across traits*

To compare the integration of environmental and genetic effects across traits (that is, do the trait responses integrate E and G, and G x E, and is there a positive correlation across traits, or not), GLMMs were fitted to standardised trait measures (i.e. z-scores: with a mean of zero and variance equal to one), for each trait in each sex. Each GLMM included food level, genetic status and their interaction, with number of eggs per petri dish included as a covariate and cross as a random effect. The model effect coefficients for environment, genetics and G x E were extracted (Bonduriansky *et al.* 2015). At one data point per trait, the

average environmental effect coefficient was regressed on the genetic effect coefficient.. As I detected G x E interactions for mean trait sizes at intermediate food levels, the intermediate environmental effect coefficient was also regressed on the intermediate G x E effect coefficient. Pearson correlation analyses were then used to test alignment between environmental and genetic (or G x E) responses (Bonduriansky *et al.* 2015). Each coefficient combination was plotted for absolute and relative (body size controlled) trait model output. Further, the absolute trait integration plots were also run both with and without male and female thorax included as data points, so as to test the prediction that such traits are canalised and will thus fall well off the line of E-G (or G x E) integration.

3.2.4.3 *Trait variance*

To elucidate the causes of variation in trait means, and to look at between line effects of both environmental and genetic stress, a number of further analyses were undertaken. First, both variances and coefficients of variation (CV = $\sigma/\bar{X}$, the ratio of the standard deviation to the mean) were calculated for absolute and relative male and female eyespans in incross and outcross flies. To do so, GLMMs were used; one per food level, per genetic treatment, per sex. Each included cross as a random effect, with thorax included as a covariate for relative traits. Least squared means for each cross were then extracted, and the between-line variances and CVs calculated. Variances were contrasted with Brown-Forsythe tests, a standard test for the equality of variances; and CVs were then used to confirm the results after trait size scale effects were taken into account.

3.2.4.4 *Across-environment genetic correlations*

To further explore the between line patterns, in addition to the mean patterns, across-environment genetic correlations (r_g) were then calculated. These were calculated in two ways. First, to detect the presence, absence and type of r_g a series of three GLMMs were contrasted per trait. The first included food level as a fixed effect; the second, food level as a fixed effect and cross as a random effect; and the third, food level as a fixed effect and cross nested within food level as a random effect. The models related to a slope and intercept for all lines, a slope and multiple intercepts, and multiple slopes and intercepts (Roff & Wilson 2014). The models were compared with likelihood ratio tests, and were repeated for contrasts between low and intermediate, and intermediate and high food levels, per genetic state, sex and trait. Direct estimates of r_g were then calculated using both variance components (Roff & Wilson 2014) and least squared means (Yamada 1962; Astles *et al.* 2006) based approaches (SI.DS1). For each method, r_g was calculated between both low and intermediate, and intermediate and high food levels.

3.2.4.5 *Mean effects of environment and genetics on male age at death*

Males were maintained for 10 weeks until mature before the dissections for testes and AG were started. Across this time, the age of males at their natural death was recorded. To test for effects of environmental and genetic stress on male age at death, a GLMM was fitted that included both forms of stress and the interaction between these as fixed effects, with each cross included as a random effect. This was done to indirectly test if E or G could have altered larval mortality. The data were square-root transformed to account for Poisson error structures (see SI.E).

All statistical analyses were conducted in JMP v 11.2.0 (SAS Institute 1989-2007) and R v 3.1.3 (R Core Development Team 2015). GLMM results tables and effects coefficients are provided in the Chapter 3 Supplementary Information.

3.3 RESULTS

3.3.1 *Mean effects of environment, genetics and their interaction*

3.3.1.1 *Absolute traits*

Male and female thorax length, absolute eyespan and absolute wing length all increased with higher food quality (all $F_{2,1019\text{-}1177} > 528.19$, $p < 0.001$). There was no difference between outcross and incross flies in male ($F_{1,26.87} = 1.49$, $p = 0.232$) nor in female thorax length ($F_{1,30.79} = 1.22$. $p = 0.278$). Outcross flies in both sexes had larger absolute eyespan (male $F_{1,26.66} = 5.34$, $p = 0.029$, Figure 2; female $F_{1,32.61} = 7.38$, $p = 0.011$) and absolute wing length (male $F_{1,28.48} = 7.06$, $p = 0.013$; female $F_{1,32.09} = 6.76$, $p = 0.014$). There were no G x E interactions (all $F_{2,1080\text{-}1244} < 1.25$, $p > 0.284$), except in one trait, male thorax length, which was larger in outcross flies at low and high food levels ($F_{2,1143} = 3.51$, $p = 0.030$; see SI.A.S1-6).

3.3.1.2 *Relative traits*

The analyses were repeated on relative trait sizes using thorax length as a covariate for body size (SI.A.S7-10). Relative eyespan and relative wing length increased with higher food quality in males (all $F_{2,1067\text{-}1130} > 143.88$, $p < 0.001$) and females (all $F_{2,1028\text{-}1128} > 87.15$, $p < 0.001$). Outcross flies had larger relative wing length in males ($F_{1,29.75} = 7.01$, $p = 0.013$) and females ($F_{1,30.57} = 11.43$, $p = 0.002$), and larger relative eyespan in females ($F_{1,24.86} = 10.09$, $p = 0.004$). Male relative

Figure 2

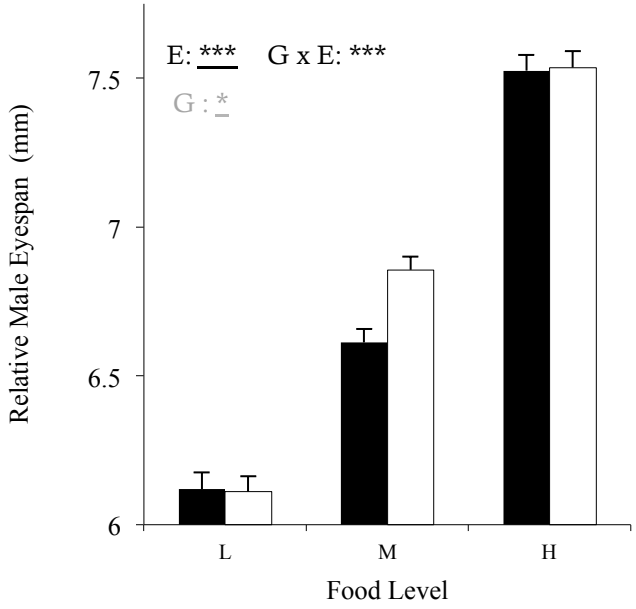


Figure 2 a, b. Absolute and relative male eyespan, least squares means for incross and outcross flies at each food level (± s.e.). Black bars = incross, white = outcross. In each case, significance is denoted by asterisks: at *, **, ***, $p < 0.05, 0.01, 0.001$. Underlined asterisks indicate that a response to E, G or G x E existed before and after control for thorax length variation, grey asterisks indicate effects lost after this control.

eyespan did not vary with genetic state ($F_{1,25.68}$ = 4.13, p = 0.053), but this effect was marginal, and in the direction of larger trait values in outcross flies. There was a G x E interaction for relative eyespan in males ($F_{2,1145}$ = 7.17, p < 0.001; Figure 2) and females ($F_{2,1240}$ = 4.22, p = 0.015), and for relative wing length in males ($F_{2,1080}$ = 5.13, p = 0.006) but not females ($F_{2,1177}$ = 1.19, p = 0.306). In all cases the largest difference (outcross > incross) was observed at intermediate food levels.

3.3.1.3 *Trait comparisons*

I then compared the scale of eyespan and wing length responses. To do so, I fitted a model for trait size, that included trait (eyespan or wing length, to control for variation due to the different basic sizes of the two traits), as well as the trait x E (i.e. food level), trait x G (i.e. incross/outcross) and trait x G x E interactions (to allow for an exploration of relative trait responses). Eyespan increased more with higher food quality than did wing length in both sexes (male $F_{2,1091}$ = 1012.92, p < 0.001, female $F_{2,1185}$ = 879.64, p < 0.001) and the same held for relative trait values after controlling for body size (male $F_{2,1114}$ = 1018.39, p < 0.001, female $F_{2,1181}$ = 874.12, p < 0.001). The same pattern was seen in relation to genetic quality as the larger trait size of outcross compared to incross flies was greater for eyespan than wing length in both sexes, for both absolute (male $F_{1,1088}$ = 14.66, p < 0.001, female $F_{1,1185}$ = 18.92, p < 0.001) and relative trait values (male $F_{1,1111}$ = 14.44, p < 0.001, female $F_{1,1180}$ = 18.74, p < 0.001). Neither absolute nor relative traits differed in their responses to the G x E interaction in either sex (all $F_{1091\text{-}1182}$ < 1.11, p > 0.329; see SI.A.S11-12 for absolute, A.S13-14 for relative contrasts).

I also compared the response in males and females. The effect of sex on trait size was controlled, and the sex x food level, sex x genetic status, and sex x G x E interactions were tested. Eyespan was more responsive to changes in food levels in males than females ($F_{2,2395}$ = 163.60, $p < 0.001$) but wing length was not ($F_{2,2255}$ = 2.39, p = 0.092). Likewise, both relative eyespan ($F_{1,2383}$ = 313.38.46, $p < 0.001$) and relative wing length ($F_{1,2258}$ = 3.34, p = 0.035) were more responsive to changes in food levels in males than females. However, sex differences in genetic status or G x E were not detectable for absolute or relative eyespan or wing length (genetic status: all $F_{1,2254\text{-}2392} < 2.30, p > 0.129$, G x E: all $F_{2,2256\text{-}2397} < 1.08$, $p > 0.339$; for full output see SI.A.S15-17 for absolute, A.S18-19 for relative trait contrasts).

I also contrasted the within sex trait relations between the sexes. The larger response to increased food level of eyespan compared to wing length (both absolute and relative) were even larger in males than in females (trait x food x sex interaction, absolute $F_{2,2277}$ = 237.06, $p < 0.001$; relative $F_{2,2307}$ = 235.24, $p < 0.001$), but did not vary with genetics (absolute $F_{1,2271}$ = 3.13, p = 0.077; relative $F_{1,2302}$ = 2.91, p = 0.088) – although the genetic effect tended in the direction of males – or G x E (absolute $F_{2,2271}$ = 0.89, p = 0.409, relative $F_{2,2304}$ = 0.68, p = 0.505; for full model output see SI.A.S20 for absolute, A.S21 for relative trait contrasts).

3.3.2 *Integration of environmental and genetic effects across traits*

I observed qualitative alignment between the effect coefficients of food level and genetic status for all absolute and relative traits (Table B.S1-2). Incross status or lower food level resulted in reduced trait sizes. But across traits the correlations were negative. For both absolute (t = -3.83, df = 5, p = 0.019, r = -0.889; SI.3.F.

A) and relative traits (t = -7.26, df = 5, p = 0.002, r = -0.964; Figure 3), the traits that responded most to food level responded *least* to genetics and vice versa. I repeated this analysis using food level and G x E at intermediate food levels (Table B.S1-2). This was done because, while the response to genetics across environments was low in certain traits, the scale of the G x E in these traits was often largest: so a large part of the effect of genetic state on trait values was tied up in the G x E; with the largest responses seen at intermediate levels of environmental stress. In this case both the absolute (t = 13.5, df = 5, p = 0.002, r = 0.989; SI.3.F.B) and relative trait (t = 4.66, df = 5, p = 0.010, r = 0.918; Figure 3) correlations were positive. Note that for both absolute and relative traits, male eyespan exhibited the greatest response to food level and G x E, and the least to genetics, and also that, for absolute traits, the patterns were lost on inclusion of male and female thorax as additional data points, as expected for canalised traits.

3.3.3 *Genetic differences in trait variance*

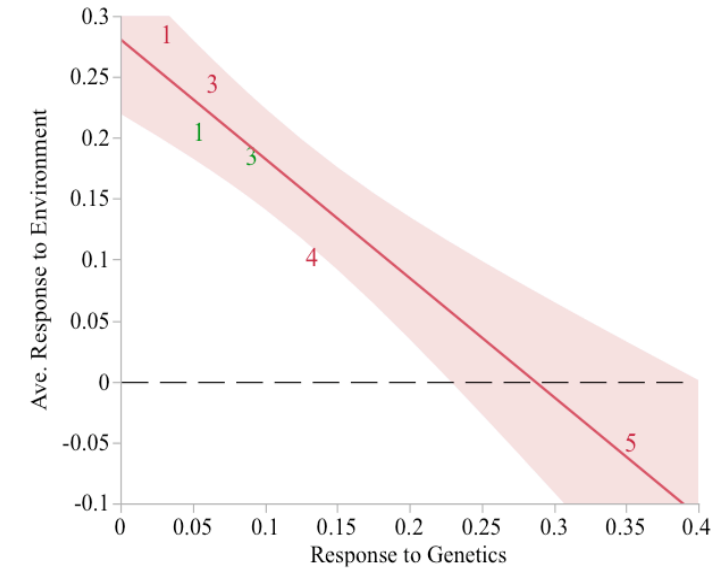
3.3.3.1 *Variance and CV*

Absolute male eyespan between line variance was lower in incross than outcross flies at high food levels ($F_{1,34}$ = 16.289, p < 0.001), but the difference was small. Variances were higher at intermediate and low food than at high, but incross and outcross lines did not differ at either intermediate ($F_{1,34}$ = 1.698, p = 0.201) or low ($F_{1,34}$ = 0.094, p = 0.761) food levels. A similar pattern was seen for CV (SI.3.F.B).

Relative male eyespan between-line variance increased markedly as food level declined. It did not differ between incross or outcross lines at either low ($F_{1,34}$ = 1.109, p = 0.299) or high ($F_{1,34}$ = 0.893, p = 0.351) food levels. But, there was a

Figure 3

a)



b)

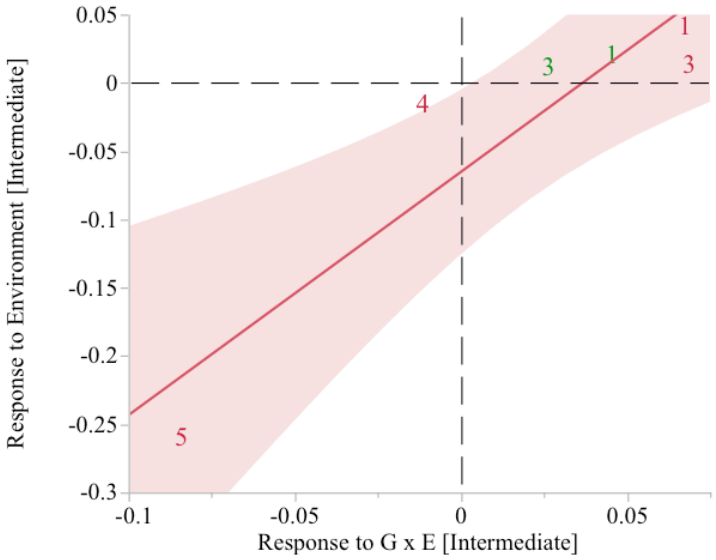


Figure 3 a, b. Regression of model coefficients for E on G (a) and for intermediate E on intermediate G x E (b) for relative trait z-scores. Numbers 1, and 3 – 5 relate to traits in order: eyespan, wing length, testes length and accessory gland length. Red = male, green = female. The red outlines show the confidence intervals at ± 95%.

large difference between incross and outcross lines at intermediate food levels ($F_{1,34}$ = 22.03, p < 0.001), where the outcross flies retained lower between line variance. As for the absolute traits, a similar pattern was seen for CV (Figure 4).

3.3.3.2 *Across-environment genetic correlations*

All across-environment genetic correlations (r_g) were positive – indicative of low crossover in all environments. For absolute male eyespan, for both incross and outcross flies, the estimates of r_g between L-I were larger than those at I-H (incross: 0.61, 0.32; outcross: 0.64, 0.15; SI.3.F.C). For relative male eyespan, the estimate of r_g for incross flies at L-I was larger than at I-H (0.29, 0.12, Figure 5a). For outcross flies the values were inestimable due to zero variance at intermediate food levels (Figure 5b). The results were backed up by the model contrasts (SI. Table D.S2-DS3). The results are represented as per line LS means for absolute eyespan and relative eyespan, in Figure 5 a, b; SI.3.F.C.

3.3.4 *Mean effects of environment and genetics on age of males at death*

Male age at death decreased with food stress ($F_{2,947.6}$ = 44.11, p < 0.001) and genetics ($F_{1,30.78}$ = 14.28), but did not vary with G x E ($F_{2,948.4}$ = 0.18, p = 0.839).

3.4 DISCUSSION

In this chapter, I test whether a sexual ornamental trait (male eyespan) responds in a heightened manner to variation in environmental and genetic stress and ask how these two forms of stress interact in altering trait development: "does environmental stress precipitate the signal of genetic condition in male eyespan?" The novelty of this approach is that I examine trait responses in explicitly low

Figure 4

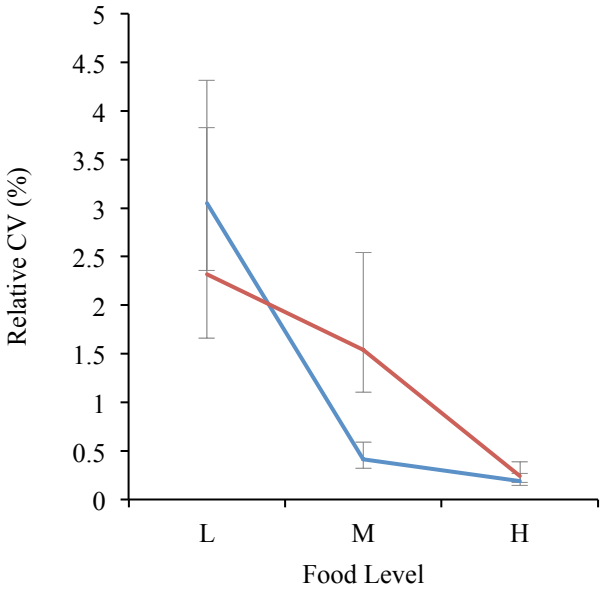


Figure 4 a, b. Variation in CV across food levels for relative male eyespan in incross versus outcross flies. Red = incross, blue = outcross (± 95% C.I.).

Figure 5

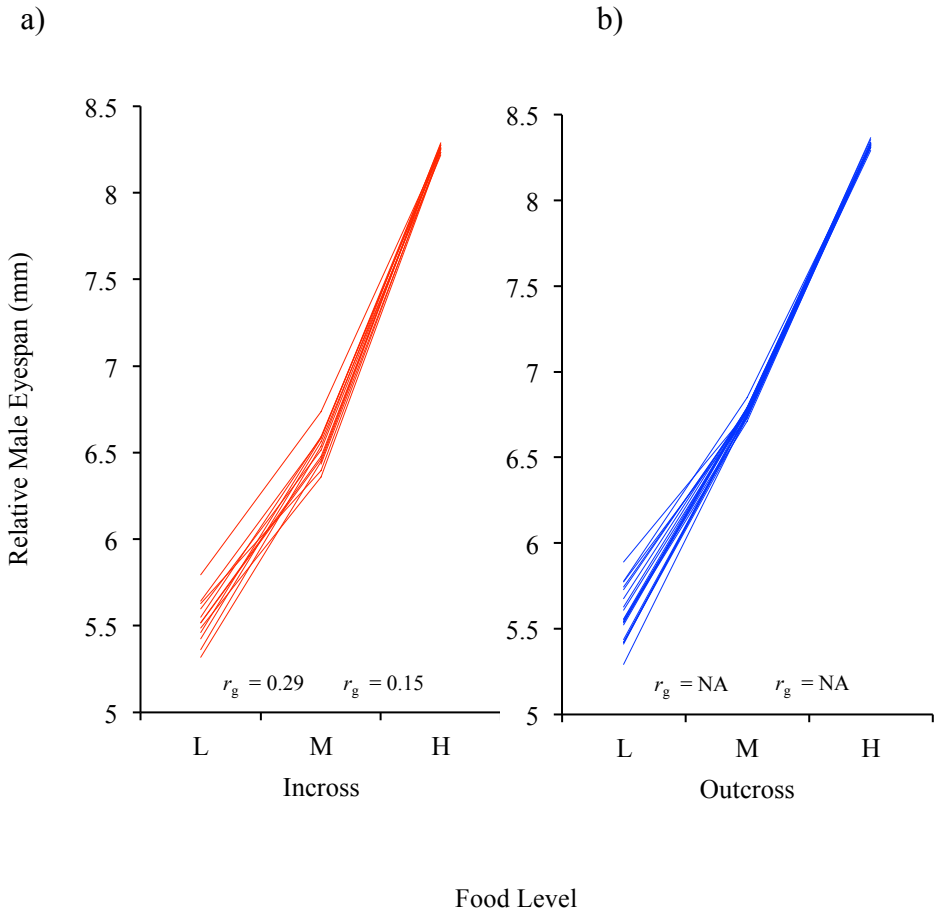


Figure 5 a, b. Across-environment genetic correlations for relative trait values. Per cross least squares means for incross (a) and outcross (b) relative male eyespan. Error bars are excluded for clarity. Across-environment genetic correlation values for the low versus intermediate and intermediate versus high food levels are also shown. For incross flies, the rank order is more variable between intermediate and high food levels. For outcross flies, the values cannot be calculated, but the rank orders do not vary between intermediate and high, while the lines 'fan out' towards low food levels. The use of LS means allows the patterns to be seen more clearly, but leaves non-zero variance at outcross, intermediate – but it was equal to zero for variance components.

and high stress genotypes across three tightly controlled larval environments that reflect a wide range of natural variation. I compare homozygotes (crosses within inbred lines) with restored heterozygotes (crosses between inbred lines) in which the effects of fixed deleterious alleles present in inbred lines are expected to be masked. This approach allows for the direct comparison of incross and outcross (high and low stress) flies whilst allelic richness is controlled. Based on this technique, I provide the first test of the interactive effects of environmental and genetic stress on the expression of sexual versus non-sexual traits. I provide new insight into the across-trait integration of these effects. I also provide an interpretation of the results in relation to the indirect genetic benefits of mate choice.

As expected, all sexual and nonsexual traits responded positively to variation in both environmental and genetic stress, and the responses persisted after control for body size. Male and female eyespan also responded in a heightened manner to each type of stress relative to male and female wing length, both before and after control for body size. Male eyespan also responded to variation in environmental stress in a heightened manner relative to female eyespan, as did male eyespan relative to male wing length in contrast to the female homologue. In line with multiple previous studies I thus show that male eyespan reveals information about environmental and genetic condition over and above that revealed by body size (environment: *T. dalmanni*: David *et al*. 1998; Cotton *et al*. 2004b; genetics: David *et al*. 2000; but not Prokop *et al*. 2010; *D. meigenii*: Bellamy *et al*. 2013) in a heightened manner relative to nonsexual traits (environment: *T. dalmanni*: Cotton *et al*. 2004a; *Sphyracephala beccarri*: Cotton *et al*. 2004b; genetics: *D. meigenii*: Bellamy *et al*. 2013). The similar responses of eyespan and wing

length also provide evidence for a potential compensatory coevolution between these traits, as implied across species in Ribak *et al.* (2009) and Husak *et al.* (2010): that is, as eyespan increases, larger wings are needed to maintain inflight stability.

However, the response of male eyespan to genetic stress did not differ from that of female eyespan, as neither did the response of male eyespan relative to male wing length in contrast to the female equivalent, either before or after control for body size. A potential reason for this is that male and female eyespan similarly reflect a wide range of loci or loci with large effects: either due to a shared historic genetic architecture and predisposition for condition dependence; to vicarious selection on female eyespan via male-female trait linkage; or due to a convergent evolution of trait condition dependence caused by male *and* female choice (for instance, males are known to prefer larger eyespan females in the related *T. dalmanni*; Cotton *et al*. 2014). Alternatively, it is possible that the lack of sex difference is due to my control of allelic richness effects (which could be an important contributor to sex differences in nature). Serial inbreeding reduces allelic richness (Falconer and Mackay 1996), which is also controlled in the outcrosses. Bellamy *et al*. (2013) found a sex difference in the response of eyespan to serial inbreeding (as allelic richness declines), but not outcrossing (where it is controlled): male relative eyespan decreased more than female relative eyespan after inbreeding (it exhibited heightened inbreeding depression), but it did not increase more than female relative eyespan on outcrossing (it did not exhibit heightened heterosis). Finally, a lack of statistical power could explain the lack of sex difference, as the trend, here and in Bellamy *et al*. (2013), was for near-significantly larger responses in males, both before and after control for body size.

A crucial finding in my analyses was the shape of the G x E interactions on trait size. Higher stress environments have been predicted to amplify genetic variation that is hidden under more benign conditions (David *et al.* 2000). Others have predicted the reverse if high stress environments create noise that overwhelms genetic variation, so that genetic differences are only apparent under benign conditions (Jia *et al.* 2000; and see meta-analysis in Fox & Reed 2011). I tested the hypothesis that the former is true: that environmental stress precipitates the signal of genetic condition in male ornamental traits. I provide partial support for this expectation as the difference in trait size between high genetic stress (i.e. incross) and low genetic stress (i.e. outcross) flies increased as environmental stress increased (i.e. low to intermediate environmental stress). This held for relative male and female eyespan, and relative male wing length. But, I did not provide full support. There was a convergence of incross and outcross trait sizes with further environmental stress (i.e. from intermediate to high environmental stress). A simple explanation of this would be if larval survival selection plays a larger part under poor environmental conditions. Now individuals in poor genetic condition have much reduced survival chances. This results in an elimination of individuals that would bear very small trait sizes and, hence, little further reduction in trait size amongst low genetic quality incross flies. The effect of environmental deterioration on trait size is felt by the better quality outcross flies whose trait size is reduced to the level equivalent to that of the incross flies (as in Figure 2). In line with this expectation, I observed that mean age at death was lower in both incross and low food level males, and lowest of all in the incross *and* low food level males. The test is on adults, not larvae; so it is not direct. But it shows that high

environmental and genetic stress affect survival: and this is in line with the interpretation given – that smaller incross or low food larvae fail to reach eclosion.

This framework of understanding also applies well to changes in male eyespan variance. Looking at male absolute eyespan, variance was limited at both low and high environmental stress (as would be expected for the combination of an upper limitation on trait size and survival selection), with wide between-line trait variance at intermediate environments obscuring any absolute trait variance G x E. In contrast, when looking at male relative eyespan, I found increasing between-line variance moving from low to high environmental stress (Figure 4). This increase occurred under weaker environmental stress for low quality incross flies (i.e. low to intermediate environmental stress) than for high quality outcross flies (i.e. intermediate to high environmental stress). As with the mean trait sizes, the difference in relative trait variance between genetic classes was therefore high in the intermediate stress environment and abolished under low and high stress environments (Figure 4). So, it appears that there are two effects: at the between line level, variance in relative trait size increases as environmental stress increases, as has been predicted. However, the level of variance increase is modulated relative to overall incross/outcross stress. In outcross flies, the overall low genetic stress levels lead to a suppression of between line genetic variation until more extreme environmental stress. Hence, there are two effects: specific alleles, and overall condition: the suppression of allele effects part of the cause of the overall condition G x E (due to a reduction in variance at intermediate stress).

A further insight can be gained by additional examination of the individual lines. To focus on male relative eyespan, all across-environmental genetic correlations were either positive or non-calculable (due to low between-line variance). Given this, lines that performed well in one environment performed well across all environments. In terms of incross lines, the difference in slopes between lines was greater between low and intermediate than between intermediate and high stress environments. For outcross lines, the difference in slopes could not be calculated, but was observed qualitatively to be lower for low to intermediate, and higher for intermediate to high, stress environments. In concordance with the between-line variance pattern, then, the "fan-out" between lines started at lower stress levels for low quality (i.e. incross) lines and at higher stress levels for higher quality (i.e. outcross) lines. Hence, it is this variance-r_g effect, in concert with the trait mean pattern, that explains the net, directional G x E for relative trait values. Two fan-shaped between-line G x E patterns are overlaid: one for incross flies, one for outcross flies, each with a different mean, variance, and fan 'start-point' (Figure 5a,b).

In simple terms, this overall directional G x E implies that the amount of genetic information about overall condition revealed by the male sexual trait (i.e. relative eyespan) is highest at intermediate environmental condition. So potential genetic benefits, and thus sexual selection, will be strongest in this environment as well. We have to tread a little carefully with this interpretation, as the genetic quality we are comparing is between highly inbred and highly heterotic individuals, and the environments are laboratory constructs, not necessarily equivalent to those existing in nature. Nevertheless, such an interpretation is not without backing. Janicke *et al.* (2015) noted that benign *as well as* harsh environments can mask genetic variation in individual condition. As sexual traits are expected to be

condition-dependent, genetic variance in sexual traits could thus plausibly be highest and sexual selection strongest at intermediate levels of stress. Hence, if intermediate rather than extreme conditions are common, then sexual selection could often be stronger than currently estimated and, in contrast to standard expectations, weaker not only in high quality environments, but in low as well. (Although, if specific alleles, rather than overall condition, are the targets of female mate choice, then selection could be stronger in ever worse environments.)

Another point of interest is dependent on the relationship between the length of individual life cycles and climatic-environmental cycles in natural populations, and how this can further modulate sexual selection. For instance, in "fine-grained" rapidly fluctuating environments, ornamental traits are likely to be less reliable signals of quality due to G x E, so mate choice will become less important (Rodríguez 2013). In contrast, in "coarse-grained" rarely fluctuating environments, signal reliability will increase, and the benefits of choice will be magnified, making sexual selection more important. In the latter "coarse grain" situation, males and females will all be 'large' (in low stress environments) or 'small' (in higher stress environments) at the same time. In *D. meigenii*, larger females are known to exert stronger preferences on male eyespan (Cotton *et al.* 2006). So, on the assumption of a mirrored G x E trait response curve in both sexes, at lower-intermediate levels of environmental stress, there will not only be high levels of genetic variation amongst males to exploit but also stronger exertion of female preference (as all will be 'large'). The net result will be stronger sexual selection.

Such variation in sexual selection could be further compounded due to covariation between population density and climatic flux. Female mate choice is

predicted to be weaker in the case that choice is plastic, male encounters rare and low sperm store status more common (Kokko & Mappes 2005). Female choosiness in *D. meigenii* has been shown to be both plastic and to vary in relation to both female mating status (Howie *et al*. Chapter 2) and sperm storage level (Howie *et al*., in prep.). Also, encounter rates are likely to be lower as environmental quality and population density falls. So, discrimination and thus sexual selection could be weaker in higher-intermediate stress environments and stronger yet in lower-intermediate stress environments due to correlated effects on male ornament genetic variation as well as female mate preference and choice, all of which will be weaker in high, and stronger in lower stress environments. Such results are also important as a demonstration that factors other than population size or operational sex ratio can modulate sexual selection, confirming the implication of recent studies and meta-analyses which have shown that these more 'traditional' factors often explain little variation in sexual selection (Mobley & Jones 2009; Serbezov *et al.* 2010; Byers & Dunn 2012; Moura & Peixoto 2013).

In this study I also investigated the integration of responses to E, G and G x E. I found integration across traits in response to both environmental and genetic (Figure 3a), as well as environmental and gene-by-environment (Figures 3b) variation. In contrast to the results found in *D. melanogaster* (Bonduriansky *et al*. 2015), traits that responded the most to environmental variation responded the least to genetic variation. In addition, and again in contrast to *D. melanogaster* (Bonduriansky *et al*. 2015), I found evidence of G x E, and an inverted relationship so that traits that responded most to environmental variation had the strongest G x E interaction. In each case, the traits fell into distinct clusters. Male

eyespan responded most to environment, least to genetics and had the largest G x E. This was followed by male wing length, female eyespan and female wing length. The results held for absolute and relative traits. A potential implication, then, is that traits under selection for condition dependence (i.e. sexual ornamental traits) evolve large responses to environmental variation. Given this, the size of their cross-environmental genetic responses, while large, can look small, unless viewed in the context of G x E. For instance, the response of relative eyespan to genetics was large at intermediate environmental stress, but the overall response to genetic stress looked small, because the genetic stress effect was lost under both low and high environmental stress. In contrast, traits under less severe directional selection (i.e. non-secondary sexual traits) evolve less dramatic responses, or responses to the genetic part of condition. Hence, their genetic responses can be viewed across environments and do not show G x E. An alternate explanation for this would be that the reproductive traits (testes and accessory gland) were free to grow post eclosion, and were therefore less limited by larval environmental stress. Irrespective, this important observation shows that the levels of environmental stress used in a study can have impacts on the perceived responses of traits to E, G and G x E as well as the integration of these responses across traits. For instance, where the low and high environmental stress conditions are far apart it will not be possible to observe G x E, or the G "locked" within it, for certain trait types. As the choice of environmental stress levels can thus alter or even reverse results, it is crucial that in the future multiple levels of environmental and genetic stresses are utilised in studies of condition dependence.

In summary, I am able to show that an exaggerated male sexual ornament responds in a heightened manner to variation in environmental and genetic stress

(after taking into account body size variation) relative to equivalent nonsexual traits. I show that the level of genetic condition dependence varies across environments in a distinct manner, so that the sexual ornament relates information about both genetic condition and environmental stress, and that the two interact to increase the information on condition available in intermediate environments. Given this, I confirm the hypothesis that male eyespan is a heightened condition dependent trait, but provide only partial support for the hypothesis that environmental stress precipitates the signal of genetic condition in sexual ornamental traits. Indeed, increased environment stress precipitates the between-line variation (associated with specific alleles); but it masks the signal of genetic condition at high levels of environmental stress. I also show that condition integrates both environmental and genetic variation, that male relative eyespan exhibits the largest responses to E and G x E, and that a number of environmental stress levels greater than two is crucial if one is to reliably test across-trait trait-response integrations. I conclude that interactions between environmental and genetic condition on male ornament expression will have important consequences for the indirect genetic benefits of female choice and the operation of sexual selection in nature. This should be studied further in the future.

CHAPTER 4

Environmental and genetic condition dependence of pre- and post-copulatory reproductive traits in the stalk-eyed fly, *Diasemopsis meigenii*

A B S T R A C T

Male reproductive, fertility and attractiveness traits are costly, related to life history, and under direct selection, similar to that experienced by secondary sexual traits. Due to this, such pre- and post-copulatory traits could covary positively with environmental and genetic condition, and have integrated responses to these factors. Alternatively, resource allocation trade-offs could lead to a lack of integration or to negative covariation between such traits, as is also the case for the resource allocations to the present and future predicted by sperm competition theory. The extent to which pre- and post-copulatory trait responses to environmental and genetic condition are integrated has important consequences for sexual selection. Yet, it has not been studied. To address this, I test the hypotheses that: reproductive, fertility and attractiveness traits are condition dependent, that these traits covary positively with the male sexual ornament (male eyespan), and that there is a broad integration of trait responses, across trait types, to variation in both environmental and genetic condition. To do so, I use a set of crosses between 17 inbred lines to generate individuals under either high (incross) or low (outcross) genetic stress, in the stalk-eyed fly species *Diasemopsis meigenii*. I manipulate their diet to exert three levels of environmental stress. I then look at the expression of a suite of pre- and post-copulatory traits (related to attractiveness and fertility) across a full factorial combination of these environmental and genetic states. I find that there is a broad qualitative alignment in trait responses to genetic and environmental stress (each tends to be positive), but a negative integration across traits (the traits that respond most to genetics respond least to environmental stress). The results have important consequences for our understanding of the operation of sexual selection in natural populations.

4.1 INTRODUCTION

An individual's condition relates to the pool of resources available for allocation to different traits (Rowe & Houle 1996), and integrates both environmental and genetic factors (Bonduriansky *et al.* 2015). Male sexual ornaments are one class of condition-dependent traits (Kotiaho *et al.* 2001), which I have examined in Chapter 3. Another class of male traits are those related to sperm production and ejaculate quality. Due to the competitive nature of male reproductive success in species in which females mate polyandrously (Parker 1970; Andersson 1994; Birkhead & Møller 1998; Parker & Ball 2005; Pizzari & Parker 2009) as is the case in the majority of taxa (Jennions & Petrie 2000; Simmons 2001), the traits involved in achieving copulation, ejaculate delivery and sperm competitive success are likely to be under similar selective pressures as sexual ornaments (Eberhard & Cordero 1995; Arnqvist 1998; Griffith 2000; Dixson & Anderson 2004; Ramm *et al.* 2005; Ramm *et al.* 2007; Martin-Coello *et al.* 2009; Wigby *et al.* 2009; Perry & Rowe 2010). Such traits are also costly, related to life history and sexual output, and are often exaggerated. They are thus likely to evolve to be condition-dependent (Alatalo *et al.* 1988; Houle 1992; Rowe & Houle 1996; Cotton *et al.* 2004b; Bonduriansky & Rowe 2005), and to integrate both environmental and genetic condition in their development (Pizzari & Birkhead 2002).

Given this view of male reproductive traits, their expression is expected to mirror that of exaggerated sexual ornaments. So reproductive trait size should relate to a wide range of loci (Pomiankowski & Møller 1995; Rowe & Houle 1996; Tomkins *et al.* 2004) and exhibit heightened environmental and genetic condition dependence (de Visser *et al.* 2003; Cotton *et al.* 2004a, c, b; Bonduriansky & Rowe

2005; Tomkins *et al.* 2004). This predicts a positive covariance between male sexual ornaments and reproductive traits as greater investment in the former will result in greater attraction and resulting mating opportunities, which will require a greater investment in male reproductive traits to turn this advantage into higher fertility. As mediated via condition, trade-offs between these traits are inter-twined and thus likely to evolve to a state of positive covariance (as in Møller 1994; Pizzari *et al.* 2004; Rogers *et al.* 2008). So, both male ornamentation and reproductive investment will increase together in relation to both environmental and genetic condition. Male sexual ornaments could thus reflect fertility and the genetics of fertility (Trivers 1972; Birkhead & Pizzari 2002; Pizzari *et al.* 2004), with important consequences for sexual selection; both in terms of the selection on males (i.e. attractive males have the most sperm which results in reinforced selection) and in the potential for direct (i.e. the phenotype-linked fertility hypothesis, Sheldon 1994) and indirect genetic (i.e. 'good' or 'sexy' genes, Kuijper *et al.* 2012) benefits for female mate choice. However, it is also notable that from an economic, life-history, resource-allocation or ejaculate-allocation perspective (Williams *et al.* 2005; Tazzyman *et al.* 2009; Engqvist 2012), one could instead expect a trade-off between ornaments and reproductive or ejaculate traits (Parker 1998; Simmons & Emelen 2006), which could lead to negative covariation between these traits (Evans 2010; Simmons *et al.* 2010; Engqvist 2011; Evans *et al.* 2015) and result in various constraints on sexual selection as well as limits on the evolvability of these traits (Lande 1979; Clark 1987; Arnold 1992).

There is a long history of the assessment of sexual ornament variation in response to environmental and genetic 'condition', or stress (Zuk *et al.* 1990; David *et al.*

1998; David *et al.* 2000; Kotiaho 2000; Holzer *et al.* 2003; Cotton *et al.* 2004a; Punzalan *et al.* 2008; McGuigan 2009; Rashed & Polak 2010; Ahuja *et al.* 2011; Weddle *et al.* 2012; Ingleby *et al.* 2013; Evans *et al.* 2015; Howie *et al*. Chapter 3). Recently, this has been mirrored by an increasing number of experimental studies that have investigated the effects of variation in environmental quality or stress on reproductive traits (testis size: Droney 1998; Jensen *et al.* 2004; Rogers *et al*. 2008; Vasudeva *et al*. 2014; accessory gland size: Fedina & Lewis 2006; Rehm *et al.* 2008; Rogers *et al.* 2008), as well as on functional correlates of ejaculate quality (spermatophore or ejaculate size: Gwynne 1990; Cerolini *et al.* 1995; Delisle & Bouchard 1995; Kast *et al.* 1998; Watanabe & Hirota 1999; Jia *et al.* 2000; Ferkau & Fisher 2006; Lewis & Wedell 2007; Blanco *et al*. 2009; sperm size, number, velocity or viability: Fedina & Lewis 2006; McGraw *et al.* 2007; Perez-Staples *et al.* 2008; Simmons 2011; Gasparini *et al.* 2013; Rahman *et al.* 2013, 2014 ; O'Dea *et al.* 2014; Cordes *et al.* 2015; Kahrl & Cox 2015; ejaculate composition: Perry & Row 2010; seminal proteins: Wigby *et al.* 2016) and resulting fertility (Vasudeva *et al.* 2014; Kahrl & Cox 2015). The studies have included arthropods (fruit flies; Droney 1998; McGraw *et al*. 2007; Perez-Staples *et al*. 2008; Rogers *et al*. 2008; Wigby *et al*. 2016, moths and butterflies: Delisle & Bouchard 1995; Watanabe & Hirota 1999; Ferkau & Fisher 2006; Lewis & Wedell 2007; Blanco *et al*. 2009; Cordes *et al*. 2015; beetles: Fedina & Lewis 2006; Perry & Rowe 2010; Vasudeva *et al*. 2015; crickets and katydids: Gwynne 1990; Jia *et al*. 2000; Simmons 2011), reptiles (Kahrl & Cox 2015), fish (Gasparini *et al*. 2013; Rahman *et al*. 2013, 2014; O'Dea *et al*. 2014), mammals (Jensen *et al*. 2004; Rehm *et al*. 2008) and birds (Cerolini *et al*. 1995; Kast *et al*. 1998), and have used a range of environmental variables such as adult diet (quantity: Cerolini *et al*. 1995; Watanabe & Hirota

1999; Fedina & Lewis 2006; Rehm *et al*. 2008; Perry & Rowe 2010; Simmons 2011; Devigili *et al*. 2012; O'Dea *et al*. 2014; quality: Gwynne 1990; Droney 1998; Lewis & Wedell 2007; Perez-Staples *et al*. 2008; Blanco *et al*. 2009; Rahman *et al*. 2013, 2014; Kahrl & Cox 2015) larval diet (quantity: Rogers *et al*. 2008; quality: Delisle & Bouchard 1995; Cordes *et al*. 2015), larval density (McGraw *et al*. 2007; Wigby *et al*. 2016), testosterone treatment (Kast *et al*. 1998) developmental temperature (Vasudeva *et al*. 2015) and the presence of smoke (Jensen *et al*. 2004). In general, these studies have demonstrated a clear pattern of increased trait size with environmental quality; or of decreased trait size with environmental stress.

In contrast to these environmental responses, the genetic and gene-by-environmental (G x E) responses have hardly been studied at all. A single consensus in the field is that reproductive and ejaculate traits have a large genetic component (Sakaluk 1988; Radwan 1998; Morrow & Gage 2001; Pitnick *et al.* 2001; Miller *et al.* 2003; Birkhead *et al.* 2005; Johns & Wilkinson 2007), which has been confirmed by several studies showing that these traits exhibit considerable additive genetic variation (Simmons & Kotiaho 2002, 2007; Moore *et al.* 2004; Engqvist 2008; Chargé *et al.* 2013; Gasparini *et al.* 2013; but see Morrow *et al*. 2008; Evans 2010 for instances of high heritability and low CV_A), as is characteristic of fitness traits and traits subject to sexual selection (reviewed in Merilä & Sheldon 1999). Additionally, a small number of studies have demonstrated that there is a genetic basis to environmental condition dependence (or environmental 'plasticity'). However, the results have been mixed, with 'ecological cross-over G x Es' (in which genotypes cross over in relative performance, or trait size, across environments) found for sperm transfer rates (in

the scorpion fly, *Panorpa cognate*: Engqvist 2008), sperm length (in *Drosophila melanogastor*: Morrow *et al.* 2008; and the fresh-water guppy *Poecilia reticulata*: Evans *et al.* 2015), and sperm defensive competitiveness (in the flour beetle *Tribolium castaneum*: Lewis *et al.* 2012), and a classic 'variance G x E' (in which the rank order of genotypes, in terms of performance or trait size, was stable across environments, while the differences between genotypes increased with environmental stress) found for sperm velocity in *P. reticulata* (Evans *et al.* 2015).

Likewise, studies on the covariation between ornamental and reproductive or ejaculate traits are also rare and have produced mixed results. Initial studies found evidence in favour of 'covariation-with-condition' models. For instance, Hosken *et al.* (2008) found a positive genetic correlation between male attractiveness and siring success in *Drosophila simulans*, while in the dung beetle *Onthophagus taurus*, soma weight – or 'condition' – was found to be positively genetically correlated with both attractiveness (Kotiaho *et al.* 2001) and testes weight (Simmons & Kotiaho 2002). In contrast, a number of recent studies of the relation between ornaments or attractiveness and ejaculates have found evidence of negative genetic correlations (e.g. in *P. reticulata*: Evans 2010; in the Australian cricket *Teleogryllus oceanicus*: Simmons *et al.* 2010; and in *P. cognata*: Engqvist 2011) and have thus provided support for the economic trade-off hypothesis. Nonetheless, a recent study by Chargé *et al*. (2013) was able to show that even ejaculate traits can exhibit positive genetic correlations with ornamental traits, as ejaculate size and sexual display characteristics were found to be positively genetically correlated in the houbara bustard *Chlamydotis undulate undulate*. Moreover, Evans *et al.* (2015), who found evidence of both positive and negative

correlations between different aspects of attractiveness and reproductive investment in the fresh-water guppy *P. reticulata*, also found that such correlations can vary across environments or even break down as environmental stress is increased. But the number of studies is limited, and little is known about direct responses of traits to explicit genetic stress, or how these vary across environments.

As noted in Chapter 3, a powerful tool for the direct manipulation of genetic condition is inbreeding (Rowe & Houle 1996; Cotton *et al*. 2004b; Tomkins *et al*. 2004; Bellamy *et al*. 2013, 2014). A small number of studies have used inbreeding to test for the effects of genetic stress on reproductive and ejaculate traits. For instance, Wildt *et al.* (1982) found that the number of sperm per ejaculate was lower in inbred than in outbred foxhounds. Using pedigree based calculations, Roldan *et al.* (1998) and Gomendio *et al.* (2000) found an inverse relationship between inbreeding coefficient and various ejaculate traits in the endangered gazelle species *Gazella cuvieri*, as did van Eldik *et al.* (2006) in the Shetland pony *Equus caballus*, and Margulis and Walsh (2002), in the oldfield mouse, *Peromyscus polionotus*. Likewise, Gage *et al.* (2006) showed that heterozygosity was positively associated with testis size and the production of normal sperm in the wild rabbit *Oryctolagus cuniculus*, while Fitzpatrick and Evans (2009) demonstrated an impairment of ejaculate quality with increased homozygosity across 20 mammalian species. Taken as one, the pattern has been for reduced reproductive trait size with increased genetic stress. Nonetheless, some variability is seen once non-mammalian studies are included. In relation to reproductive and ejaculate traits, Gasparini *et al.* (2013), found inbreeding depression in sperm number, but not size or velocity in the fresh-water guppy *P. reticulata* after two generations of

full-sib inbreeding; and Michalczyk *et al.* (2010) found a reduction in sperm competitiveness, but not fertilization potential, after 8 generations of inbreeding in the flour beetle *Tribolium castaneum*. Yet, despite the clear potential for important effects of both environmental and genetic stress on male ejaculate allocation and the direct benefits of female mate choice, nothing has been done to assess the across-environmental shape of genetic condition dependence for reproductive or ejaculate traits, as neither has the across-trait coordination of environmental and genetic responses of these traits been studied in any species.

To address this, I conducted a series of interrelated studies using the African stalk-eyed fly, *Diasemopsis meigenii*. As in many stalk-eyed fly species (Wilkinson 2001; Chapman *et al.* 2005), male *D. meigenii* have larger eyespans than females, even after controlling for body size (Baker & Wilkinson 2001). Females exhibit strong mate preferences for males with larger eyespans (Cotton *et al.* 2006; Howie *et al*. Chapter 2). Across species, male eyespan shows heightened condition dependence in response to both environmental (David *et al.* 1998; Cotton *et al.* 2004a, c; Bellamy *et al.* 2013) and genetic stress (David *et al.* 2000; Bellamy *et al.* 2013; Howie *et al*. Chapter 3). Male eyespan also correlates with internal reproductive organ size – testes and accessory gland length – in both *D. meigenii* (Harley *et al.* 2013) and the related *Teleopsis dalmanni* (Rogers *et al.* 2008; Cotton *et al.* 2010). In *T. dalmanni*, accessory gland size (and testis length to a lesser degree) is also known to be both phenotypically (Baker *et al.* 2003; Rogers *et al.* 2005b) and genetically (Rogers *et al.* 2005a) correlated with male mating frequency. Accessory gland size is also related to the age at first reproduction (Baker *et al*. 2003), and testis length to the number of sperm stored in a female's spermathecae

after copulation (Fry 2006). In *D. meigenii*, male ejaculate size (spermatophore area and the area of sperm within it) does not relate to male eyespan (Harley *et al.* 2013). Males of all sizes allocate larger ejaculates to larger females, as expected due to their higher reproductive value (Harley *et al.* 2013). However, larger eyespan males have been found to deplete ejaculates less rapidly over multiple matings (Harley 2013) so that larger eyespan males have both larger reproductive organs and provide, on average, a larger number of sperm to a female per mating.

Here, I investigate the interactions between inbred and outbred status and environmental condition on the expression of a range of pre- and post-copulatory traits. I test the hypotheses that: pre- and post-copulatory traits exhibit heightened condition dependent response to variation in environmental and genetic stress, that such traits covary positively with male sexual ornamental traits, and that there is a positive integration of trait response to each type of stress, across traits. To do so, I use the same design as in Chapter 3 that utilises a series of crosses within and between a suite of 17 highly inbred lines ($f \sim 0.908$; Bellamy *et al*. 2013) to induce two distinct levels of genetic stress. I raise offspring on three tightly controlled larval diets that reflect a wide range of natural variation (Cotton *et al*. 2004b; Howie *et al*. Chapter 3). I then assess the consequences for the male sexual ornament (male eyespan), for reproductive investment (testis length and accessory gland length), for ejaculate allocation in a single mating (spermatophore size and area filled with sperm) and for ejaculate production across multiple matings (sperm stored in female ventral receptacle). As the implications for sexual selection could be compounded due to the effects of genetic or environmental condition on fertility or attractiveness, I then record

several aspects of male mate behaviour (mating latency and length) and attractiveness (number of mating attempts prior to acceptance), as well as the number of offspring produced by each male under each level of stress. I examine the variation in the relationships between absolute and relative male eyespan and each reproductive trait, across environmental and genetic states. I measure the extent to which the responses to both environmental and genetic stress are integrated and coordinated across traits. This enables me to ask how environmental and genetic condition affect male reproductive investment, ejaculate allocation, attraction and reproductive success, to test my hypotheses, and to explore the across-environmental genetic condition dependence of each trait. The results have important implications for the direct selection on and reproductive strategies of males, for the relative scale of the direct and indirect benefits of female mate choice, and for the operation of sexual selection in nature.

4.2 MATERIAL AND METHODS

4.2.1 *Production of experimental flies, variation in genetic and environmental stress, and adult and reproductive morphology*

Experimental flies were produced, with both genetic state and environmental condition manipulated as recorded in Chapter 3. Incross and outcross larvae were raised on controlled low, intermediate or high corn diets. After eclosion, males and females were housed in 1.5l pots, with 8 – 10 flies per pot, and fed *ad libitum* with puréed sweet corn. Flies were separated by sex at two weeks. Males were kept at a density of 8 – 10 flies per pot until mature, and were then transferred to

individual 500ml pots. Each adult male was measured for eyespan, thorax length and wing length, testis length and accessory gland length as described in Chapter 3.

4.2.2 *Male fertility*

Three features of an individual male's fertility were measured: spermatophore size (and the area of sperm within it), the number of sperm stored in the female's ventral receptacle and the number of offspring produced by a male. In addition, I scored a number of properties relating to male attractiveness. This was achieved using a complex mating scheme (Figure 1), involving a focal male mating to two females. This scheme provided an efficient use of females and experimenter time.

An individual focal male *i* was mated to a single stock virgin female *j*, which was added to the male's pot. Female size was standardised to a mid-range size (eyespans ≥5.4mm, ≤6.2mm) in order to minimise any effects of female size on male sperm allocation, as males are known to allocate more sperm to larger females (Harley *et al.* 2013). Female age was also standardized (8-14 weeks old) to avoid variation due to changes in female reproductive quality through time (as shown in the related *Teleopsis dalmanni*; Reguera *et al.* 2004). Each male *i* x female *j* pair was allowed to mate freely for 3 days, and was then separated. All eggs laid by female *j* were collected during this period and over the following 1-5 days (see below for an explanation of this variation). The eggs were placed in a single petri dish per female with two moist cotton pads, a blue paper liner and *ad libitum* corn. The number of subsequent eclosed adults was counted as a measure of the focal male *i* offspring productive output per diem (larvae and pupae were also counted).

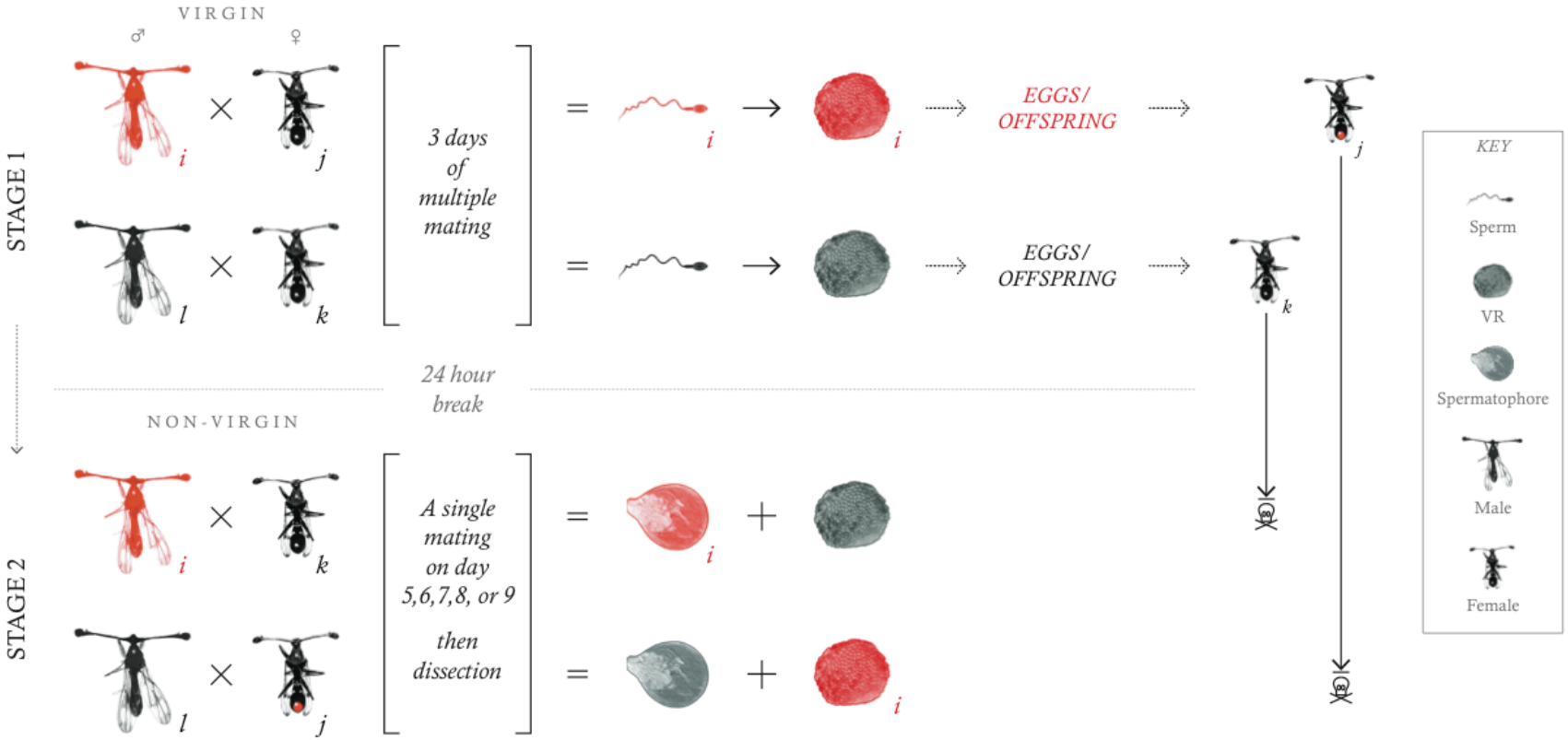


Figure 1. Experimental workflow used to measure male fertility traits. At stage 1, the focal male ***i*** is housed with and can mate with the virgin female *j* for a period of 3-days. Across this period, the sperm of male ***i*** enter the ventral receptacle (VR) of female *j*. Female *j* lays fertilised eggs (until she is dissected at the subsequent stage 2) all of which are collected, and assigned to male ***i***. At stage 2, after a 24 hour break, male ***i*** is placed in a container with the non-virgin female *k* (who has mated with another male, *l*, at stage 1). Male ***i*** and female *k* are allowed to complete a single full mating in which he delivers a spermatophore to the reproductive tract of female *k*. Female *k* is then dissected, her reproductive tract removed, and the spermatophore within it is assigned to male ***i***. At a similar point, and after a single mating to another male (such as male *l*), female *j* is dissected, her reproductive tract removed, and the VR within it – and the VR counts – are also assigned to male ***i***.

After 24 h of separation, on day 5, male *i* was placed with a second female *k* who had already been subject to the mating regime above, but with a different male, *l*. The focal male *i* was allowed 15 minutes to mate. A mating attempt was considered successful if it lasted for ≥150 seconds (Harley *et al.* 2013). If no successful mating attempt occurred after 15 minutes, the male was removed and allocated to another female. This was repeated to a maximum of 15 females, over 5 days (3 per day). As soon as a successful mating attempt had occurred female *k* was immediately removed, anaesthetised on ice, and her reproductive tract dissected. The spermatophore of male *i* was immediately located and photographed at x 200. The visualisations were enhanced with DIC microscopy (differential interference contrast: polarized light is used to increase contrast in unstained transparent samples). The area of the spermatophore was then measured to the nearest 0.0001 mm^2 with ImageJ by tracing the circumference (Harley *et al.* 2013). The area of the spermatophore filled with sperm was also measured in a similar manner. If no spermatophore was present, or if the spermatophore was clearly ruptured during dissection, male *i* was given another female, and the process was repeated until he had successfully mated (subject to the maximum, described above, of three mating opportunities per day for a maximum of 5 consecutive days). The number of days between removal and dissection was recorded and accounted for in later models (see section 2.3.1 below).

I also recorded a number of additional parameters as gauges of male *i*'s attractiveness and mating behaviour. These included a) the latency (length of time) until the first mating attempt, b) an inverse measure of 'male attractiveness', that is the number of mating attempts rejected prior to an accepted copulation (as

the males often made mating attempts that the females rejected via the vigorous shaking of their abdomens, Cotton *et al.* 2006), and c) the duration of the successful copulation in which a spermatophore was transferred, or 'mating length'.

As well as recording properties of the male spermatophore at dissection, the ventral receptacle (VR) was removed to count sperm. The VR is the single functional sperm storage organ in *D. meigenii*, and is formed of between 150 – 300 pouches, each able to store a single sperm (Kotrba 1995; JH, *pers. obs.*). The spermathecae are degenerate in *D. meigenii,* and do not store sperm (Kotrba 1995). The VR was removed from each female and placed on a slide with a drop of PBS photographed at x100, and the number of filled and unfilled pouches were counted with ImageJ image capture software. The VR counts of female *j* were assigned to male *i* (i.e. female *j* was the first female that male *i* mated with, during days 1-3), and represent his sperm output over 3-days. Female *j* was also used in the second phase of the study, being mated to a second male (other than male *i*) to gather information on the spermatophore size of this second male. Therefore, she was dissected between 1-5 days after the initial 3-day mating period with male *i*. This 1-5 day period was taken into account in models of the male *i* VR counts.

4.2.3 *Statistical analysis*

4.2.3.1 *Adult and reproductive morphology*

To test for effects of food level (environmental variation, E), genetic status (genetic variation, G) and G x E on morphological trait variation, I fitted general linear mixed effects models (GLMMs) via REML to the trait data. In each model, food level, genetic status (incross/outcross) and their interactions were

included as fixed effects. The rearing density (the number of eggs on the petri dish that the individuals were reared on) was included as a covariate to control for variation in size due to larval competition. Each cross was included in the models as a random effect. The GLMMs were fitted for absolute thorax length, eyespan, wing length, testis length, and accessory gland length. Further GLMMs were then fitted for relative trait values with the inclusion of thorax length as a covariate.

Further GLMMs were fitted to test for effects on the spermatophore area, sperm area, the number of sperm in the ventral receptacle, and the offspring counts. All models included food level, genetic status and their interaction. In each, cross was included as a random effect, with the number of days over which eggs were laid included as an additional random effect in the sperm-in-ventral-receptacle and offspring count models. For sperm in the VR and offspring counts GLMMs, the data were also square root transformed. However, as count data transformations can be unreliable (O'Hara & Kotze 2010), equivalent generalized linear mixed effects models (GLME in lme4, Bates *et al.* 2015) with Poisson error structures and log link functions (i.e. 'Poisson lognormal models') were also fitted via Laplace approximation to the non-transformed data; with observation level random effects included as required to account for overdispersion (Harrison 2014), and *p*-values obtained via stepwise effect removal and model comparison with likelihood ratio tests. As reproductive output can covary with body size and reproductive organ size, all GLMMs were then repeated with thorax length, testis length and accessory gland length included, in turn, as covariates (to test for residual effects E and G on each trait after relevant variation had been removed).

GLMMS were also fitted to test for effects on the length of time until the first mating attempt (model 1), the number of mating attempts before a successful mating attempt (model 2), and the length of the successful mating (model 3). Each test included food level, genetic status, and their interaction, with cross included as a random effect. A log transformation was used for model 1 and model 3, a square-root transformation for model 2. Equivalent Poisson GLMEs were also fitted for each variable. As male attractiveness and mating characteristics could relate to body size and reproductive organ size, GLMMs were also fitted with thorax length, testis length, accessory gland length included in turn as covariates in order to test for residual variation related to E, G or G x E.

4.2.3.2 *Relationships between eyespan and reproductive traits*

A further two sets of GLMMs were then fitted to test pairwise relationships between male eyespan and the reproductive traits. First, to test for overall relationships, a GLMM was fitted per trait, with male eyespan included as a fixed effect and cross as a random effect. As such global relationships can be unrepresentative the models were re-run after the data were split by environmental and then genetic treatment. To test for quantitative variation in these relationships across environments and in relation to genetic state, a final GLMM was then run for each trait, with male eyespan, food level, genetic status and all two- and three-way interactions as fixed effects, and with cross as a random effect. The GLMMs were run in turn for testis length, accessory gland length, spermatophore area, sperm area, VR count, offspring count, mating latency, mating length and male attractiveness. To test trait relationships with male relative eyespan, each GLMM was then re-run with thorax included as an

extra covariate. These latter models also provided a test for residual effects of E, G and G x E on each trait after control for relative eyespan associated variation.

4.2.3.3 *Integration of environmental and genetic effects across traits*

To investigate the integration of environmental and genetic effects into trait responses, across traits, GLMMs were also fitted to standardised trait measures (i.e. z-scores: with a mean of zero and variance equal to one), for each trait. Each GLMM included food level, genetic status and their interaction, with cross as a random effect. The model effect coefficients for environment, genetics and G x E were extracted (Bonduriansky *et al.* 2015). As a single data point per trait, the average environmental effect coefficient was regressed on the genetic effect coefficient. Pearson correlation analyses were then also used to test the alignment between and coordination of environmental, genetic responses (Bonduriansky *et al.* 2015) (the G x E coefficients were not used in these analyses as the responses to G x E were significant for only one trait: the ventral receptacle sperm count). Each coefficient combination was plotted for absolute and relative trait z-scores. Each analysis was also run both with and without the inclusion of the key outliers – sperm area and mate length. This was done to test the prediction that reproductive investment traits would exhibit 'covariation with condition' with the ornamental traits, while allocation traits would exhibit 'trade-offs' and therefore fall off the line of correlation. Mating latency was not included in any of these analyses as it responded to neither G nor E; but results held when it was included.

All statistical analyses were conducted in JMP v 11.2.0 (SAS Institute 1989-2007) and R v 3.1.3 (R Core Development Team 2015). GLMM results tables and effects coefficients are provided in the Chapter 4 Supplementary Information.

4.3 RESULTS

4.3.1 ***Responses to environmental and genetic variation***

4.3.1.1 *Adult morphology*

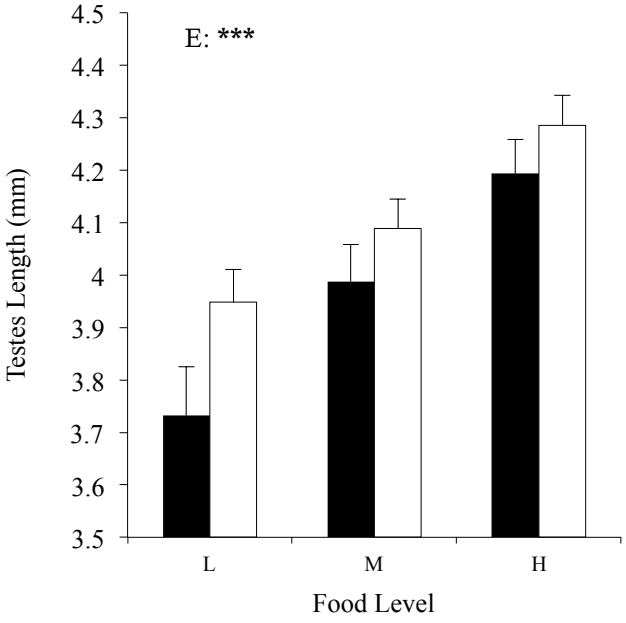
A subset of males were investigated compared to those in Chapter 3. Males were only included in the analyses if they survived until reproductive maturity. In line with the findings of Chapter 3, thorax length ($F_{2,398.2} = 209.11$, $p < 0.001$), eyespan ($F_{2,401.7} = 421.66$, $p < 0.001$) and wing length ($F_{2,389.9} = 261.40$, $p < 0.001$) increased with food level. Thorax length was also larger in outcross flies ($F_{1,23.97} = 8.12$, $p = 0.009$). However, unlike these previous results, neither eyespan ($F_{1,29.24} = 1.65$, $p = 0.209$) nor wing length varied with genetic status ($F_{1,29.27} = 0.053$, $p = 0.819$), and no traits showed G x E (all: $F_{2,392\text{-}404} = 0.802 – 2.14$, $p > 0.11$) when this mature subset was investigated. All results held after control for body size (SI.A1-3, 6-7).

4.3.1.2 *Adult reproductive morphology*

Testis and accessory gland lengths increased with higher levels of food (testis $F_{2,388.1} = 20.46$, $p < 0.001$, accessory gland $F_{2,323.6} = 10.48$, $p < 0.001$). Testis length did not vary with genetics ($F_{1,36.6} = 2.77.$, $p = 0.105$; Figure 2 a), but accessory glands were larger in outcross flies ($F_{1,38.36} = 23.85$, $p < 0.001$; Figure 2 b). Neither testis nor accessory gland lengths showed G x E (testes $F_{2,388.6} = 0.64$, $p = 0.527$, accessory gland $F_{2,325} = 1.81$, $p = 0.165$). After the inclusion of thorax to control for body size, the effect of food levels on testis length was lost ($F_{2,353} = 1.48$, $p =$

Figure 2:

a)



b)

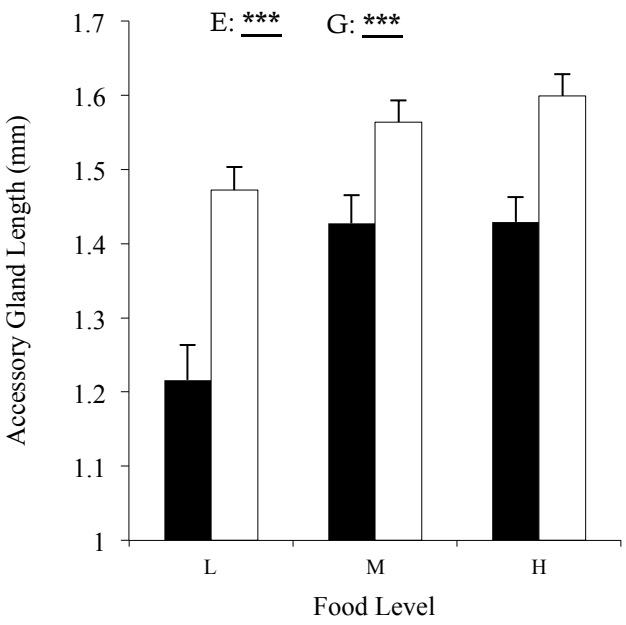


Figure 2. a) Absolute testis and b) absolute accessory gland length, for incross and outcross flies at each food level (LSM ± S.E.). Black bars denote incross males, white bars denote outcross males. In each case, significance is denoted by asterisks: at *, **, ***, $p < 0.05, 0.01, 0.001$. Underlined asterisks indicate that a response to E or G also persisted after controlling for variation in thorax length.

0.228). But the positive response of accessory gland to food levels ($F_{2,294.6}$ = 6.97, p = 0.001) and the larger size in outcross flies persisted ($F_{1,39.14}$ = 16.01, p < 0.001). As in the absolute case, there was no G x E for relative testes ($F_{2,383.8}$ = 0.78, p = 0.461) or relative accessory gland length ($F_{2,319.8}$ = 2.09, p = 0.126: SI.A4-5, 8-9).

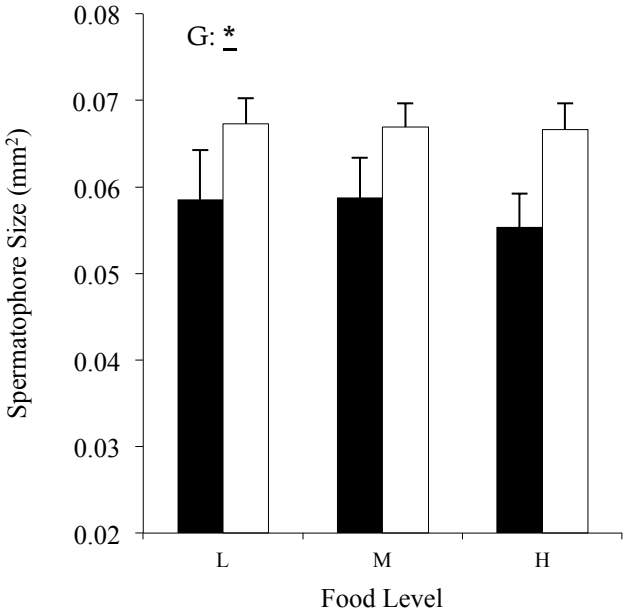
4.3.1.3 *Male fertility*

Spermatophore area did not vary with food level ($F_{2,155.3}$ = 0.81, p = 0.447) but was larger in outcross flies ($F_{1,32.32}$ = 6.34, p = 0.017; Figure 3 a). There was no G x E for spermatophore area ($F_{2,154.2}$ = 0.13, p = 0.877). The results held in repeated analyses with failed transfers included as zeros. The response to genetics persisted after controlling for thorax length ($F_{1,31.49}$ = 5.88, p = 0.021) and testis length ($F_{1,31.64}$ = 5.88, p = 0.021), but not for accessory gland length ($F_{1,32.66}$ = 3.99, p = 0.054). In contrast, absolute sperm area decreased with higher food level ($F_{2,150.6}$ = 4.89, p = 0.009), but did not vary with genetic status ($F_{1,28.78}$ = 0.03, p = 0.959; Figure 3 b). Neither was there a G x E for absolute sperm area ($F_{2,148.4}$ = 0.15, p = 0.860). The results held in repeated analyses with failed transfers included as zeros. The response to food level was lost after controlling for thorax length ($F_{2,147.8}$ = 2.93, p = 0.057), but persisted after control for both testis length ($F_{2,146.4}$ = 5.09, p = 0.007) and accessory gland length ($F_{2,140.2}$ = 4.69, p = 0.012: SI.B1-2, 5-6, 9-10, 13-14).

The number of sperm filled VR pouches increased overall with higher food levels ($F_{2,182}$ = 6.42, p = 0.002) and there were more sperm filled pouches in outcross flies ($F_{1,81.4}$ = 16.70, p < 0.001). There was also a G x E interaction ($F_{2,178.7}$ = 6.65, p = 0.002; Figure 4 a); incross counts increased, whereas outcross counts

Figure 3:

a)



b)

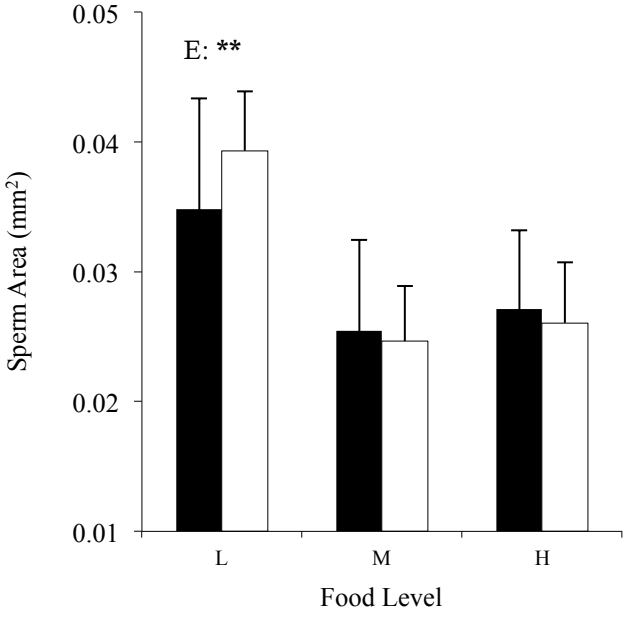
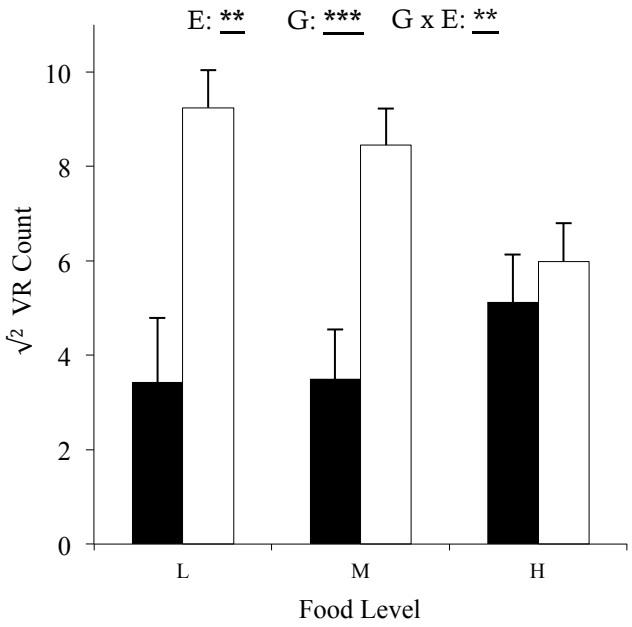


Figure 3. a) Absolute spermatophore area and b) the absolute area of the spermatophore that was filled with sperm, for incross and outcross flies at each food level (LSM ± S.E.). Black bars denote incross males, white bars denote outcross males. In each case, significance is denoted by asterisks: at *, **, ***, $p < 0.05$, 0.01, 0.001). Underlined asterisks indicate that a response to E or G persisted after controlling for variation in thorax length.

Figure 4:

a)



b)

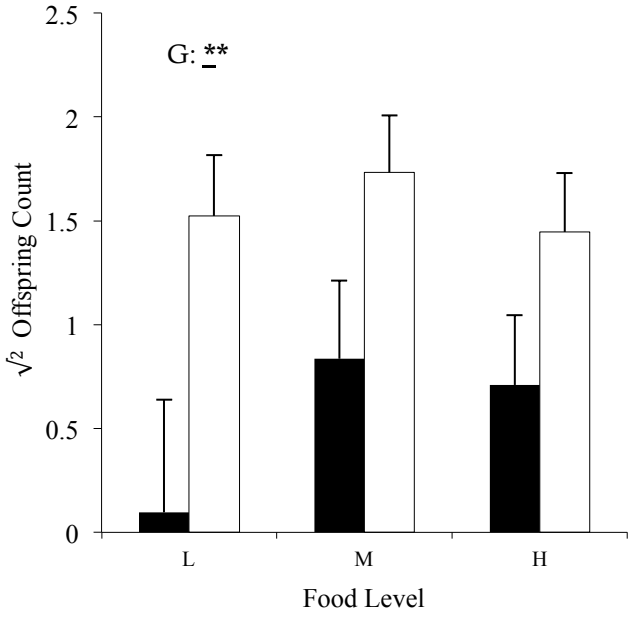


Figure 4. a) Absolute number of sperm stored in the female's ventral receptacle after three-days of mating with a focal male, and b) the absolute number of F2 progeny, each after square root transformation, for incross and outcross flies at each food level (LSM ± S.E.). Black bars denote incross males, white bars denote outcross males. In each case, significance is denoted by asterisks: at *, **, ***, $p < 0.05, 0.01, 0.001$). Underlined asterisks indicate that a response to E, G or G x E persisted after controlling for variation in thorax length.

decreased with food level (they converged at high food level). The G x E held in the Poisson GLME. All results also persisted after the inclusion of thorax length, testes length, and accessory gland length as covariates (E: $F_{1,164\text{-}174} > 6.46$, $p < 0.002$; G: $F_{1,80\text{-}86} > 12.24, p < 0.001$; GxE: $F_{2,160\text{-}168} > 6.15, p < 0.002$; SI.BS3, 7, 11, 15).

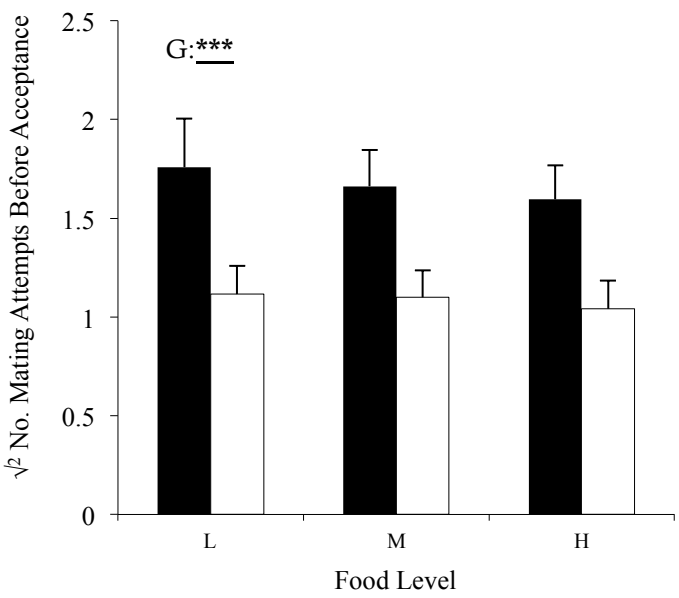
In contrast, the number of offspring sired was only sensitive to genetics. The counts did not vary across food levels ($F_{2,177.6} = 0.87$, $p = 0.606$), but were higher in outcross flies ($F_{1,77.9} = 8.09$, $p = 0.006$; Figure 4 b). There was no G x E ($F_{2,166.6} = 0.52$, $p = 0.596$). All results persisted after the inclusion of thorax length, testes length, and accessory gland length as covariates (G: all $F_{1,75\text{-}78} > 6.59$, $p < 0.012$; SI.BS4, 8, 12, 16). The offspring count pattern was weaker in a repeated analysis with zero counts excluded, but the trend remained in the same direction ($F_{1,54.53} = 2.61$, $p = 0.112$). The first results held using a Poisson GLME, with the exception that now there was a significant G x E (l-r test: $\chi^2 = 34.31$, $df = 2$, $p < 0.001$). Incross counts increased while outcross counts were flat across food levels.

4.3.1.4 *Male attractiveness*

The latency (time taken) until the first mating attempt did not vary with food level ($F_{2,187.4} = 0.16$, $p = 0.851$), genetic status ($F_{1,22.97} = 1.123$, $p = 0.464$) or G x E ($F_{2,188.4} = 0.77$, $p = 0.464$). The number of mating attempts made prior to acceptance was greater for incross flies ($F_{1,27.53} = 15.43$, $p < 0.001$), but did not vary with either food level ($F_{2,203.5} = 0.02$, $p = 0.985$) or G x E ($F_{2,204} = 0.04$, $p = 0.965$; Figure 5 a). The length of the successful mating attempt decreased with higher food level ($F_{2,167.4} = 4.01$, $p = 0.019$), but did not vary with genetics ($F_{1,19.97}$

Figure 5:

a)



b)

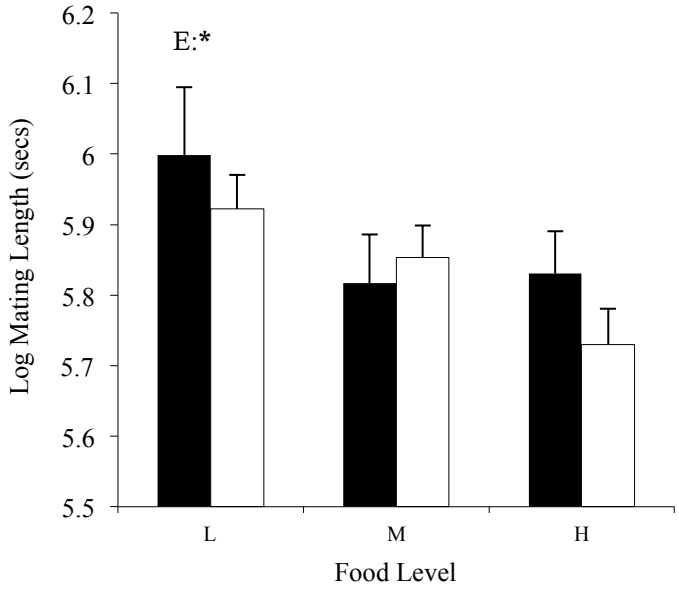


Figure 5 a) Absolute number of mating attempts made by a male prior to acceptance, or 'inverse attractiveness', after square-root transformation, and b) absolute mating length in seconds, after log transformation, for incross and outcross flies at each food level (LSM ± S.E.). Black bars denote incross males, white bars denote outcross males. In each case, significance is denoted by asterisks: at *, **, ***, $p < 0.05$, 0.01, 0.001). Underlined asterisks indicate that a response to E or G persisted after controlling for variation in thorax length.

= 0.79, p =0.384) or G x E ($F_{2,166.6}$ = 0.778, p = 0.461, Figure 5 b). All results held in the Poisson GLMEs and after the inclusion of thorax length, testis length, and accessory gland length as covariates, with one exception: the effect of food level on mating length was lost after controlling for male body size ($F_{2,144.5}$ = 0.097, p = 0.907: see SI.CS1-12).

4.3.2 *Variation in the relationships between absolute and relative eyespan with reproductive, fertility and attractiveness traits*

4.3.2.1 *Basic eyespan-trait relationships*

Given that eyespan and relative eyespan responded to environmental and genetic effects, I tested the degree to which these traits used in female mate choice were associated with the responses seen in reproductive, fertility and attractiveness traits. I only did this for those reproductive, fertility and attractiveness traits that showed significant responses to environmental or genetic effects. These were: testis length, accessory gland length, spermatophore area, sperm area, VR count, offspring count, mating length and attractiveness (but not latency, see Table 1).

Both testis length and accessory gland length increased with eyespan (testis: R^2 = 0.30, $F_{1,401.8}$ = 76.97, p < 0.001; AG: R^2 = 0.38, $F_{1,331.8}$ = 51.23, p < 0.001). In contrast, sperm area and mating length decreased with eyespan (sperm area: R^2 = 0.30, $F_{1,144.1}$ = 6.15, p = 0.014; mating length: R^2 = 0.06, $F_{1,166.2}$ = 16.78, p < 0.001). None of the other fertility traits nor male attractiveness (inverse number of mating attempts) showed any relationship with eyespan (all $F_{1,145\text{-}190}$ < 1.33, p > 0.249). After controlling for thorax length, all the same relationships held (testis:

Table 1:

	Absolute Trait Responses			Relative Trait Responses			
	E	G	GxE	E	G	GxE	*Notes*
Thorax Length	+	O>I					
Eyespan	+			+			
Wing Length	+			+			
Testes Length	+						weak
Accessory Gland Length	+	O>I		+	O>I		
Spermatophore Area		O>I			O>I		
Sperm Area	-			-			
VR Count	+	O>I	YES	+	O>I	YES	
Offspring Count		O>I			O>I		
Mating Latency							
Mating Length		O>I			O>I		weak
Attractiveness	-						

Table 1. Absolute and relative (after controlling for variation in thorax length) trait responses to environmental (food level) and genetic (incross/outcross) variation (based on full models, not z-scores). All responses shown are significant. A positive response to environmental variation is denoted as '+', a negative response as '-'. A positive response to outcrossing is denoted as O>I, and negative one as I>O.

R^2 = 0.30, $F_{1,404.2}$ = 11.75, p < 0.001; mating length: R^2 = 0.06, $F_{1,167.4}$ = 5.06, p = 0.029; all other fertility traits: $F_{1,151\text{-}186}$ < 0.17, p > 0.207), except that the positive relationship with accessory gland length was much weaker (R^2 = 0.39, $F_{1,334.1}$ = 2.85, p = 0.092), and that the negative relationship with sperm area was lost (R^2 =0.30, $F_{1,148.6}$ = 1.61, p = 0.206), and that male attractiveness was positively associated with male relative eyespan (R^2 = 0.22, $F_{1,195.5}$ = 10.79, p = 0.001: SI.DS1-16).

4.3.2.1 *Variation in eyespan relationships across environmental and genetic states*

I next examined whether trait relationships with eyespan changed across environments (i.e. eyespan x E interaction) or with genetic status (i.e. eyespan x G interaction). For absolute trait size there was no difference in the strength of the trait relationships with eyespan across food level or with genetic status (for E x eyespan, all traits: $F_{2,137\text{-}392}$ < 2.216, p > 0.113; for G: $F_{1,143\text{-}400}$ < 2.62, p > 0.108), with two exceptions. Firstly, the relationship between absolute male eyespan and the area of sperm in the spermatophore varied with food level (for ES x E: $F_{2,137}$ = 3.14, p = 0.0463): it was positive in intermediate, but negative in high and low stress environments (high: *est*. = -0.07; inter: *est*. = 0.006; low: *est*. = -0.005). Secondly, the equivalent relationship between absolute eyespan and attractiveness varied with genetic status (ES x G, $F_{1,190.2}$ = 11.25, p < 0.001), and was less steep in outcross flies (incross: *est*. = 0.252; outcross: *est*. = 0.004: SI.ES17-24, 33-34).

As for absolute eyespan, the relationships between each trait and relative eyespan did not vary with environment (for E x relative eyespan, all traits: $F_{2,144\text{-}392}$ < 2.25, p > 0.108), with the exception of sperm area (for E x relative eyespan: $F_{136.2}$ = 3.133, p = 0.047), which was positive in intermediate and negative in high and

low stress environments (high: *est*. = -0.008; inter: *est*. = 0.011; low: *est*. = -0.014). All absolute trait relations held for relative traits for genetics (for G x relative eyespan, all traits: $F_{142\text{-}400} < 2.63$, $p > 0.107$; for G x relative eyespan, on male attractiveness: $F_{1,189.7} = 15.22$, $p < 0.001$: incross: *est*. = 1.208; outcross: *est*. = 0.049). However it is also notable that, when the data were split by environmental state, the overall positive relationships for testis length, accessory gland length and male attractiveness were all far stronger at, and driven by, the patterns seen at the intermediate and low stress environments (testis: high: *est*. = -0.090, $F_{1,90.99} = 0.65$, $p = 0.042$; inter: *est*. = 0.232, $F_{1,150} = 14.99$, $p < 0.002$; low: *est*. = 0.114, $F_{1,158} = 1.27$, $p = 0.263$; AG: high: *est*. = 0.031, $F_{1,76.22} = 0.55$, $p = 0.459$; inter: *est*. = 0.054, $F_{1,116.6} = 3.14$, $p = 0.079$; low: *est*. = 0.076, $F_{1,134.8} = 1.62$, $p = 0.207$; attractiveness: high: *est*. = 0.472, $F_{1,53.48} = 2.68$, $p = 0.107$; inter: *est*. = 0.653, $F_{1,69.68} = 7.11$, $p = 0.001$; low: *est*. = 0.64, $F_{1,65.84} = 2.05$, $p = 0.157$; SI.DS25-32, 35-39).

4.3.3 *Integration of environmental and genetic effects across traits*

I observed qualitative alignment between the effect coefficients of, or trait responses to, food level and genetic status for all absolute and relative traits (Table 1 for standard responses, SI.E for z-score responses). Both lower food level and incross status resulted in reduced trait sizes. But across traits the general relationship was negative: traits that responded most to food level responded *least* to genetics and vice versa for eyespan, thorax, wing length, testis length, accessory gland length, spermatophore area, VR count, offspring count, mating latency and attractiveness. This negative relationship was not significant when all traits were included, for absolute ($R^2 < 0.01$, $r = -0.12$, $t = 0.25$, $df = 10$, $p = 0.805$; correlation = -0.08, $p = 0.805$; Figure 6 a) or relative ($R^2 = 0.16$, $r = -0.29$, $t = 1.25$,

Figure 6:

a)

b)

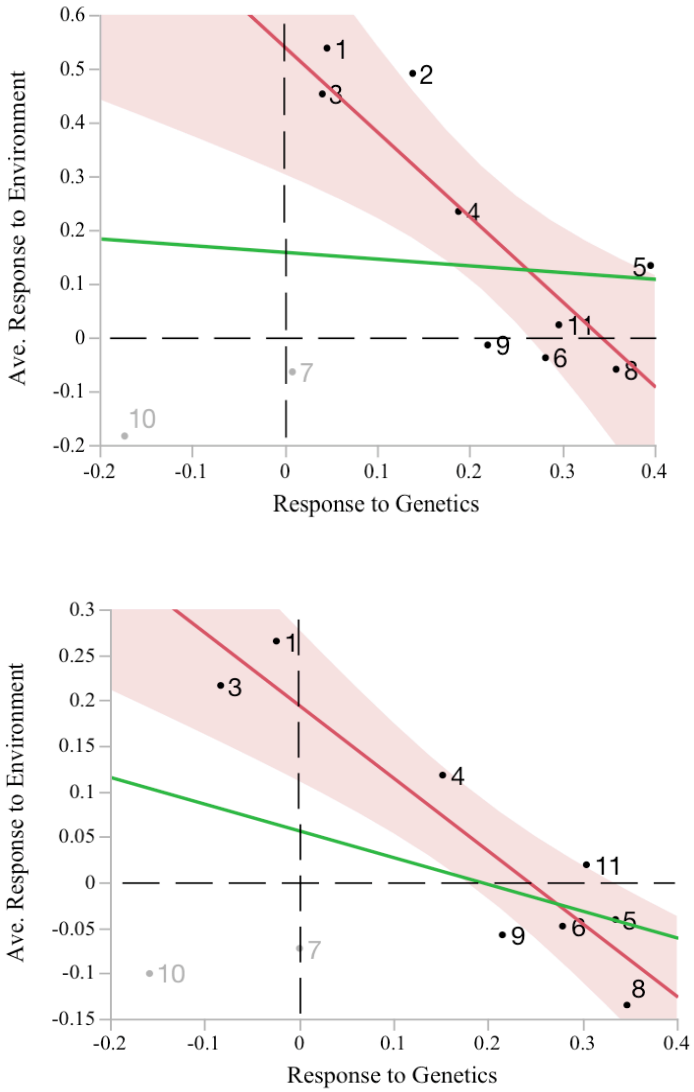


Figure 6. Integration of trait responses to environmental and genetic variation for absolute (a) and (b) relative, thorax controlled, traits. Values on the Y axis reflect model effect coefficients for the responses of each trait to food level; that is, the average value of low versus high and intermediate versus high responses (inverted for clarity). Values on the X axis reflect model effect coefficients for the responses of each trait to genetic status; that is, for incross versus outcross (inverted for clarity). Models were run per trait based on non-transformed z-scores. Numbers are labels for the traits: 1 eyespan, 2 thorax, 3 wing length, 4 testes length, 5 accessory gland length, 6 spermatophore area, 7 sperm area in spermatophore, 8 sperm number in ventral receptacle, 9 offspring count, 10 mating length, 11 male attractiveness. Green line denotes linear regression across all traits. Red line denotes linear regression, with key outliers, sperm area in spermatophore and mating length, excluded (± 95% C.I. for expected values). In each case, the linear regression provided an identical pattern to that provided by the equivalent correlational analyses in terms of direction and significance.

df = 9, *p* = 0.248; correlation = -0.40, *p* = 0.248; Figure 6 b) trait values. However, if the pair of traits (sperm area and mating length) that responded negatively to environmental condition was excluded (to test the prediction that the rest of the reproductive traits exhibited a covariation with condition response, while the allocation traits cluster off the line, to trade-off), there was a negative relationship for absolute (R^2 = 0.69, *r* = -1.58, *t* = 3.93 *df* = 8, *p* = 0.006; correlation = -0.83, *p* = 0.006) and relative trait values (R^2 = 0.85, *r* = -0.79, *t* = 5.77, *df* = 7, *p* = 0.001; correlation = -0.92, *p* = 0.001: for full tables of responses see SI Tables ES1-2).

4.4 DISCUSSION

In this chapter, I investigate how the size of male reproductive traits (testis length and accessory gland length), ejaculate traits and fertility (spermatophore size, sperm area, sperm stored in the ventral receptacle and offspring counts) and aspects of male mating behaviour (mating latency, attractiveness and duration of copulation) vary in relation to different levels of environmental and genetic stress. I ask how these two forms of stress interact in altering trait development, and I link the results to a previous in-depth study of male ornamentation (Chapter 3). I also ask how each of these traits relate to absolute and relative male eyespan, and how these relationships vary across environmental and genetic states. Finally, I examine the integration of responses to variation in environmental and genetic condition across traits. I test the hypotheses that pre- and post-copulatory traits are condition dependent, covary positively with male ornaments, and have integrated responses to E and G. My approach is novel in that it uses explicitly low and high quality genotypes and examines trait responses to these genetic states across three tightly controlled larval environments.

Examining a wide range of pre- and post-copulatory traits also provides a broad picture of the effects of environmental and genetic condition on male attractiveness, ejaculate allocation and reproductive output, with implications for the benefits of female mate choice and the operation of sexual selection in nature.

I found a complex picture, but one that could be broken down into a number of simple results. First, I was able to confirm that male eyespan responded in a heightened manner to variation in environmental stress. In contrast to Chapter 3, I did not find a response to genetic stress. But the trends of the genetic and G x E responses were in the same direction as those seen in Chapter 3. So these results are not in conflict, and are most likely explained by decreased sample size. The sample size used in Chapter 3 was n = 1154, whereas here it was reduced to n = 434. Another more interesting possibility is that, in the current study, male ornament size was assessed at reproductive maturity, 10-14 weeks after eclosion. In this case, death prior to adulthood could have preferentially removed flies with smaller eyespans. However, while there was an effect of absolute and relative eyespan, and of environmental and genetic condition on male age at death, these effects did not arise in a manner that would explain this loss of signal (see SI.F).

A new aspect of this work was the test of the prediction that male reproductive traits exhibit heightened condition dependent responses to both environmental and genetic variation. In line with this hypothesis I found that testis and accessory gland length increased in size with environmental condition. But, testis length did not differ between the genetic conditions, while the environmental response was accounted for by changes in body size (Figure 2 a). This is likely because body

size is fixed at eclosion, with testis then growing on *ad libitum* food to fill the available body cavity, with a consequent limited effect of genetic status on absolute testis size, if not functionality (in *D. meigenii* testes are very large and fill up a large proportion of the abdomen). In contrast, accessory glands exhibited a heightened response to both environmental *and* genetic condition, with larger trait sizes in outcross flies, and at higher food levels. The responses persisted after controlling for body size, and the increase in relative accessory gland size with genetic condition was present across all environments (Figure 2 b). This pattern differed from that seen in male eyespan, which was only genetically differentiated at the intermediate levels of environmental stress (in Chapter 3) or not at all (in Chapter 4). A likely reason for this is that, unlike eyespan, accessory gland size reflects both larval and adult environmental quality. After eclosion, *ad libitum* food allows the accessory gland to reach a maximum size as limited by genetic quality (accessory glands are far smaller than testes and do not fill the abdomen), with a degree of stunting due to larval food restriction. In contrast, for relative eyespan, low larval food levels lead to survival selection and limited investment in fixed ornaments irrespective of genetics, while high food levels blur the signal of genetics in a trait that reflects environmental condition (as seen in Chapter 3).

As each trait was found to be condition dependent, male absolute and relative eyespan must – in line with the covariation with condition hypothesis – reflect some information about reproductive investment. Yet neither was an ideal indicator. As noted above, relative eyespan, in particular, was shown to be a poor indicator of both accessory gland size and the genetic basis of accessory gland size across the range of environments examined in our study. Both highly stressful

(very restricted food) and weakly stressful (excess food) environments diminished genetic differences in eyespan, but such a change was not evident for accessory gland size (Figure 2 b). And a large residual effect of genetics on relative accessory gland size also remained after control for both body size and relative eyespan. Nonetheless, absolute eyespan did reveal some information about reproductive investment. It was positively related to both testis length and accessory gland length in each environment. Further, in an overall test, relative eyespan related positively to testis size, and there was also a positive trend in respect of accessory gland size (at $p = 0.092$). Interestingly, however, the positive relationship and trend were both driven by the relationships at intermediate food levels. That is, at intermediate food/stress levels, relative eyespan reflected information about testis length, and likely accessory gland length, above that revealed by body size. The result accords with those in Chapter 3, for relative eyespan and genetic condition. A consequence of this is that in intermediate environments, large relative eyespan, attractive males that obtain more mating opportunities could also provide more reproductive resources and contribute disproportionally to the next generation; with female preference also increased via the dual direct and genetic benefits availed of in relation to relative eyespan.

In concordance with the accessory gland results above, spermatophore size was also found to decrease with genetic stress across all food levels, both before and after control for body size; although, no variation with food level (environmental stress level) was observed (Figure 3 a). A simple explanation for this is that accessory glands are involved in the production of the accessory proteins used in the construction and filling of the spermatophore (Kotrba 1993; Kotrba 1996).

Larger accessory glands may be associated with the ability to produce larger spermatophores or a larger number of spermatophores (Baker *et al.* 2003; Rogers *et al.* 2005b). In line with this, the negative effect of genetic stress on spermatophore size was lost after controlling for accessory gland size, while it persisted after controlling for testis size and body size. A consequence of this is that males with higher genetic condition will have larger accessory glands and produce larger spermatophores. These males are also likely to be able to produce a greater number of spermatophores and to mate more often than rivals, because accessory gland is known to covary with male re-mating rates (Baker *et al.* 2003; Rogers *et al.* 2005a; Rogers *et al.* 2005b) and with the rate of spermatophore depletion (Harley 2013). In accordance with this interpretation, the volume of accessory products that fill a spermatophore is known to relate to the size of a spermatophore in stalk-eyed flies (Kotrba 1996). Furthermore, accessory products, such as seminal fluid proteins, are known to increase male fertilisation success (Fricke *et al.* 2009; Wigby *et al.* 2009), to decrease female receptivity to additional male advances, to decrease female re-mating rates and egg-laying rates (Kalb *et al.* 1993; Chapman *et al.* 2003; Ram & Wolfner 2007) and to lower post-copula attractiveness (Tram & Wolfner 1998) in related fruit flies (reviews: Wolfner 1997, 2007; Chapman 2001; Gillot 2003). So, the results have clear implications for male reproductive success related to ejaculate production and competition.

In contrast, the absolute area of the spermatophore filled with sperm was found to *increase* with environmental stress before controlling for body size, and to be insensitive to both environmental and genetic stress after controlling for body size (Figure 3 b). As testes are involved in the production of sperm, larger testes might

have been expected to be associated with an increase in sperm area (i.e. in sperm *per se*) per spermatophore (Birkhead & Møller 1998). But this was not borne out by my experiments. In fact, testis size and sperm delivery appeared to be only weakly condition-dependent traits. A potential explanation for this is that, in a single mating context, the smaller, high environmental stress males were able to compensate by increasing their ejaculate output (or, that larger males reduced their per mating sperm allocation due to an expectation of more future mating opportunities). Previous experimental work in *D. meigenii* has shown that small males are able to allocate similar numbers of sperm in a single mating as larger males (Harley *et al.* 2013). Further, sperm competition theory predicts that smaller males will invest equivalent numbers of sperm as larger males because such unattractive (small) males expect fewer mating opportunities, while more attractive males expect a larger number of such opportunities (Tazzyman *et al.* 2009). In line with this interpretation, males that developed under high environmental stress (i.e. smaller males) mated for longer than those raised under intermediate or higher environmental stress, presumably to enable them to deliver equivalent or increased numbers of sperm. Mating length and sperm area were also found to be negatively associated with absolute male eyespan; and both the absolute and relative trait forms clustered as outliers in the across-trait integrations (Figures 6 a, b). This further highlights the potential role of trade-offs between present and future allocation as a driver of the patterns observed for mating length and sperm area. And it implies that, in a single mating context, male eyespan could act as a poor indicator of ejaculate size. It is, nonetheless, notable that at intermediate stress levels the relative eyespan-sperm area correlation became *positive*, with implications for the strength of sexual selection in such intermediate conditions.

In contrast to the low condition dependence of sperm delivery in single matings, the number of sperm stored in the female ventral receptacle after three days of multiple mating decreased with environmental stress in incross flies and increased with environmental stress in outcross flies, with convergence between genetic statuses at high environmental stress levels (Figure 4 a). The effects persisted after controlling for body size. From these patterns under multiple mating, I can infer aspects of the sperm allocation strategy in *D. meigenii.* In single matings (as captured by the sperm area per spermatophore, after a single mating), males allocate sperm in a largely condition-independent manner, with allocation varying with male size, while under multiple mating (as captured by the number of sperm in the VR after three days of mating), male ejaculate allocation becomes strongly condition-dependent. In incross males, environmental condition (stress) is paramount, and allocation increases with food level. But, in outcross flies, environmental conditions are less important, and smaller (low and intermediate food level) males allocate larger numbers of sperm than the larger (high food level) males. As the outcross pattern matches those seen in the single mating context, and the incross and outcross males have similar counts at high food level, the results imply that outcross males are able to use optimal allocation across multiple matings. In contrast, the allocation effect is lost in incross flies; which accords well with a similar rapid reduction in spermatophore size and sperm area across multiple mates seen in smaller versus larger males, by Harley (2013). Across multiple matings, then, the trade-off changes to covariation with condition.

In nature, the presence of multiple males and females could blur the effect of increased across-mate allocation in smaller outcross males, as smaller males will be outcompeted in direct contests over mates. Nonetheless, under these experimental conditions the greater delivery of sperm should be reflected in higher male reproductive success, as reflected in my offspring counts under the multiple mating regime. These revealed a positive effect of genetics in which outcross flies had larger offspring counts across all environments, with an increase with food level within the incross males (Figure 4 b). The effects also persisted after controlling for body size variables (thorax length and eyespan), and reproductive traits (testes and accessory gland length). The picture was thus similar to that of the VR counts (Figure 4 a), but without convergence between genetic states at high food levels, and without a decrease in counts with increased food level for the outcross males. A potential reason for this is that outcross males have higher quality sperm than incross males or that such males have higher quality or larger volumes of accessory products. It is possible that this is the case. Accessory glands – which relate to spermatophore size and the production of accessory products in stalk-eyed flies (Kotrba 1993; Kotrba 1996) – also varied with environmental and genetic condition even after control for body size; and accessory gland length, spermatophore size and offspring count were also highly clustered on the E-G integration. However, no studies have been conducted on the role of accessory products or sperm quality on fertilisation success or lay-rate manipulation in the *D. meigenii* system. Irrespective of these explanations, though, it is notable that the outcross males derived from high quality environments achieved similar offspring counts to the outcross males from the low and intermediate environments, despite the provision of fewer sperm. This implies

that the low and intermediate males are able to compensate in terms of sperm numbers, but also indicates that the high environment outcross males can use fewer sperm to the same effect. In the case that larger outcross males also have larger sperm reserves (they have larger testes) then this could be crucial in nature.

Another factor that could contribute to male reproductive success and sexual selection is male attractiveness. In line with the pattern seen for offspring counts, attractiveness (the inverse of the number of mating attempts that a male made prior to acceptance) decreased with genetic stress across all environments, and the effects persisted after control for body size. Attractiveness was also related to relative eyespan at intermediate environments. Both outcrossed and larger relative eyespan flies required far fewer mating attempts to obtain a successful copulation. Relative eyespan also positively related to testis size, accessory gland size, and sperm area – and negatively with mating length – at intermediate food levels. Likewise, the effect of genetic condition on relative eyespan was greatest under such conditions (see Chapter 3). So it possible that sexual selection could be reinforced in intermediate environments. Nonetheless, while relative eyespan covaried with attractiveness, it did not explain all the variation in attractiveness. The positive effect of genetic condition on attractiveness persisted after control for relative eyespan. An implication of this, then, is that male attractiveness is determined via a suite of traits. In related species, CHCs (Chenoweth & Blows 2003; Howard & Blomquist 2005; Rundle *et al.* 2008; Delcourt *et al.* 2010; Ingleby *et al.* 2014), behavioural displays (Spieth 1974; Griffith & Ejima 2009), 'songs' (Kyriacou & Hall 1980; Hoikkala *et al.* 1998; Blankers *et al.* 2015) and refractive indexes (Katayama *et al.* 2014) are all known to influence multivariate

attractiveness. As male *D. meigenii* are known to complement their courtship with a variety of behaviours (Chapman *et al.* unpublished; and see Kotrba 1996 for related stalk-eyed fly species), and as the prior presence of male *T. dalmanni* on lengths of twine is known to influence female mate decisions even after the males have been removed (Cotton, S., pers. comm.), it is plausible that multiple traits are used in stalk-eyed mate choices as well; a point to which weight is added by the observation that the relationship between relative eyespan and attractiveness was less positive in outcross flies than in incross flies (implying that eyespan is a better determinate of attractiveness when other signals are worse). Irrespective of the composition of attractiveness, outcross males were more attractive, and had larger offspring counts, VR counts, spermatophore sizes, and accessory gland sizes across all environments (Table 1). So, in nature, it is possible that males with superior genetic quality will be able to obtain more matings, deliver larger volumes of higher quality ejaculate, and increase their representation across generations; with females deriving both indirect genetic and direct fertility benefits in relation to mate choice; with clear consequences for sexual selection.

A final point of interest relates to the pairwise eyespan-trait relationships and across-trait integration of trait responses to environmental and genetic condition. A potential expectation is that trait responses will be coordinated, as each trait is dependent upon condition (as noted in Evans 2010; Engqvist 2011). An alternate expectation is that finite resources will lead to trade-offs in investment, for instance, between ornamental and reproductive traits (Parker 1998; Simmons & Emelen 2006). I tested the hypothesis that reproductive investment traits and sexual ornaments would exhibit integrated response to E an G. As noted above,

all absolute as well as most relative traits responded in a positive direction to increases in both environmental and genetic condition. There was a qualitative integration of trait responses. As both absolute and relative eyespans were also associated with key reproductive trait sizes (such as testis length and accessory gland length), this provides partial evidence in favour of a 'condition duality' model of across-trait investment. However, while individual trait responses were mostly positive, the across-trait integration was negative (Figure 6 a, b). The reason for this is that there were two, overlapping, clusters of traits. In one cluster, the morphological traits (eyespan, thorax length and wing length) responded to environmental condition. In another, the reproductive traits (testis length, accessory gland length, spermatophore size, VR count, offspring counts and attractiveness) responded to genetic condition. At first glance, this implies support for the economic trade-off hypothesis. But, as neither absolute nor relative eyespan was negatively associated with any of these traits, this rather implies that there is simply a limitation to condition duality in which traits pool either environmental *or* genetic variation, or, more weakly, a mixture of both. Taken as one, these results imply that the selection on each trait class is coordinated to an extent, which could lead to the partial reinforcement of sexual selection: the level of re-enforcement limited due to the non-linear integration of environmental and genetic condition dependence across traits classes (Lande 1979). It is, nonetheless, notable that a third class of trait, including mating length and sperm area, fell well off the across trait correlation, and so provides evidence for another type of trade-off: the trade-off between present and future resource allocation, based on the potential for mating opportunities, which is predicted by sperm competition theory (reviewed in Wedell *et al.* 2002; Parker & Pizzari 2010).

The results back up Bonduriansky *et al*. (2015) in implying that there could be more than one type of 'condition dependence': related, here, to E, or G, or both. In conclusion, then, I conducted a complex set of interrelated experiments in which incross and outcross flies were raised on low, intermediate and high quality larval diets, and in which the responses of a wide range of pre- and post copulatory traits – related to ornamentation and reproduction – were recorded. I tested the hypotheses that pre- and post-copulatory reproductive, fertility and attractiveness traits would exhibit heightened condition dependence, would covary with the male sexual trait (eyespan), and would exhibit integrated responses to variation in environmental and genetic stress. I found that multiple reproductive traits exhibited heightened condition dependent responses to environmental and genetic stress, and that there was limited similarity between the responses of the reproductive and ornamental trait classes. I also found a complex qualitative integration of trait responses across traits, with a negative across trait correlation. As such, I was able to infer that direct selection on one class of traits will lead to tangential, correlated selection on the other class, and could lead to a limited reinforcement of sexual selection in certain environments. The results have implications for male driven sexual selection, as well as for the suite of benefits available to female mate choice; and also imply that male attractiveness could comprise of a multivariate matrix rather than a univariate trait. As relative eyespan reflected increased information about reproductive traits in intermediate environments, the results also add weight to my Chapter 3 conclusion that sexual selection could be stronger in less extreme environments. In the future, profitable insight will likely be gained via a consideration of the effects of genetic condition on precise across-mate ejaculate depletion rates, as

well as through the characterisation of seminal proteins so as to determine the manner in which genetic condition leads to increased reproductive success. Further studies that use new alternate techniques to induce finer grades of genetic condition, and which look at environmental variation in adults as well as larvae, could also provide valuable insights. For instance, such studies could shed light on the extent that ornamental and reproductive trait integration varies in nature.

CHAPTER 5

GENERAL DISCUSSION

5.1 OVERVIEW

As was realised by Darwin (1859, 1871), an explanation for the evolution of the bright male displays that cannot be explained by natural selection could lie in inter-sexual selection via female mate choice. An issue with Darwin's proposal, though, was that he provided no explanation for the evolution of female mate choice itself. In certain cases, to be sure, a clear direct benefit, such as parental care, could be seen to be associated with the male ornament. However, in other cases, no such direct benefits were evident. To address this, Fisher (1915, 1930, 1958) developed a verbal model based on an indirect genetic female benefit related to 'sexy sons', in which male sexual ornaments and female mate preferences would become associated genetically, and would become exaggerated in spite of the costs. Later, Zahavi (1975) added a further concept. In Zahavi's Handicap Hypothesis, ornaments and preference would evolve because of the costs. These initial ideas have been developed into an array of models explaining the evolution of condition-dependent male ornaments and female preference for these ornaments. Yet, the manner in which female mate choice and preference varies is not understood, as neither are the effects of environmental variation on the extent of genetic condition dependence of male ornaments. The understanding of the effects of both environmental and genetic condition on the covariation between male ornaments and reproductive traits is also incomplete. It is crucial to understand each of these points if we are to understand the variability, dynamics and operational strength of sexual selection in nature. Hence, the core themes of this thesis have been variation in female mate choice and the condition dependence of the ornaments that females prefer. I will now recapitulate the principal results, discuss the relevance of these results to stalk-eyed flies and sexual selection, and provide some directions for future experiments.

5.2 SUMMARY OF PRINCIPAL FINDINGS

5.2.1 Chapter 2. **Mating status affects components of female mating behaviour and sexual selection in the stalk-eyed fly, *Diasemopsis meigenii*.**

An important aspect of sexual selection is female mate choice and the preferences that drive it (Darwin 1859, 1871; Fisher 1915). It is this that drives the evolution of the diverse ornamental traits seen in nature (Fisher 1915, 1930; Zahavi 1975; Andersson 1994). The last two decades have seen an explosion in research related to the shape of the preference functions (Ritchie 1996), on their variation between individuals (Wagner *et al.* 1995) and within individuals (Fowler-Finn & Rodriguez 2012), and on the delineation of mate preference into different components (reviewed in Edward 2015). Yet little is known about the way that the different components of preference – such as choosiness and the preference function – respond to different factors, and interact to influence sexual selection. In part this is because preference is complex (Heisler *et al.* 1987), and difficult to measure (Wagner 1998). But is it also because studies have tended to standardise mating status of females, as either virgin or singly mated females, to increase the control of measurements (for instance: Ritchie 1996; Rosenqvist & Houde 1997; Cotton *et al.* 2006; Bailey 2008; Judge *et al.* 2014). An issue with this is that virgins are both rare in nature (Bateman 1948; Burns 1968; Trivers 1972), and likely to behave differently to mated females due to their greater reproductive cost of mate choice (Kokko & Mappes 2005). Given this, it is important to study the effects of female mating status both on components of preference and selection. Yet few studies have done this (Bateman *et al.* 2001; Lynch *et al.* 2005; Peretti & Carrera 2005; Uetz & Norton 2007; Judge *et al.* 2010; Gershman *et al.* 2014), while those

that have can be criticised in relation to weak assay design, low sample size or both. There is a need for better studies on the effects of female mating status on components of female preference and selection if we are to better understand the way that female driven sexual selection varies with the costs of choice in nature.

To address this, I conducted a set of two related experiments on the effects of female mating status (virgin or mated) on components of preference and selection in the stalk-eyed fly species, *D. meigenii*. I tested the hypotheses that virgin studies: underestimate choosiness and selection, lower the resolution of estimates of the preference function, and increase the variation in preference function estimates within and between females. To do so, I first manipulated female mating status by placing individual females in pots, either with, or without, two males. I allowed the females to mate with the males for two weeks (in the first experiment) or one week (in the second experiment). After this, I assayed female mating responses to sequentially presented males drawn from two or five ornament size classes (in the first and second experiments respectively; the two studies used to increase the reliability of the results and to compare the methods). Female *D. meigenii* exhibit an unequivocal rejection response towards undesired suitors, via the extension of their ovipositors, and shaking of the their abdomens (Cotton *et al.* 2006). As such I was able to record a series of direct responses, per female, to the sequential presentation of males from each phenotype; with each phenotype presented 2-3 times to each female (and with the responses separable from male effects). I then used mathematical and statistical techniques to deconstruct these responses into individual level estimates of choosiness, the preference function and selection. I used statistical models to visualise preference functions, and I

investigated the shape of these preference functions via the use of *post-hoc* interrogative tests. Finally, I used further statistical tests to contrast the levels of variation in each component of preference and selection, and to ask how each aspect of preference varied between individuals and across time (days, or weeks).

The key results of these two experiments were that: 1] choosiness was underestimated in virgin females; 2] preference functions were robust to variation in mate status (although the resolution of estimates was reduced in virgin females); 3] that sexual selection on the male ornament (eyespan) exerted by female mate choice was weaker in virgins; 4] that variation in choosiness was robust to mating status, both within and between individuals, and 5] that variation in the preference function was higher in virgin females, both within and between individuals. In addition to these key results, I also showed that the overall female preference function in female *D. meigenii* is a near open-ended, plateauing curve, with a potential peak positioned well beyond the population mean, towards larger eyespans. Finally, I provided direct evidence in favour of an interpretation of female preference as a composite of at least two components – choosiness and the preference function – which are independent in their responses to variation in the reproductive costs of mate choice, and which interact to influence sexual selection.

From these results, I concluded that the use of virgins in studies of female mating preferences should be treated with caution, and that the variation in female choosiness with the natural costs of mate choice warranted further investigation. I can now add to this with a brief discussion of the wider implications of these results.

As just noted, the first point is that previous studies of the strength of female preference could well have underestimated the strength of female driven sexual selection in nature. This point is backed up by this Chapter 2 pair of studies, but also by the 5 prior studies on the effects of variation in the costs of choice associated with female mating status on female choosiness (Bateman *et al.* 2001; Lynch *et al.* 2005; Peretti & Carrera 2005; Uetz & Norton 2007), overall preference (which pools choosiness and the preference function), and selection (Judge 2010). All show that choosiness increases as the costs of choice are reduced, and vice versa. An implication, then, is that studies that have used virgin females – which includes most prior studies on female mate choice, preference, and selection – could have underestimated female choosiness, overall preference and selection. Those that looked at the preference function alone are likely to be unaffected, even if the resolution of estimates could have been lowered. Taken together, this implies that the strength of sexual selection in nature could have been underestimated due to the use of virgin studies in the lab. To the extent that models of sexual selection rely on empirical estimates of choosiness or pooled overall preference, this must be considered in future models.

A related point is that mathematical models of sexual selection need to consider the variability in choosiness that likely exists in nature. The costs of choice likely vary in real time with multiple factors. In insects, it is common for females to store sperm in specialised organs. In some cases, this store will last until the insect dies, due to the vast number of sperm stored. However, in other cases this number is likely to drop over time (as is the case in *D. meigenii*, J. H. pers. obs.). Likewise, population density and sex ratio could well affect male-female

encounter rates, and thus the costs of mate choice. Variation in sex ratios could have similar effects. In these cases the effects should operate in mammals, birds, fish and other animals as well. And such factors will themselves be likely to vary in response to environmental or climatic conditions, and to depend on the dispersal range of the species in question, or even on the spatial location of the sub-population or individual in question within the total range of the wider population (in the centre, density will often be higher, while at the edges of population ranges or habitats density can often be lower). Female attractiveness to males – dependent on environmental and genetic effects – could also alter the cost of choice, with variation in attractiveness covarying in complex ways with density, sex ratio and other cost of choice altering factors. As can be seen, this variation could be taxonomically widespread. Hence, variation in choosiness and overall preference, and the selection exerted on male ornaments by female mate choices could well vary to large extents in nature. Empirically, there is thus a need to examine these forms of variation. Conceptually, there is a need to consider that mate choice will be variable. And theoretically, there is a need to include complex, multiple component, variable preference in models of sexual selection. This will require a lot of work, but is necessary if we are to understand the evolution of female preferences and male ornamentation – and it could provide useful insights into new resolutions to old questions like the lek paradox. With this in mind, I provide some directions for future studies in the next main section, 'Future Directions', with a focus on future studies in the stalk-eyed flies.

5.2.2 Chapter 3. **Environmental variation can amplify or mask the signal of genetic condition in sexual ornaments, in stalk-eyed flies.**

An area of sexual selection that remains unresolved is the nature of the indirect genetic benefits that females obtain via mate choice. Male sexual ornaments are known to reveal information about the environmental and genetic condition of the bearer (Pomiankowski & Møller 1995; Rowe & Houle 1996; Tomkins *et al.* 2004), and can do so in a heightened manner relative to non-sexual traits (environmental: Zuk *et al*. 1990; David *et al*. 1998; Kotiaho 2000; genetic: Aspi 2000; van Oosterhout *et al*. 2003; Bellamy *et al*. 2013). But how does environmental variation alter the genetic condition dependence of these ornaments; does it enhance or mask the genetic signal? Despite a spate of recent studies on the broad genetic basis of environmental condition dependence in sexual traits, for instance via the use of gene-by-environment interactions (G x Es) (Greenfield & Rodríguez 2004; Bussière *et al.* 2008; Evans *et al.* 2015), little is known about the effects of environmental variation on the genetic condition dependence of sexual versus nonsexual traits (Cotton *et al.* 2004c). In fact, to date, only two studies have looked at the effects of environmental variation on the explicit genetic condition dependence of sexual traits. Zajitschek and Brooks (2010) used inbreeding to conduct such a study in guppies, but used only two non-controlled environmental levels and did not control for body size. In contrast, Bonduriansky *et al.* (2015) conducted a commendable study of G x E in sexual and nonsexual traits in *Drosophila melanogaster*. Here, Bonduriansky *et al.* (2015) used mutation accumulation lines and controlled diet treatments to alter condition, or stress. However, the use of two environmental levels limited

interpretations about the directionality and shape of cross-environmental genetic condition dependence in each trait. Moreover, the sexual trait used was a multivariate CHC complex, with no *a priori* expectation of condition-dependence.

As such, there was a need for well-conducted G x E studies to look at the cross-environmental shape of genetic condition dependence in male sexual ornaments relative to non-sexual traits (Cotton *et al.* 2004b; Tomkins *et al.* 2004). To address this, I conducted a study based on the male sexual ornament (eyespan) and a suite of non-sexual morphological traits in *D. meigenii*. I tested the hypotheses that: environmental stress would precipitate the signal of genetic condition in the male sexual ornamental traits in this species; that the response to environmental and genetic variation would be heightened for the sexual trait relative to a suite of non-sexual traits; and that there would be an across trait integration of trait responses to E, G and G x E. To exert two distinct levels of genetic stress I used a series of incrosses and outcrosses between a suite of 17 inbred lines ($f \sim 0.908$, Falconer & Mackay 1996) that were produced by Bellamy *et al*. (2013). An advantage of the use of inbred lines is that inbreeding increases homozygosity (Wright 1977), exposes deleterious alleles (Roff 1997) and reduces fitness at loci subject to the heterozygote advantage (Bulmer 1980; Charlesworth & Charlesworth 1987). Hence, the inbreeding coefficient was a useful and easily manipulated measure of genetic quality, or 'condition' (Rowe & Houle; Tomkins *et al*. 2004). It could be manipulated via incrosses (within lines) and outcrosses (between lines) to produce homo- and heterotic individuals (Prokop *et al.* 2010). To induce environmental stress I used three levels of larval diet, which spanned a wide range of variation and were tightly controlled (based on Cotton *et al.* 2004a).

I then measured the eyespan, thorax length, and wing length of all males and females, after eclosion (all external morphological traits are fixed at eclosion in stalk-eyed flies). I used statistical tests to contrast environmental, genetic, and G x E trait responses, and repeated these analyses on relative trait measures after control for variation in body sizes via the inclusion of thorax length as a covariate in each model. I also contrasted the scale of the responses in males and females. I looked at the cross-environmental patterns of between-line variance, and at across-environmental genetic correlations, for absolute and relative male eyespan, in incross and outcross flies. Finally, I looked at the across-trait integration of trait responses to E, G, and G x E for the range of morphological traits mentioned, as well as for key male reproductive traits – testes length, and accessory gland length.

The key results of this experiment were: 1] that male eyespan exhibited a heighted response to variation in environmental condition relative to body size, wing length, and the female homolog; 2] that male eyespan also exhibited a heightened genetic and G x E response relative to male body size, and wing length, but not female eyespan; 3] that there was a novel G x E for male (and, to a lesser extent, female) relative eyespan, in which the effects of genetic stress on relative trait size were masked in both high and low, but not intermediate, stress environments; 4] that between-line variance in male relative eyespan increased at less severe levels of environmental stress in incross, relative to outcross males; and, 5] that there was a positive across-trait integration of trait responses to intermediate E and G x E, but a negative integration in relation to overall E and G.

From these results, I concluded that male eyespan and relative eyespan exhibit

heightened condition-dependent responses to both environmental and genetic stress, as predicted by the condition-dependent handicap hypothesis of sexual selection. I concluded that, in contrast to standard expectations, extreme environmental conditions can blur the signal of genetic condition in sexual traits at both high and low levels, rather than at high alone. I discussed the potential consequences of this for the benefits of female choice, and the depletion of additive genetic variation (V_A) in variable, natural populations. I discussed how an implication of this G x E could be that sexual selection varies in a condition-dependent manner. I also described how the overall condition G x E pattern arose as a composite of two standard 'variance G x Es', one for incross males, one for outcross, each with a different mean, variance and 'fan-out' start point. I note now that this implies that specific alleles or genetic make-ups can lead to across environmental variation in a 'classical' sense (that is, the rank orders are stable across environments, while the variance between best and worst increases with environmental stress); but that, overall genetic condition (hetero- versus homozygosity) can then alter the point at which such allele or line specific effects are seen. I also note now that, in nature, it is thus possible that multiple forms of genetic quality are signalled, in different ways, in different environments (i.e. 'overall' quality versus specific 'good' alleles). Finally, I discussed the way that a positive across-trait integration of trait responses to environmental and genetic condition was seen only when the genetic variation 'locked' in the G x E was included. The pattern that was observed implies that, as expected under the condition dependent handicap hypothesis of sexual selection, male eyespan and relative eyespan have evolved to pool the largest amount of environmental and genetic variation (relative to female eyespan, and male morphological and

reproductive traits). The pattern adds weight to the ideas either that female eyespan is also selected for heightened condition dependence (female eyespan clustered near to male eyespan), or that the eyespan trait is 'pre-adapted' for condition dependence (as was suggested in Cotton *et al.* 2004c, and which could explain the origin of the sexual preference for eyespan). I also discussed how the positive across-trait integration of trait responses to intermediate E and G x E hints at the potential for male eyespan to reveal information about direct reproductive benefits to female mate choice in an environmentally-dependent manner (eyespan integrated E and G x E responses in a similar, but more dramatic manner than the reproductive traits, testis length and accessory gland length). As a final point, I noted that the overall directional G x E provides a clear demonstration of the importance of using multiple levels of environmental variation in studies of genetic condition dependence. An equivalent three studies that had used, in turn, the two extreme food levels, the low and intermediate levels, or the intermediate and high food levels, would have reported dramatically different patterns. In each case, the observers would have been led to erroneous or incomplete conclusions. The use of two environmental levels is thus insufficient.

Above, I have recapitulated the main points discussed in Chapter 3, and added some additional discussion points about the types of genetic information (and thus female benefits) revealed by the male ornament, and about prior limitations to experimental methodology. However, the focus was, in the main, on sexual selection in stalk-eyed flies, and *D. meigenii* in particular. I now provide a brief discussion of the wider consequences of the results for sexual selection in general.

The first point to note is that, across species, studies of the levels of environmental and genetic condition dependence (and condition G x Es) in traits must use at least three levels of environmental stress – otherwise the across-environmental patterns of trait responses to variation in genetic condition that are observed can be simple artefact of the levels of environmental condition (or stress) that are used in the study. Most recent studies that have looked at environmental variation have used at least three levels of stress. And, to date, only two other studies have looked at condition G x Es (Zajitschek and Brooks 2010, in guppies, and Bonduriansky *et al*. 2015, in *Drosophila*). This is fortunate, and each provides novel insight into trait condition dependence. Nonetheless, further studies are needed, and in the design of these studies consideration needs to be given to the number of levels of environmental (and genetic) condition or stress that are used. I provide directions for some such studies in the next section, 'Future Directions'.

Another area that deserves additional discussion is that related to the levels of signal of genetic condition in male sexual ornamental traits across environments. As expected, I found that the male sexual trait (relative eyespan) varied with both environmental and genetic stress – it revealed information about both. A crucial result, however, was that the level of information about overall genetic stress (or condition) in the sexual trait was obscured at both environmental extremes – that is, at low, but also at high, levels of environmental stress. Yet, this was not the case for between line (genetic) variation, which increased with environmental stress. As noted before, this implies that male sexual ornaments reflect information about both the overall genetic condition of the male, and about specific alleles associated with particular genotypes that allow males to perform

well or less well over all environments (the rank orders of the different crosses did not vary across environments). Yet, these two types of information were revealed, and affected, in different ways, across the different environments. So, on the assumption that such effects exist across species, this could have serious consequences for our understanding of sexual selection under variable conditions.

For instance, where environmental stress is low, little information about the genetics of a male will be revealed in his ornament. Where environmental stress is high, a large amount of information about specific sexual trait associated alleles will be visible. And where environmental stress is intermediate information about both overall male genetic condition and sexual trait associated alleles will be visible in male ornaments. At first sight, then, sexual selection could be expected to be strongest at intermediate, or even high, levels of environmental stress – dependent on the type of indirect genetic benefit that is more important to females. However, for male sexual ornaments to be reliable environmental conditions need to be relatively stable, as rapid fluctuations – relative to the lifespan of the organism – can blur out ornament reliability (Rodríguez 2013). Hence, where female mate choices are based on indirect genetic benefits (rather than environmental factors, such as ornament-testes covariation etc.), females and males will often experience similar environmental conditions to one another [over the course of their ontogenetic development]. All will be 'large' (due to low stress), or small (due to high stress) at the same time. A common observation about female mate choice is that the preference functions that drive it are often dependent on the condition of the female (Gray 1999; Hingle *et al.* 2001a; Hunt *et al.* 2005a; Cotton *et al.* 2006a; reviewed in Cotton *et al.* 2006b Holveck & Riebel

2010; Holveck *et al.* 2011). Across species, better condition females exert stronger preferences for more ornamented males. A consequence of this, then, is that, while the total allelic benefits to female mate choice could be high under high environmental stress, females are likely to exert weak mate choice under these conditions – so sexual selection could remain weak. Likewise, at low environmental stress, while female preferences are expected to be strong, the information about male genetic condition (or allelic variants) will be low, so sexual selection based indirect genetic benefits will be weak. Indeed, this latter point could provide a partial resolution to the lek paradox: depletion of V_A will be low in low stress environments, due to weak genetic signal, and could also be low in high stress environments, due to weak female preference functions (or due to the weak expression of preference functions if choosiness is low due to low population densities and consequence increases in the costs of female mate choice). Another point of interest, here, is that, if female preference and choosiness are often weak in high stress environments, where the most information about allelic variants is available (and where little information about overall condition is available) in male ornaments, then it could imply that females have evolved preference not for small sets of 'good alleles' at a specific locus, but rather for the overall condition of a male across his genome. Irrespective, the next point is that, at intermediate environments, a large amount of information about a male's overall condition, and some information about line specific allelic sets, will be available to female mate choice, and female mate choice should be strong and in the direction of more ornamented males (as female condition should be good, and as the costs of choice should, due to the environment and her condition, be relatively low). Hence, it could be that sexual selection is both more

variable than often expected, that it is weak at extreme environment stresses, low and high, and that it is even stronger than expected at intermediate environments.

In short, it is important to realise that it is the interaction between available genetic benefits and female choice, which will vary with environmental stress, that will alter the strength of sexual selection, and the rate of erosion of V_A in male traits: and that such interactions could result in weaker than expected selection at high environmental stress due to weak mate choice, to lower selection than expected at low environmental stress due to low genetic signal in male ornaments, and to stronger than expected selection at intermediate environmental stress due to a maximal genetic signal (for condition and alleles) as well as strong female preference and choosiness. It is likewise crucial to realise that all this will, of course, be further modulated by rate of environmental flux. However, the extent to which this is true will depend on the extent to which these patterns hold across species. For instance, while it could be common in insects, it is not clear whether this would hold in mammals. There is no *a priori* reason to expect that this is not the case, but mammals (birds, fish etc.) cannot fix traits post eclosion. Hence, the specific patterns could well depend on the species in question, and on the type of trait that is used as a sexual signal. Moreover, the rate at which low and high genetic stress male trait sizes converge as environmental stress increases will be important – the effects of high environmental stress on the availability of information about male genetic condition will be less important if the convergence is rapid and present only at maximal stress. At present, the effect of extreme or graduated environmental stress on the expression of sexual ornaments is known only in one species – *D. meigenii*. Further experimental work is required.

5.2.3 Chapter 4. **Environmental and genetic condition dependence of pre- and post-copulatory reproductive traits in the stalk-eyed fly, *Diasemopsis meigenii*.**

Due to the competitive nature of male reproductive success in species in which females mate polyandrously (Parker 1970; Andersson 1994; Parker & Ball 2005), male reproductive, fertility and attractiveness traits are likely to be under direct selection, similar to that experienced by secondary sexual traits (Arnqvist 1998; Ramm *et al.* 2007; Perry & Rowe 2010). They are also costly, related to life history and reproduction, and often exaggerated. Given this, such traits can be expected to evolve to be condition-dependent (Alatalo *et al.* 1988; Rowe & Houle 1996; Bonduriansky & Rowe 2005), and to integrate environmental and genetic condition in their development (Pizzari & Birkhead 2002 Chapter 3). A direct prediction of this is 'covariation-with-condition', where sexual pre-copulatory and reproductive post-copulatory traits exhibit positive covariance (Hosken *et al.* 2008; Chargé *et al.* 2013; Evans *et al.* 2015). An alternate expectation is that based on an economic, life-history, resource-allocation or ejaculate-allocation perspective (Williams *et al.* 2005; Tazzyman *et al.* 2009; Engqvist 2012). Here, one could instead expect an ornament-reproductive investment, or ornament-ejaculate trade-off (Parker 1998; Simmons & Emelen 2006), which could lead to negative covariation between these trait classes. The extent to which the responses of these traits to environmental and genetic condition are integrated or not, and do or do not covary, has important consequences for sexual selection. A number of studies have looked at the responses of reproductive or ejaculate traits to environmental variation (Jensen *et al.* 2004; McGraw *et al.* 2007; Vasudeva *et al.* 2014), or genetic variation (Wildt *et al.* 1982; Fitzpatrick & Evans 2009; Michalczyk *et al.* 2010),

have shown that there is a genetic basis to environmental responsiveness (Engqvist 2008; Morrow *et al.* 2008; Evans *et al.* 2015), and even that phenotypic and genetic correlations between such trait classes exist (Hosken *et al.* 2008; Evans *et al.* 2015). Yet, to date, the full coordination of pre- and post-copulatory trait responses to variation in environmental and genetic condition has not been studied.

To address this, I conducted a series of interrelated studies using the African stalk-eyed fly species, *Diasemopsis meigenii* to test the hypotheses that: pre- and post-copulatory traits exhibit heightened condition-dependent response to variation in environmental and genetic stress, that such traits covary positively with male sexual ornamental traits, and that there is an integration of trait response to each type of stress, across traits. To do so, I used a set of crosses between 17 inbred lines to generate individuals with either low (incross) or high (outcross) genetic quality. I manipulated diet to exert three levels of environmental stress, and then raised males to sexual maturity (~ 10 weeks post-eclosion). I then used various techniques to obtain measurements for a suite of pre- and post-copulatory traits (related to attractiveness and fertility) across a full factorial combination of these environmental and genetic states. These traits included: 1] male eyespan, 2] thorax length and 3] wing length; 4] testis length and 5] accessory gland length; 6] spermatophore size and 7] the volume of sperm in the spermatophore (in a single mating); 8] the number of sperm stored in the female ventral receptacle, after 3 days of free-mating (the ventral receptacle is the single used female sperm storage organ in *D. meigenii*); 9] the number of F_2 progeny that a male sired (this related to the 3-day period of free mating with a single female); 10] a male's latency to mate with a female (before the single

mating); 11] the number of times a male was rejected by a female before copulation (an inverse measure of attractiveness); and 12] the duration of the successful copulation (in which a spermatophore was transferred to the female). I used statistical tests to contrast environmental, genetic, and G x E trait responses, and repeated these analyses on relative trait measures after control for variation in body sizes via the inclusion of thorax length as a covariate in each model. I examined the relationships between absolute and relative male eyespan, and each of the reproductive, fertility (and attractiveness) traits in turn, for overall patterns, and for variation in these patterns associated with environmental and genetic states. Finally, I looked at the across-trait integration of trait responses to E and G for each of the traits that exhibited significant response to either E, G (or G x E).

The key results of this experiment were: 1] that there was a qualitative alignment of traits responses to E and G (most were positive – and a large number of traits exhibited [often heightened] condition dependence), 2] there was a negative across-trait integration of trait response to E and G (traits that responded most to E responded least to G, and vice versa) (intermediate E and G x E was not examined, as just one trait, VR count, exhibited G x E); 3] that there was a positive association between male relative eyespan and testis length (almost accessory gland length) and attractiveness, each of which was stronger in intermediate environments; 4] that there was a negative relationship between relative eyespan and mating length (and between absolute eyespan and the area of sperm in the spermatophore); 5] that the relationship between relative eyespan and sperm area in the spermatophore became positive in intermediate environments; 6] that there was evidence that ejaculate and mating length traits clustered away from the

other classes of trait on the across-trait trait-response integration; and, 7] that male attractiveness (a multivariate trait?) was positively associated with male eyespan, even if the association was weaker in outcross flies relative to incross flies.

From these results, I concluded that there is some support for the covariation-with-condition expectation, especially in intermediate environments. I also concluded that there was clear evidence in favour of an ejaculate allocation trade-off in a single mating context; with larger males allocating a smaller area of sperm under these conditions, likely due to the expectation of a larger number of future mates. I noted that the environmental stress related ejaculate allocation effect was diminished in the incross state across multiple matings, but that it persisted even over multiple matings for the outcross males. Nonetheless, I realised that the outcross, low environmental stress males obtained an equal number of offspring as the outcross intermediate and high environmental stress males, despite the delivery of fewer sperm. Across all environments outcross males also obtained a larger number of offspring than the incross equivalents. I noted that both larger eyespan *and* outcrossed males required fewer mating attempts before they were accepted to copulate. Hence, I concluded that outcross and high food level (low stress) males have larger ornaments, attract females, and are able to obtain similar numbers of offspring per female as lower food level or incross equivalents, even while fewer sperm were allocated by them per mate attempt and per female. I now note that this could mean that the rate of depletion of V_A in intermediate environments is higher than expected, as the depletive consequences of female choice could be compounded by male reproductive performance (in an environmentally condition-dependent manner) such that F_2 genomes derive from

a small of a subset of F_1 males. Another point of interest was that the relationship between eyespan and attractiveness was weaker in outcross males, with implications for multivariate male attractiveness and female mate choice (eyespan seem less important as a determinant of attractiveness when genetic condition is high). I discussed the potential for limited correlated selection across traits, which could reinforce sexual selection, to some extent (integration existed, but the slope was negative), and that this could occur at a more powerful level in intermediate environments: Male relative eyespan was related to attractiveness, testis size, accessory gland size and even sperm area in spermatophore – which was usually negatively related to male absolute and relative eyespan – more positively in intermediate environments; hence females were more attracted to males with larger testis, more sperm, and larger accessory glands in intermediate environments and could thus obtain larger direct benefits. This latter point adds weight to the idea of environment-dependent sexual selection, raised in Chapter 3.

To discuss the wider implications of these results for sexual selection, the first point is to note that secondary covariation between male ornamental traits and male reproductive, fertility and attractiveness traits due to primary covariation of each with environmental and genetic condition could be common in nature. Males with large ornaments could thus be more attractive, have better genetic condition, and also be more fecund or fertile. Given this, females could use ornaments to obtain information about direct benefits to their reproductive output as well as about indirect genetic benefits to their offspring. It could be the case that these direct benefits, like the indirect benefits, are also more visible at intermediate environments. And it could be that, due to environmental effects on

both condition and the reproductive information in male, fixed, morphological, ornamental traits, that females use multiple traits in different contexts to obtain a wider, fuller picture of male quality relative to their own 'desires' (which could vary in a condition dependent manner as well). The extent to which these patterns of increased direct benefit visibility in intermediate environmental conditions persist across species is unclear. However, if this is common then it adds to the points made in the previous Chapter discussions, that sexual selection could – on the assumption that intermediate, low fluctuation relative to lifespan environments are common in nature – be more powerful than is often thought, as well as more variable across environments. The moderate covariance of the reproductive and fertility traits with condition also highlights the point that traits that reveal condition in a heightened manner relative to less condition-dependent traits will be useful guides to female mate choice, and could often be the traits that are preferred by females (in which case the use of condition-dependence as a resolution to the lek paradox will become an ever more relevant explanation). Nonetheless, the extent to which these patterns of direct and indirect benefits, of ornamental and reproductive trait covariation with condition, persist across species is unknown. And it is important to realise that the extent to which insect studies, where morphological traits are fixed at eclosion, are relevant to other species (and to insect traits that are not fixed at eclosion such as CHCs), will depend on the extent to which larval and adult environmental stresses covary or are separated in nature. Where these are closely related, covariation with condition could be even stronger than I measured. But where the two are not related, it could lead to unreliability of ornamental traits as indicators of current adult condition, and obfuscate the information about male reproductive quality.

As in Chapter 2 and 3, these results show that further studies of the effects of environmental and genetic variation on mate ornamentation and female mate choice are required if we are to better understand the operation of sexual selection.

5.3.4 *A brief meta-discussion and summary*

In short, the results of the empirical sections in this thesis draw attention to the importance of variation in female choosiness, preference and selection, and of variation in the type and extent of direct and indirect genetic benefits signalled by male eyespan in difference environments. The results imply that an interaction between the potential benefits to choice (direct, indirect) and the strength of female choice could lead to considerable variation in the strength of sexual selection in nature, as well as to an amplification of sexual selection in intermediate environments. There is, thus, a need for further studies. However, these studies need to focus on testable questions. I now provide a number of directions for future studies that could build on these results, using stalk-eyed flies.

5.3 FUTURE DIRECTIONS

5.3.1 *Female mate choice – the reproductive costs of choice*

A key result of Chapter 2 was that female choosiness varies with the costs of choice associated with female mating status (virgin versus mated). An implication of this is that female choosiness is 'plastic', rather than 'fixed'. If so, this could have important implications for the evolution of female mate preferences, and for the robustness of populations to climatic variation (Kokko & Mappes 2005). However, the transition between a virgin and mated state happens, per female,

only once. Virgins are also rare (Burns 1968; Trivers 1972; Burkhardt & de la Motte 1983). Furthermore, it is possible that choosiness becomes fixed in a non-virgin state, because females have sufficient sperm stores to remove the reproductive costs of choice. Yet it is also possible that choosiness continues to fluctuate with variation in the costs of mate choice. For instance, such fluctuations could arise due to female sperm limitation (Kokko & Mappes 2005).

A large proportion of sexual selection literature assumes that females have few difficulties obtaining enough sperm to fertilise their eggs. This is because sperm are often viewed as cheap to produce (Dawkins 1976), while females and female egg laying rates are thought to be the limiting factor in reproduction (Bateman 1948; Trivers 1972; Clutton-Brock & Parker 1992; Andersson 1994). However, the costs of sperm production are not trivial (Nakatsuru & Kramer 1982), and a range of factors have been shown to lead to female sperm limitation. For instance, males that mate with multiple females often allocate sperm strategically (Parker 1982); and this can, in turn, lead to female sperm limitation (Tazzyman *et al.* 2009; Harley 2013). Another set of factors that can lead to female sperm limitation are those that can alter male encounter rates (Kokko & Mappes 2005): such as population structure (Willis *et al.* 2011; Ryder *et al.* 2012), sex ratio (Fawcett *et al.* 2011) and mating system (Beehler & Foster 1988). Finally, this limitation can be compounded further by factors such as male choice (Dewsbury 1982; Chenoweth *et al.* 2007), female fecundity competition (Le Boeuf 1974; Cremer *et al.* 2012), or any factor that decreases the number of sperm per egg that a female is able to obtain. In short, females may often be sperm limited in nature.

A consequence of variable reproductive costs for mate choice is that the extent to which choosiness is 'fixed' or 'plastic' becomes important. For instance, where choosiness is 'fixed', females will be less likely to evolve strong preferences in the face of variable mate encounter rates, as the females with the strongest preference will obtain fewer mates where encounter rates drop. In contrast, where choosiness is 'plastic' females will be able to evolve or maintain strong mate preferences in the face of variable mate encounter rates, as the females with the strongest preferences will still mate with lower quality males in situations where males are rare (and will thus not be selected against, Kokko & Mappes 2005). Likewise, populations with such 'plastic' choosiness are predicted to be robust to environmental variation, while populations with 'fixed' choosiness could have increased extinction risks in the face of environmental variation, or habitat fragmentation (for instance, due to anthropic deforestation or climate change).

Female stalk-eyed flies are known to be sperm limited (Harley 2013). Female *D. meigenii* store sperm in the ventral receptacle (VR). This is the single sperm storage organ that is used in this species, and is composed of ~150-300 pouches (Kotrba 1996). After a female has mated for the first time, her reproductive cost of choice is reduced, and her choosiness increases (Chapter 2). A potential cause of this increase in choosiness is that virgin females are naïve, while mated females are not. However, this is unlikely important, as virgin females were found to have lower choosiness across all three weeks of the five size experiment in Chapter 2. A more likely reason for this difference is that virgin females do not have sperm stored in their VR, while mated females do. If this is the case, then female choosiness will be expected to vary in real time as sperm stores decline, or are

replenished. In nature this could arise due to the various mechanisms noted above – for instance, due to male encounter rates, or population structures, or due to male ejaculate allocation strategies. A simple way to manipulate this effect in the lab would be to mate females, and wait for sperm stores to decline. It would thus be possible to manipulate the reproductive costs of mate choice in a biologically meaningful way, and on a graded scale – dependent on sperm depletion rates.

To address this, I have – in addition to the doctoral work presented in this thesis – conducted two (recent) studies to start to look at this issue. First, I looked at sperm depletion. I mated 100 females for 1 week. I then dissected females at 7 intervals over a 3-week timespan. I found that sperm stores declined rapidly over days 1 – 11, and had run out (or were close to running out) after about 18-21 days. To build on this, I then looked at the effects of female sperm stores on female choosiness, the slope of the preference function, and selection. To do this, I set up a 5-block experiment. Females were mated to males for one week, and the males were then removed. This was set up in a staggered manner, so that all females were the same age, but had not mated for different lengths of time. I recorded female mate responses based on the two-size protocol. I found (in a recent preliminary analysis) that female choosiness and selection declined in line with sperm depletion, while – as seen in Chapter 2, for virgin-mated contrasts – the preference function was robust to this variation in the costs of female mate choice.

These initial results show that real time variation in the reproductive costs of female mate choice can influence female choosiness and selection. In the future, after these analyses have been confirmed, it would be useful to build on this work.

For instance, studies could be conducted in which a more natural variation in male encounter rates was utilised. But another direction to be investigated is that of the interactions between context and condition on mate choices and selection.

5.3.2 *Female mate choice – context and condition*

As has been discussed in Chapter 1 and Chapter 2, female choosiness is expected to vary with the costs of mate choice, while female preferences functions are expected to relate to 'intrinsic' properties of the female. In an unpublished study, Small (2009) used adult dietary stress to manipulate female condition in *D. meigenii*. The key result was that females on better diets exhibited stronger preference for larger eyespan males. The experiment was conducted with virgins.

To build on this, I repeated this experiment – but used mated females. In addition, I varied both female diet, and the length of time since the females had mated. The idea of this was to manipulate condition (and thus the preference function), as well as the costs of choice (and thus choosiness), so as to test the interactive effects of these factors on female mate choice and selection. I completed this experiment as I wrote this thesis. Given this, the data have not been subjected to a full analysis. However, to shed some light on the issue, I have conducted a preliminary analysis. The result of this was a surprise. As expected, choosiness was lower in less recently mated females. But it was also lower in high condition females. The preference function was robust to both forms of variation (though there was a trend towards stronger preferences in high condition females) and there was an interactive effect on selection. In low condition females, selection did not vary with sperm stores. But, in high condition females, there was

a large effect – selection was far stronger in high sperm store females. A crucial point, though, is that high condition females had larger egg stores. As an initial interpretation, then, it seems that, in the virgin state, variation in condition leads to variation in the preference function. But, once mated, the effect of condition on egg stores means that such females need more sperm. Hence, the effect of sperm stores becomes more important for high condition females, and leads to increased variation in the strength of sexual selection exerted on the male ornaments. A full interpretation will require that more complex and detailed analyses be conducted. But, the results do imply that sexual selection could be weakened in situations where sperm is not abundant, as the most fecund females will suffer reduced choosiness and therefore fail to exhibit the strong underlying preference as choice. In contrast, where sperm is abundant, small variations in underlying preference functions associated with condition could lead to large differences in sexual selection, because the more fecund females will be choosy and lay the most eggs.

5.3.3 *Female mate choice – genetics, and G x E*

As a final future direction related to female mate choice, it would be useful to look at the effects of female genetic condition on components of preference and selection. I conducted a pilot study in this direction. To do so, I used a similar cross protocol to that in Chapters 3 and 4. I then used a two-size test to record female mate choices. I found a weak effect on choosiness: outcross females were choosier. But I found no effect on the preference function. This experiment did not have enough power to detect subtle variations in preference. However, it was also conducted in a benign environment, in which all females were fed on full diets. Hence, it would be of interest to test for gene-by-environmental variation in

female mating preferences. In the case that female preferences are, like male ornaments, expressed more strongly in intermediate environments, this could have serious consequences for our estimates of sexual selection. Likewise, the potential for 'high G' females to exhibit increased preference for 'high G' males under intermediate environments could provide a basis for extreme trait evolution. To determine whether this is the case would require further studies.

5.3.4 *Male ornamentation – levels of G x E*

As was noted in Chapter 3 and Chapter 4, there is a need for studies that look at the shape of across-environmental genetic condition dependence of sexual versus non-sexual traits. Likewise, there is a need for studies that look at the effects of environmental and genetic condition on the pairwise relationships between traits. Finally, there is a need for studies on the integration of trait responses across traits.

To date, and to the author's knowledge, there have been only three studies that have manipulated both environmental and genetic condition and contrasted sexual and non-sexual traits. First, Zajitschek and Brooks (2010), in guppies. Second, Bonduriansky *et al.* (2015), in *Drosophila*. Finally, the studies that I present on *D. meigenii*, in Chapters 3 and 4. Likewise, to the author's knowledge, Evans *et al.* (2015) have provided the only study on the effects of environmental variation on the genetic correlations between pre- and post-copulatory traits. Bonduriansky *et al.* (2015) and Chapter 3 and 4 are the only studies that look at an integration for trait responses, across traits, to variation in both environmental and genetic stress.

To take this field further, a wide array of studies could be utilised. However, I will outline, in brief, a few studies that relate to the research that I have presented in this thesis. In Chapter 3, I presented evidence for a novel G x E, in which variation in male relative eyespan associated with genetic condition was reduced in both high and low, but not intermediate environments. A simple extension of this would be to use a wider range of environmental conditions. In Cotton *et al.* (2004a), five levels of food variation were used. I have run a pilot study that shows that it is possible to use nine food levels to exert controlled environmental stress on (stock population) male eyespans. Moreover, it would be possible to achieve the sample sizes required, as an experiment could be set up to focus on the collection of as many males as possible, with no requirement to maintain the males for future behavioural traits or dissections to measure reproductive organs. The results of such an investigation could provide insight into the fine-resolution shape of across-environmental genetic condition dependence. It would be valuable to know whether low and high genetic condition flies ornamental trait sizes converge at once (in a steep curve), or in a graduated manner as environmental stress, as this would have direct implications for intra-sexual selection, in relation to the indirect genetic benefits of female mate choice in variable situations – that is, in situations that start to resemble nature in complexity.

A simple extension to this, with similar output, would be instead to vary the levels of genetic condition; for instance, via the use of mutation accumulation, or inbred lines. It would be possible to start lines – inbred, or mutation accumulation – and then place the lines in population cages at different levels of stress (that is, after different numbers of generations of inbreeding or accumulation). Likewise, a

complex cross protocol could achieve similar ends. This type of investigation would show whether the genetic divergence across environments is sudden, or not.

As a final example, it would be useful to look at variation in larval and adult environmental stress (diet stress), or at different types of stress. It would then be possible to test the effects of each type of stress, or condition, on pairwise trait relationships – for instance, between male eyespan and reproductive traits. In real life, stress levels will vary in both the larval and adult stages. The stress experienced at each stage could covary, or could not. This would also be useful to determine, but would require field work. Irrespective, a laboratory test would be able to test the extent to which such trait classes are or become associated or disassociated in semi-realistic situations. Moreover, with the addition of a genetic condition element (obtained via inbreeding, mutation accumulation, direct stress [i.e. radiation], or outcrosses), it would also be possible to test the integration of across-trait trait responses in relation to larval and adult stress. This could be especially valuable in insects, as the larval and adult stages are distinct, and as it is often the case that some traits are fixed at eclosion, while others are free to vary.

5.3.5 *Field studies*

As a short, final section, I will note that, in addition to more realistic scenarios in the laboratories, there is a need to take studies of sexual selection into the field. In the species that I have worked with throughout this thesis – that is, the African *D. meigenii* – such field studies would have to start at a basic level. A research area, or station, would need to be established. An ideal location to start could be in Maputo in Mozambique, where large numbers of this species are often observed

(H. Feijen, pers. comm.). Initial studies should be observational. It would be useful to determine the details of the mating system. After this, it would be possible to conduct field manipulation experiments. For instance, females of different sizes could be collected, and presented with males of different sizes. It would thus be possible to test the variation in (and type and strength of) female preference (choosiness and the preference function; and selection) in the field, or even between sites. A study of this type would be feasible and would require very few resources. The flies would need to be captured, maintained, and observed in standard 500ml experimental pots. A brief acclimatisation phase would be required, but the observations would need to be made within a short space of time, to minimise chances of the imposed diets affecting the results of the observations.

Another example of a simple field experiment would be to dissect a sample of females, and to use a field microscope to count the number of sperm in their VRs. This could start to build a picture of the natural levels of variation in the reproductive costs of choice. It could also be extended to look at fluctuations over time, or across space – or both. Likewise, a simple (if somewhat cruel) test of the condition dependent handicap hypothesis would be to capture males with different size ornaments, and observe the time taken until death via starvation.

As a final field test, one could to switch to the Malaysian *Teleopsis dalmanni*. In a pilot experiment that I conducted in the forests in the Gombak valley in Malaysia (near Kuala Lumpur), I observed that larger eyespan females roosted with larger males, mated more often, and with more different males than the smaller eyespan females. These females also had larger egg stores. Thus, I found field evidence in

line with the idea that higher condition females exhibit stronger preferences, but also have lower choosiness than smaller females, due to their higher fecundity. Further studies on this line could confirm or contradict this idea with more power.

5.3.6 *Notes – to future students*

In addition to the work presented here, I have observed several points about *D. meigenii* that could be of interest to investigate. First, the males use their wings in male-male contests; and the wings, when held at a similar angle to that which the males hold them at, refract or reflect light in a dramatic red colour. Second, the male sperm are, for the most part, monomorphic. However, larger sperm similar to those described by Kotrba *et al.* (2016) – in the related, African stalk-eyed fly, *Diasemopsis comoroensis* – can also be seen. It could be useful to find out why.

5.3.7 *Final thoughts*

As was realised by Darwin (1859, 1871) intra-sexual selection is driven by female mate choice. These mate choices can arise in relation to direct or indirect genetic benefits. Over the last decades, a large amount of research has been conducted on male sexual ornamental traits. However, the effect of interactions between environmental and genetic condition on the expression of these ornaments, and on their relation to other traits are still areas of active research in which a lot is yet to be discovered and understood. Likewise, a lot is left to learn about female preference and its components. I would urge any reader to take up the challenge, and try to make a step forward in this difficult direction. In the future, we will, I hope, learn ever more about sexual selection, and stalk-eyed flies.

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APPENDIX 1 - A

CHAPTER 2. SUPPLEMENTARY MATERIAL

CHAPTER 2. SUPPLEMENTARY MATERIAL

Here, I present all GLM, GLMz, GLME tables and effect size estimates for full models, for the tests of mean effects. I also present the equivalent output for variance tests where used. The tables are split into 10 sections:

1) Two-size assay of female mate choices

A1) Effects of female mating status on components of preference and selection [GLM]

B1) Relationships between components of preference and selection [GLM]

C1) Effects of order and day, female morphology, fecundity female mating status and male size on female mating responses [GLME]

D1) Female mating status and fecundity [GLM]

2) Five-size assay of female mate choices

A2) Effects of mating status on components of preference and selection [GLM, GLMz]

B2) Relationships between components of preference and selection [GLM]

C2) Effects of day, week and female mating status on female mating responses [GLME]

D2) Female mating status and fecundity [GLM]

E2) Effect of female mating status on between-individual variance in components of preference and selection [Brown-Forsythe tests]

F2) Effect of female mating status on within-individual variation in female mating responses [GLME]

1) Two-size assay of female mate choices

A1) Effects of female mating status on components of preference and selection [GLM]

Here, I provide model output tables for GLMs on the effects of female mating status [virgin/mated] on components of preference and selection: rejection, preference slope, and selection.

A1.S1) Effect of female mating status on rejection [*R*]

Rejection [*R*] = Female Eyespan + Female Thorax + Female Eyespan * Female Thorax + SQRT Fecundity + Mating Status

Df Model = 5, Error = 85, Total = 90; $R^2 = 0.24$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Female Eyespan	1	1	0.0001188	0.0015	0.9689
Female Thorax	1	1	0.0186382	0.2393	0.626
Female Eyespan*Female Thorax	1	1	0.0019909	0.0256	0.8734
SQRT Fecundity	1	1	0.0247005	0.3171	0.5748
Mating Status	1	1	2.0132066	25.8467	<.0001

Term	Estimate	Std Error
Intercept	0.4747153	1.313208
Female Eyespan	0.1176523	0.267665
Female Thorax	-0.164142	0.340312
(Female Eyespan-6.14066)*(Female Thorax-3.71571)	-0.022373	1.842902
SQRT FEC	-0.129341	0.052037
Mating Status[Mated]	0.1680402	0.033053

A1.S2) Effect of female mating status on preference slope [P_1]

Preference Slope [P_1] = Female Eyespan + Female Thorax + Female Eyespan * Female Thorax + SQRT Fecundity + Mating Status

Df Model = 5, Error = 85, Total = 90; $R^2 = 0.05$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Female Eyespan	1	1	0.41991866	2.2885	0.134
Female Thorax	1	1	0.07061327	0.3848	0.5367
Female Eyespan*Female Thorax	1	1	0.0536761	0.2925	0.59
SQRT Fecundity	1	1	0.12290114	0.6698	0.4154
Mating Status	1	1	0.12326849	0.6718	0.4147

Term	Estimate	Std Error
Intercept	3.6265002	2.015554
Female Eyespan	-0.36437	0.410821
Female Thorax	-0.316101	0.522322
(Female Eyespan-6.14066)*(Female Thorax-3.71571)	1.0104286	2.828546
SQRT Fecundity	-0.085598	0.079869
Mating Status[Mated]	0.041581	0.050731

A1.S3) Effect of female mating status on selection [S_1]

Selection [S_1] = Female Eyespan + Female Thorax + Female Eyespan * Female Thorax + SQRT Fecundity + Mating Status

Df Model = 5, Error = 75, Total = 80; $R^2 = 0.14$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Female Eyespan	1	1	0.07985111	1.7415	0.191
Female Thorax	1	1	0.00159558	0.0348	0.8525
Female Eyespan*Female Thorax	1	1	0.02390734	0.5214	0.4725
SQRT Fecundity	1	1	0.00239893	0.0523	0.8197
Mating Status	1	1	0.43507413	9.4888	0.0029

Term	Estimate	Std Error
Intercept	2.1286153	1.051624
Female Eyespan	-0.150509	0.211509
Female Thorax	-0.142329	0.27526
(Female Eyespan-6.13864)*(Female Thorax-3.71716)	0.7158007	1.535426
SQRT Fecundity	-0.072465	0.044459
Mating Status[Mated]	0.0896067	0.029089

B1) Relationships between components of preference and selection [GLM]

Here, I provide model output for GLMs on the relationships between the components of preference and selection, and on the variation in these relationships across female mating status categories.

B1.S1) Relationship between rejection [*R*] and preference slope [P_1]

Preference Slope [P_1] = Rejection [*R*]

Df Model = 1, Error = 91, Total = 92; R^2 = < 0.01

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Rejection	1	1	0.03135006	0.1738	0.6777

Term	Estimate	Std Error
Intercept	0.1064732	0.073151
Rejection	0.0593102	0.142263

B1.S2) Relationship between rejection [*R*] and selection [S_1]

Selection [S_1] = Rejection [*R*]

Df Model = 1, Error = 81, Total = 82; R^2 = 0.22

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Rejection	1	1	0.85608804	22.1794	<.0001

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.4390835	0.036765	11.94	<.0001
Rejection	0.4129889	0.087693	4.71	<.0001

B1.S3) Relationship between preference slope [P_1] and selection [S_1]

Selection [S_1] = Preference Slope [P_1]

Df Model = 1, Error = 81, Total = 82; R^2 = 0.55

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Preference	1	1	2.1926128	99.2223	<.0001

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.5159075	0.017515	29.46	<.0001
Preference Slope	0.3860402	0.038755	9.96	<.0001

B1.S4) Relationship between rejection [*R*] and preference slope [P_1] – across female mating status

Preference Slope [P_1] = Rejection [*R*] + Mating Status + Rejection [*R*] * Mating Status

Df Model = 3, Error = 89, Total = 92; $R^2 = 0.01$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Rejection	1	1	0.03135006	0.1716	0.6797
Treatment	1	1	0.04961869	0.2717	0.6035
Mating Status*Rejection	1	1	0.10851102	0.5941	0.4429

Term	Estimate	Std Error
Intercept	0.1391352	0.084726
Rejection	0.0333956	0.157135
Mating Status[Mated]	0.02905	0.050251
Mating Status[Mated]*(Rejection-0.41057)	-0.121117	0.157135

B1.S5) Relationship between rejection [*R*], preference [P_1] and selection [S_1] – across female mating status

Selection [S_1] = Rejection [*R*] + Preference Slope [P_1] + Mating Status + Rejection [*R*] * Mating Status + Preference Slope [P_1] * Mating Status + Rejection [*R*] * Preference Slope [P_1] * Mating Status

Df Model = 7, Error = 75, Total = 82; $R^2 = 0.70$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Rejection	1	1	0.856088	53.9194	<.0001
Preference	1	1	1.6271909	102.4862	<.0001
Mating Status	1	1	0.0522044	3.288	0.0738
Mating Status*Rejection	1	1	0.1573899	9.913	0.0024
Mating Status*Preference Slope	1	1	0.0592055	3.729	0.0573
Rejection*Preference	1	1	0.0328949	2.0718	0.1542
Mating Status*Rejection*Pref Slope	1	1	0.0067883	0.4276	0.5152

Term	Estimate	Std Error
Intercept	0.4363409	0.027443
Rejection	0.2553123	0.061936
Preference Slope	0.2879414	0.04365
Mating Status[Mated]	0.0259001	0.016243
Mating Status[Mated]*(Rejection-0.33956)	0.2079025	0.061936
Mating Status[Mated]*(Pref Slope-0.16426)	-0.071484	0.04365
(Rejection-0.33956)*(Pref Slope-0.16426)	0.2485729	0.177709
Mating Status[Mated]*(*R*-0.33956)*(P_1-0.16426)	-0.116199	0.177709

C1) Effect of order and day, female morphology, fecundity, female mating status and male size on female mating responses [GLMM]

Here, I provide output for GLME model comparisons used to assess the significance of terms in the GLMEs. I show likelihood ratio tests in all cases. Where relevant, I also show bootstrap comparisons as well as estimates for overdispersion and R^2GLME. I also show Tukey Contrasts where relevant.

C1.S1) Effects of order of male presentation and experimental day on female mating responses

Mating Response = Presentation Order [p] * Experimental Day [d] + Female ID & Random [R] [[with binomial error + logit link]]

Interaction [p*d]

```
pdm2: y ~ p + d + (1 | R)
pdm1: y ~ p * d + (1 | R)
     Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
pdm2  5 644.06 665.17 -317.03   634.06
pdm1  7 645.88 675.44 -315.94   631.88 2.1765      2     0.3368
```

Presentation order

```
pdm3a: y ~ d + (1 | R)
pdm2: y ~ p + d + (1 | R)
      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
pdm3a  4 643.68 660.57 -317.84   635.68
pdm2   5 644.06 665.17 -317.03   634.06 1.6219      1     0.2028
```

Experimental day

```
pdm3b: y ~ p + (1 | R)
pdm2: y ~ p + d + (1 | R)
      Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
pdm3b  3 643.79 656.46 -318.90   637.79
pdm2   5 644.06 665.17 -317.03   634.06 3.7359      2     0.1544
```

C1.S2) Effects of female eyespan and thorax on female mating responses

Mating Response = Female Eyespan [ES] + Female Thorax [T] + Female ID & Random [R] [[with binomial error + logit link]]

Eyespan

```
STm2a: y ~ ES + (1 | R)
ESTm1: y ~ ES + T + (1 | R)
       Df    AIC    BIC  logLik deviance Chisq Chi Df Pr(>Chisq)
ESTm2a  3 645.35 658.01 -319.67   639.35
ESTm1   4 647.35 664.24 -319.67   639.35 2e-04      1     0.9876
```

Thorax

```
ESTm2b: y ~ T + (1 | R)
ESTm1: y ~ ES + T + (1 | R)
       Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
ESTm2b  3 645.41 658.08 -319.70   639.41
ESTm1   4 647.35 664.24 -319.67   639.35 0.0614      1     0.8043
```

C1.S3) Effects of fecundity on female mating responses

Mating Response = Fecundity [F] + Female ID & Random [R] [[with binomial error + logit link]]

Fecundity

```
Fm2: y ~ (1 | R)
Fm1: y ~ F + (1 | R)
    Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
Fm2  2 643.45 651.90 -319.73   639.45
Fm1  3 645.27 657.94 -319.64   639.27 0.1758      1      0.675
```

C1.S4) Full Model for female mating status and male size on female mating responses

Mating Response = Mating Status [z] + Male Size [x] + Mating Status*Male Size + Female ID & Random [R] [[with binomial error + logit link + estimation = Gauss-Hermite Quadrature, nAGQ optimization = 4]]

Interaction [x * z]

Likelihood ratio test [LR test – contrast with bootstrap below]

```
fm5: y ~ x + z + (1 | R)
fm4: y ~ x * z + (1 | R)
    Df    AIC    BIC  logLik deviance Chisq Chi Df Pr(>Chisq)
fm5  4 621.24 638.13 -306.62   613.24
fm4  5 623.13 644.24 -306.56   613.13 0.112      1     0.7379
```

```
parametric bootstrap test; samples: 10000
fm4 : y ~ x * z + (1 | R)
fm5 : y ~ x + z + (1 | R)
        stat df p.value
LRT    0.112  1  0.7379
PBtest 0.112     0.7399 ##LR p-value is reliable, as expected
```

Female mating status [z]

```
fm6a: y ~ x + (1 | R)
fm5: y ~ x + z + (1 | R)
     Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm6a  3 634.98 647.65 -314.49   628.98
fm5   4 621.24 638.13 -306.62   613.24 15.742      1  7.258e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Male size [x]

```
m6b: y ~ z + (1 | R)
fm5: y ~ x + z + (1 | R)
     Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm6b  3 629.45 642.12 -311.72   623.45
fm5   4 621.24 638.13 -306.62   613.24 10.209      1   0.001398 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

```
R.SQUARED.GLME

Model with interaction [fm4]
     R2m       R2c
0.1078500 0.3472212

Model without interaction [fm5]
      R2m       R2c
0.1077807 0.3460834


OVERDISPERSION

Model with interaction [fm4]
      chisq       ratio         rdf           p
379.3714041   0.7602633 499.0000000   0.9999810

Model without interaction [fm5]
      chisq       ratio         rdf           p
379.3714041   0.7602633 499.0000000   0.9999810
```

Full Model output for female mating status and male size on female mating responses

1] Full Model [With Interaction [fm4]] y ~ x * z + (1 | R)

```
Generalized linear mixed model fit by maximum likelihood (Adaptive
  Gauss-Hermite Quadrature, nAGQ = 4) [glmerMod]
 Family: binomial  ( logit )
Formula: y ~ x * z + (1 | R)
   Data: data1

     AIC      BIC   logLik deviance df.resid
   623.1    644.2   -306.6    613.1      499

Scaled residuals
    Min      1Q  Median      3Q     Max
-2.2649 -0.7302  0.4386  0.6269  2.0416
```

```
Random effects
 Groups Name        Variance Std.Dev.
 R      (Intercept) 1.349    1.161
Number of obs: 504, groups:  R, 92

Fixed effects
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   0.6179     0.2459   2.513 0.011987 *
x1            0.6254     0.2747   2.277 0.022792 *
z1           -1.4278     0.4105  -3.478 0.000505 ***
x1:z1         0.1473     0.4405   0.334 0.738144
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

2] Minimal Model [without interaction [fm5] y ~ x + z + (1 | R)

```
Generalized linear mixed model fit by maximum likelihood (Adaptive
  Gauss-Hermite Quadrature, nAGQ = 4) [glmerMod]
 Family: binomial  ( logit )
Formula: y ~ x + z + (1 | R)
   Data: data1

     AIC      BIC   logLik deviance df.resid
   621.2    638.1   -306.6    613.2      500

Scaled residuals
    Min      1Q  Median      3Q     Max
-2.2997 -0.7182  0.4348  0.6314  1.9975



Random effects
 Groups Name        Variance Std.Dev.
 R      (Intercept) 1.34     1.157
Number of obs: 504, groups:  R, 92

Fixed effects
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   0.5904     0.2310   2.555  0.01061 *
x1            0.6819     0.2170   3.143  0.00167 **
z1           -1.3519     0.3405  -3.971 7.16e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of fixed effects
   (Intr) x1
x1 -0.416
z1 -0.547 -0.067

Correlation of fixed effects
      (Intr) x1     z1
x1    -0.517
z1    -0.614  0.296
x1:z1  0.335 -0.612 -0.556
```

D1) Female mating status and fecundity (GLM)

Here, I provide model output tables for GLMs on fecundity. Fecundity was calculated as the number of eggs laid per day by a female. Fecundity was assayed in order to confirm that the mated females really had mated, and thus laid more eggs while the virgins laid fewer. For the 2-size assays, the estimates were derived from a minimum of 3 counts over 11 days, and a maximum of 5 counts over 18 days. To test for an effect of female mating status, a Wilcoxon/Kruskal Wallis test was used. As female size can influence female fecundity, a GLM was then fitted to SQRT fecundity. The transformation was used to account for the Poisson distribution associated with the count data. The GLM included as fixed effects: female eyespan, female thorax, female eyespan x thorax interaction, and female mating status. As expected, virgin females had far lower fecundity.

D1.S1) Effect of female mating status on fecundity [Wilcoxon/Kruskal Wallis]

Wilcoxon/Kruskal-Wallis test: $z = 3.62$, $p < 0.001$

D1.S2) Effect of female mating status on fecundity [GLM]

SQRT Fecundity [*F*] = Female Eyespan + Female Thorax + Female Eyespan * Female Thorax + Mating Status

Df Model = 4, Error = 86, Total = 90; $R^2 = 0.20$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Female Eyespan	1	1	0.9335665	2.7912	0.0984
Female Thorax	1	1	0.0023445	0.007	0.9335
Female Eyespan*Female Thorax	1	1	1.4084362	4.211	0.0432
Mating Status	1	1	5.0590879	15.1258	0.0002

Term	Estimate	Std Error
Intercept	4.0736112	2.68556
Female Eyespan	-0.415903	0.552842
Female Thorax	-0.110639	0.705099
(Female Eyespan-6.14066)*(Female Thorax-3.71571)	-6.68088	3.75032
Mating Status[Mated]	0.2456536	0.063163

2) Five-size assay of female mate choices

A2) Effects of female mating status on components of preference and selection [GLM/GLMz]

Here, I provide model output tables for GLM and GLMz on the effects of female mating status [virgin/mated] on the components of preference and selection: rejection, preference slope, and selection.

A2.S1) Effect of female mating status on rejection [R]

Rejection [R] = Female Eyespan + Female Thorax + Female Eyespan * Female Thorax + SQRT Fecundity + Mating Status

Df Model = 5, Error =199, Total = 205; $R^2 = 0.25$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Female ES	1	1	0.0077547	0.2897	0.591
Female T	1	1	0.109353	4.0847	0.0446
Female ES*Female T	1	1	0.0390301	1.4579	0.2287
SQRT Fecundity	1	1	0.2918293	10.9008	0.0011
Mating Status	1	1	1.355257	50.6236	<.0001

Term	Estimate	Std Error
Intercept	0.1493192	0.520936
Female ES	-0.065932	0.090043
Female T	0.1981955	0.108554
(Female ES-6.11698)*(Female T-3.63307)	-1.049102	0.65656
SQRT Fecundity	-0.011487	0.018614
Mating Status[Mated]	0.0937211	0.013172

A2.S2) Effect of female mating status on preference slope [P_2]

Preference Slope [P_2] = Female Eyespan + Female Thorax + Female Eyespan * Female Thorax + SQRT Fecundity + Mating Status

Df Model = 5, Error = 174, Total = 179; $R^2 = 0.03$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Female ES	1	1	0.128569	1.1704	0.2808
Female T	1	1	0.16650395	1.5158	0.2199
Female ES*Female T	1	1	0.26417084	2.4049	0.1228
SQRT Fecundity	1	1	0.00112084	0.0102	0.9197
Mating Status	1	1	0.09625031	0.8762	0.3505

Term	Estimate	Std Error
Intercept	-0.479324	1.115962
Female ES	0.2641125	0.200618
Female T	-0.254824	0.24274
(Female ES-6.11767)*(Female T-3.63539)	2.1366064	1.426834
SQRT Fecundity	-0.021788	0.040551
Mating Status[Mated]	0.0265206	0.028332

A2.S3) Effect of female mating status on selection [*SD*] [GLMz]

Selection [*SD*] = Female Eyespan + Female Thorax + Female Eyespan * Female Thorax + SQRT Fecundity + Mating Status [[Exponential Error, Reciprocal Link]]

Df Model = 5, Error = 126, Total = 131

Source	DF	L-R ChiSquare	Prob>ChiSq
Female ES	1	0.0732039	0.7867
Female T	1	0.3297447	0.5658
Female ES*Female T	1	0.1038695	0.7472
SQRT Fecundity	1	0.5522125	0.4574
Mating Status	1	7.1567041	0.0075

Term	Estimate	Std Error
Intercept	11.380143	23.124235
Female ES	1.0871437	4.01835
Female T	-2.836935	4.9433723
(Female ES-6.11729)*(Female T-3.63394)	9.7199411	30.767175
SQRT Fecundity	-0.608764	0.8176125
Mating Status[Mated]	-1.654069	0.644622

[Note LS GLM = Df 5, 196, 201; $F_{1,196} = 5.43$, $p = 0.021$ [mate status]; $R^2 = 0.05$]

B2) Relationships between components of preference and selection [GLM]

Here, I provide model output for GLMs on the pairwise relationships between the components of preference and selection, and on the variation in these relationships across female mating status categories.

B2.S1) Relationship between rejection [*R*] and preference slope [P_2]

Preference Slope [P_2] = Rejection [*R*]

Df Model = 1, Error = 177, Total = 178; $R^2 < 0.1$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Rejection	1	1	0.11561527	1.0387	0.3095

Term	Estimate	Std Error
Intercept	0.2594969	0.06577
Rejection	-0.140163	0.137527

B2.S2) Relationship between rejection [*R*] and selection [*SD*]

Selection [*SD*] = Rejection [*R*]

Df Model = 1, Error = 201, Total = 202; $R^2 = 0.05$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Rejection	1	1	0.33184682	11.937	0.0007

Term	Estimate	Std Error
Intercept	-0.03117	0.030557
Rejection	0.2207816	0.063902

B2.S3) Relationship between preference slope [P_1] and selection [*SD*]

Selection [*SD*] = Preference Slope [P_2]

Df Model = 1, Error = 177, Total = 178; $R^2 = 0.51$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Preference	1	1	2.7086151	203.2028	<.0001

Term	Estimate	Std Error
Intercept	0.0134356	0.010063
Preference	0.3707475	0.026008

B2.S4) Relationship between rejection [*R*] and preference slope [P_2] – across female mating status

Preference Slope [P_2] = Rejection [*R*] + Mating Status + Rejection [*R*] * Mating Status

Df Model = 3, Error = 174, Total = 177; $R^2 = 0.03$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Rejection	1	1	0.11561527	1.0488	0.3072
Mating Status	1	1	0.27534636	2.4978	0.1158
Mating Status*Rejection	1	1	0.1338403	1.2141	0.272

Term	Estimate	Std Error
Intercept	0.3046472	0.075616
Rejection	-0.277882	0.159358
Mating Status[Mated]	0.0464224	0.028943
Mating Status[Mated]*(Rejection-0.44232)	0.1755928	0.159358

B2.S5) Relationship between rejection [*R*], preference slope [P_2] and selection [*SD*] – across female mating status

Selection [*SD*] = Rejection [*R*] + Preference Slope [P_2] + Mating Status + Rejection [*R*] * Mating Status + Preference Slope [P_2] * Mating Status + Rejection [*R*] * Preference Slope [P_2] * Mating Status

Df Model = 7, Error = 170, Total = 177; $R^2 = 0.89$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Rejection	1	1	0.4890835	156.1817	<.0001
Preference Slope	1	1	2.90485	927.6218	<.0001
Mating Status	1	1	0.0037282	1.1906	0.2768
Mating Status*Rejection	1	1	0.0323728	10.3378	0.0016
Mating Status*Preference Slope	1	1	0.3602299	115.0342	<.0001
Rejection*Preference Slope	1	1	0.7132492	227.7658	<.0001
Mating Status*Rejection*Pref Slope	1	1	0.0187579	5.9901	0.0154

Term	Estimate	Std Error
Intercept	-0.13527	0.013822
Rejection	0.3000577	0.027514
Preference Slope	0.4558544	0.017105
Mating Status[Mated]	0.0020518	0.005037
Mating Status[Mated]*(Rejection-0.44232)	0.0507741	0.027514
Mating Status[Mated]*(Preference-0.1975)	-0.006863	0.017105
(Rejection-0.44232)*(Preference Slope-0.1975)	1.3421336	0.087984
Mating Status[Mated]*(Rejection-0.44232)*(Pref Slope-0.1975)	0.2153378	0.087984

C2) Effect of day, week, and female mate status on female mating responses [GLME]

Here, I provide output for GLME model comparisons used to assess the significance of terms in the GLMEs. I show likelihood ratio tests in all cases. Where relevant, I also show bootstrap comparisons as well as estimates for overdispersion and R^2GLME. We also show Tukey Contrasts where relevant.

C2.S1) Effects of experimental day and experimental week on female mating responses

Mating Response = Experimental Day [d] * Experimental Week [w] + Female ID & Random [R] [[with binomial error + logit link]]

Interaction [d*w]

```
fm2: y ~ d + w + (1 | R)
fm1: y ~ d * w + (1 | R)
    Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm2  8 3665.4 3712.7 -1824.7   3649.4
fm1 16 3624.6 3719.2 -1796.3   3592.6 56.739      8  2.025e-09 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Experimental day [d]

```
fm3a: y ~ w + (1 | R)
fm2: y ~ d + w + (1 | R)
     Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm3a  4 3681.0 3704.6 -1836.5   3673.0
fm2   8 3665.4 3712.7 -1824.7   3649.4 23.574      4   9.72e-05 ***
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Experimental week [w]

```
fm3: y ~ d + (1 | R)
fm2: y ~ d + w + (1 | R)
    Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm3  6 3668.0 3703.5 -1828.0   3656.0
fm2  8 3665.4 3712.7 -1824.7   3649.4 6.6665      2    0.03568 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TUKEY CONTRAST for FULL Model: y ~ d * w + (1 | R)

Day

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

```
Fit: glmer(formula = y ~ d * w + (1 | R), data = data1, family =
binomial)

Linear Hypotheses:
                          Estimate Std. Error z value Pr(>|z|)
Monday - Friday == 0      -1.13564    0.21978  -5.167  < 0.001 ***
Thursday - Friday == 0    -0.04069    0.21539  -0.189  0.99972
Tuesday - Friday == 0     -0.30609    0.21445  -1.427  0.60988
Wednesday - Friday == 0    0.01549    0.21674   0.071  0.99999
Thursday - Monday == 0     1.09495    0.21712   5.043  < 0.001 ***
Tuesday - Monday == 0      0.82955    0.21611   3.838  0.00115 **
Wednesday - Monday == 0    1.15113    0.21862   5.266  < 0.001 ***
Tuesday - Thursday == 0   -0.26541    0.21188  -1.253  0.72025
Wednesday - Thursday == 0  0.05618    0.21424   0.262  0.99896
Wednesday - Tuesday == 0   0.32159    0.21330   1.508  0.55732
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)
```

Week [w]

Simultaneous Tests for General Linear Hypotheses

Multiple Comparisons of Means: Tukey Contrasts

```
Fit: glmer(formula = y ~ d * w + (1 | R), data = data1, family =
binomial)

Linear Hypotheses:
           Estimate Std. Error z value Pr(>|z|)
2 - 1 == 0 -0.25364    0.21909  -1.158    0.478
3 - 1 == 0 -0.06943    0.23915  -0.290    0.955
3 - 2 == 0  0.18421    0.23977   0.768    0.722
(Adjusted p values reported -- single-step method)
```

Full Model Output: y ~ d * w + (1 | R)

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: y ~ d * w + (1 | R)
   Data: data1

     AIC      BIC   logLik deviance df.resid
  3624.6   3719.2  -1796.3   3592.6     2710
```

```
Scaled residuals
    Min      1Q  Median      3Q     Max
-2.0741 -0.9637  0.5894  0.8110  1.7883

Random effects
 Groups Name        Variance Std.Dev.
 R      (Intercept) 0.3739   0.6115
Number of obs: 2726, groups:  R, 209

Fixed effects
              Estimate Std. Error z value Pr(>|z|)
(Intercept)    0.53391    0.15995   3.338 0.000844 ***
dMonday       -1.13564    0.21978  -5.167 2.38e-07 ***
dThursday     -0.04069    0.21539  -0.189 0.850169
dTuesday      -0.30609    0.21445  -1.427 0.153485
dWednesday     0.01549    0.21674   0.071 0.943015
w2            -0.25364    0.21909  -1.158 0.246996
w3            -0.06943    0.23915  -0.290 0.771571
dMonday:w2     0.53698    0.31425   1.709 0.087492 .
dThursday:w2   0.14712    0.30679   0.480 0.631556
dTuesday:w2    0.34116    0.30540   1.117 0.263944
dWednesday:w2 -0.29717    0.30495  -0.974 0.329818
dMonday:w3     1.36343    0.33087   4.121 3.78e-05 ***
dThursday:w3  -0.52939    0.32579  -1.625 0.104176
dTuesday:w3   -0.10307    0.32169  -0.320 0.748652
dWednesday:w3  0.44674    0.34044   1.312 0.189434
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

C2.S2) Effects of female eyespan and thorax on female mating responses

Mating Response = Female Eyespan [ES] + Female Thorax [T] + Female ID & Random [R] [[with binomial error + logit link]]

Interaction [ES*T]

```
Data: data
Models:
fm2: y ~ ES + T + (1 | R)
fm1: y ~ ES * T + (1 | R)
    Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm2  4 3680.2 3703.8 -1836.1   3672.2
fm1  5 3681.9 3711.4 -1835.9   3671.9 0.3169      1     0.5735
```

Female eyespan [ES]

```
Data: data
Models:
fm3a: y ~ T + (1 | R)
fm2: y ~ ES + T + (1 | R)
     Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm3a  3 3678.6 3696.4 -1836.3   3672.6
fm2   4 3680.2 3703.8 -1836.1   3672.2 0.4399      1     0.5072
```

Female thorax [T]

```
fm3b: y ~ ES + (1 | R)
fm2: y ~ ES + T + (1 | R)
     Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm3b  3 3684.2 3701.9 -1839.1   3678.2
fm2   4 3680.2 3703.8 -1836.1   3672.2 6.0074      1    0.01425 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

C2.S3) Effects of fecundity on female mating responses

Mating Response = Fecundity [F] + Female ID & Random [R] [[with binomial error + logit link]]

```
fm2: y ~ (1 | R)
fm1: y ~ f + (1 | R)
    Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm2  2 3682.4 3694.2 -1839.2   3678.4
fm1  3 3676.6 3694.3 -1835.3   3670.6 7.7991      1   0.005227 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

C2.S4) Effects of thorax and fecundity on female mating responses

Mating Response = Female Thorax [T] + Fecundity [F] + Female Thorax [T]*Fecundity[F] + Female ID & Random [R] [[with binomial error + logit link]]

Interaction [T*F]

```
fm2: y ~ T + f + (1 | R)
fm1: y ~ T * f + (1 | R)
    Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm2  4 3674.2 3697.8 -1833.1   3666.2
fm1  5 3672.5 3702.0 -1831.2   3662.5 3.6704      1    0.05539 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

C2.S5) Maximal model for female mating status and male size

Mating Response = Mating Status [z] + Male Size [x] + Mating Status*Male Size + Female Thorax [T] + Female Fecundity [F] + Experimental Day / Experimental Week / Female ID & Random [R] [[with binomial error + logit link + estimation = Laplace Approximation, nAGQ = 1]]

Remove female fecundity [F]

```
fm2: y ~ x + z + T + (1 | d/w/R) + x:z
fm1: y ~ x * z + T + f + (1 | d/w/R)
    Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm2 14 3597.5 3680.3 -1784.8   3569.5
fm1 15 3598.3 3687.0 -1784.2   3568.3 1.1624      1      0.281
```

C2.S6) Full Model: for female mating status and male size on female mating responses

Mating Response = Mating Status [z] + Male Size [x] + Mating Status*Male Size + Female Thorax [T] + Experimental Day / Experimental Week / Female ID & Random [R] [with binomial error + logit link + estimation = Laplace Approximation, nAGQ = 1]

Interaction [z*x]

```
fm3: y ~ x + z + T + (1 | d/w/R)
fm2: y ~ x + z + T + (1 | d/w/R) + x:z
    Df    AIC    BIC  logLik deviance Chisq Chi Df Pr(>Chisq)
fm3 10 3592.4 3651.5 -1786.2   3572.4
fm2 14 3597.5 3680.3 -1784.8   3569.5  2.93      4     0.5696
Bootstrap: at 1000 repetitions


         stat df p.value
LRT    3.0034  4  0.5696
PBtest 3.0034     0.4770   # basic p-value is reliable
```

Mating status [z]

```
fm4: y ~ x + T + (1 | d/w/R)
fm3: y ~ x + z + T + (1 | d/w/R)
    Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm4  9 3679.8 3733.0 -1830.9   3661.8
fm3 10 3592.4 3651.5 -1786.2   3572.4 89.329      1  < 2.2e-16 ***
```

Male size [x]

```
fm5: y ~ z + T + (1 | d/w/R)
fm3: y ~ x + z + T + (1 | d/w/R)
    Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
fm5  6 3610.3 3645.7 -1799.1   3598.3
fm3 10 3592.4 3651.5 -1786.2   3572.4 25.835      4  3.417e-05 ***
```

Female thorax [T]

```
fm6: y ~ x + z + (1 | d/w/R)
fm3: y ~ x + z + T + (1 | d/w/R)
    Df    AIC    BIC  logLik deviance Chisq Chi Df Pr(>Chisq)
fm6  9 3597.7 3650.9 -1789.8   3579.7
fm3 10 3592.4 3651.5 -1786.2   3572.4 7.263      1   0.007039 **
```

```
R.SQUARED.GLME

Model with interaction [fm2]
      R2m       R2c
0.0546907 0.0832067

Model without interaction [fm3]
       R2m        R2c
0.05343711 0.08179542
```

```
OVERDISPERSION

Model with interaction [fm2]
       chisq        ratio          rdf            p
2697.9927070    0.9944684 2713.0000000    0.5773253

Model without interaction [fm3]
       chisq        ratio          rdf            p
2697.6334255    0.9928721 2717.0000000    0.6003671


Full Model Output: y ~ x + z + T + (1 | d/w/R) + x:z [fm2]

Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: y ~ x + z + T + (1 | d/w/R) + x:z
   Data: data1

     AIC      BIC   logLik deviance df.resid
  3597.5   3680.3  -1784.8   3569.5     2712

Scaled residuals
    Min      1Q  Median      3Q     Max
-2.0552 -0.9862  0.6000  0.8517  1.7464


Random effects
 Groups  Name        Variance  Std.Dev.
 R:(w:d) (Intercept) 7.450e-09 8.632e-05
 w:d     (Intercept) 1.037e-01 3.220e-01
 d       (Intercept) 7.379e-09 8.590e-05
Number of obs: 2726, groups:  R:(w:d), 2726; w:d, 15; d, 5


Fixed effects
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  3.85892    1.20918   3.191  0.00142 **
xB          -0.40020    0.18182  -2.201  0.02773 *
xC          -0.12218    0.17887  -0.683  0.49458
xD           0.30126    0.18413   1.636  0.10182
xE           0.04314    0.18024   0.239  0.81082
z1          -1.00135    0.17914  -5.590 2.27e-08 ***
T           -0.88298    0.33035  -2.673  0.00752 **
xB:z1        0.42401    0.25778   1.645  0.10000
xC:z1        0.29252    0.24989   1.171  0.24176
xD:z1        0.26757    0.25559   1.047  0.29516
xE:z1        0.27353    0.25113   1.089  0.27607
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1


Correlation of Fixed Effects
      (Intr) xB     xC     xD     xE     z1     T      xB:z1  xC:z1
xB    -0.085
xC    -0.085  0.504
xD    -0.078  0.487  0.506
xE    -0.094  0.499  0.521  0.501
z1    -0.046  0.494  0.501  0.487  0.497
T     -0.992  0.011  0.008  0.004  0.018 -0.029
xB:z1  0.046 -0.697 -0.344 -0.335 -0.342 -0.695  0.006
xC:z1  0.045 -0.356 -0.702 -0.351 -0.359 -0.716  0.009  0.498
xD:z1  0.051 -0.346 -0.349 -0.710 -0.346 -0.699  0.001  0.485  0.500
xE:z1  0.064 -0.353 -0.358 -0.348 -0.702 -0.712 -0.011  0.495  0.510
```

Full Model 2 – without Interaction [fm3] `y ~ x + z + T + (1 | d/w/R)`

```
Generalized linear mixed model fit by maximum likelihood (Laplace
  Approximation) [glmerMod]
 Family: binomial  ( logit )
Formula: y ~ x + z + T + (1 | d/w/R)
   Data: data1

     AIC      BIC   logLik deviance df.resid
  3592.4   3651.5  -1786.2   3572.4     2716

Scaled residuals
    Min      1Q  Median      3Q     Max
-1.9926 -0.9834  0.6040  0.8525  1.7914

Random effects
 Groups  Name        Variance  Std.Dev.
 R:(w:d) (Intercept) 6.333e-09 7.958e-05
 w:d     (Intercept) 1.031e-01 3.210e-01
 d       (Intercept) 0.000e+00 0.000e+00
Number of obs: 2726, groups:  R:(w:d), 2726; w:d, 15; d, 5

Fixed effects
            Estimate Std. Error z value Pr(>|z|)
(Intercept)  3.74889    1.20641   3.107 0.001887 **
xB          -0.19126    0.13005  -1.471 0.141390
xC           0.02313    0.12673   0.183 0.855186
xD           0.43482    0.12884   3.375 0.000739 ***
xE           0.17924    0.12769   1.404 0.160391
z1          -0.75238    0.08033  -9.366  < 2e-16 ***
T           -0.88688    0.33031  -2.685 0.007253 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Correlation of fixed effects
   (Intr) xB     xC     xD     xE     z1
xB -0.072
xC -0.074  0.495
xD -0.058  0.484  0.514
xE -0.069  0.490  0.523  0.512
z1  0.027  0.007  0.000 -0.015 -0.003
T  -0.994  0.021  0.020  0.006  0.016 -0.061
```

Tukey Contrasts for y ~ x:

```
Fit: glmer(formula = y ~ x + T + (1 | d/w/R), data = data1, family =
binomial, nAGQ = 1)

Linear hypotheses
           Estimate Std. Error z value Pr(>|z|)
B - A == 0  -0.1886     0.1278  -1.476  0.57829
C - A == 0   0.0235     0.1245   0.189  0.99972
D - A == 0   0.4307     0.1268   3.397  0.00607 **
E - A == 0   0.1816     0.1254   1.448  0.59642
C - B == 0   0.2121     0.1268   1.673  0.45060
D - B == 0   0.6193     0.1294   4.786  < 0.001 ***
E - B == 0   0.3702     0.1279   2.895  0.03103 *
D - C == 0   0.4072     0.1240   3.284  0.00912 **
E - C == 0   0.1581     0.1221   1.295  0.69412
E - D == 0  -0.2491     0.1247  -1.998  0.26689
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

TUKEY CONTRASTS FOR VIRGINS – Mate size on female mating response

```
Simultaneous tests for general linear hypotheses

Multiple comparisons of means: Tukey contrasts


Fit: glmer(formula = y1 ~ x1 + T1 + (1 | d1/w1/R1), data =
dataVIRGIN,
    family = binomial, nAGQ = 1)

Linear hypotheses
           Estimate Std. Error z value Pr(>|z|)
B - A == 0 -0.37892    0.18315  -2.069  0.23352
C - A == 0 -0.10478    0.18009  -0.582  0.97778
D - A == 0  0.31055    0.18488   1.680  0.44632
E - A == 0  0.07903    0.18139   0.436  0.99252
C - B == 0  0.27414    0.18114   1.513  0.55362
D - B == 0  0.68946    0.18672   3.692  0.00204 **
E - B == 0  0.45794    0.18296   2.503  0.08982 .
D - C == 0  0.41533    0.18044   2.302  0.14411
E - C == 0  0.18381    0.17567   1.046  0.83365
E - D == 0 -0.23152    0.18202  -1.272  0.70845
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)
```

TUKEY CONTRASTS FOR MATED – Mate size on female mating response

```
Simultaneous tests for general linear hypotheses

Multiple comparisons of means: Tukey Contrasts


Fit: glmer(formula = y2 ~ x2 + T2 + (1 | d2/w2/R2), data = dataMATED,
    family = binomial, nAGQ = 1)

Linear Hypotheses:
            Estimate Std. Error z value Pr(>|z|)
B - A == 0 -0.002906   0.187674  -0.015   1.0000
C - A == 0  0.158245   0.180469   0.877   0.9054
D - A == 0  0.560635   0.182819   3.067   0.0184 *
E - A == 0  0.300883   0.181686   1.656   0.4613
C - B == 0  0.161151   0.183974   0.876   0.9057
D - B == 0  0.563541   0.186999   3.014   0.0216 *
E - B == 0  0.303790   0.185526   1.637   0.4731
D - C == 0  0.402390   0.177781   2.263   0.1567
E - C == 0  0.142638   0.176057   0.810   0.9276
E - D == 0 -0.259752   0.177787  -1.461   0.5878
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
(Adjusted p values reported -- single-step method)
```

D2) Female mating status and fecundity

Here, I provide model output tables for GLMs on fecundity. Fecundity was calculated as the number of eggs laid per day by a female. Fecundity was assayed in order to confirm that the mated females really had mated, and thus laid more eggs while the virgins laid fewer. For the 5-size assays, the estimates were derived from a minimum of 6 counts over 21 days, and a maximum of 9 counts over 30 days. To test for an effect of female mating status, a two-tailed t-test was used for SQRT fecundity. As female size can influence female fecundity, a GLM was then fitted to SQRT fecundity. The transformation was used to account for the Poisson distribution associated with the count data. The GLM included as fixed effects: female eyespan, female thorax, eyespan x thorax interaction, and female mating status. Virgin females had far lower fecundity.

D2.S1) Effect of female mating status on fecundity [Wilcoxon/Kruskal Wallis]

Two-tailed t-test: $T = -8.04$, $df = 202.7$, $p < 0.001$, power = 1.0

D2.S2) Effect of female mating status on fecundity [GLM]

SQRT Fecundity [*F*] = Female Eyespan + Female Thorax + Female Eyespan * Female Thorax + Mating Status

Df Model = 4, Error = 199, Total = 203; $R^2 = 0.25$

Source	Nparm	DF	Seq SS	F Ratio	Prob > F
Female ES	1	1	0.520513	1.3372	0.2489
Female T	1	1	0.358056	0.9199	0.3387
Female ES*Female T	1	1	0.140911	0.362	0.5481
Mating Status	1	1	23.961805	61.5583	<.0001

Term	Estimate	Std Error
Intercept	-0.263293	1.987618
Female ES	0.0874241	0.344086
Female T	0.2984247	0.414231
(Female ES-6.11739)*(Female T-3.63266)	-2.307612	2.501237
Mating Status[Mated]	0.3449423	0.043965

E2) Effect of female mating status on between-individual variance in components of preference and selection

E2.S1) Effect of female mating status on between-individual variance in rejection [$\boldsymbol{R}$]

Brown-Forsythe test: $F_{1,201} = 1.079$, $p = 0.300$

E2.S2) Effect of female mating status on between-individual variance in preference slope [$\boldsymbol{P_2}$]

Brown-Forsythe test: $F_{1,176} = 5.382$, $p = 0.022$

E2.S3) Effect of female mating status on between-individual variance in selection [$\boldsymbol{SD}$]

Brown-Forsythe test: $F_{1,200} = 13.34$, $p < 0.001$

F2) Effect of female mating status on within-individual variation in female mating responses [GLME]

Here, I provide output for GLME model comparisons used to assess the significance of terms in the GLMEs. I show likelihood ratio tests in all cases. Where relevant, I also show overdispersion, R^2GLME and Tukey Contrasts.

F2.S1) Full Model for within-female mating response variation

Mating Response = Mating Status [z] + Male Size [x] + Experimental Week [w] + Mating Status*Male Size + Mating Status*Experimental Week + Male Size*Experimental Week + Mating Status*Male Size*Experimental Week + Female ID & Random [R] [[with binomial error + logit link + est = Gauss-Hermite Quadrature, nAGQ = 10]]

Three-way interaction [z*x*w]

```
varis2: y ~ z + x + w + (1 | R) + z:x + z:w + x:w
varis1: y ~ z * x * w + (1 | R)
       Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
varis2 23 3594.6 3730.5 -1774.3   3548.6
varis1 31 3594.3 3777.5 -1766.2   3532.3 16.288      8    0.03845 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Two-way interaction [x*w] [male size x week]

```
varis2a: y ~ z + x + w + (1 | R) + z:x + z:w
varis2: y ~ z + x + w + (1 | R) + z:x + z:w + x:w
        Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
varis2a 15 3610.7 3699.4 -1790.3   3580.7
varis2   23 3594.6 3730.5 -1774.3    3548.6 32.095       8   8.957e-05
***
```

Two-way interaction [z*w] [mate status x week]

```
varis2b: y ~ z + x + w + (1 | R) + z:x + x:w
varis2: y ~ z + x + w + (1 | R) + z:x + z:w + x:w
        Df    AIC    BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
varis2b 21 3590.9 3715.0 -1774.4   3548.9
varis2  23 3594.6 3730.5 -1774.3   3548.6 0.2845      2     0.8674
```

```
OVERDISPERSION

Full Model
       chisq        ratio          rdf            p
2543.3887184    0.9437435 2695.0000000    0.9819591
```

Full Model 1 – with 3-way interaction y ~ z * x * w + (1 | R)

```
Generalized linear mixed model fit by maximum likelihood (Adaptive
  Gauss-Hermite Quadrature, nAGQ = 10) [glmerMod]
 Family: binomial  ( logit )
Formula: y ~ z * x * w + (1 | R)
   Data: data1

     AIC      BIC   logLik deviance df.resid
  3594.3   3777.5  -1766.2   3532.3     2695

Scaled residuals
    Min      1Q  Median      3Q     Max
-3.2753 -0.9225  0.5410  0.8283  1.7566

Random effects
 Groups Name        Variance Std.Dev.
 R      (Intercept) 0.2324   0.482
Number of obs: 2726, groups:  R, 209


Fixed effects
            Estimate Std. Error z value Pr(>|z|)
(Intercept)   1.0405     0.2306   4.512 6.44e-06 ***
z1           -1.4386     0.3159  -4.555 5.25e-06 ***
xB           -0.4679     0.3184  -1.470 0.141685
xC           -0.8343     0.3044  -2.741 0.006133 **
xD           -0.6778     0.3063  -2.213 0.026921 *
xE           -0.1365     0.3147  -0.434 0.664457
w2           -0.6763     0.3079  -2.196 0.028071 *
w3           -0.5678     0.3301  -1.720 0.085409 .
z1:xB         0.6491     0.4373   1.484 0.137710
z1:xC         0.8368     0.4273   1.959 0.050167 .
z1:xD         1.2520     0.4252   2.944 0.003236 **
z1:xE         0.6924     0.4332   1.598 0.109975
z1:w2         0.4740     0.4314   1.099 0.271928
z1:w3         0.8029     0.4609   1.742 0.081546 .
xB:w2        -0.2725     0.4455  -0.612 0.540713
xC:w2         1.5271     0.4355   3.506 0.000454 ***
xD:w2         1.3975     0.4388   3.184 0.001450 **
xE:w2         0.3196     0.4338   0.737 0.461182
xB:w3         0.4387     0.4631   0.947 0.343566
xC:w3         0.7665     0.4502   1.702 0.088692 .
xD:w3         1.9975     0.4895   4.081 4.49e-05 ***
xE:w3         0.4594     0.4589   1.001 0.316803
z1:xB:w2      0.2631     0.6320   0.416 0.677192
z1:xC:w2     -1.0622     0.6089  -1.744 0.081099 .
z1:xD:w2     -1.2572     0.6093  -2.063 0.039078 *
z1:xE:w2     -0.6406     0.6064  -1.056 0.290823
z1:xB:w3     -1.0492     0.6498  -1.615 0.106358
z1:xC:w3     -0.6534     0.6367  -1.026 0.304740
z1:xD:w3     -1.7769     0.6727  -2.641 0.008261 **
z1:xE:w3     -0.6847     0.6465  -1.059 0.289527
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

APPENDIX 1 - B

CHAPTER 3. SUPPLEMENTARY MATERIAL

CHAPTER 3. SUPPLEMENTARY MATERIAL

I present all GLM, GLMz, and GLMM tables and effect size estimates for full models for the tests of mean effects. I also provide model effect coefficients and standard errors for integration of E and G and G x E, as well as key output for CVs and variance tests. I provide an extended methods section for the calculation of across-environmental genetic correlations (r_g), as well as tables of the r_g scores. Finally, I provide model output for GLMMs run to test for effects of E and G on death rates.

The tables are split into 5 sections:

A) Effect of G x E on morphological traits
B) Integration of E, G and G x E for morphological and reproductive traits
C) Variance and CV patterns for morphological traits
D) Across-environment genetic correlations of morphological traits
E) Effect of G x E on male age at death
F) Figures

A) Effect of G x E on morphological traits

A.S1) Response of absolute male thorax to G and E

Thorax = Cross & Random + Number of Eggs per Petri Dish Reared on + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	508.8	7.9393	0.005
FOOD.TREATMENT	2	1144	528.1901	<.0001
GENETIC.STATUS	1	26.87	1.4946	0.2321
GENETIC.STATUS*FOOD.TREATMENT	2	1143	3.5126	0.0301

Term	Estimate	Std Error
Intercept	2.1911411	0.066027
EGGS.IN.PETRI.REARED.ON	-0.007346	0.013932
FOOD.TREATMENT[LOW]	-0.252217	0.009712
FOOD.TREATMENT[MEDIUM]	-0.044746	0.008686
GENETIC.STATUS[INCROSSED]	-0.01724	0.012132
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	-0.00052	0.00967
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	0.0203819	0.008688

A.S2) Response of absolute female thorax to G and E

Thorax = Cross & Random + Number of Eggs per Petri Dish Reared on + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	548.6	25.7993	<.0001
FOOD.TREATMENT	2	1112	621.4264	<.0001
GENETIC.STATUS	1	30.79	1.2217	0.2776
GENETIC.STATUS*FOOD.TREATMENT	2	1238	0.5433	0.581

Term	Estimate	Std Error
Intercept	2.1227354	0.061564
EGGS.IN.PETRI.REARED.ON	0.0070945	0.013084
FOOD.TREATMENT[LOW]	-0.258822	0.008649
FOOD.TREATMENT[MEDIUM]	-0.022493	0.008146
GENETIC.STATUS[INCROSS]	-0.011532	0.012764
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.005788	0.008561
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0083798	0.008147

A.S3) Response of absolute male eyespan to G and E

Eyespan = Cross & Random + Number of Eggs per Petri Dish Reared on + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	513	19.747	<.0001
FOOD.TREATMENT	2	1153	1117.73	<.0001
GENETIC.STATUS	1	26.66	5.3417	0.0288
GENETIC.STATUS*FOOD.TREATMENT	2	1154	1.2591	0.2843

Term	Estimate	Std Error
Intercept	6.8861632	0.223658
EGGS.IN.PETRI.REARED.ON	-0.020438	0.047293
FOOD.TREATMENT[LOW]	-1.278898	0.033074
FOOD.TREATMENT[MEDIUM]	-0.16555	0.029652
GENETIC.STATUS[INCROSSED]	-0.083439	0.038672
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0493933	0.032933
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.034977	0.029653

A.S4) Response of absolute female eyespan to G and E

Eyespan = Cross & Random + Number of Eggs per Petri Dish Reared on + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	613.5	49.1162	<.0001
FOOD.TREATMENT	2	1177	955.2257	<.0001
GENETIC.STATUS	1	32.61	7.3752	0.0105
GENETIC.STATUS*FOOD.TREATMENT	2	1244	0.4535	0.6355

Term	Estimate	Std Error
Intercept	5.4301351	0.137299
EGGS.IN.PETRI.REARED.ON	0.0110943	0.029251
FOOD.TREATMENT[LOW]	-0.755044	0.01949
FOOD.TREATMENT[MEDIUM]	-0.046631	0.018329
GENETIC.STATUS[INCROSSED]	-0.06917	0.0262
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.000514	0.019297
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.015837	0.018331

A.S5) Response of absolute male wing length to G and E

Wing Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	531	10.8018	0.0011
FOOD.TREATMENT	2	1083	716.3649	<.0001
GENETIC.STATUS	1	28.48	7.0614	0.0128
GENETIC.STATUS*FOOD.TREATMENT	2	1080	0.3598	0.6979

Term	Estimate	Std Error
Intercept	2.4485768	0.045427
EGGS.IN.PETRI.REARED.ON	-0.009366	0.00956
FOOD.TREATMENT[LOW]	-0.212797	0.006778
FOOD.TREATMENT[MEDIUM]	-0.022891	0.006
GENETIC.STATUS[INCROSSED]	-0.023287	0.009075
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0024432	0.006748
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.005079	0.006002

A.S6) Response of absolute female wing length to G and E

Wing Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	522	44.3448	<.0001
FOOD.TREATMENT	2	1019	719.4557	<.0001
GENETIC.STATUS	1	32.09	6.7618	0.014
GENETIC.STATUS*FOOD.TREATMENT	2	1173	0.3508	0.7042

Term	Estimate	Std Error
Intercept	2.3191908	0.04952
EGGS.IN.PETRI.REARED.ON	0.0094077	0.010475
FOOD.TREATMENT[LOW]	-0.234598	0.006887
FOOD.TREATMENT[MEDIUM]	-0.014793	0.006383
GENETIC.STATUS[INCROSSED]	-0.028616	0.011196
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	-0.001624	0.006825
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.003833	0.006382

A.S7) Response of relative male eyespan to G and E

Eyespan = Cross & Random + Number of Eggs per Petri Dish Reared on + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	512.4	40.7564	<.0001
THORAX	1	1146	4795.541	<.0001
FOOD.TREATMENT	2	1130	302.6452	<.0001
GENETIC.STATUS	1	25.68	4.1279	0.0526
GENETIC.STATUS*FOOD.TREATMENT	2	1145	7.1733	0.0008

Term	Estimate	Std Error
Intercept	1.7065062	0.225417
EGGS.IN.PETRI.REARED.ON	-0.012102	0.033926
THORAX	2.3839536	0.072433
FOOD.TREATMENT[LOW]	-0.678423	0.030007
FOOD.TREATMENT[MEDIUM]	-0.058974	0.021542
GENETIC.STATUS[INCROSSED]	-0.041044	0.025951
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0450627	0.023699
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.080845	0.02135

A.S8) Response of relative female eyespan to G and E

Eyespan = Cross & Random + Number of Eggs per Petri Dish Reared on + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	575.7	131.9991	<.0001
THORAX	1	1246	5681.168	<.0001
FOOD.TREATMENT	2	1128	157.857	<.0001
GENETIC.STATUS	1	24.86	10.0973	0.0039
GENETIC.STATUS*FOOD.TREATMENT	2	1240	4.2231	0.0149

Term	Estimate	Std Error
Intercept	1.6477079	0.130394
EGGS.IN.PETRI.REARED.ON	0.0187349	0.019229
THORAX	1.7372904	0.04373
FOOD.TREATMENT[LOW]	-0.295084	0.017445
FOOD.TREATMENT[MEDIUM]	-0.015587	0.012266
GENETIC.STATUS[INCROSSED]	-0.039793	0.013763
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0129392	0.012928
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.035388	0.012252

A.S9) Response of relative male wing length to G and E

Wing Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	565	21.3113	<.0001
THORAX	1	1084	2775.668	<.0001
FOOD.TREATMENT	2	1067	143.8801	<.0001
GENETIC.STATUS	1	29.75	7.0052	0.0129
GENETIC.STATUS*FOOD.TREATMENT	2	1080	5.1334	0.006

Term	Estimate	Std Error
Intercept	1.5150449	0.049884
EGGS.IN.PETRI.REARED.ON	-0.00611	0.007439
THORAX	0.4249594	0.016055
FOOD.TREATMENT[LOW]	-0.107429	0.006622
FOOD.TREATMENT[MEDIUM]	-0.004085	0.004737
GENETIC.STATUS[INCROSSED]	-0.015339	0.006601
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0040239	0.005269
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.014512	0.004699

A.S10) Response of relative female wing length to G and E

Wing Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	584.5	101.3339	<.0001
THORAX	1	1188	4012.944	<.0001
FOOD.TREATMENT	2	1028	87.1458	<.0001
GENETIC.STATUS	1	30.57	11.429	0.002
GENETIC.STATUS*FOOD.TREATMENT	2	1177	1.187	0.3055

Term	Estimate	Std Error
Intercept	1.1122168	0.049488
EGGS.IN.PETRI.REARED.ON	0.0078166	0.007389
THORAX	0.5615842	0.016442
FOOD.TREATMENT[LOW]	-0.087935	0.006518
FOOD.TREATMENT[MEDIUM]	-0.002822	0.004564
GENETIC.STATUS[INCROSSED]	-0.021669	0.006645
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0015835	0.004867
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.00676	0.004551

A.S11) Comparison of males of responses in absolute eyespan and absolute wing length to G and E

Trait length = Cross & Random + Male ID & Random + Number of Eggs per Petri Dish Reared on + Trait Type + Food Treatment + Genetic Status + Trait Type * Food Treatment + Trait Type * Genetic Status + Food Treatment * Genetic Status + Trait Type * Food Treatment [E] * Genetic Status [G]

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	518.3	20.0301	<.0001
TRAIT.TYPE	1	1089	52938.56	<.0001
FOOD.TREATMENT	2	1108	1079.665	<.0001
GENETIC.STATUS	1	26.12	5.5339	0.0265
FOOD.TREATMENT*TRAIT.TYPE	2	1091	1012.917	<.0001
GENETIC.STATUS*TRAIT.TYPE	1	1088	14.6608	0.0001
GENETIC.STATUS*FOOD.TREATMENT	2	1109	1.2263	0.2938
GENETIC.STATUS*FOOD.TREATMENT*TRAIT.TYPE	2	1091	1.1106	0.3297

Term	Estimate	Std Error
Intercept	4.6685589	0.135103
EGGS.IN.PETRI.REARED.ON	-0.016169	0.028554
TRAIT.TYPE[EYESPAN]	2.1869905	0.009731
FOOD.TREATMENT[LOW]	-0.744381	0.020099
FOOD.TREATMENT[MEDIUM]	-0.093507	0.017936
GENETIC.STATUS[INCROSSED]	-0.049053	0.023687
FOOD.TREATMENT[LOW]*TRAIT.TYPE[EYESPAN]	-0.522875	0.014418
FOOD.TREATMENT[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.073416	0.012911
GENETIC.STATUS[INCROSSED]*TRAIT.TYPE[EYESPAN]	-0.034571	0.009731
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0264306	0.020011
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.023461	0.017938
G[INCROSSED]*E[LOW]*TRAIT.TYPE[EYESPAN]	0.0209516	0.014419
G[INCROSSED]*E[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.012867	0.012912

A.S12) Comparison of females of responses in absolute eyespan and absolute wing length to G and E

Trait length = Cross & Random + Female ID & Random + Number of Eggs per Petri Dish Reared on + Trait Type + Food Treatment + Genetic Status + Trait Type * Food Treatment + Trait Type * Genetic Status + Food Treatment * Genetic Status + Trait Type * Food Treatment [E] * Genetic Status [G]

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	591.4	38.0465	<.0001
TRAIT.TYPE	1	1188	101756.5	<.0001
FOOD.TREATMENT	2	1127	920.6444	<.0001
GENETIC.STATUS	1	31.96	8.0729	0.0078
FOOD.TREATMENT*TRAIT.TYPE	2	1185	879.6436	<.0001
GENETIC.STATUS*TRAIT.TYPE	1	1185	18.9152	<.0001
GENETIC.STATUS*FOOD.TREATMENT	2	1213	0.4102	0.6636
GENETIC.STATUS*FOOD.TREATMENT*TRAIT.TYPE	2	1182	0.3516	0.7037

Term	Estimate	Std Error
Intercept	3.8779758	0.093102
EGGS.IN.PETRI.REARED.ON	0.0085463	0.019812
TRAIT.TYPE[EYESPAN]	1.5676297	0.004984
FOOD.TREATMENT[LOW]	-0.495342	0.013189
FOOD.TREATMENT[MEDIUM]	-0.031573	0.012353
GENETIC.STATUS[INCROSSED]	-0.050261	0.018448
FOOD.TREATMENT[LOW]*TRAIT.TYPE[EYESPAN]	-0.257611	0.007124
FOOD.TREATMENT[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.016993	0.006717
GENETIC.STATUS[INCROSSED]*TRAIT.TYPE[EYESPAN]	-0.020887	0.004984
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.001039	0.013063
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.010329	0.012353
G[INCROSSED]*E[LOW]*TRAIT.TYPE[EYESPAN]	-0.000621	0.007124
G[INCROSSED]*E[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.004751	0.006717

A.S13) Comparison of males of responses in relative eyespan and relative wing length to G and E

Trait length = Cross & Random + Male ID & Random + Number of Eggs per Petri Dish Reared on + Thorax + Trait Type + Food Treatment + Genetic Status + Trait Type * Food Treatment + Trait Type * Genetic Status+ Food Treatment * Genetic Status + Trait Type * Food Treatment [E] * Genetic Status [G]

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	498.7	45.9901	<.0001
THORAX	1	1120	4401.621	<.0001
TRAIT.TYPE	1	1111	53803.06	<.0001
FOOD.TREATMENT	2	1087	290.1415	<.0001
GENETIC.STATUS	1	24.21	5.7139	0.025
FOOD.TREATMENT*TRAIT.TYPE	2	1114	1018.385	<.0001
GENETIC.STATUS*TRAIT.TYPE	1	1111	14.4407	0.0002
GENETIC.STATUS*FOOD.TREATMENT	2	1110	7.8619	0.0004
GENETIC.STATUS*FOOD.TREATMENT*TRAIT.TYPE	2	1114	0.8596	0.4236

Term	Estimate	Std Error
Intercept	1.5327281	0.134158
EGGS.IN.PETRI.REARED.ON	-0.01003	0.020093
THORAX	1.4408299	0.043337
TRAIT.TYPE[EYESPAN]	2.1890278	0.009678
FOOD.TREATMENT[LOW]	-0.383687	0.017929
FOOD.TREATMENT[MEDIUM]	-0.029802	0.012868
GENETIC.STATUS[INCROSSED]	-0.024645	0.014491
FOOD.TREATMENT[LOW]*TRAIT.TYPE[EYESPAN]	-0.521503	0.01432
FOOD.TREATMENT[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.073498	0.012843
GENETIC.STATUS[INCROSSED]*TRAIT.TYPE[EYESPAN]	-0.034335	0.009678
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0250162	0.01424
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.050147	0.012761
G[INCROSSED]*E[LOW]*TRAIT.TYPE[EYESPAN]	0.0181049	0.01432
G[INCROSSED]*E[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.011767	0.012843

A.S14) Comparison of females of responses in relative eyespan and relative wing length to G and E

Trait length = Cross & Random + Female ID & Random + Number of Eggs per Petri Dish Reared on + Thorax + Trait Type + Food Treatment + Genetic Status + Trait Type * Food Treatment + Trait Type * Genetic Status + Food Treatment * Genetic Status + Trait Type * Food Treatment [E] * Genetic Status [G]

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	564.3	124.8072	<.0001
THORAX	1	1203	5162.71	<.0001
TRAIT.TYPE	1	1182	101796.9	<.0001
FOOD.TREATMENT	2	1091	150.3084	<.0001
GENETIC.STATUS	1	24.47	14.6994	0.0008
FOOD.TREATMENT*TRAIT.TYPE	2	1181	874.1186	<.0001
GENETIC.STATUS*TRAIT.TYPE	1	1180	18.7383	<.0001
GENETIC.STATUS*FOOD.TREATMENT	2	1184	3.201	0.0411
GENETIC.STATUS*FOOD.TREATMENT*TRAIT.TYPE	2	1178	0.665	0.5145

Term	Estimate	Std Error
Intercept	1.3358541	0.085968
EGGS.IN.PETRI.REARED.ON	0.012138	0.012712
THORAX	1.1712583	0.028922
TRAIT.TYPE[EYESPAN]	1.5682475	0.005006
FOOD.TREATMENT[LOW]	-0.186984	0.011526
FOOD.TREATMENT[MEDIUM]	-0.007872	0.008115
GENETIC.STATUS[INCROSSED]	-0.030136	0.008621
FOOD.TREATMENT[LOW]*TRAIT.TYPE[EYESPAN]	-0.2567	0.007148

FOOD.TREATMENT[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.018972	0.006746
GENETIC.STATUS[INCROSSED]*TRAIT.TYPE[EYESPAN]	-0.020685	0.005006
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0078017	0.008597
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.020282	0.008099
G[INCROSSED]*E[LOW]*TRAIT.TYPE[EYESPAN]	0.0001947	0.007148
G[INCROSSED]*E[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.00705	0.006747

A.S15) Comparison between males and females of responses in absolute thorax to G and E

Thorax = Cross & Random + Number of Eggs per Petri Dish Reared on + Sex + Food Treatment + Genetic Status + Sex * Food Treatment + Sex * Genetic Status + Food Treatment * Genetic Status + Sex * Food Treatment [E] * Genetic Status [G]

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	465.1	26.8088	<.0001
SEX	1	2386	0.0494	0.8242
FOOD.TREATMENT	2	2028	1121.155	<.0001
GENETIC.STATUS	1	32.22	1.3758	0.2494
SEX*FOOD.TREATMENT	2	2380	2.4882	0.0833
SEX*GENETIC.STATUS	1	2378	0.0583	0.8092
GENETIC.STATUS*FOOD.TREATMENT	2	2387	3.4151	0.033
SEX*GENETIC.STATUS*FOOD.TREATMENT	2	2382	0.8377	0.4328

Term	Estimate	Std Error
Intercept	2.1688877	0.045111
EGGS.IN.PETRI.REARED.ON	-0.002836	0.009416
SEX[FEMALE]	0.0045263	0.004322
FOOD.TREATMENT[LOW]	-0.25683	0.006379
FOOD.TREATMENT[MEDIUM]	-0.033099	0.005821
GENETIC.STATUS[INCROSSED]	-0.015529	0.011652
SEX[FEMALE]*FOOD.TREATMENT[LOW]	-0.006297	0.006272
SEX[FEMALE]*FOOD.TREATMENT[MEDIUM]	0.0131761	0.005807
SEX[FEMALE]*GENETIC.STATUS[INCROSSED]	-0.000199	0.004318
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	-0.003487	0.006336
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	0.0146378	0.005822
SEX[FEMALE]*G[INCROSSED]*E[LOW]	-0.001155	0.006273
SEX[FEMALE]*G[INCROSSED]*E[MEDIUM]	-0.006109	0.005807

A.S16) Comparison between males and females of responses in absolute eyespan to G and E

Eyespan = Cross & Random + Number of Eggs per Petri Dish Reared on + Sex + Food Treatment + Genetic Status + Sex * Food Treatment + Sex * Genetic Status + Food Treatment * Genetic Status + Sex * Food Treatment [E] * Genetic Status [G]

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	609.5	82.1905	<.0001
SEX	1	2397	2828.265	<.0001
FOOD.TREATMENT	2	2209	1980.195	<.0001
GENETIC.STATUS	1	31.12	9.1887	0.0049
SEX*FOOD.TREATMENT	2	2395	166.599	<.0001
SEX*GENETIC.STATUS	1	2392	1.1285	0.2882
GENETIC.STATUS*FOOD.TREATMENT	2	2404	1.3327	0.264
SEX*GENETIC.STATUS*FOOD.TREATMENT	2	2397	0.61	0.5434

Term	Estimate	Std Error
Intercept	6.1509854	0.130558
EGGS.IN.PETRI.REARED.ON	-0.002981	0.027509
SEX[FEMALE]	-0.639164	0.012701
FOOD.TREATMENT[LOW]	-1.0138	0.018706
FOOD.TREATMENT[MEDIUM]	-0.108681	0.017127
GENETIC.STATUS[INCROSSED]	-0.075696	0.028593
SEX[FEMALE]*FOOD.TREATMENT[LOW]	0.2627721	0.018405
SEX[FEMALE]*FOOD.TREATMENT[MEDIUM]	0.0590898	0.017087
SEX[FEMALE]*GENETIC.STATUS[INCROSSED]	0.0117398	0.012692
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0264025	0.018582
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.025504	0.01713
SEX[FEMALE]*G[INCROSSED]*E[LOW]	-0.020227	0.018406
SEX[FEMALE]*G[INCROSSED]*E[MEDIUM]	0.0069666	0.017088

A.S17) Comparison between males and females of responses in absolute wing length to G and E

Wing Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Sex + Food Treatment+ Genetic Status + Sex * Food Treatment + Sex * Genetic Status + Food Treatment * Genetic Status + Sex * Food Treatment [E] * Genetic Status [G]

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	437	52.0204	<.0001
SEX	1	2258	55.3176	<.0001
FOOD.TREATMENT	2	1797	1386.644	<.0001
GENETIC.STATUS	1	32.89	7.7563	0.0088
SEX*FOOD.TREATMENT	2	2255	2.3915	0.0917
SEX*GENETIC.STATUS	1	2252	0.6303	0.4273
GENETIC.STATUS*FOOD.TREATMENT	2	2260	0.6212	0.5374
SEX*GENETIC.STATUS*FOOD.TREATMENT	2	2256	0.0188	0.9814

Term	Estimate	Std Error
Intercept	2.3741568	0.034255
EGGS.IN.PETRI.REARED.ON	0.0021147	0.007114
SEX[FEMALE]	-0.018106	0.003251
FOOD.TREATMENT[LOW]	-0.222809	0.004843
FOOD.TREATMENT[MEDIUM]	-0.019487	0.004371
GENETIC.STATUS[INCROSSED]	-0.02604	0.00949
SEX[FEMALE]*FOOD.TREATMENT[LOW]	-0.010655	0.004762
SEX[FEMALE]*FOOD.TREATMENT[MEDIUM]	0.0038856	0.004361
SEX[FEMALE]*GENETIC.STATUS[INCROSSED]	-0.002617	0.003249
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0001323	0.004812
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.004348	0.004371
SEX[FEMALE]*G[INCROSSED]*E[LOW]	-0.000922	0.004762
SEX[FEMALE]*G[INCROSSED]*E[MEDIUM]	0.000436	0.004361

A.S18) Comparison between males and females of responses in relative eyespan to G and E

Eyespan = Cross & Random + Number of Eggs per Petri Dish Reared on + Thorax + Sex + Food Treatment + Genetic Status + Sex * Food Treatment + Sex * Genetic Status + Food Treatment * Genetic Status [E] + Sex * Food Treatment [E] * Genetic Status [G]

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	721.9	183.12	<.0001
THORAX	1	2387	9455.599	<.0001
SEX	1	2380	5547.799	<.0001
FOOD.TREATMENT	2	2120	417.8059	<.0001
GENETIC.STATUS	1	28.24	11.0195	0.0025
SEX*FOOD.TREATMENT	2	2383	313.3811	<.0001
SEX*GENETIC.STATUS	1	2378	2.2964	0.1298
GENETIC.STATUS*FOOD.TREATMENT	2	2391	10.7852	<.0001
SEX*GENETIC.STATUS*FOOD.TREATMENT	2	2385	0.9173	0.3997

Term	Estimate	Std Error
Intercept	1.6452258	0.130735
EGGS.IN.PETRI.REARED.ON	0.0052935	0.019467
THORAX	2.0712974	0.042775
SEX[FEMALE]	-0.651028	0.009078
FOOD.TREATMENT[LOW]	-0.480907	0.017321
FOOD.TREATMENT[MEDIUM]	-0.042515	0.012291
GENETIC.STATUS[INCROSSED]	-0.039936	0.016617
SEX[FEMALE]*FOOD.TREATMENT[LOW]	0.2751986	0.013164
SEX[FEMALE]*FOOD.TREATMENT[MEDIUM]	0.0306867	0.0122
SEX[FEMALE]*GENETIC.STATUS[INCROSSED]	0.0107879	0.009069
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0314683	0.013275
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.057314	0.012237
SEX[FEMALE]*G[INCROSSED]*E[LOW]	-0.013606	0.013161
SEX[FEMALE]*G[INCROSSED]*E[MEDIUM]	0.0152687	0.012187

A.S19) Comparison between males and females of responses in relative wing length to G and E

Wing Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Thorax + Sex + Food Treatment + Genetic Status + Sex * Food Treatment + Sex * Genetic Status + Food Treatment * Genetic Status + Sex * Food Treatment [E] * Genetic Status [G]

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	605.9	112.4543	<.0001
THORAX	1	2276	6595.43	<.0001
SEX	1	2257	101.8607	<.0001
FOOD.TREATMENT	2	1763	221.8238	<.0001
GENETIC.STATUS	1	32.85	11.6005	0.0018
SEX*FOOD.TREATMENT	2	2258	3.3446	0.0354
SEX*GENETIC.STATUS	1	2254	1.2627	0.2613
GENETIC.STATUS*FOOD.TREATMENT	2	2264	6.5515	0.0015
SEX*GENETIC.STATUS*FOOD.TREATMENT	2	2259	1.0817	0.3392

Term	Estimate	Std Error
Intercept	1.3075338	0.035684
EGGS.IN.PETRI.REARED.ON	0.003438	0.005304
THORAX	0.4906051	0.011594
SEX[FEMALE]	-0.02057	0.002441
FOOD.TREATMENT[LOW]	-0.098612	0.004668
FOOD.TREATMENT[MEDIUM]	-0.002873	0.003302

GENETIC.STATUS[INCROSSED]	-0.018725	0.005932
SEX[FEMALE]*FOOD.TREATMENT[LOW]	-0.00704	0.003574
SEX[FEMALE]*FOOD.TREATMENT[MEDIUM]	-0.002214	0.003274
SEX[FEMALE]*GENETIC.STATUS[INCROSSED]	-0.00337	0.002439
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0027207	0.003609
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.011457	0.003283
SEX[FEMALE]*G[INCROSSED]*E[LOW]	-0.001132	0.003573
SEX[FEMALE]*G[INCROSSED]*E[MEDIUM]	0.004634	0.003272

A.S20) Comparison between males and females of responses in absolute eyespan and absolute wing length to G and E

Trait length = Cross & Random + Fly ID & Random + Number of Eggs per Petri Dish Reared on + Sex + Trait Type + Food Treatment + Genetic Status + Sex * Trait Type + Sex * Food Treatment + Sex * Genetic Status + Trait Type * Food Treatment + Food Treatment * Genetic Status + Sex * Trait Type * Food Treatment [E] + Sex * Trait Type * Genetic Status [G] + Trait Type * Food Treatment [E] * Genetic Status [G] + Sex * Trait Type [T] * Food Treatment [E] * Genetic Status [G]

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	557.7	70.0779	<.0001
SEX	1	2314	2085.046	<.0001
TRAIT.TYPE	1	2278	126864.3	<.0001
FOOD.TREATMENT	2	2066	1938.958	<.0001
GENETIC.STATUS	1	30.62	9.0018	0.0053
SEX*GENETIC.STATUS	1	2306	0.5603	0.4542
SEX*FOOD.TREATMENT	2	2315	102.8534	<.0001
GENETIC.STATUS*FOOD.TREATMENT	2	2319	0.9566	0.3844
SEX*GENETIC.STATUS*FOOD.TREATMENT	2	2314	0.6184	0.5389
SEX*TRAIT.TYPE	1	2274	3758.286	<.0001
GENETIC.STATUS*TRAIT.TYPE	1	2271	1.7653	0.1841
SEX*GENETIC.STATUS*TRAIT.TYPE	1	2271	3.1305	0.077
FOOD.TREATMENT*TRAIT.TYPE	2	2277	1792.697	<.0001
SEX*FOOD.TREATMENT*TRAIT.TYPE	2	2277	237.0603	<.0001
G *E*TRAIT.TYPE	2	2274	0.9212	0.3982
SEX*G*E*TRAIT.TYPE	2	2274	0.8923	0.4099

Term	Estimate	Std Error
Intercept	4.2678415	0.082229
EGGS.IN.PETRI.REARED.ON	-0.00248	0.017268
SEX[FEMALE]	-0.327724	0.007955

TRAIT.TYPE[EYESPAN]	1.8772213	0.005342
FOOD.TREATMENT[LOW]	-0.617983	0.011763
FOOD.TREATMENT[MEDIUM]	-0.06427	0.010718
GENETIC.STATUS[INCROSSED]	-0.049052	0.019188
SEX[FEMALE]*GENETIC.STATUS[INCROSSED]	0.002624	0.007949
SEX[FEMALE]*FOOD.TREATMENT[LOW]	0.1255485	0.011571
SEX[FEMALE]*FOOD.TREATMENT[MEDIUM]	0.0307074	0.010691
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0145415	0.011686
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.016969	0.010718
SEX[FEMALE]*G[INCROSSED]*E[LOW]	-0.010415	0.011572
SEX[FEMALE]*G[INCROSSED]*E[MEDIUM]	0.0053543	0.010691
SEX[FEMALE]*TRAIT.TYPE[EYESPAN]	-0.310382	0.005342
GENETIC.STATUS[INCROSSED]*TRAIT.TYPE[EYESPAN]	-0.028074	0.005342
SEX[FEMALE]*G[INCROSSED]*TRAIT.TYPE[EYESPAN]	0.0067838	0.005342
FOOD.TREATMENT[LOW]*TRAIT.TYPE[EYESPAN]	-0.390299	0.007781
FOOD.TREATMENT[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.045024	0.007142
SEX[FEMALE]*E[LOW]*TRAIT.TYPE[EYESPAN]	0.132436	0.007781
SEX[FEMALE]*E[MEDIUM]*TRAIT.TYPE[EYESPAN]	0.0281203	0.007142
G[INCROSSED]*E[LOW]*TRAIT.TYPE[EYESPAN]	0.0099102	0.007781
G[INCROSSED]*E[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.008601	0.007142
SEX[FEMALE]*G[INCROSSED]*E[LOW]*T[EYESPAN]	-0.010375	0.007781
SEX[FEMALE]*G[INCROSSED]*E[MEDIUM]*T[EYESPAN]	0.0039194	0.007142

A.S21) Comparison between males and females of responses in relative eyespan and relative wing length to G and E

Trait length = Cross & Random + Fly ID & Random + Number of Eggs per Petri Dish Reared on + Thorax + Sex + Trait Type + Food Treatment + Genetic Status + Sex * Trait Type + Sex * Food Treatment + Sex * Genetic Status + Trait Type * Food Treatment + Food Treatment * Genetic Status + Sex * Trait Type * Food Treatment [E] + Sex * Trait Type * Genetic Status [G] + Trait Type * Food Treatment [E] * Genetic Status [G] + Sex * Trait Type [T] * Food Treatment [E] * Genetic Status [G]

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	713.9	186.9306	<.0001
THORAX	1	2325	8962.078	<.0001
SEX	1	2292	4273.988	<.0001
TRAIT.TYPE	1	2305	128210.7	<.0001
FOOD.TREATMENT	2	2047	412.7982	<.0001
GENETIC.STATUS	1	26.32	15.2421	0.0006
SEX*GENETIC.STATUS	1	2286	0.8212	0.3649
SEX*FOOD.TREATMENT	2	2299	201.8126	<.0001

GENETIC.STATUS*FOOD.TREATMENT	2	2304	9.9105	<.0001
SEX*GENETIC.STATUS*FOOD.TREATMENT	2	2298	1.4775	0.2284
SEX*TRAIT.TYPE	1	2305	3764.21	<.0001
GENETIC.STATUS*TRAIT.TYPE	1	2302	1.5358	0.2154
SEX*GENETIC.STATUS*TRAIT.TYPE	1	2302	2.9149	0.0879
FOOD.TREATMENT*TRAIT.TYPE	2	2307	1794.728	<.0001
SEX*FOOD.TREATMENT*TRAIT.TYPE	2	2307	235.2426	<.0001
G*E*TRAIT.TYPE	2	2304	1.0166	0.362
SEX*G*E*TRAIT.TYPE	2	2304	0.6826	0.5054

Term	Estimate	Std Error
Intercept	1.4180545	0.079695
EGGS.IN.PETRI.REARED.ON	0.0025487	0.011861
THORAX	1.3103117	0.026151
SEX[FEMALE]	-0.335593	0.005549
TRAIT.TYPE[EYESPAN]	1.8786345	0.005334
FOOD.TREATMENT[LOW]	-0.283082	0.010576
FOOD.TREATMENT[MEDIUM]	-0.020798	0.007505
GENETIC.STATUS[INCROSSED]	-0.02725	0.009679
SEX[FEMALE]*GENETIC.STATUS[INCROSSED]	0.0025089	0.005544
SEX[FEMALE]*FOOD.TREATMENT[LOW]	0.1333716	0.008085
SEX[FEMALE]*FOOD.TREATMENT[MEDIUM]	0.0143114	0.007445
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[LOW]	0.0172961	0.008151
GENETIC.STATUS[INCROSSED]*FOOD.TREATMENT[MEDIUM]	-0.034877	0.007468
SEX[FEMALE]*G[INCROSSED]*E[LOW]	-0.007444	0.008083
SEX[FEMALE]*G[INCROSSED]*E[MEDIUM]	0.0120463	0.007439
SEX[FEMALE]*TRAIT.TYPE[EYESPAN]	-0.310324	0.005333
GENETIC.STATUS[INCROSSED]*TRAIT.TYPE[EYESPAN]	-0.027633	0.005333
SEX[FEMALE]*G[INCROSSED]*TRAIT.TYPE[EYESPAN]	0.0067621	0.005333
FOOD.TREATMENT[LOW]*TRAIT.TYPE[EYESPAN]	-0.389077	0.007759
FOOD.TREATMENT[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.046461	0.00713
SEX[FEMALE]*E[LOW]*TRAIT.TYPE[EYESPAN]	0.1326521	0.007759
SEX[FEMALE]*E[MEDIUM]*TRAIT.TYPE[EYESPAN]	0.026984	0.00713
G[INCROSSED]*E[LOW]*TRAIT.TYPE[EYESPAN]	0.0091833	0.007759
G[INCROSSED]*E[MEDIUM]*TRAIT.TYPE[EYESPAN]	-0.009685	0.00713
SEX[FEMALE]*G[INCROSSED]*E[LOW]*T[EYESPAN]	-0.008793	0.007759
SEX[FEMALE]*G[INCROSSED]*E[MEDIUM]*T[EYESPAN]	0.001962	0.00713

B) Integration of E, G and G x E for morphological and reproductive traits

B.S1) Model effect coefficients for absolute trait values

		Effect Coefficients						Standard Error					
Trait	*Sex*	E1	E2	E3	G	GxE1	GxE2	E1	E2	E3	G	GxE1	GxE2
ES	F	0.989	0.061	0.525	0.092	0.000	0.020	0.025	0.024	0.025	0.034	0.025	0.024
T	F	0.899	0.068	0.484	0.052	0.020	-0.027	0.028	0.027	0.028	0.043	0.028	0.027
W	F	0.950	0.061	0.505	0.120	0.009	0.015	0.027	0.026	0.027	0.045	0.027	0.026
ES	M	1.004	0.118	0.561	0.069	-0.039	0.035	0.026	0.023	0.024	0.031	0.026	0.023
T	M	0.835	0.135	0.485	0.061	0.001	-0.062	0.032	0.028	0.030	0.040	0.032	0.028
W	M	0.942	0.089	0.515	0.102	-0.013	0.031	0.029	0.026	0.028	0.040	0.029	0.026
Testes	M	0.443	0.001	0.222	0.169	0.077	-0.018	0.078	0.066	0.072	0.080	0.078	0.066
AG	M	0.468	-0.221	0.123	0.412	0.134	-0.081	0.079	0.070	0.074	0.082	0.079	0.070

Table B.S1. Model effect coefficients for E, G and G x E from GLMMs fitted to absolute trait z-scores. Trait abbreviations ES, T, W, Testes and AG relate to eyespan, thorax length, wing length, testes length and accessory gland length. Model effects abbreviations E1, E2, E3, G, GxE1 and GxE2 relate to the coefficients for low, intermediate and averaged food level effects, genetic status effects, and GxE at low and GxE at intermediate food level effects. All output are shown to 3 decimal points.

B.S2) Model effect coefficients for relative trait values

		Effect Coefficients						Standard Error					
Trait	*Sex*	E1	E2	E3	G	GxE1	GxE2	E1	E2	E3	G	GxE1	GxE2
ES	F	0.388	0.021	0.204	0.055	-0.016	0.046	0.023	0.016	0.019	0.018	0.017	0.016
W	F	0.357	0.012	0.184	0.091	-0.005	0.027	0.026	0.018	0.022	0.027	0.020	0.018
ES	M	0.529	0.041	0.285	0.033	-0.035	0.068	0.023	0.017	0.020	0.020	0.018	0.016
W	M	0.475	0.013	0.244	0.065	-0.020	0.069	0.029	0.020	0.025	0.029	0.023	0.020
Testes	M	0.220	-0.015	0.102	0.133	0.082	-0.011	0.098	0.066	0.082	0.079	0.077	0.066
AG	M	0.159	-0.260	-0.050	0.354	0.145	-0.084	0.094	0.067	0.081	0.085	0.076	0.067

Table B.S2. Model effect coefficients for E, G and G x E from GLMMs fitted to relative trait z-scores. Trait abbreviations ES, W, Testes and AG relate to eyespan, wing length, testes length and accessory gland length. Model effects abbreviations E1, E2, E3, G, GxE1 and GxE2 relate to the coefficients for low, intermediate and averaged food level effects, genetic status effects, and GxE at low and GxE at intermediate food level effects. All output are shown to 3 decimal points.

C) Variance and CV patterns for morphological traits

C.S1) Between-line variance of absolute eyespan for males and females

		SD		Brown-Forsythe				
Food Level	Sex	Incross	Outcross	*F* Ratio	*DF*Num	*DF*Den	*p*	In v Out
L	F	0.098	0.162	2.501	1	36	0.123	I = O
M	F	0.134	0.133	0.096	1	35	0.758	I = O
H	F	0.089	0.078	0.456	1	36	0.504	I = O
L	M	0.140	0.104	0.078	1	34	0.781	I = O
M	M	0.150	0.242	1.728	1	34	0.198	I = O
H	M	0.000	0.021	16.289	1	34	0.000	I < O

Table C.S1. Between-line variance contrasts for absolute eyespan at each food level for incross versus outcross flies. Food levels L, M, H relate to low, intermediate and high.

C.S2) Between-line variance of relative eyespan for males and females

		SD		Brown-Forsythe				
Food Level	Sex	Incross	Outcross	*F* Ratio	*DF*Num	*DF*Den	*p*	In v Out
L	F	0.027	0.037	0.027	1	36	0.157	I = O
M	F	0.050	0.033	0.050	1	35	0.290	I = O
H	F	0.056	0.074	0.056	1	35	0.517	I = O
L	M	0.128	0.171	0.128	1	34	0.299	I = O
M	M	0.100	0.028	0.100	1	34	0.000	I > O
H	M	0.019	0.016	0.019	1	34	0.351	I = O

Table C.S2. Between-line variance contrasts for relative eyespan at each food level for incross versus outcross flies. Food levels L, M, H relate to low, intermediate and high.

C.S3) CV of absolute eyespan for males and females

		CV%	C.I.		CV%	C.I.		
Food Level	Sex	Incross	5%	95%	Outcross	5%	95%	In v Out
L	F	2.101	1.523	3.385	3.397	2.640	4.768	I = O
M	F	2.501	1.813	4.031	2.396	1.853	3.393	I = O
H	F	1.435	1.050	2.263	1.229	0.950	1.739	I = O
L	M	2.530	1.814	4.178	1.861	1.439	2.634	I = O
M	M	2.303	1.651	3.803	3.573	2.763	5.060	I = O
H	M	0.001	0.001	0.002	0.251	0.193	0.359	I < O

Table C.S3. CV and C.I. for absolute eyespan at each food level for incross versus outcross flies. L, M, H denote low, intermediate and high food levels.

C.S4) CV of relative eyespan for males and females

Food Level	Sex	CV% Incross	C.I. 5%	95%	CV% Outcross	C.I. 5%	95%	In v Out
L	F	0.586	0.425	0.944	0.768	0.597	1.078	I = O
M	F	0.931	0.675	1.501	0.587	0.454	0.831	I = O
H	F	0.901	0.660	1.421	1.163	0.895	1.662	I = O
L	M	2.318	1.662	3.829	3.048	2.357	4.316	I = O
M	M	1.539	1.103	2.540	0.416	0.322	0.589	I > O
H	M	0.240	0.174	0.386	0.189	0.145	0.270	I = O

Table C.S4. CV and C.I. for relative eyespan at each food level for incross versus outcross flies. L, M, H denote low, intermediate and high food levels.

D) Across-environment genetic correlation (r_g) of morphological traits

D.S1) Statistical Methods

Across-environment genetic correlations were calculated in two ways. For each, an $\boldsymbol{r_g}$ value or comparison was calculated for low versus intermediate (L – I) and for intermediate versus high food levels (I – H). This was done per genetic status (in/outcross), per sex (male/female), and for absolute and relative trait size (eyespan).

D.S1.1) Method 1: Multiple model contrasts and r_g

First, to detect the presence, absence and type of r_g a series of three GLMMs were contrasted, per genetic status, sex and trait type and for absolute and relative traits. The first GLMM included food level; the second, food level and cross as a random effect; the third, food level and cross nested within food level as a random effect. The models relate to a slope and intercept, a slope and multiple intercepts (aka each line with a different intercept), and multiple slopes and intercepts (aka each line with a different slope and intercept) (Roff & Wilson 2014). The models were compared with likelihood ratio tests. A single slope and intercept (model 1) or a single slope and multiple intercepts relates to an $\boldsymbol{r_g}$ value of 1. That is, all lines that are high in one environment are high in another. Multiple slopes and intercepts relates to a value < 1.

That is, either lines cross over, or respond to different extents to environmental variation. This is evidence for the traditional G x E that has been used to demonstrate the existence, scale and type of genetic variation in sexual and nonsexual traits.

D.S1.2) Method 2: Estimates of r_g from variance component and LS Means

A number of methods can be used to estimate values for r_g (Astles *et al.* 2006). The values can range from – 1 to + 1. At + 1 there is no crossover in the performance of lines between environments. At -1 there is perfect crossover (that is, the order in environment 1 is reversed in environment 2). For the purposes of this study, the actual numbers are not important. But the broad scale of the r_g estimates is informative, as is the comparison of r_g at low versus intermediate (L – I) with that at intermediate versus high (I – H) for each genetic status and sex, and for absolute and relative traits.

To calculate estimates of r_g two methods were used: the first, based on variance components (Roff & Wilson 2014); the second, based on least square means (Yamada 1962). For the first method, for the given sex, and for the given genetic status, a GLMM that included cross as a random effect was fitted to the absolute trait size at each food level (low, intermediate, high) and the random effect variance component for cross was extracted. Another GLMM that included the interaction between food level and cross as a random effect was then fitted, for both low versus intermediate and intermediate versus high food levels (subsets of the data) and the covariance of cross across food level was extracted. An estimate of r_g was then calculated for low versus intermediate, and for intermediate versus high food levels as:

$$r_g = \sigma_{1,2} / \sqrt{\sigma^2_{1,1}\, \sigma^2_{2,2}}$$

where $\sigma_{1,2}$ is equal to the genetic covariance between the two environments, and where $\sigma^2_{1,1}$ and $\sigma^2_{2,2}$ are equal to the genetic variance in environments 1 and 2 respectively. To calculate values for relative traits, the residuals of a GLMM that modeled trait as a function of thorax were used in place of the absolute trait sizes.

An issue with the above method is that variance components that equal zero lead to inestimable r_g. To compensate (in part) for this, I also calculated least squared means based estimates (Yamada 1962). To do so, LS means per cross were extracted from GLMs of trait size that included cross as a fixed effect. The genetic variance per environment was estimated as the between-line variance per food level. Finally, the genetic covariance between environments was estimated as the correlation coefficient in a Pearson's correlation test on the genetic cross LS means at each of two food levels.

As noted above, values at 1 or -1 relate to zero and full crossover. In either case, evolution to the two optimal trait values will not be possible (Lande & Via 1985). At zero, optima can be achieved, but there is no G x E. For optima to be achieved in general requires that $0 \leq |r_g| < |1|$ (Roff & Wilson 2014). For the purposes of this study, the direction (+ or -) and rough scale of the estimates at L – I and I – H are useful, as is the qualitative difference in r_g estimates at L – I versus M – H, for each sex and genetic status. The exact quantifications are not reliable nor are they of interest.

D.S2) Estimates of r_g for absolute eyespan

Sex	G	Contrast	Model	LR	*p*	Best Fit	r_g	Inc/Dec	r_g LS	Inc/Dec LS
F	I	L - I	1 v 2	14.52	< 0.001	2	0.169		1.283	
F	I	L - I	2 v 3	0.35	0.8401			INC		INC
F	I	I - H	1 v 2	9.97	< 0.001	3	0.275		8.080	
F	I	I - H	2 v 3	0.03	0.025					
F	O	L - I	1 v 2	5.67	0.0172	3	0.438		0.037	
F	O	L - I	2 v 3	10.36	0.0056			INC		EQUAL
F	O	I - H	1 v 2	7.86	0.0051	3	0.889		0.036	
F	O	I - H	2 v 3	10.76	0.0046					
M	I	L - I	1 v 2	5.47	0.0193	2	0.612		0.540	
M	I	L - I	2 v 3	1.24	0.5367			DEC		DEC
M	I	I - H	1 v 2	2.40	0.0475	3	Inf.		0.325	
M	I	I - H	2 v 3	6.89	0.0319					
M	O	L - I	1 v 2	9.13	0.0025	2	0.641		0.566	
M	O	L - I	2 v 3	1.78	0.4116			DEC		DEC
M	O	I - H	1 v 2	6.04	0.014	3	0.150		0.052	
M	O	I - H	2 v 3	12.00	0.0025					

Table D.S2. Model contrasts and r_g estimates for absolute eyespan. Sex, F, M relates to female and male. G, in, out relates to incross and outcross flies. Contrast, L – I, I – H relates to low versus intermediate and intermediate versus high food levels. Model,

1 v 2, 2, v 3 relates to the contrast of GLMMs with a single slope and intercept (1), a slope and multiple intercepts (2), and slopes and intercepts (3). LR is the likelihood ratio for the model comparison and *p* is the related *p* value. Best fit 1, 2, 3 relates to the model that best fits the data. r_g is the variance components estimate, r_g LS is the LS means equivalent. Inc/Dec and Inc/Dec LS relate to an increase or decrease in r_g from L – I to I – H for r_g and LS r_g in turn. Inf = inestimable value due to zero variance at one or both food levels. r_g LS > 1 arises for similar reasons.

D.S3) Estimates of r_g for relative eyespan

Sex	G	Contrast	Model	LR	*p*	Best Fit	r_g	Inc/Dec	r_g LS	Inc/Dec LS
F	I	L – I	1 v 2	1.42	0.234	1	-	-	4.273	INC
F	I	L - I	2 v 3	1.41	0.493					
F	I	I - H	1 v 2	1.81	0.178	1	-		38.712	
F	I	I - H	2 v 3	0.01	0.997					
F	O	L – I	1 v 2	2.9	0.089	2	-	-	0.01	INC
F	O	L - I	2 v 3	0.03	0.597					
F	O	I - H	1 v 2	6	0.014	2	-		2.858	
F	O	I - H	2 v 3	0.63	0.73					
M	I	L – I	1 v 2	11.11	< 0.0001	2	0.29	DEC	2.543	DEC
M	I	L - I	2 v 3	0	0.998					
M	I	I - H	1 v 2	3.84	0.05	1	0.12		0.002	
M	I	I - H	2 v 3	2.15	0.341					
M	O	L – I	1 v 2	1.81	0.178	1	Inf.	NA	0.333	INC
M	O	L - I	2 v 3	0.27	0.874					
M	O	I - H	1 v 2	0	1	1	Inf.		1.129	
M	O	I - H	2 v 3	1.98	0.371					

Table D.S3. Model contrasts and r_g estimates for relative eyespan. Sex, F, M relates to female and male. G, in, out relates to incross and outcross flies. Contrast, L – I, I – H relates to low versus intermediate and intermediate versus high food levels. Model, 1 v 2, 2, v 3 relates to the contrast of GLMMs with a single slope and intercept (1), a slope and multiple intercepts (2), and slopes and intercepts (3). LR is the likelihood ratio for the model comparison and *p* is the related *p* value. Best fit 1, 2, 3 relates to the model that best fits the data. r_g is the variance components estimate, r_g LS is the LS means equivalent. Inc/Dec and Inc/Dec LS relate to an increase or decrease in r_g from L – I to I – H for r_g and LS r_g in turn. Inf = inestimable value due to zero variance at one or both food levels. r_g LS > 1 arises for similar reasons.

E) Effect of G x E on male age at death

Mean Age at Death [Weeks] [SQRT] = Cross & Random + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
FOOD.TREATMENT	2	947.6	44.1126	<.0001
GENETIC.STATUS	1	30.78	14.2829	0.0007
GENETIC.STATUS*FOOD.TREATMENT	2	948.4	0.1761	0.8386

Term	Estimate	Std Error
Intercept	4.0603284	0.129702
FOOD.TREATMENT[LOW]	-1.010696	0.129879
FOOD.TREATMENT[MEDIUM]	-0.103538	0.115194
GENETIC.STATUS[INCROSS]	-0.476978	0.129702
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0201235	0.129879
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.066444	0.115194

R E F E R E N C E S

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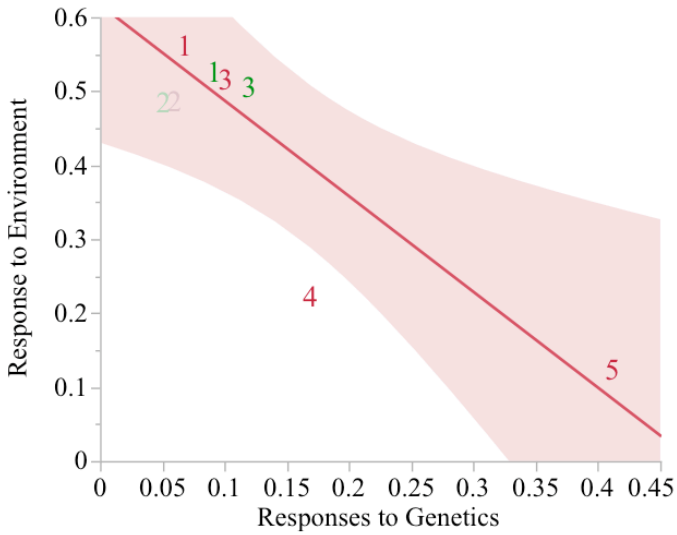
Yamada, Y. (1962). Genotype x environment interaction and genetic correlation of the same trait under different environments. *Japanese Journal of Genetics*, 37, 498-509.

F) Figures

F.A)

a)



b)

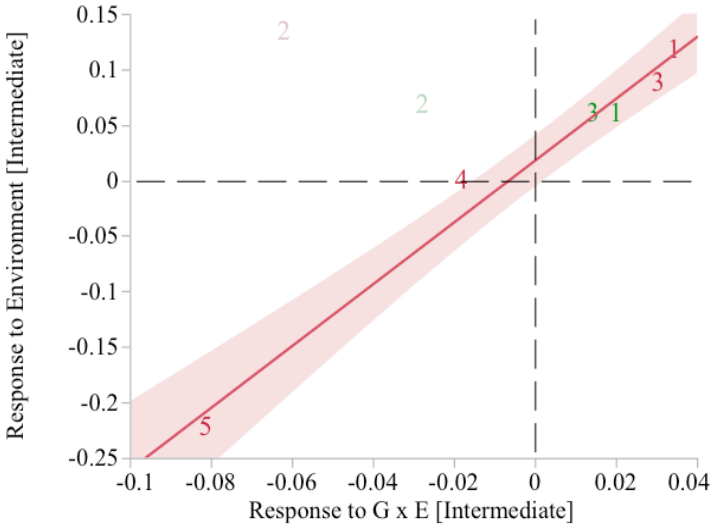


Figure F.A. Regression of model coefficients for average E on G (a) and for intermediate E on intermediate G x E (b) for absolute trait z-scores. Numbers 1 – 5 relate to traits, in order: eyespan, thorax length, wing length, testes length and accessory gland length. Red = males, green = females. In each case, the line was fitted without the inclusion of male or female thorax as data points. But both male and female thorax are shown: near the line for E on G; and far from the line for E on G x E. The red outlines show the confidence intervals at ± 95% for the expected values.

F.B)

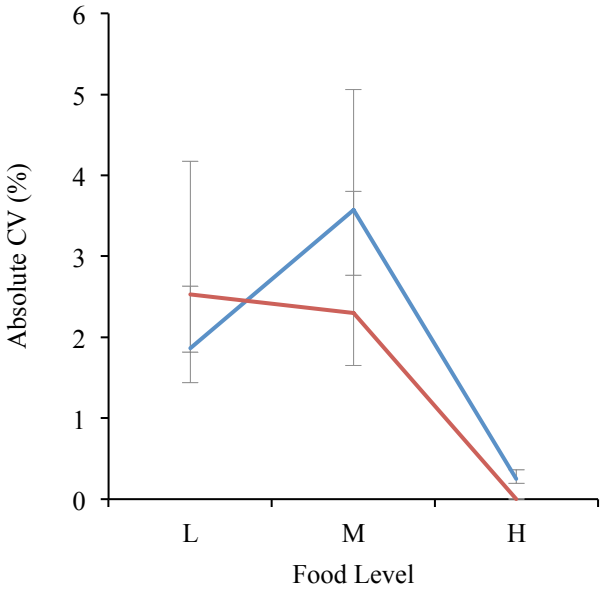


Figure F.B. Variation in CV across food levels for absolute male eyespan in incross versus outcross flies. Red = incross, blue = outcross (± 95% C.I.).

F.C)

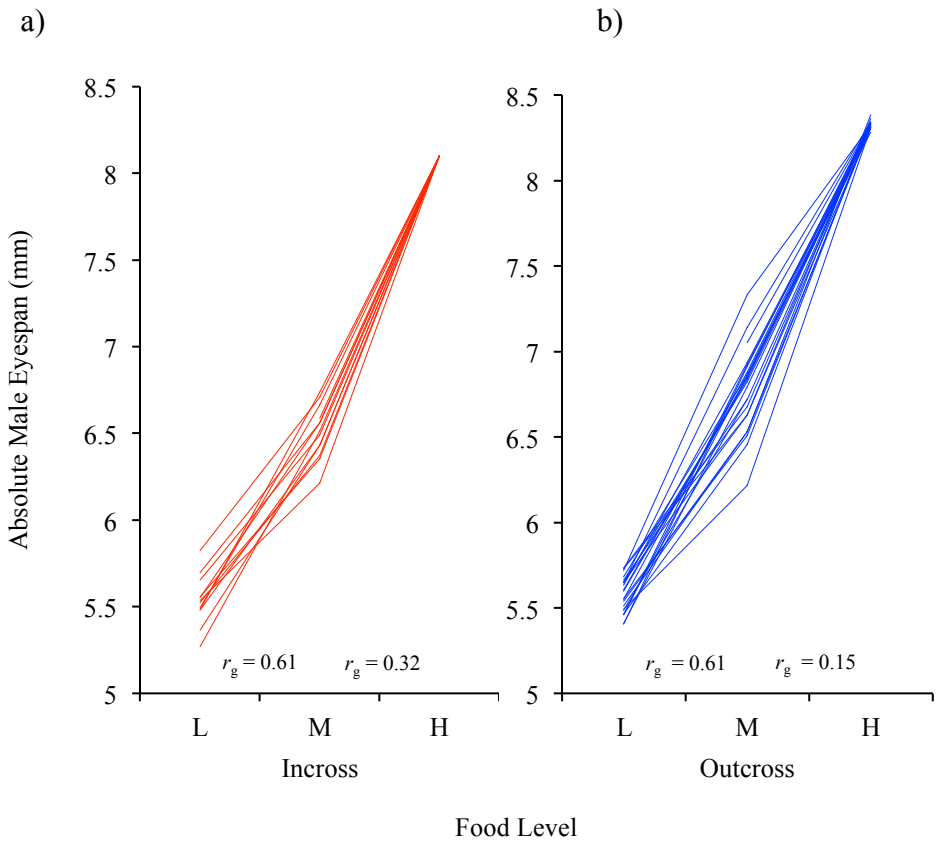


Figure F.C. Across-environment genetic correlations for absolute trait values. Per cross least squares means for incross (a) and outcross (b) absolute male eyespan. Error bars are excluded for clarity. Across-environment genetic correlation values for the low versus intermediate and intermediate versus high food levels are also shown. In each case, the rank order of lines is less stable between intermediate and high food.

APPENDIX 1 - C

CHAPTER 4. SUPPLEMENTARY MATERIAL

CHAPTER 4. SUPPLEMENTARY MATERIAL

I present all GLM, GLMz, GLMM and GLME tables and effect size estimates for full models for the tests of mean effects. I also provide model effect coefficients and standard errors for integration of E (food level) and G (genetic status) and G x E. I then provide an extended methods section for the calculation of effects of E and G and male absolute and relative eyespan on male age at death, so as to investigate potential causes for the different patterns seen in Chapter 3 and in Chapter 4. Finally, I provide model output for GLMMs run to test for effects of E and G on death rates.

The tables are split into 6 sections:

A) Effect of G x E on morphological and reproductive traits
B) Effect of G x E on fertility traits
C) Effect of G x E on attractiveness and behavioural traits
D) Absolute and relative eyespan-trait relationships, and the effects of E and G
E) Integration of E, G and G x E for morphological and reproductive traits
F) Effect of E, G, G x E and absolute and relative eyespan on male age at death

A) Effect of G x E on morphological and reproductive traits

A.S1) Response of absolute male thorax length to G and E

Thorax = Cross & Random + Number of Eggs per Petri Dish Reared on + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	307	22.4882	<.0001
FOOD.TREATMENT	2	398.2	209.1147	<.0001
GENETIC.STATUS	1	23.97	8.121	0.0088
GENETIC.STATUS*FOOD.TREATMENT	2	403.6	1.1165	0.3284

Term	Estimate	Std Error
Intercept	2.1824565	0.084577
EGGS.IN.PETRI.REARED.ON	0.0110905	0.018207
FOOD.TREATMENT[LOW]	-0.213391	0.014513
FOOD.TREATMENT[MEDIUM]	-0.026235	0.012243
GENETIC.STATUS[INCROSS]	-0.032419	0.011766
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.002666	0.014401
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0156942	0.012311

A.S2) Response of absolute male eyespan to G and E

Eyespan = Cross & Random + Number of Eggs per Petri Dish Reared on + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	331.3	20.9569	<.0001
FOOD.TREATMENT	2	401.7	421.6571	<.0001
GENETIC.STATUS	1	29.24	1.6463	0.2095
GENETIC.STATUS*FOOD.TREATMENT	2	404.9	0.8022	0.449

Term	Estimate	Std Error
Intercept	7.1349431	0.321131
EGGS.IN.PETRI.REARED.ON	-0.011203	0.069056
FOOD.TREATMENT[LOW]	-1.203871	0.054799
FOOD.TREATMENT[MEDIUM]	-0.00943	0.046291
GENETIC.STATUS[INCROSSED]	-0.048275	0.047159
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0425819	0.054372
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0134058	0.046537

A.S3) Response of absolute male wing length to G and E

Wing Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	313.7	8.4751	0.0039
FOOD.TREATMENT	2	389.9	261.4037	<.0001
GENETIC.STATUS	1	21.27	0.0533	0.8196
GENETIC.STATUS*FOOD.TREATMENT	2	392.9	2.1387	0.1192

Term	Estimate	Std Error
Intercept	2.4902581	0.064966
EGGS.IN.PETRI.REARED.ON	-0.005906	0.01396
FOOD.TREATMENT[LOW]	-0.192385	0.011137
FOOD.TREATMENT[MEDIUM]	0.0031632	0.009402
GENETIC.STATUS[INCROSS]	0.0011431	0.009724
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0138189	0.011059
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0048099	0.009449

A.S4) Response of absolute male testes length to G and E

Testes Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	350.2	2.1632	0.1422
FOOD.TREATMENT	2	388.1	20.4609	<.0001
GENETIC.STATUS	1	36.6	2.7652	0.1049
GENETIC.STATUS*FOOD.TREATMENT	2	388.6	0.6418	0.5269

Term	Estimate	Std Error
Intercept	4.0495862	0.217119
EGGS.IN.PETRI.REARED.ON	-0.002259	0.046502
FOOD.TREATMENT[LOW]	-0.198942	0.036044
FOOD.TREATMENT[MEDIUM]	-0.001104	0.030703
GENETIC.STATUS[INCROSS]	-0.068257	0.036698
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.040021	0.035719
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0175094	0.030863

A.S5) Response of absolute accessory gland length to G and E

Accessory Gland Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	280.4	0.2052	0.6509
FOOD.TREATMENT	2	323.6	10.4838	<.0001
GENETIC.STATUS	1	38.36	23.8546	<.0001
GENETIC.STATUS*FOOD.TREATMENT	2	325	1.8103	0.1652

Term	Estimate	Std Error
Intercept	1.6397617	0.116912
EGGS.IN.PETRI.REARED.ON	-0.040442	0.025113
FOOD.TREATMENT[LOW]	-0.107077	0.018671
FOOD.TREATMENT[MEDIUM]	0.0442742	0.01657
GENETIC.STATUS[INCROSS]	-0.093675	0.018396
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.034199	0.018537
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0252798	0.016773

A.S6) Response of relative male eyespan to G and E [1 - thorax controlled]

Eyespan = Cross & Random + Number of Eggs per Petri Dish Reared on + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	313.5	42.1408	<.0001
THORAX	1	403.2	1615.579	<.0001
FOOD.TREATMENT	2	369.5	109.6286	<.0001
GENETIC.STATUS	1	31.28	0.2266	0.6374
GENETIC.STATUS*FOOD.TREATMENT	2	402.5	0.8573	0.4251

Term	Estimate	Std Error
Intercept	1.5998363	0.389453
EGGS.IN.PETRI.REARED.ON	-0.034639	0.051237
THORAX	2.5262376	0.141328
FOOD.TREATMENT[LOW]	-0.658256	0.05094
FOOD.TREATMENT[MEDIUM]	0.0529881	0.034794
GENETIC.STATUS[INCROSS]	0.0261118	0.032069
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0533694	0.040757
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.028207	0.03485

A.S7) Response of relative male wing length to G and E [1 - thorax controlled]

Wing Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	307.9	14.8288	0.0001
THORAX	1	390.2	881.9203	<.0001
FOOD.TREATMENT	2	351.6	54.46	<.0001
GENETIC.STATUS	1	23.67	2.6505	0.1168
GENETIC.STATUS*FOOD.TREATMENT	2	392.7	1.7995	0.1667

Term	Estimate	Std Error
Intercept	1.5308831	0.08606
EGGS.IN.PETRI.REARED.ON	-0.009847	0.011341
THORAX	0.4378817	0.031082
FOOD.TREATMENT[LOW]	-0.099532	0.011203
FOOD.TREATMENT[MEDIUM]	0.0152296	0.00771
GENETIC.STATUS[INCROSS]	0.0151822	0.007639
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0141253	0.00902
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.001049	0.007708

A.S8) Response of relative male testes length to G and E [1 - thorax controlled]

Testis Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	347.8	2.5192	0.1134
THORAX	1	379.5	58.0607	<.0001
FOOD.TREATMENT	2	353	1.485	0.2279
GENETIC.STATUS	1	38.12	1.5646	0.2186
GENETIC.STATUS*FOOD.TREATMENT	2	383.8	0.7769	0.4605

Term	Estimate	Std Error
Intercept	3.0609884	0.348635
EGGS.IN.PETRI.REARED.ON	-0.003975	0.0459
THORAX	0.446117	0.124887
FOOD.TREATMENT[LOW]	-0.10519	0.044672
FOOD.TREATMENT[MEDIUM]	0.0074577	0.030436
GENETIC.STATUS[INCROSS]	-0.053403	0.036202
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.041972	0.035339
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0133966	0.0305

A.S9) Response of relative accessory gland length to G and E [1 - thorax controlled]

Accessory Gland Length = Cross & Random + Number of Eggs per Petri Dish Reared on + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EGGS.IN.PETRI.REARED.ON	1	278.3	0.503	0.4788
THORAX	1	318.1	58.5149	<.0001
FOOD.TREATMENT	2	294.6	6.9723	0.0011
GENETIC.STATUS	1	39.14	16.0101	0.0003
GENETIC.STATUS*FOOD.TREATMENT	2	319.8	2.088	0.1256

Term	Estimate	Std Error
Intercept	0.905131	0.178924
EGGS.IN.PETRI.REARED.ON	-0.038087	0.024333
THORAX	0.3241625	0.061456
FOOD.TREATMENT[LOW]	-0.040312	0.022151
FOOD.TREATMENT[MEDIUM]	0.0544502	0.016099
GENETIC.STATUS[INCROSS]	-0.081174	0.019112
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.035772	0.017903
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0253389	0.016168

B) Effect of G x E on fertility traits

B.S1) Response of absolute spermatophore area to G and E

Spermatophore Area = Cross & Random + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
FOOD.TREATMENT	2	155.3	0.8093	0.447
GENETIC.STATUS	1	30.32	6.3439	0.0173
GENETIC.STATUS*FOOD.TREATMENT	2	154.2	0.1311	0.8772

Term	Estimate	Std Error
Intercept	0.0622504	0.001953
FOOD.TREATMENT[LOW]	0.0006844	0.002179
FOOD.TREATMENT[MEDIUM]	0.000572	0.00195
GENETIC.STATUS[INCROSS]	-0.004677	0.001953
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0003214	0.002179
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.000601	0.00195

B.S2) Response of absolute sperm area in spermatophore to G and E

Sperm Area = Cross & Random + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
FOOD.TREATMENT	2	150.6	4.8979	0.0087
GENETIC.STATUS	1	28.78	0.0027	0.9589
GENETIC.STATUS*FOOD.TREATMENT	2	148.4	0.1505	0.8604

Term	Estimate	Std Error
Intercept	0.0295724	0.00299
FOOD.TREATMENT[LOW]	0.0074955	0.003314
FOOD.TREATMENT[MEDIUM]	-0.004509	0.002966
GENETIC.STATUS[INCROSS]	-0.000439	0.00299
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.001813	0.003314
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0008471	0.002966

B.S3) Response of absolute number of sperm in ventral receptacle to G and E

VR Count [SQRT] = Cross & Random + Number of Days Eggs Laid Over & Random + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
FOOD.TREATMENT	2	194.9	3.3848	0.0359
GENETIC.STATUS	1	25.28	14.4735	0.0008
GENETIC.STATUS*FOOD.TREATMENT	2	193.6	4.7884	0.0093

Term	Estimate	Std Error
Intercept	5.9569987	0.480084
FOOD.TREATMENT[LOW]	0.3853581	0.540266
FOOD.TREATMENT[MEDIUM]	0.0168075	0.489043
GENETIC.STATUS[INCROSS]	-1.934459	0.480084
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.968142	0.540266
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.538345	0.489043

B.S4) Response of absolute offspring counts to G and E

Offspring Count [SQRT] = Cross & Random + Number of Days Eggs Laid Over & Random + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
FOOD.TREATMENT	2	177.6	0.6059	0.5467
GENETIC.STATUS	1	77.9	8.0815	0.0057
GENETIC.STATUS*FOOD.TREATMENT	2	166.6	0.5192	0.596

Term	Estimate	Std Error
Intercept	1.0569512	0.172194
FOOD.TREATMENT[LOW]	-0.247021	0.210712
FOOD.TREATMENT[MEDIUM]	0.2265596	0.183683
GENETIC.STATUS[INCROSS]	-0.509873	0.172194
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.203484	0.210712
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0613602	0.183683

B.S5) Response of relative spermatophore area to G and E [1 - thorax controlled]

Spermatophore Area = Cross & Random + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	148.2	0.1358	0.713
FOOD.TREATMENT	2	152.7	1.1084	0.3327
GENETIC.STATUS	1	31.49	5.8754	0.0213
GENETIC.STATUS*FOOD.TREATMENT	2	150.2	0.1294	0.8787

Term	Estimate	Std Error
Intercept	0.0584503	0.021293
THORAX	0.0016737	0.009295
FOOD.TREATMENT[LOW]	0.0010422	0.00294
FOOD.TREATMENT[MEDIUM]	0.000557	0.001979
GENETIC.STATUS[INCROSS]	-0.004628	0.001995
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0003226	0.002212
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0006152	0.001979

B.S6) Response of relative sperm area in spermatophore to G and E [1 - thorax controlled]

Sperm Area = Cross & Random + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	143.2	4.3839	0.038
FOOD.TREATMENT	2	147.8	2.9304	0.0565
GENETIC.STATUS	1	30.41	0.0005	0.9818
GENETIC.STATUS*FOOD.TREATMENT	2	143.8	0.2426	0.7849

Term	Estimate	Std Error
Intercept	0.0326073	0.032469
THORAX	-0.001466	0.014145
FOOD.TREATMENT[LOW]	0.0073883	0.004397
FOOD.TREATMENT[MEDIUM]	-0.004182	0.00294
GENETIC.STATUS[INCROSS]	-0.000279	0.003072
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.002122	0.003285
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0004681	0.00294

B.S7) Response of relative number of sperm in ventral receptacle to G and E [1 - thorax controlled]

VR Count [SQRT] = Cross & Random + Number of Days Eggs Laid Over & Random + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	174.3	1.5868	0.2095
FOOD.TREATMENT	2	173.2	7.1472	0.001
GENETIC.STATUS	1	81.6	15.4505	0.0002
GENETIC.STATUS*FOOD.TREATMENT	2	167.7	6.4805	0.0019

Term	Estimate	Std Error
Intercept	-0.135472	5.484633
THORAX	2.715059	2.413231
FOOD.TREATMENT[LOW]	1.0938057	0.818564
FOOD.TREATMENT[MEDIUM]	0.0631967	0.529958
GENETIC.STATUS[INCROSS]	-2.032642	0.460115
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-1.622174	0.608386
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.174966	0.530489

B.S8) Response of relative offspring counts to G and E [1 - thorax controlled]

Offspring Count [SQRT] = Cross & Random + Number of Days Eggs Laid Over & Random + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	169.4	0.5752	0.4492
FOOD.TREATMENT	2	167.1	1.9533	0.145
GENETIC.STATUS	1	77.18	7.1682	0.0091
GENETIC.STATUS*FOOD.TREATMENT	2	150.3	0.7351	0.4812

Term	Estimate	Std Error
Intercept	-1.135549	1.92366
THORAX	0.9792615	0.844854
FOOD.TREATMENT[LOW]	0.0069194	0.280198
FOOD.TREATMENT[MEDIUM]	0.2016284	0.183563
GENETIC.STATUS[INCROSS]	-0.508625	0.179395
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.239105	0.210168
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0651847	0.18382

B.S9) Response of relative spermatophore area to G and E [2 – testis controlled]

Spermatophore Area = Cross & Random + Testis Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVE.TESTES	1	154.5	0.0016	0.9684
FOOD.TREATMENT	2	151.3	1.032	0.3588
GENETIC.STATUS	1	31.64	5.8824	0.0212
GENETIC.STATUS*FOOD.TREATMENT	2	149.2	0.1833	0.8327

Term	Estimate	Std Error
Intercept	0.0607282	0.012956
AVE.TESTES	0.0004144	0.003063
FOOD.TREATMENT[LOW]	0.001132	0.002339
FOOD.TREATMENT[MEDIUM]	0.0003897	0.001998
GENETIC.STATUS[INCROSS]	-0.004507	0.001998
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0007134	0.002299
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0004661	0.001998

B.S10) Response of relative sperm area in spermatophore to G and E [2 - testis controlled]

Sperm Area = Cross & Random + Testis Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVE.TESTES	1	148.9	0.1797	0.6722
FOOD.TREATMENT	2	146.4	5.0892	0.0073
GENETIC.STATUS	1	28.93	0.0064	0.9366
GENETIC.STATUS*FOOD.TREATMENT	2	143.3	0.0457	0.9553

Term	Estimate	Std Error
Intercept	0.0273304	0.01955
AVE.TESTES	0.0006107	0.004622
FOOD.TREATMENT[LOW]	0.0084716	0.00351
FOOD.TREATMENT[MEDIUM]	-0.00478	0.003005
GENETIC.STATUS[INCROSS]	5.04E-05	0.003012
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.00104	0.003459
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0004527	0.003005

B.S11) Response of the relative number of sperm in ventral receptacle to G and E [2 -testis controlled]

VR Count [SQRT] = Cross & Random + Number of Days Eggs Laid Over & Random + Testis Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVE.TESTES	1	170.3	0.0343	0.8532
FOOD.TREATMENT	2	172.1	6.4635	0.002
GENETIC.STATUS	1	80.16	13.9919	0.0003
GENETIC.STATUS*FOOD.TREATMENT	2	168.7	6.8617	0.0014

Term	Estimate	Std Error
Intercept	3.3102563	3.491721
AVE.TESTES	0.6873086	0.832834
FOOD.TREATMENT[LOW]	0.5331179	0.638343
FOOD.TREATMENT[MEDIUM]	0.1545816	0.553775
GENETIC.STATUS[INCROSS]	-1.99674	0.460105
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-1.660976	0.614238
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.193182	0.549712

B.S12) Response of relative offspring counts to G and E [2 - testis controlled]

Offspring Count [SQRT] = Cross & Random + Number of Days Eggs Laid Over & Random + Testis Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVE.TESTES	1	167.3	1.1126	0.293
FOOD.TREATMENT	2	172.1	1.1339	0.3242
GENETIC.STATUS	1	72.57	6.5945	0.0123
GENETIC.STATUS*FOOD.TREATMENT	2	165.3	0.6192	0.5396

Term	Estimate	Std Error
Intercept	0.0570845	1.247014
AVE.TESTES	0.246271	0.296817
FOOD.TREATMENT[LOW]	-0.204355	0.22747
FOOD.TREATMENT[MEDIUM]	0.2624928	0.197776
GENETIC.STATUS[INCROSS]	-0.473499	0.174318
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.225817	0.21874
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0605038	0.196653

B.S13) Response of relative spermatophore area to G and E [3 - AG controlled]

Spermatophore Area = Cross & Random + Accessory Gland Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVERAGE.AG	1	135.5	2.7685	0.0984
FOOD.TREATMENT	2	146.2	1.4507	0.2378
GENETIC.STATUS	1	32.66	3.9944	0.054
GENETIC.STATUS*FOOD.TREATMENT	2	144.8	0.4217	0.6568

Term	Estimate	Std Error
Intercept	0.0489585	0.013943
AVERAGE.AG	0.0090156	0.008911
FOOD.TREATMENT[LOW]	0.0023896	0.002492
FOOD.TREATMENT[MEDIUM]	-0.000201	0.0021
GENETIC.STATUS[INCROSS]	-0.003701	0.002095
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0018097	0.002322
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.000168	0.002063

B.S14) Response of relative of sperm area in spermatophore to G and E [3 - AG controlled]

Sperm Area = Cross & Random + Accessory Gland Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVERAGE.AG	1	134.4	1.1467	0.2862
FOOD.TREATMENT	2	140.2	4.6869	0.0107
GENETIC.STATUS	1	31.81	0.0029	0.9572
GENETIC.STATUS*FOOD.TREATMENT	2	137.2	0.2543	0.7758

Term	Estimate	Std Error
Intercept	0.0352493	0.020876
AVERAGE.AG	-0.003403	0.013273
FOOD.TREATMENT[LOW]	0.0094905	0.003652
FOOD.TREATMENT[MEDIUM]	-0.006635	0.003071
GENETIC.STATUS[INCROSS]	-0.000177	0.003459
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0008655	0.003394
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.00208	0.003021

B.S15) Response of relative number of sperm in ventral receptacle to G and E [3 - AG controlled]

VR Count [SQRT] = Cross & Random + Number of Days Eggs Laid Over & Random + Accessory Gland Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVERAGE.AG	1	164.8	6.151	0.0141
FOOD.TREATMENT	2	163.7	7.1021	0.0011
GENETIC.STATUS	1	86.61	12.2416	0.0007
GENETIC.STATUS*FOOD.TREATMENT	2	160.9	6.1553	0.0027

Term	Estimate	Std Error
Intercept	-0.645674	3.548617
AVERAGE.AG	4.3552568	2.275222
FOOD.TREATMENT[LOW]	0.8653897	0.662087
FOOD.TREATMENT[MEDIUM]	-0.363822	0.570999
GENETIC.STATUS[INCROSS]	-1.939222	0.488732
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-1.463726	0.620716
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.359924	0.557555

B.S16) Response of relative offspring counts to G and E [3 - AG controlled]

Offspring Count [SQRT] = Cross & Random + Number of Days Eggs Laid Over & Random + Accessory Gland Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVERAGE.AG	1	173.6	0.905	0.3428
FOOD.TREATMENT	2	167.4	0.5803	0.5609
GENETIC.STATUS	1	75.01	8.1771	0.0055
GENETIC.STATUS*FOOD.TREATMENT	2	161.1	0.4845	0.6169

Term	Estimate	Std Error
Intercept	1.2806779	1.281231
AVERAGE.AG	-0.16295	0.822914
FOOD.TREATMENT[LOW]	-0.232643	0.239509
FOOD.TREATMENT[MEDIUM]	0.1944937	0.205667
GENETIC.STATUS[INCROSS]	-0.53331	0.181925
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.144345	0.22352
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.038375	0.200258

C) Effect of G x E on attractiveness and behavioural traits

C.S1) Response of absolute latency to mate to G and E

Latency to Mate [Log] = Cross & Random + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
FOOD.TREATMENT	2	187.4	0.1611	0.8514
GENETIC.STATUS	1	22.97	1.2342	0.2781
GENETIC.STATUS*FOOD.TREATMENT	2	188.4	0.7702	0.4644

Term	Estimate	Std Error
Intercept	5.148436	0.089042
FOOD.TREATMENT[LOW]	-0.039649	0.139679
FOOD.TREATMENT[MEDIUM]	-0.016304	0.124378
GENETIC.STATUS[INCROSS]	0.0705915	0.089042
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.173353	0.139679
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0898614	0.124378

C.S2) Response of absolute number of mating attempts made by a male until accepted by a female [i.e. inverse male attractiveness] to G and E

Inv. Male Attractiveness [SQRT] = Cross & Random + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
FOOD.TREATMENT	2	203.5	0.015	0.9851
GENETIC.STATUS	1	27.53	15.4267	0.0005
GENETIC.STATUS*FOOD.TREATMENT	2	204	0.0354	0.9653

Term	Estimate	Std Error
Intercept	1.3783646	0.075492
FOOD.TREATMENT[LOW]	0.0585852	0.105378
FOOD.TREATMENT[MEDIUM]	0.0019173	0.094348
GENETIC.STATUS[INCROSS]	0.2937133	0.075492
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0278764	0.105378
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.012106	0.094348

C.S3) Response of absolute length of successful mating to G and E

Mating Length [Log] = Cross & Random + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
FOOD.TREATMENT	2	167.4	4.0114	0.0199
GENETIC.STATUS	1	19.97	0.7914	0.3843
GENETIC.STATUS*FOOD.TREATMENT	2	166.6	0.7781	0.461

Term	Estimate	Std Error
Intercept	5.8586417	0.026031
FOOD.TREATMENT[LOW]	0.1017065	0.04043
FOOD.TREATMENT[MEDIUM]	-0.023545	0.03537
GENETIC.STATUS[INCROSS]	0.0234459	0.026031
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0149152	0.04043
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.041698	0.03537

C.S4) Response of relative latency to mate to G and E [1 - thorax controlled]

Latency to Mate [Log] = Cross & Random + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	182	0.2924	0.5893
FOOD.TREATMENT	2	173.6	0.4803	0.6194
GENETIC.STATUS	1	23.43	0.6498	0.4283
GENETIC.STATUS*FOOD.TREATMENT	2	181.6	0.7165	0.4899

Term	Estimate	Std Error
Intercept	6.3346683	1.375561
THORAX	-0.518733	0.60116
FOOD.TREATMENT[LOW]	-0.158453	0.195224
FOOD.TREATMENT[MEDIUM]	-0.005206	0.12694
GENETIC.STATUS[INCROSS]	0.0474913	0.091226
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.168225	0.142362
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.1044983	0.126789

C.S5) Response of relative number of mating attempts made by a male until accepted by a female [i.e. inverse of male attractiveness] to G and E [1 - thorax controlled]

Inv. Male Attractiveness [SQRT] = Cross & Random + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	193	0.0411	0.8396
FOOD.TREATMENT	2	191.8	0.032	0.9685
GENETIC.STATUS	1	27.29	17.4996	0.0003
GENETIC.STATUS*FOOD.TREATMENT	2	195.1	0.0553	0.9463

Term	Estimate	Std Error
Intercept	0.2558491	1.036197
THORAX	0.4955935	0.453939
FOOD.TREATMENT[LOW]	0.1687233	0.147446
FOOD.TREATMENT[MEDIUM]	-0.01455	0.095932
GENETIC.STATUS[INCROSS]	0.3169107	0.076649
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0221465	0.107046
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.031776	0.095899

C.S6) Response of relative length of successful mating to G and E [1 - thorax controlled]

Mating Length [Log] = Cross & Random + Thorax + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	163.9	11.2864	0.001
FOOD.TREATMENT	2	144.5	0.0974	0.9072
GENETIC.STATUS	1	18.11	0.7506	0.3976
GENETIC.STATUS*FOOD.TREATMENT	2	161.4	1.0102	0.3665

Term	Estimate	Std Error
Intercept	6.5273263	0.396113
THORAX	-0.293905	0.173471
FOOD.TREATMENT[LOW]	0.0422694	0.054915
FOOD.TREATMENT[MEDIUM]	-0.019709	0.035601
GENETIC.STATUS[INCROSS]	0.0228511	0.025682
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0134757	0.040744
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.046851	0.035592

C.S7) Response of relative latency to mate to G and E [2 - testis controlled]

Latency to Mate [Log] = Cross & Random + Testis Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVE.TESTES	1	162.7	0.0015	0.9696
FOOD.TREATMENT	2	177	0.6532	0.5216
GENETIC.STATUS	1	23.54	0.7552	0.3936
GENETIC.STATUS*FOOD.TREATMENT	2	178.4	0.1014	0.9036

Term	Estimate	Std Error
Intercept	4.9834546	0.841491
AVE.TESTES	0.0428674	0.200961
FOOD.TREATMENT[LOW]	0.068801	0.147123
FOOD.TREATMENT[MEDIUM]	-0.121646	0.129985
GENETIC.STATUS[INCROSS]	0.0689372	0.09205
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.054259	0.142732
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0033142	0.129444

C.S8) Response of relative number of mating attempts made by a male until accepted by a female [i.e. inverse of male attractiveness] response to G and E [2 - testis controlled]

Inv. Male Attractiveness [SQRT] = Cross & Random + Testis Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVE.TESTES	1	187.4	4.8302	0.0292
FOOD.TREATMENT	2	190.7	0.2052	0.8146
GENETIC.STATUS	1	27.86	11.7137	0.0019
GENETIC.STATUS*FOOD.TREATMENT	2	190.9	0.456	0.6345

Term	Estimate	Std Error
Intercept	2.5115171	0.639051
AVE.TESTES	-0.274585	0.152939
FOOD.TREATMENT[LOW]	0.0540283	0.108212
FOOD.TREATMENT[MEDIUM]	-0.059527	0.095159
GENETIC.STATUS[INCROSS]	0.2556682	0.074418
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0632574	0.104392
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.090319	0.094857

C.S9) Response of relative length of successful mating to G and E [2 - testis controlled]

Mating Length [Log] = Cross & Random + Testis Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVE.TESTES	1	127.8	1.8026	0.1818
FOOD.TREATMENT	2	161.9	2.8267	0.0621
GENETIC.STATUS	1	23.61	0.5592	0.462
GENETIC.STATUS*FOOD.TREATMENT	2	162	0.9561	0.3865

Term	Estimate	Std Error
Intercept	5.9937846	0.240844
AVE.TESTES	-0.034125	0.057341
FOOD.TREATMENT[LOW]	0.0927174	0.043194
FOOD.TREATMENT[MEDIUM]	-0.026057	0.036537
GENETIC.STATUS[INCROSS]	0.0188934	0.026211
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.009597	0.042291
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.044857	0.036509

C.S10) Response of relative latency to mate to G and E [3 - AG controlled]

Latency to Mate [Log] = Cross & Random + Accessory Gland Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVERAGE.AG	1	71.32	0.1992	0.6567
FOOD.TREATMENT	2	163.1	0.3694	0.6917
GENETIC.STATUS	1	26.09	1.4978	0.232
GENETIC.STATUS*FOOD.TREATMENT	2	168.6	0.8213	0.4416

Term	Estimate	Std Error
Intercept	4.2294182	0.877387
AVERAGE.AG	0.5562247	0.563827
FOOD.TREATMENT[LOW]	0.051337	0.15846
FOOD.TREATMENT[MEDIUM]	-0.038316	0.138438
GENETIC.STATUS[INCROSS]	0.0945725	0.099228
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.189242	0.147746
GENETIC.STATUS[INCROSSEFOOD.TREATMENT[MEDIUM]	0.0948838	0.136072

C.S11) Response of the relative number of mating attempts made by a male until accepted by a female [i.e. inverse male attractiveness] response to G and E [3 - AG controlled]

Inv. Male Attractiveness [SQRT] = Cross & Random + Accessory Gland Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVERAGE.AG	1	107.2	6.0216	0.0157
FOOD.TREATMENT	2	179.5	0.072	0.9305
GENETIC.STATUS	1	16.27	5.5961	0.0307
GENETIC.STATUS*FOOD.TREATMENT	2	177.8	0.4191	0.6583

Term	Estimate	Std Error
Intercept	2.1895771	0.634616
AVERAGE.AG	-0.553884	0.406678
FOOD.TREATMENT[LOW]	0.0674997	0.108055
FOOD.TREATMENT[MEDIUM]	-0.01865	0.092849
GENETIC.STATUS[INCROSS]	0.1982764	0.081625
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0718123	0.098135
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.078184	0.090163

C.S12) Reponses of the relative length of successful mating to G and E [3 - AG controlled]

Mating Length [Log] = Cross & Random + Accessory Gland Length + Food Treatment + Genetic Status + Food Treatment * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
AVERAGE.AG	1	16.3	8.1899	0.0111
FOOD.TREATMENT	2	133.6	1.3726	0.257
GENETIC.STATUS	1	14.19	0.2283	0.6401
GENETIC.STATUS*FOOD.TREATMENT	2	157	1.2058	0.3022

Term	Estimate	Std Error
Intercept	6.3523639	0.237841
AVERAGE.AG	-0.325012	0.154775
FOOD.TREATMENT[LOW]	0.0537037	0.044481
FOOD.TREATMENT[MEDIUM]	-0.013172	0.036926
GENETIC.STATUS[INCROSS]	0.0072387	0.025133
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.017319	0.041851
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.035658	0.036627

D) Absolute and relative eyespan-trait relationships, and the effects of E and G

D.S1) Absolute eyespan effect on testes length

Testes Length = Cross & Random + Eyespan

$R^2 = 0.30$

Variance Ratio [Cross/Residual] = 0.15

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	2.9678595	0.133197	412.2	22.28	<.0001*
EYESPAN	0.1546924	0.017632	401.8	8.77	<.0001*

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	401.8	76.9727	<.0001*

D.S2) Absolute eyespan effect on accessory gland length

Accessory Gland Length = Cross & Random + Eyespan

$R^2 = 0.38$

Variance Ratio [Cross/Residual] = 0.33

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	1.0196575	0.070073	345.3	14.55	<.0001*
EYESPAN	0.06556	0.00916	331.8	7.16	<.0001*

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	331.8	51.2294	<.0001*

D.S3) Absolute eyespan effect on spermatophore area

Spermatophore Area = Cross & Random + Eyespan

$R^2 = 0.32$

Variance Ratio [Cross/Residual] = 0.35

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.0647541	0.00873	156.1	7.42	<.0001*
EYESPAN	-0.000156	0.001165	145.2	-0.13	0.8938

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	145.2	0.0179	0.8938

D.S4) Absolute eyespan effect on sperm area

Sperm Area = Cross & Random + Eyespan

$R^2 = 0.30$

Variance Ratio [Cross/Residual] = 0.25

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.0615108	0.013409	151.4	4.59	<.0001*
EYESPAN	-0.004465	0.0018	144.1	-2.48	0.0143*

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	144.1	6.1528	0.0143*

D.S5) Absolute eyespan effect on number of sperm in ventral receptacle

VR Count [SQRT] = Cross & Random + Eyespan

$R^2 = 0.32$

Variance Ratio [Cross/Residual] = 0.33

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	7.679783	2.303731	192.1	3.33	0.0010*
EYESPAN	-0.153223	0.309432	178.1	-0.50	0.6211

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	178.1	0.2452	0.6211

D.S6) Absolute eyespan effect on offspring count

Offspring Count [SQRT] = Cross & Random + Eyespan

$R^2 = 0.17$

Variance Ratio [Cross/Residual] = 0.13

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.8534532	0.847425	186.2	1.01	0.3152
EYESPAN	0.0429691	0.114426	178	0.38	0.7077

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	178	0.1410	0.7077

D.S7) Absolute eyespan effect on number of mating attempts made by a male until accepted by a female [i.e. inverse male attractiveness]

Inv. Male Attractiveness [SQRT] = Cross & Random + Eyespan

$R^2 = 0.16$

Variance Ratio [Cross/Residual] = 0.11

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	1.8022964	0.447937	196.8	4.02	<.0001*
EYESPAN	-0.069955	0.060609	189.9	-1.15	0.2499

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	189.9	1.3322	0.2499

D.S8) Absolute eyespan effect on length of successful mating

Mating Length [log] = Cross & Random + Eyespan

$R^2 = 0.06$

Variance Ratio [Cross/Residual] = 0.02

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	6.4834497	0.157323	167.5	41.21	<.0001*
EYESPAN	-0.088047	0.021492	166.2	-4.10	<.0001*

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	166.2	16.7838	<.0001*

D.S9) Relative eyespan effect on testis length

Testis Length = Cross & Random + Thorax + Eyespan

$R^2 = 0.30$

Variance Ratio [Cross/Residual] = 0.15

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	2.78027	0.200824	410.7	13.84	<.0001*
THORAX	0.2007463	0.160293	407	1.25	0.2112
EYESPAN	0.1177066	0.03434	404.2	3.43	0.0007*

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	403.6	65.5294	<.0001*
EYESPAN	1	404.2	11.7487	0.0007*

D.S10) Relative eyespan effect on accessory gland length

Accessory Gland Length = Cross & Random + Thorax + Eyespan

$R^2 = 0.39$

Variance Ratio [Cross/Residual] = 0.33

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.8433931	0.102659	346.2	8.22	<.0001*
THORAX	0.191046	0.081712	336.3	2.34	0.0200*
EYESPAN	0.0299497	0.017737	334.1	1.69	0.0922

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	333.6	53.9073	<.0001*
EYESPAN	1	334.1	2.8512	0.0922

D.S11) Relative eyespan effect on spermatophore area

Spermatophore Area = Cross & Random + Thorax + Eyespan

$R^2 = 0.32$

Variance Ratio [Cross/Residual] = 0.34

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.065144	0.015788	154.1	4.13	<.0001*
THORAX	-0.000361	0.013091	153.8	-0.03	0.9781
EYESPAN	-0.000095	0.002614	151.1	-0.04	0.9710

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	147.8	0.0177	0.8943
EYESPAN	1	151.1	0.0013	0.9710

D.S12) Relative eyespan effect on sperm area

Sperm Area = Cross & Random + Thorax + Eyespan

$R^2 = 0.30$

Variance Ratio [Cross/Residual] = 0.25

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.0583352	0.024078	152.1	2.42	0.0166*
THORAX	0.0031874	0.019813	151.6	0.16	0.8724
EYESPAN	-0.005039	0.003971	148.6	-1.27	0.2064

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	147.3	4.3984	0.0377*
EYESPAN	1	148.6	1.6102	0.2064

D.S13) Relative eyespan effect on number of sperm in ventral receptacle

VR Count [SQRT] = Cross & Random + Thorax + Eyespan

$R^2 = 0.32$

Variance Ratio [Cross/Residual] = 0.33

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	8.9280946	4.146761	185.7	2.15	0.0326*
THORAX	-1.31097	3.593748	184.9	-0.36	0.7157
EYESPAN	0.0899505	0.732773	184.3	0.12	0.9024

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	177.7	0.3615	0.5484
EYESPAN	1	184.3	0.0151	0.9024

D.S14) Relative eyespan effect on offspring count

Offspring Count [SQRT] = Cross & Random + Thorax + Eyespan

$R^2 = 0.17$

Variance Ratio [Cross/Residual] = 0.13

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.2083346	1.543385	186.1	0.13	0.8928
THORAX	0.6734546	1.345784	186.6	0.50	0.6174
EYESPAN	-0.081707	0.274323	185.4	-0.30	0.7662

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	179	0.3081	0.5795
EYESPAN	1	185.4	0.0887	0.7662

D.S15) Relative eyespan effect on number of mating attempts made by a male until accepted by a female [i.e. inverse male attractiveness]

Inv. Male Attractiveness [SQRT] = Cross & Random + Thorax + Eyespan

$R^2 = 0.22$

Variance Ratio [Cross/Residual] = 0.15

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	-0.171598	0.781014	196.3	-0.22	0.8263
THORAX	2.0349388	0.663549	197.1	3.07	0.0025*
EYESPAN	-0.442896	0.134791	195.5	-3.29	0.0012*

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	189.3	0.1086	0.7421
EYESPAN	1	195.5	10.7964	0.0012*

D.S16) Relative eyespan effect on length of successful mating

Mating Length [log] = Cross & Random + Thorax + Eyespan

$R^2 = 0.06$

Variance Ratio [Cross/Residual] = 0.02

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	6.3756063	0.287869	166	22.15	<.0001*
THORAX	0.1071346	0.238574	164.4	0.45	0.6540
EYESPAN	-0.10716	0.047631	167.4	-2.25	0.0258*

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	167.7	11.8375	0.0007*
EYESPAN	1	167.4	5.0615	0.0258*

D.S17) Absolute eyespan, food level and genetic status effects on testis length

Testis Length = Cross & Random + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	394.4	75.5828	<.0001
FOOD.TREATMENT	2	385.8	0.2019	0.8173
GENETIC.STATUS	1	37.83	2.9188	0.0957
GENETIC.STATUS*FOOD.TREATMENT	2	398.6	0.9616	0.3832
GENETIC.STATUS*EYESPAN	1	399.5	0.1526	0.6963
FOOD.TREATMENT*EYESPAN	2	392.2	0.3958	0.6734
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	393.8	0.5016	0.6059

Term	Estimate	Std Error
Intercept	2.9898095	0.319699
EYESPAN	0.1447891	0.042115
FOOD.TREATMENT[LOW]	-0.070335	0.080236
FOOD.TREATMENT[MEDIUM]	0.0310299	0.054446
GENETIC.STATUS[INCROSS]	-0.068378	0.056356
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.090568	0.080236
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0193278	0.054446
GENETIC.STATUS[INCROSS]*(EYESPAN-7.32085)	-0.01142	0.042115
FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	-0.050504	0.056844
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.32085)	0.0378058	0.047962
GENETIC.STA[INC]*FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	-0.020128	0.056844
GENETIC.STA[INC]*FOOD.TREATMENT[MED]*(EYESPAN-7.32085)	0.0466958	0.047962

D.S18) Absolute eyespan, food level and genetic status effects on accessory gland length

Accessory Gland Length = Cross & Random + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	329.6	52.8558	<.0001
FOOD.TREATMENT	2	322.2	10.193	<.0001
GENETIC.STATUS	1	36.53	20.5499	<.0001

GENETIC.STATUS*FOOD.TREATMENT	2	334.3	2.7234	0.0671
GENETIC.STATUS*EYESPAN	1	332	0.3751	0.5406
FOOD.TREATMENT*EYESPAN	2	327.9	0.2796	0.7563
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	330.5	1.3765	0.2539

Term	Estimate	Std Error
Intercept	0.8333678	0.15864
EYESPAN	0.0889606	0.02085
FOOD.TREATMENT[LOW]	0.0094166	0.03842
FOOD.TREATMENT[MEDIUM]	0.0454647	0.027096
GENETIC.STATUS[INCROSS]	-0.070785	0.028235
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.079983	0.03842
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0093965	0.027096
GENETIC.STATUS[INCROSS]*(EYESPAN-7.32085)	-0.027818	0.02085
FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	0.0117938	0.027983
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.32085)	0.0011157	0.024168
GENETIC.STA[INC]*FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	0.0110878	0.027983
GENETIC.STA[INC]*FOOD.TREATMENT[MED]*(EYESPAN-7.32085)	0.0374934	0.024168

D.S19) Absolute eyespan, food level and genetic status effects on spermatophore area

Spermatophore Area = Cross & Random + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	141.6	0.2318	0.6309
FOOD.TREATMENT	2	147.7	1.1208	0.3288
GENETIC.STATUS	1	29.74	5.8741	0.0217
GENETIC.STATUS*FOOD.TREATMENT	2	145.3	0.0844	0.9191
GENETIC.STATUS*EYESPAN	1	150.7	1.5426	0.2162
FOOD.TREATMENT*EYESPAN	2	145.2	1.1583	0.3169
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	143.2	0.5942	0.5534

Term	Estimate	Std Error
Intercept	0.084	0.021902
EYESPAN	-0.002978	0.002943
FOOD.TREATMENT[LOW]	-0.005602	0.004998
FOOD.TREATMENT[MEDIUM]	0.0004415	0.00346
GENETIC.STATUS[INCROSS]	-0.007936	0.003387

GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.005655	0.004998
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0040527	0.00346
GENETIC.STATUS[INCROSS]*(EYESPAN-7.32085)	-0.003133	0.002943
FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	-0.002199	0.004071
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.32085)	0.006019	0.003458
GENETIC.STA[INC]*FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	-0.004396	0.004071
GENETIC.STA[INC]*FOOD.TREATMENT[MED]*(EYESPAN-7.32085)	0.0013408	0.003458

D.S20) Absolute eyespan, food level and genetic status effects on sperm area

Sperm Area = Cross & Random + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	135.3	6.4283	0.0124
FOOD.TREATMENT	2	142.2	2.1166	0.1242
GENETIC.STATUS	1	29.8	0.001	0.9745
GENETIC.STATUS*FOOD.TREATMENT	2	138.8	0.3977	0.6727
GENETIC.STATUS*EYESPAN	1	143.8	2.623	0.1075
FOOD.TREATMENT*EYESPAN	2	137	3.1416	0.0463
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	135.8	2.1944	0.1154

Term	Estimate	Std Error
Intercept	0.0719741	0.031781
EYESPAN	-0.006914	0.004265
FOOD.TREATMENT[LOW]	-0.011139	0.007232
FOOD.TREATMENT[MEDIUM]	0.0036582	0.005001
GENETIC.STATUS[INCROSS]	-0.004246	0.004971
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.020615	0.007232
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0046981	0.005001
GENETIC.STATUS[INCROSS]*(EYESPAN-7.32085)	-0.010139	0.004265
FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	-0.014723	0.005886
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.32085)	0.012898	0.004988
GENETIC.STA[INC]*FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	-0.007874	0.005886
GENETIC.STA[INC]*FOOD.TREATMENT[MED]*(EYESPAN-7.32085)	0.0092728	0.004988

D.S21) Absolute eyespan, food level and genetic status effects on number of sperm in ventral receptacle

VR Count [SQRT] = Cross & Random + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	172	0.6417	0.4242
FOOD.TREATMENT	2	182.8	6.2468	0.0024
GENETIC.STATUS	1	23.06	14.4467	0.0009
GENETIC.STATUS*FOOD.TREATMENT	2	179.1	5.3144	0.0057
GENETIC.STATUS*EYESPAN	1	186.2	1.2707	0.2611
FOOD.TREATMENT*EYESPAN	2	175.8	0.7101	0.493
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	175	0.4984	0.6084

Term	Estimate	Std Error
Intercept	1.784126	5.477409
EYESPAN	0.6975867	0.716644
FOOD.TREATMENT[LOW]	1.7969078	1.177941
FOOD.TREATMENT[MEDIUM]	-0.872023	0.859418
GENETIC.STATUS[INCROSS]	-1.658259	0.847654
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-2.305418	1.177941
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.804516	0.859418
GENETIC.STATUS[INCROSS]*(EYESPAN-7.32085)	-0.969691	0.716644
FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	1.0061151	0.924774
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.32085)	0.230385	0.822748
GENETIC.STA[INC]*FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	0.1703777	0.924774
GENETIC.STA[INC]*FOOD.TREATMENT[MED]*(EYESPAN-7.32085)	0.8017093	0.822748

D.S22) Absolute eyespan, food level and genetic status effects on offspring count

Offspring Count [SQRT] = Cross & Random + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	171	0.1126	0.7376
FOOD.TREATMENT	2	174	1.606	0.2036

GENETIC.STATUS	1	24.53	5.16	0.0322
GENETIC.STATUS*FOOD.TREATMENT	2	175.6	0.4989	0.6081
GENETIC.STATUS*EYESPAN	1	179	1.2771	0.26
FOOD.TREATMENT*EYESPAN	2	175.2	0.9877	0.3745
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	173.5	0.6723	0.5119

Term	Estimate	Std Error
Intercept	-2.703284	2.070949
EYESPAN	0.4604619	0.275484
FOOD.TREATMENT[LOW]	0.1596144	0.515606
FOOD.TREATMENT[MEDIUM]	0.6033543	0.346612
GENETIC.STATUS[INCROSS]	-0.446884	0.336131
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.347538	0.515606
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0647643	0.346612
GENETIC.STATUS[INCROSS]*(EYESPAN-7.32085)	0.1215342	0.275484
FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	-0.429305	0.369925
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.32085)	-0.204655	0.325738
GENETIC.STA[INC]*FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	-0.222778	0.369925
GENETIC.STA[INC]*FOOD.TREATMENT[MED]*(EYESPAN-7.32085)	0.344135	0.325738

D.S23) Absolute eyespan, food level and genetic status effects on number of mating attempts made by a male until accepted by a female [i.e. inverse male attractiveness]

Inv. Male Attractiveness [SQRT] = Cross & Random + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	185.3	1.3007	0.2556
FOOD.TREATMENT	2	180.6	1.3229	0.2689
GENETIC.STATUS	1	25.29	16.5912	0.0004
GENETIC.STATUS*FOOD.TREATMENT	2	190.2	0.0321	0.9684
GENETIC.STATUS*EYESPAN	1	190.2	11.2485	0.001
FOOD.TREATMENT*EYESPAN	2	189.4	0.4907	0.613
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	189.6	0.6049	0.5472

Term	Estimate	Std Error
Intercept	2.8798646	1.055361
EYESPAN	-0.23437	0.137927

FOOD.TREATMENT[LOW]	-0.353237	0.228764
FOOD.TREATMENT[MEDIUM]	0.1908971	0.165651
GENETIC.STATUS[INCROSS]	0.1037563	0.15426
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.464176	0.228764
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.1747195	0.165651
GENETIC.STATUS[INCROSS]*(EYESPAN-7.32085)	-0.321076	0.137927
FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	-0.217747	0.178924
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.32085)	-0.004842	0.162551
GENETIC.STA[INC]*FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	-0.182574	0.178924
GENETIC.STA[INC]*FOOD.TREATMENT[MED]*(EYESPAN-7.32085)	-0.059626	0.162551

D.S24) Absolute eyespan, food level and genetic status effects on length of successful mating

Mating Length [log] = Cross & Random + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	155.2	16.7777	<.0001
FOOD.TREATMENT	2	136.4	0.3138	0.7312
GENETIC.STATUS	1	18.55	0.4042	0.5327
GENETIC.STATUS*FOOD.TREATMENT	2	155.5	0.9652	0.3832
GENETIC.STATUS*EYESPAN	1	156.1	0.994	0.3203
FOOD.TREATMENT*EYESPAN	2	153	2.216	0.1125
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	157.1	1.3206	0.2699

Term	Estimate	Std Error
Intercept	6.9089224	0.382378
EYESPAN	-0.13066	0.050679
FOOD.TREATMENT[LOW]	0.0617749	0.086876
FOOD.TREATMENT[MEDIUM]	-0.122903	0.061054
GENETIC.STATUS[INCROSS]	0.0783825	0.056244
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0682312	0.086876
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.109069	0.061054
GENETIC.STATUS[INCROSS]*(EYESPAN-7.32085)	-0.02303	0.050679
FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	0.1785797	0.068473
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.32085)	-0.041681	0.060028
GENETIC.STA[INC]*FOOD.TREATMENT[LOW]*(EYESPAN-7.32085)	0.1076806	0.068473
GENETIC.STA[INC]*FOOD.TREATMENT[MED]*(EYESPAN-7.32085)	-0.038629	0.060028

D.S25) Relative eyespan, food level and genetic status effects on testis length

Testis Length = Cross & Random + Thorax + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	398.4	66.4058	<.0001
EYESPAN	1	402	9.7675	0.0019
FOOD.TREATMENT	2	384.3	0.1204	0.8866
GENETIC.STATUS	1	38.74	2.4999	0.122
GENETIC.STATUS*FOOD.TREATMENT	2	398.5	0.941	0.3911
GENETIC.STATUS*EYESPAN	1	400	0.1029	0.7486
FOOD.TREATMENT*EYESPAN	2	392.4	0.4904	0.6128

Term	Estimate	Std Error
Intercept	2.8199482	0.351558
THORAX	0.1716409	0.165565
EYESPAN	0.1158275	0.050722
FOOD.TREATMENT[LOW]	-0.059163	0.074258
FOOD.TREATMENT[MEDIUM]	0.0225646	0.052297
GENETIC.STATUS[INCROSS]	-0.062036	0.036174
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.036054	0.054699
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0098322	0.029794
GENETIC.STATUS[INCROSS]*(EYESPAN-7.31824)	0.0066487	0.034127
FOOD.TREATMENT[LOW]*(EYESPAN-7.31824)	-0.039156	0.053538
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.31824)	0.0324711	0.04627

D.S26) Relative eyespan, food level and genetic status effects on accessory gland length

Accessory Gland Length = Cross & Random + Thorax + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	334.8	64.0324	<.0001
EYESPAN	1	335.4	0.572	0.45
FOOD.TREATMENT	2	320.2	11.807	<.0001
GENETIC.STATUS	1	37.19	16.7305	0.0002

GENETIC.STATUS*FOOD.TREATMENT	2	332.1	2.4367	0.089
GENETIC.STATUS*EYESPAN	1	330	0.5428	0.4618
FOOD.TREATMENT*EYESPAN	2	326.2	0.1229	0.8844
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	328.3	1.3388	0.2636

Term	Estimate	Std Error
Intercept	0.6533991	0.177323
THORAX	0.1821709	0.081818
EYESPAN	0.0562129	0.025354
FOOD.TREATMENT[LOW]	0.0059344	0.038192
FOOD.TREATMENT[MEDIUM]	0.0544661	0.027219
GENETIC.STATUS[INCROSS]	-0.06457	0.028563
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.078923	0.038171
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.005706	0.026967
GENETIC.STATUS[INCROSS]*(EYESPAN-7.27732)	-0.030091	0.020719
FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	0.0062613	0.027893
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.27732)	0.0006329	0.023992
GENETIC.ST[INCRO]*FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	0.0133496	0.027797
GENETIC.S[INCROSS]*FOOD.TREATMENT[ME]*(EYESPAN-7.27732)	0.0358477	0.023999

D.S27) Relative eyespan, food level and genetic status effects on spermatophore area

Spermatophore Area = Cross & Random + Thorax + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	142.7	0.1678	0.6827
EYESPAN	1	147.8	0.0633	0.8017
FOOD.TREATMENT	2	146.6	1.1359	0.3239
GENETIC.STATUS	1	30.42	5.9374	0.0209
GENETIC.STATUS*FOOD.TREATMENT	2	144.4	0.0812	0.922
GENETIC.STATUS*EYESPAN	1	149.4	1.489	0.2243
FOOD.TREATMENT*EYESPAN	2	144.4	1.1293	0.3261
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	142.4	0.5964	0.5522

Term	Estimate	Std Error
Intercept	0.0859268	0.025702
THORAX	-0.001959	0.013552
EYESPAN	-0.002631	0.003808

FOOD.TREATMENT[LOW]	-0.005624	0.005018
FOOD.TREATMENT[MEDIUM]	0.0004577	0.003473
GENETIC.STATUS[INCROSS]	-0.007974	0.003408
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.00569	0.005022
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.004046	0.003472
GENETIC.STATUS[INCROSS]*(EYESPAN-7.27732)	-0.003136	0.002952
FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	-0.002249	0.004097
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.27732)	0.0060074	0.00347
GENETIC.ST[INCRO]*FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	-0.004416	0.004086
GENETIC.S[INCROSS]*FOOD.TREATMENT[ME]*(EYESPAN-7.27732)	0.0013941	0.003492

D.S28) Relative eyespan, food level and genetic status effects on sperm area

Sperm Area = Cross & Random + Thorax + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	137.2	4.7922	0.0303
EYESPAN	1	141.6	1.4468	0.231
FOOD.TREATMENT	2	141.1	2.1069	0.1254
GENETIC.STATUS	1	30.39	0.0012	0.9729
GENETIC.STATUS*FOOD.TREATMENT	2	137.8	0.3946	0.6747
GENETIC.STATUS*EYESPAN	1	142.3	2.628	0.1072
FOOD.TREATMENT*EYESPAN	2	136.2	3.1333	0.0467
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	135.1	2.149	0.1206

Term	Estimate	Std Error
Intercept	0.0717598	0.037538
THORAX	0.0002625	0.019916
EYESPAN	-0.006966	0.005535
FOOD.TREATMENT[LOW]	-0.011129	0.007267
FOOD.TREATMENT[MEDIUM]	0.0036534	0.005023
GENETIC.STATUS[INCROSS]	-0.00424	0.005005
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.020611	0.007269
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0047072	0.005019
GENETIC.STATUS[INCROSS]*(EYESPAN-7.27732)	-0.010142	0.004281
FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	-0.014714	0.005931
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.27732)	0.0128959	0.005008
GENETIC.ST[INCRO]*FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	-0.007876	0.005913
GENETIC.S[INCROSS]*FOOD.TREATMENT[ME]*(EYESPAN-7.27732)	0.0092657	0.00504

D.S29) Relative eyespan, food level and genetic status effects on number of sperm in ventral receptacle

VR Count [SQRT] = Cross & Random + Thorax + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	171.8	0.4885	0.4855
EYESPAN	1	181	0.1429	0.7058
FOOD.TREATMENT	2	181.7	6.2469	0.0024
GENETIC.STATUS	1	23.33	14.6122	0.0009
GENETIC.STATUS*FOOD.TREATMENT	2	178.1	5.3088	0.0058
GENETIC.STATUS*EYESPAN	1	185.2	1.1391	0.2872
FOOD.TREATMENT*EYESPAN	2	174.7	0.7191	0.4886
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	173.3	0.5518	0.5769

Term	Estimate	Std Error
Intercept	3.73506	6.34674
THORAX	-2.143828	3.512818
EYESPAN	1.106794	0.982705
FOOD.TREATMENT[LOW]	1.8181059	1.180344
FOOD.TREATMENT[MEDIUM]	-0.873697	0.860613
GENETIC.STATUS[INCROSS]	-1.656433	0.849472
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-2.305416	1.179671
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.827904	0.861272
GENETIC.STATUS[INCROSS]*(EYESPAN-7.27732)	-0.955877	0.718051
FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	1.0207349	0.926387
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.27732)	0.2105169	0.824533
GENETIC.ST[INCRO]*FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	0.1990899	0.927195
GENETIC.S[INCROSS]*FOOD.TREATMENT[ME]*(EYESPAN-7.27732)	0.8441827	0.826486

D.S30) Relative eyespan, food level and genetic status effects on offspring count

Offspring Count [SQRT] = Cross & Random + Thorax + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	171.9	0.3002	0.5845
EYESPAN	1	177.8	0.1421	0.7067
FOOD.TREATMENT	2	172.9	1.6564	0.1938
GENETIC.STATUS	1	24.38	4.9061	0.0363
GENETIC.STATUS*FOOD.TREATMENT	2	174.4	0.4847	0.6167
GENETIC.STATUS*EYESPAN	1	177.8	1.2165	0.2715
FOOD.TREATMENT*EYESPAN	2	174.2	0.9708	0.3808
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	172.1	0.6665	0.5148

Term	Estimate	Std Error
Intercept	-2.844744	2.394073
THORAX	0.1610701	1.366168
EYESPAN	0.4288114	0.385919
FOOD.TREATMENT[LOW]	0.154353	0.519028
FOOD.TREATMENT[MEDIUM]	0.6058131	0.348081
GENETIC.STATUS[INCROSS]	-0.448152	0.337308
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.349796	0.517406
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.0674442	0.34845
GENETIC.STATUS[INCROSS]*(EYESPAN-7.27732)	0.1201744	0.27643
FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	-0.431765	0.371468
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.27732)	-0.201656	0.327634
GENETIC.ST[INCRO]*FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	-0.225736	0.372065
GENETIC.S[INCROSS]*FOOD.TREATMENT[ME]*(EYESPAN-7.27732)	0.3421594	0.326961

D.S31) Relative eyespan, food level and genetic status effects on number of mating attempts made by a male until accepted by a female [i.e. inverse male attractiveness]

Inv. Male Attraction [SQRT] = Cross & Random + Thorax + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	185.7	0.0527	0.8187
EYESPAN	1	189.6	9.6412	0.0022
FOOD.TREATMENT	2	181.2	1.3829	0.2535
GENETIC.STATUS	1	23.25	19.8738	0.0002
GENETIC.STATUS*FOOD.TREATMENT	2	188.6	0.1041	0.9012

GENETIC.STATUS*EYESPAN	1	189.7	15.2241	0.0001
FOOD.TREATMENT*EYESPAN	2	187.8	0.5017	0.6063
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	187.8	1.0638	0.3472

Term	Estimate	Std Error
Intercept	0.5167852	1.173385
THORAX	2.5892198	0.644931
EYESPAN	-0.725939	0.180666
FOOD.TREATMENT[LOW]	-0.378921	0.220406
FOOD.TREATMENT[MEDIUM]	0.1999209	0.159234
GENETIC.STATUS[INCROSS]	0.1004409	0.149456
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.463792	0.220275
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	0.2087616	0.159454
GENETIC.STATUS[INCROSS]*(EYESPAN-7.27732)	-0.341834	0.132764
FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	-0.215969	0.172541
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.27732)	0.025375	0.156316
GENETIC.ST[INCRO]*FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	-0.217259	0.172848
GENETIC.S[INCROSS]*FOOD.TREATMENT[ME]*(EYESPAN-7.27732)	-0.107842	0.156515

D.S32) Relative eyespan, food level and genetic status effects on length of successful mating

Mating Length [log] = Cross & Random + Thorax + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Food Treatment + Eyespan * Genetic Status + Eyespan * Food Level * Genetic Status

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	157.6	11.8559	0.0007
EYESPAN	1	157.4	4.9478	0.0275
FOOD.TREATMENT	2	135.7	0.3101	0.7339
GENETIC.STATUS	1	18.53	0.4761	0.4987
GENETIC.STATUS*FOOD.TREATMENT	2	154.6	0.9544	0.3873
GENETIC.STATUS*EYESPAN	1	155.4	1.0889	0.2983
FOOD.TREATMENT*EYESPAN	2	152	2.2513	0.1088
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	156.1	1.3212	0.2698

Term	Estimate	Std Error
Intercept	6.7569709	0.44824
THORAX	0.1611201	0.245482
EYESPAN	-0.160446	0.067908
FOOD.TREATMENT[LOW]	0.060939	0.087029

FOOD.TREATMENT[MEDIUM]	-0.123343	0.061161
GENETIC.STATUS[INCROSS]	0.0783686	0.056314
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0683569	0.08703
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.106747	0.061257
GENETIC.STATUS[INCROSS]*(EYESPAN-7.27732)	-0.023978	0.050777
FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	0.1803575	0.068659
FOOD.TREATMENT[MEDIUM]*(EYESPAN-7.27732)	-0.039357	0.060242
GENETIC.ST[INCRO]*FOOD.TREATMENT[LOW]*(EYESPAN-7.27732)	0.1067532	0.068582
GENETIC.S[INCROSS]*FOOD.TREATMENT[ME]*(EYESPAN-7.27732)	-0.041925	0.060338

D.S33) Effect of absolute eyespan SPLIT BY environmental state on sperm area

Sperm Area = Cross & Random + Thorax + Eyespan, at:

LOW

$R^2 = 0.51$

Variance Ratio [Cross/Residual] = 0.54

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.0871778	0.037143	39.25	2.35	0.0241*
EYESPAN	-0.00785	0.006031	38.58	-1.30	0.2008

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	38.58	1.6940	0.2008

INTERMEDIATE

$R^2 = 0.35$

Variance Ratio [Cross/Residual] = 0.32

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	-0.021019	0.034114	58.89	-0.62	0.5402
EYESPAN	0.0062693	0.004663	58.95	1.34	0.1839

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	58.95	1.8077	0.1839

HIGH

$R^2 = 0.11$

Variance Ratio [Cross/Residual] = 0.06

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.0701617	0.072163	50.35	0.97	0.3356
EYESPAN	-0.005499	0.008727	50.29	-0.63	0.5315

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	50.29	0.3970	0.5315

D.S34) Effect of absolute eyespan SPLIT BY genetic status on number of mating attempts made by a male until accepted by a female [i.e. inverse male attractiveness]

Inv. Male Attractivness = Cross & Random + Thorax + Eyespan: at:

INCROSS

$R^2 = 0.06$

Variance Ratio [Cross/Residual] = 0.02

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	3.5237122	1.240226	69.95	2.84	0.0059*
EYESPAN	-0.252167	0.16646	68.73	-1.51	0.1344

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	68.73	2.2948	0.1344

OUTCROSS

$R^2 = 0.22$

Variance Ratio [Cross/Residual] = 0.23

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	1.0554205	0.225762	123.2	4.67	<.0001*
EYESPAN	0.0046817	0.030607	111.3	0.15	0.8787

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	111.3	0.0234	0.8787

D.S35) Effect of relative eyespan SPLIT BY environmental state on testis length

Testis Length = Cross & Random + Thorax + Eyespan, at:

LOW

$R^2 = 0.23$

Variance Ratio [Cross/Residual] = 0.09

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	2.3854138	0.478866	90.79	4.98	<.0001*
THORAX	0.9675188	0.356192	90.96	2.72	0.0079*
EYESPAN	-0.080097	0.099108	90.99	-0.81	0.4211

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	90.75	10.3622	0.0018*
EYESPAN	1	90.99	0.6531	0.4211

INTERMEDIATE

$R^2 = 0.30$

Variance Ratio [Cross/Residual] = 0.17

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	3.0560531	0.418896	153.3	7.30	<.0001*
THORAX	-0.29669	0.259035	153.9	-1.15	0.2538
EYESPAN	0.231977	0.059914	150	3.87	0.0002*

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	153.5	4.5750	0.0340*
EYESPAN	1	150	14.9909	0.0002*

HIGH

$R^2 = 0.22$

Variance Ratio [Cross/Residual] = 0.15

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	2.4533047	0.790896	158.6	3.10	0.0023*
THORAX	0.3457826	0.277338	158.5	1.25	0.2143
EYESPAN	0.1138113	0.101314	158	1.12	0.2630

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	159.7	4.3426	0.0388*
EYESPAN	1	158	1.2619	0.2630

D.S36) Effect of relative eyespan SPLIT BY environmental state on accessory gland length

Accessory Gland = Cross & Random + Thorax + Eyespan, at:

LOW

R^2 = 0. 69

Variance Ratio [Cross/Residual] = 1.11

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.5465324	0.193168	73.51	2.83	0.0060*
THORAX	0.3355477	0.147866	74.36	2.27	0.0262*
EYESPAN	0.0314348	0.04229	76.22	0.74	0.4596

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	70.68	20.5811	<.0001*
EYESPAN	1	76.22	0.5525	0.4596

INTERMEDIATE

R^2 = 0.43

Variance Ratio [Cross/Residual] = 0.35

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.6649529	0.195219	122.3	3.41	0.0009*
THORAX	0.218433	0.127918	120.7	1.71	0.0903
EYESPAN	0.0537636	0.030344	116.6	1.77	0.0790

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	121.6	19.3913	<.0001*
EYESPAN	1	116.6	3.1393	0.0790

HIGH

R^2 = 0.20

Variance Ratio [Cross/Residual] = 0.09

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.2538587	0.475584	131	0.53	0.5944
THORAX	0.2589527	0.16011	135	1.62	0.1081
EYESPAN	0.0755985	0.059617	134.8	1.27	0.2070

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	132.7	6.1919	0.0141*
EYESPAN	1	134.8	1.6080	0.2070

D.S37) Effect of relative eyespan SPLIT BY environmental state on sperm area

Sperm Area = Cross & Random + Thorax + Eyespan, at:

LOW

$R^2 = 0.51$

Variance Ratio [Cross/Residual] = 0.53

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.083896	0.059165	37.79	1.42	0.1644
THORAX	0.0031597	0.044076	41.46	0.07	0.9432
EYESPAN	-0.008399	0.009831	41.87	-0.85	0.3978

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	36.33	0.9376	0.3393
EYESPAN	1	41.87	0.7298	0.3978

INTERMEDIATE

$R^2 = 0.38$

Variance Ratio [Cross/Residual] = 0.36

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.0161931	0.050269	55.18	0.32	0.7486
THORAX	-0.032592	0.031434	56.87	-1.04	0.3042
EYESPAN	0.0114219	0.006674	56.78	1.71	0.0925

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	54.92	0.0722	0.7891
EYESPAN	1	56.78	2.9285	0.0925

HIGH

$R^2 = 0.02$

Variance Ratio [Cross/Residual] = 0.01

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.0437196	0.078305	49.96	0.56	0.5791
THORAX	0.0400032	0.036778	45.21	1.09	0.2825
EYESPAN	-0.014391	0.01175	49.42	-1.22	0.2265

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	48.34	0.1559	0.6947
EYESPAN	1	49.42	1.5000	0.2265

D.S38) Effect of relative eyespan SPLIT BY environmental state on number of mating attempts made by a male until accepted by a female [i.e. inverse male attractiveness]

Inv. Male Attractiveness = Cross & Random + Thorax + Eyespan, at:

LOW

$R^2 = 0.26$

Variance Ratio [Cross/Residual] = 0.15

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	1.7936544	1.559826	50.73	1.15	0.2556
THORAX	1.1218005	1.229969	53.08	0.91	0.3659
EYESPAN	-0.471731	0.287898	53.48	-1.64	0.1072

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	51.27	0.5504	0.4615
EYESPAN	1	53.48	2.6848	0.1072

INTERMEDIATE

$R^2 = 0.44$

Variance Ratio [Cross/Residual] = 0.44

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	-0.033803	1.594026	71.4	-0.02	0.9831
THORAX	2.580138	1.052743	64.33	2.45	0.0170*
EYESPAN	-0.625652	0.234663	69.68	-2.67	0.0095*

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	72.36	0.3040	0.5831
EYESPAN	1	69.68	7.1085	0.0095*

HIGH

$R^2 = 0.21$

Variance Ratio [Cross/Residual] = 0.11

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	1.7572404	3.089413	66.16	0.57	0.5714
THORAX	1.9226771	1.282593	66.63	1.50	0.1386
EYESPAN	-0.640442	0.447591	65.84	-1.43	0.1572

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	66.62	0.6430	0.4255
EYESPAN	1	65.84	2.0474	0.1572

D.S39) Effect of relative eyespan SPLIT BY genetic status on number of mating attempts made by a male until accepted by a female [i.e. inverse male attractiveness]

Inv. Male Attractiveness = Cross & Random + Thorax + Eyespan, at:

INCROSS

$R^2 = 0.25$

Variance Ratio [Cross/Residual] = 0.07

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	-0.501207	1.689423	68.44	-0.30	0.7676
THORAX	4.8103726	1.444412	67.66	3.33	0.0014*
EYESPAN	-1.208395	0.323285	66.28	-3.74	0.0004*

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	67.45	0.0227	0.8807
EYESPAN	1	66.28	13.9716	0.0004*

OUTCROSS

$R^2 = 0.22$

Variance Ratio [Cross/Residual] = 0.21

Term	Estimate	Std Error	DFDen	t Ratio	Prob>|t|
Intercept	0.7450253	0.469448	122.9	1.59	0.1151
THORAX	0.3035741	0.39996	123.7	0.76	0.4493
EYESPAN	-0.049265	0.077173	122.7	-0.64	0.5244

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	112.8	0.2056	0.6511
EYESPAN	1	122.7	0.4075	0.5244

The traits included in these splits were those with a significant or near significant ES x E or ES x G interaction in the full models, or in which notable patterns were found.

E) Integration of E, G and G x E for morphological and reproductive traits

E.S1) Model effect coefficients for absolute trait values

	Effect Coefficients						Standard Error					
Trait	E1	E2	E3	G	GxE1	GxE2	E1	E2	E3	G	GxE1	GxE2
ES	1.088	-0.012	0.538	0.046	-0.044	0.001	0.05	0.04	0.04	0.04	0.05	0.04
T	0.900	0.082	0.491	0.138	0.011	-0.066	0.06	0.05	0.05	0.05	0.06	0.05
W	0.977	-0.072	0.453	0.041	-0.110	-0.040	0.08	0.07	0.07	0.06	0.08	0.07
Testes	0.410	0.059	0.234	0.188	0.044	0.040	0.09	0.07	0.08	0.09	0.09	0.07
AG	0.503	-0.235	0.134	0.396	0.096	-0.066	0.08	0.08	0.08	0.08	0.08	0.08
Spermatophore	-0.041	-0.034	-0.038	0.282	-0.019	-0.036	0.13	0.12	0.12	0.12	0.13	0.12
Sperm Area	-0.305	0.177	-0.064	0.008	0.077	-0.030	0.13	0.12	0.13	0.12	0.13	0.12
VR Count	-0.115	-0.004	-0.059	0.358	0.186	0.104	0.10	0.09	0.10	0.09	0.10	0.09
OFF	0.105	-0.134	-0.014	0.220	0.039	0.017	0.12	0.11	0.12	0.10	0.12	0.11
M. Latency	0.021	-0.034	-0.007	0.113	-0.190	0.071	0.12	0.11	0.11	0.08	0.12	0.11
M. Length	-0.414	0.047	-0.184	-0.173	-0.125	0.112	0.13	0.12	0.12	0.09	0.13	0.12
Attractiveness	0.055	-0.008	0.023	0.296	-0.011	0.017	0.11	0.10	0.11	0.08	0.11	0.10

Table E.S1. Model effect coefficients for E, G and G x E from GLMMs fitted to absolute trait z-scores. Trait abbreviations ES, T, W, Testes and AG, Spermatophore, Sperm Area, VR Count, OFF, M. Latency, M. Length, and Attractiveness relate to eyespan, thorax length, wing length, testes length, accessory gland length, spermatophore area, sperm area, VR counts, offspring counts, latency to mate, mating length, and the inverse of the number of mate attempts required before a male was accepted by a female. E1, E2, E3, G, GxE1 and GxE2 relate to the coefficients for low, intermediate and averaged food level effects, genetic status effects, and G x E at low intermediate food level effects. Numbers rounded for clarity.

E.S2) Model effect coefficients for relative trait values

	Effect Coefficients						Standard Error					
Trait	E1	E2	E3	G	GxE1	GxE2	E1	E2	E3	G	GxE1	GxE2
ES	0.583	-0.053	0.265	-0.024	-0.055	0.039	0.04	0.03	0.04	0.03	0.04	0.03
W	0.516	-0.084	0.216	-0.083	-0.084	0.021	0.06	0.04	0.05	0.04	0.04	0.04
Testes	0.192	0.043	0.118	0.152	0.049	0.046	0.11	0.07	0.09	0.08	0.09	0.07
AG	0.192	-0.274	-0.041	0.335	0.104	-0.067	0.10	0.07	0.09	0.09	0.08	0.07
Spermatophore	-0.063	-0.034	-0.048	0.279	-0.019	-0.037	0.18	0.12	0.15	0.12	0.13	0.12
Sperm Area	-0.309	0.163	-0.073	0.001	0.090	-0.015	0.18	0.12	0.15	0.12	0.13	0.12
VR Count	-0.284	0.014	-0.135	0.347	0.189	0.108	0.14	0.09	0.12	0.09	0.10	0.09
OFF	0.006	-0.122	-0.058	0.215	0.048	0.021	0.17	0.11	0.14	0.11	0.13	0.11
M. Latency	-0.211	-0.022	-0.117	0.076	-0.194	0.090	0.17	0.11	0.14	0.08	0.12	0.11
M. Length	-0.227	0.026	-0.100	-0.158	-0.122	0.124	0.18	0.12	0.15	0.09	0.13	0.12
Attractiveness	0.055	-0.017	0.019	0.304	-0.015	0.007	0.16	0.10	0.13	0.08	0.12	0.10

Table E.S1. Model effect coefficients for E, G and G x E from GLMMs fitted to relative trait z-scores. Trait abbreviations ES, T, W, Testes and AG, Spermatophore, Sperm Area, VR Count, OFF, M. Latency, M. Length, and Attractiveness relate to eyespan, thorax length, wing length, testes length, accessory gland length, spermatophore area, sperm area, VR counts, offspring counts, latency to mate, mating length, and the inverse of the number of mate attempts required before a male was accepted by a female. E1, E2, E3, G, GxE1 and GxE2 relate to the coefficients for low, intermediate and averaged food level effects, genetic status effects, and G x E at low intermediate food level effects. Numbers rounded for clarity.

F) Effect of E, G, G x E and absolute and relative eyespan on male age at death

Here I looked at potential causes of differences between the patterns observed for adult eyespan (Chapter 4, males that survived until sexual maturity) and juvenile eyespan (Chapter 3, all males, irrespective of age at death) in the effects of absolute and relative eyespan, environment and genetics on the age of males at death.

FS.1) Methods:

As the effects of genetic state on absolute eyespan and the G x E on relative eyespan were lost in this chapter (relative to Chapter 3) a number of additional analyses were conducted. First, the mean absolute and relative eyespan for the juvenile and adult data sets were calculated and contrasted for each genetic status and food level category. A GLMM was then fitted to test for the effects of male absolute eyespan, as well as environmental and genetic state, on male age at death. It included male eyespan, food level, genetic status and all two- and three-way interactions as fixed effects, with cross included as a random effect. Male thorax was then included as an additional covariate to control for body size. Tests for the effects of absolute and relative eyespan on age at death relationships were also run with the data split via environmental, genetic and environmental and genetic states. Male age at death was square-root transformed to normalise residual error. All males were kept alive until natural death or until use in an experiment (at the age of 10 weeks). Hence, each model is fitted to data related to the males that died up until 10 weeks (males killed by dissection were omitted).

FS.2) Results:

Across all environmental and genetic levels, mean absolute and relative eyespans were larger for adult trait distributions (Chapter 4) than for juvenile distributions (Chapter 3). Mean absolute male eyespan (mm), adult = 5.87, 7.04, 8.19, 5.88, 7.11, 8.40 (incross, outcross, low, intermediate, high), juvenile = 5.45, 6.50, 8.14, 5.54, 6.74, 8.31 (incross, outcross, low, intermediate, high). Mean relative male eyespan (mm), adult = 6.62, 7.25, 7.80, 6.46, 7.25, 7.80 (incross, outcross, low, intermediate, high), juvenile = 6.01, 6.61, 7.52, 6.11, 6.85, 7.72 (incross, outcross, low, intermediate, high). For absolute and relative eyespan, the increases (juvenile versus adult distributions) were largest at low and intermediate food levels. The increases (juvenile versus adult distributions) were also larger for incross flies at intermediate food levels for absolute eyespan (increase per food level, outcross - incross: 0.080, 0.171, -0.075 mm) and at low food levels for relative eyespan (increase per food level, outcross - incross: 0.258, 0.239, 0.004 mm).

[As in Chapter 3,] incross males died sooner than outcross males ($F_{1,26.39}$ = 17.05, p < 0.001). Lower food level flies died sooner than higher food level flies ($F_{2,1345}$ = 82.02, p < 0.001). There was no G x E ($F_{2,1353}$ = 1.81, p = 0.164). Male absolute and relative eyespan were positively related to male age at death (absolute: R^2 = 0.33, r = 0.33, $F_{1,1353}$ = 579.46, p < 0.001; relative: R^2 = 0.35, r = 0.42, $F_{1,1342}$ = 423.34, p < 0.001). Across environments, the relationships between absolute and then relative eyespan and male age at death increased with food level (absolute: $F_{2,932.8}$ = 5.42, p = 0.005; relative: $F_{2,922.2}$ = 5.81, p = 0.003). Neither the relationship between absolute nor relative eyespan and male age at death varied

with genetic status (absolute: $F_{1,937.8} = 0.02$, $p = 0.879$; relative: $F_{1,925.7} = 3.48$, $p = 0.063$). For absolute eyespan, there was no ES x G x E interaction ($F_{2,932.4} = 2.28$, $p = 0.102$). But for relative eyespan there was a clear G x E trend, with the increases in slope across food levels less severe in incross males ($F_{2,922.2} = 2.84$, $p = 0.058$). Here, the slope at low food levels was steeper in incross males ($r = 0.174$ v 0.162), while at high food levels it was steeper for outcross males ($r = 0.249$ v 0.328): but at intermediate the values were similar ($r = 0.256, 0.250$; in, outcross).

F.S3) Discussion

I found a complex picture, but one that could be broken down into a number of simple results. First, I was able to confirm that male eyespan responded in a heightened manner to variation in environmental stress. In contrast to Chapter 3, I did not find a response to genetic stress. But the trends of the genetic and G x E responses were in the same direction as those seen in Chapter 3. So these results are not in conflict, and are most likely explained by decreased sample size. The sample size in Chapter 3 was $n = 1154$, whereas here it was reduced to $n = 434$. Another more interesting possibility is that, in the current study, male ornament size was assessed at reproductive maturity, 10-14 weeks after eclosion. In this case, death prior to adulthood could have preferentially removed flies with smaller eyespans. We tested for this with an examination of male age at death. A crucial result was that both absolute and relative male eyespan correlated positively with male age at death. As incross males tend to be smaller than outcross, this could provide evidence for selective death. However, while incross, low food and intermediate food flies died sooner, the overall relationships between both absolute and relative eyespan and age at death did not vary with

genetic state. Across environments the relative eyespan relationships did vary, but with similar slopes for incross and outcross flies at intermediate food levels. So, while selective death did occur, its extent did not vary with eyespan and genetics in the manner required to explain the loss of genetic and G x E effects observed.

F.S.4) Model Output:

FS.4a) Effect of absolute eyespan on male age at death

Male Age at Death [SQRT] = Cross & Random + Eyespan

$R^2 = 0.22$
Var Ratio [Cross/Resid] = 0.06

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	941.6	181.6079	<.0001

Term	Estimate	Std Error
Intercept	0.2049498	0.129093
EYESPAN	0.2457003	0.018232

FS.4b) Effects of environmental and genetic state on male age at death

Male Age at Death [SQRT] = Cross & Random + Food Level + Genetic Status + Food Level * Genetic Status

$R^2 = 0.14$
Var Ratio [Cross/Resid] = 0.03

Source	Nparm	DF	F Ratio	Prob > F
FOOD.TREATMENT	2	940.4	46.3171	<.0001
GENETIC.STATUS	1	30.27	11.6619	0.0018
GENETIC.STATUS*FOOD.TREATMENT	2	941.2	0.6107	0.5432

Term	Estimate	Std Error
Intercept	1.8549448	0.035216
FOOD.TREATMENT[LOW]	-0.296008	0.037336
FOOD.TREATMENT[MEDIUM]	-0.030164	0.033166
GENETIC.STATUS[INCROSS]	-0.114166	0.035216
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0137549	0.037336
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.036157	0.033166

FS.4c) Effects of absolute male eyespan, environmental and genetic state on male age at death

Male Age at Death [SQRT] = Cross & Random + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Genetic Status + Eyespan * Food Level + Eyespan * Genetic Status * Food Level

$R^2 = 0.24$
Var Ratio [Cross/Resid] = 0.04

Source	Nparm	DF	F Ratio	Prob > F
EYESPAN	1	935.8	188.7804	<.0001
FOOD.TREATMENT	2	920.9	2.2533	0.1056
GENETIC.STATUS	1	30.16	7.8218	0.0089
GENETIC.STATUS*FOOD.TREATMENT	2	935.5	0.449	0.6384
GENETIC.STATUS*EYESPAN	1	935.9	0.0216	0.8831
FOOD.TREATMENT*EYESPAN	2	930.8	5.4994	0.0042
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	932.4	2.2847	0.1024

Term	Estimate	Std Error
Intercept	-0.848016	0.320015
EYESPAN	0.3697062	0.042223
FOOD.TREATMENT[LOW]	0.1716048	0.080864
FOOD.TREATMENT[MEDIUM]	0.23523	0.065694
GENETIC.STATUS[INCROSS]	0.0242732	0.067645
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.113648	0.080864
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.150048	0.065694
GENETIC.STATUS[INCROSS]*(EYESPAN-6.74621)	-0.067981	0.042223
FOOD.TREATMENT[LOW]*(EYESPAN-6.74621)	-0.173536	0.053813
FOOD.TREATMENT[MEDIUM]*(EYESPAN-6.74621)	-0.083361	0.048202
GENETIC.ST[INCROSS]*FOOD.TREATMENT[LOW]*(EYESPAN-6.74621)	0.077765	0.053813
GENETIC.ST[INCROSS]*FOOD.TREATMENT[MED]*(EYESPAN-6.74621)	0.07794	0.048202

FS.4d) Effect of relative eyespan on male age at death

Male Age at Death [SQRT] = Cross & Random + Thorax + Eyespan

$R^2 = 0.22$
Var Ratio [Cross/Resid] = 0.06

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	936	136.6881	<.0001
EYESPAN	1	933.7	42.6985	<.0001

Term	Estimate	Std Error
Intercept	0.1096219	0.171195
THORAX	0.1225294	0.142486
EYESPAN	0.2215345	0.033903

FS.4e) Effects of environmental and genetic state on male age-at-death

Male Age at Death [SQRT] = Cross & Random + Thorax + Food Level + Genetic Status + Food Level * Genetic Status

$R^2 = 0.19$
Var Ratio [Cross/Resid] = 0.04

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	931	134.6831	<.0001
FOOD.TREATMENT	2	930.1	3.302	0.0372
GENETIC.STATUS	1	31.41	10.1554	0.0032
GENETIC.STATUS*FOOD.TREATMENT	2	931.5	1.4836	0.2274

Term	Estimate	Std Error
Intercept	0.3131382	0.236549
THORAX	0.7223731	0.109325
FOOD.TREATMENT[LOW]	-0.115371	0.046022
FOOD.TREATMENT[MEDIUM]	0.0067972	0.032963
GENETIC.STATUS[INCROSS]	-0.108794	0.036669
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	0.0077769	0.036673
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.052183	0.032572

FS.4f) Effects of absolute male eyespan, environmental and genetic state on male age at death

Male Age at Death [SQRT] = Cross & Random + Thorax + Eyespan + Food Level + Genetic Status + Food Level * Genetic Status + Eyespan * Genetic Status + Eyespan * Food Level + Eyespan * Genetic Status * Food Level

$R^2 = 0.24$
Var Ratio [Cross/Resid] = 0.05

Source	Nparm	DF	F Ratio	Prob > F
THORAX	1	925.7	140.3808	<.0001
EYESPAN	1	924.1	46.2948	<.0001
FOOD.TREATMENT	2	914.9	1.8748	0.154
GENETIC.STATUS	1	30.81	7.6421	0.0095
GENETIC.STATUS*FOOD.TREATMENT	2	925.5	0.5954	0.5515
GENETIC.STATUS*EYESPAN	1	926	0.1011	0.7506
FOOD.TREATMENT*EYESPAN	2	920.9	4.8663	0.0079
GENETIC.STATUS*FOOD.TREATMENT*EYESPAN	2	922.2	2.8423	0.0588

Term	Estimate	Std Error
Intercept	-0.926505	0.345081
THORAX	0.1724758	0.143297
EYESPAN	0.3283414	0.050957
FOOD.TREATMENT[LOW]	0.1585451	0.081443
FOOD.TREATMENT[MEDIUM]	0.2285493	0.066369
GENETIC.STATUS[INCROSS]	0.0397546	0.068355
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[LOW]	-0.126339	0.081499
GENETIC.STATUS[INCROSS]*FOOD.TREATMENT[MEDIUM]	-0.170621	0.066338
GENETIC.STATUS[INCROSS]*(EYESPAN-6.74837)	-0.079521	0.042648
FOOD.TREATMENT[LOW]*(EYESPAN-6.74837)	-0.16629	0.054219
FOOD.TREATMENT[MEDIUM]*(EYESPAN-6.74837)	-0.078184	0.048647
GENETIC.ST[INCROSS]*FOOD.TREATMENT[LOW]*(EYESPAN-6.74837)	0.091238	0.054177
GENETIC.ST[INCROSS]*FOOD.TREATMENT[MED]*(EYESPAN-6.74837)	0.085377	0.048696

FS.5) FIGURES

FS.5a]

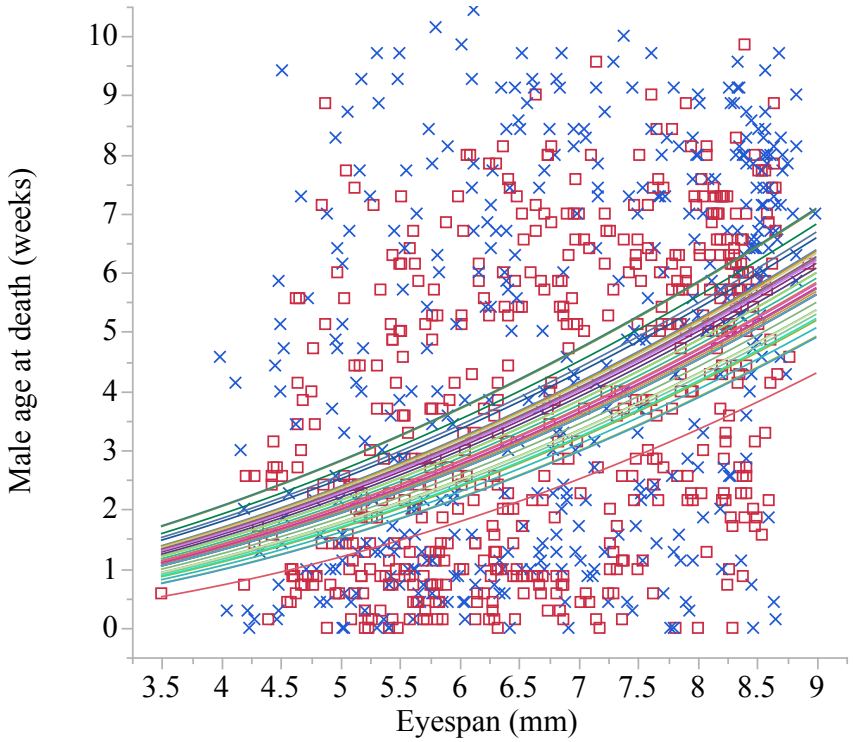


Figure FS.5a. Effect of male absolute eyespan (shown untransformed for clarity) age at death (weeks). Each linear fit represents an incross or outcross line. Red = incross data. Blue = outcross data. The overall effect of eyespan on male age at death was positive and significant for the pooled incross and outcross categories ($p < 0.001$).

FS.5b]

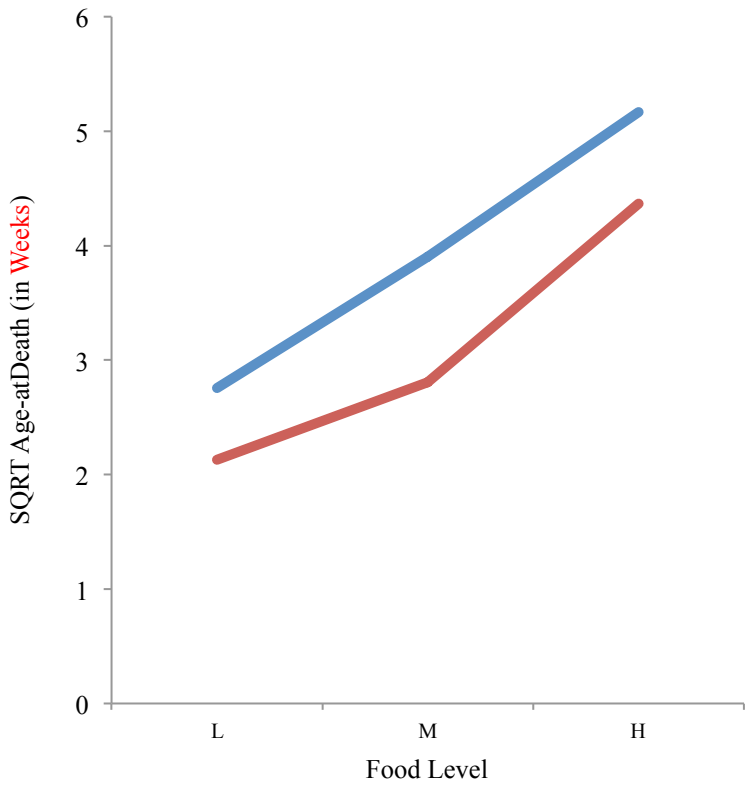


Figure FS.5b. Effect of environmental variation (food level) and genetic variation (incross/outcross) on absolute male square root transformed age at death. Red denotes the incross pattern. Blue denotes the outcross pattern. The lines connect the peaks of bars (not shown). Lines rather than bars were used for ease of comparison. Error bars are omitted for clarity. The effect of E ($p < 0.001$) and G ($p = 0.003$) but not G x E ($p = 0.543$) were significant.

Note: The relationship between absolute [and relative] eyespan and male age at death was steep at low E for incross and outcross, less steep at intermediate E for incross and outcross, and steeper at high E for incross, and especially for outcross.

Hence, in all, while age at death likely accounted for some of the reduced genetic effect (for absolute eyespan) and the loss of the G x E at intermediate E (for relative eyespan), it is unlikely to have explained the pattern in full because the slopes were most similar at intermediate levels of E, and as there was no G x E for age at death.

Nonetheless, the fact that absolute and relative eyespan have such strong effects on age at death is of interest in and of itself. It implies that high condition relates to both larger eyespan and an enhanced ability to survive. On the one hand, this could be viewed as evidence if favour of condition dependent handicaps as lower condition males appear to have substantially higher mortality costs. Alternatively, this could be viewed as evidence that the costs of ornamentation are quite low to maintain once eclosed (hence low death for larger ES males), but are hard to grow at the larval stage (hence smaller ES for both lower E and lower G males). This should be studied.

APPENDIX 2

FEMALE SNEAK COPULATION

In press, for publication in the Encyclopedia of Evolutionary Psychology

James Howie and Andrew Pomiankowski (2016)

Female Sneak Copulation

Synonyms

sneaky mating, undetected extra-pair copulation

Definition

undetected copulation with the partner of another female, or in defiance of the dominant female; often punished if detected

Introduction

A female sits with her partner, or with a dominant male. She is able to reproduce, and benefits due to her partner's parental care and protection. Another female – of low rank, in a female driven dominance hierarchy – has failed to find a partner, and has yet to mate. In both cases, the females can increase their reproductive fitness by mating with an additional male (or with additional males), or by mating for the first time. But in order to do so, each must overcome various forms of resistance. In the first case, the partner of the female, or the dominant male, will not want her to 'cheat' on him. Likewise, the partners of her additional mates will not want 'their' males to cheat on them. Finally, in the case of the low rank female, the dominant female will not want her to win over reproductive access, because this could lead to a dilution of the benefits that she receives both in terms of parental care and protection.

Here then is the basis for two broad forms of female sneak copulation. One is based on a female's attempt to 'have her cake and eat it', in which a female tries to obtain the benefits of additional mates – such as additional parental care, or genetically diverse offspring – whilst also attempting to avoid the costs of discovery, which are likely to result in lower parental investment by her partner, or even physical punishment. Another arises when a female attempts to break rank, to the same end but with similar risks. Yet cut across these two forms is another division. In the first case, the punishment (i.e. fitness loss) is meted out not only by the male partner, but also by the *female* partners of her additional mates. In the second, punishment is administered by the dominant *female*. The rest of this section focuses on these female-female interactions linked to female sneak copulation (FSC), where a female tries to ensure additional, secretive reproduction, in defiance of other females.

The Lay of the Land

Many aspects of the female-female contest for copulation are understudied, and the same is true of FSC (Neff & Svensson 2013). Indeed, there is practically no direct evidence on this topic at all. Nonetheless, it is plausible that FSC could be common. And if so, it could well have been important in evolution of animal behavior, cognition and psychology, and maybe in particular in humans. Given the dearth of direct evidence, the approach taken here is to first provide an overview of the evolutionary requirements of FSC. This is followed by a brief review of the direct and indirect evidence in favour of each form of FSC (in relation to the partners of other males, and in defiance

of dominant females). A discussion of the behavioral mechanisms that could facilitate the operation of FSC in nature is then provided, along with a discussion of the relevance of FSC to human evolution. A conclusion is then provided that looks to the future, at the questions that remain to be addressed.

Female Multiple Mating

FSC requires that there are benefits to female multiple mating. In males, multiple mating is expected to arise due to the linear relation between the number of matings that a male secures and a male's total paternity. Just such a relationship was found in Bateman's classic study on the fruit fly, *Drosophila melanogaster* (Bateman 1948). In contrast, females have been viewed as the limited sex, with fitness being determined by reproductive output rather than the mating rate. Yet even in 1948, Bateman was able to find evidence that females increased their reproductive success with the addition of extra mates. Moreover, females have been observed to mate multiple times with multiple partners in multiple species across multiple taxa. Ascribing this all to male activity with the implication that the female is just a passive vehicle now seems absurd (Trivers 1972), and recent work has rightly rejected this simplistic dichotomy. Multiple mating is as much a female mating strategy arising from female action as it is a male mating strategy based on male action.

But why do females mate multiple times? The act of copulation is associated with a number of costs (Jennions & Petrie 2000; Forstmeier *et al*. 2014). Copulation itself can be associated with direct damage to the female

reproductive tract, for example in species where the male penis carries spines; or it may simply use up a crucial resource – time. Likewise, pre-copulatory behavior can injure females. Post-copula, there are further risks resulting from the transmission of sexual diseases by multiple mating, while female longevity has been shown to decline due to the transfer of male derived ejaculate products that raise a male's paternity at the costs of female survival. Mixed ejaculates are also associated with increased risk of embryo mortality via polyspermy. And there may be additional dangers if predation risk is increased in copula (or if a female suffers costs due to attacks by her partner or by other females). Given this, there must be clear benefits to FMM.

The most obvious direct benefits arise when the female herself benefits from mating because the male provides a nuptial gift, such that more mating results in greater resource acquisition. Another example is the increased parental care that a female can obtain if she mates with more males, as a larger number of males will have a stake in her brood. Mating may also cause a reduction in male harassment or a decrease in the risk of male driven infanticide. A final – special – example is that of fertility assurance. Females may become sperm limited in situations where some males have reduced fertility or where males invest few sperm per copulation (due to the partitioning of their resources across multiple females) or where there are limits to the length of time sperm remain viable. All of these benefits provide reasons for females to mate multiple times: to accrue benefits to themselves in terms of resources, or to ensure all of their eggs are fertilized (Jennions & Petrie 2000).

In addition to these direct benefits to the female, females can obtain indirect genetic benefits to their offspring via multiple mating. A female can mate with a more ornamented male to obtain 'attractiveness genes' (in so far as the male ornament is preferred by other females) or 'viability genes' (where ornament size correlates with male genetic quality). A female can thus trade up genetically by multiple mating if she mated first with a low attractiveness or low genetic quality male. In other cases 'compatible genes' will be more important. Here, multiple mating can open up post-copulatory mechanisms that can select for compatibility (based on relatedness, MHC complexes, selfish element suppressors; Forstmeier *et al*. 2014). Alternately, multiple mating can be a simple bet-hedging strategy to increase the genetic diversity of a female's offspring. In this case, multiple mating can either compensate for non-perfect female choice or increase the chances that some offspring fit their environment (Fox & Rauter 2003). As a final indirect genetic benefit, it is notable that, post copula, 'cryptic female choice' and 'male sperm competition' are enabled by multiple mating, and may simply select for sons that are good at fertilization – as such sons will inherit their father's more competitive sperm.

Extra-Pair Copulation and FSC

As seen above then, there are various benefits to multiple mating that are necessary for FSC. However, FSC also requires that multiple mating takes place in a social context. It is not possible to 'sneak' in isolation. A simple social context is the monogamous pair bond. Here, a female is joined in a

social pair with a male, and they jointly raise their offspring. However, the benefits of female multiple mating remain in this monogamous context. This can lead to selection for female alternate mating strategies, such as extra-pair copulation (EPC), as well as FSC, which is in this context a sub-class of EPC.

EPC arises when a male or female mates with an individual other than their partner. It can be driven by males or females. Males can force females to mate with them in the face of true female resistance (forced copulation, coercion) or females may solicit males to mate with them (Griffith 2007; active female solicitation). Usually the interaction between the sexes reveals that males search and display to females, and that the females passively accept such advances, or put up a threshold of resistance (Westneat *et al*. 1990). The females' behavior is presumably a reflection of the costs and benefits associated with extra pair paternity. It is in this context that FSC might evolve.

A classic example of this FSC-EPC was provided by Kempenaers *et al*. (1992), who combined behavioral and genetic data to show that female driven EPC was common in monogamous pairs of the blue tit, *Parus caeruleus.* In the wild population studied, 31% of clutches included extra-pair paternity. Further, of the 7 EPCs (out of 90 copulations) observed, more than 70% were classified as female driven. In these cases, females moved onto the territory of neighbors and were either chased off by the resident female, or – if undetected – were sometimes able to solicit and take part in an EPC. The observation that resident females showed aggression toward intruders

highlights the extra costs to this form of EPC, which must nonetheless be outweighed by the benefits, and provides evidence of a selective pressure that could promote the evolution of costly female sneak behaviors leading to copulation.

Primate Tactical Deception, and FSC

Another class of species in which FSC-EPC could be important is primates. Exclusive pair bonds are rare in most mammals (around 3%). But they are relatively common in primates, with 14-18% of species forming monogamous sexual-bonds (Drea 2005); while the percentage is even higher if non-exclusive "pair" bonds nested within hierarchies are included (such as when a male has exclusive access to several females, see Hierarchies and Defiant FSC below). Female driven EPC has been observed in several primate species, and male mate guarding behavior is known to be common (Drea 2005). Females are also known to take part in competitive interactions with other females, as well as to use various forms of physical and social punishments (Stockley & Campbell 2013). Hence, the FSC forms of EPC could be common in primates.

An advantage to recent studies on EPC in primates is that a number have started to provide insight into the psychological processes and cognitive mechanisms that underlie EPC. For instance, Overduin-de Vries *et al*. (2015) found evidence of EPC based on tactical deception in captive populations of the macaque species *Macaca mulatta* and *Macaca fascicularis*. They observed that females appear to deliberately create distance between

themselves and other females before EPC events – a more complex level of cognitive processing than chance exploitation of a peripheral location, but less complex than taking the perspective of other females into account (e.g. by deliberately hiding out of view behind screens). Similar tactical concealment was also observed in a wild Ethiopian population of the gelada monkeys, *Theropithecus gelada*, in recent study by le Roux *et al*. (2013). As tactical deception is known to occur in relation to food and other nonsexual contexts in fish, corvids, apes, and monkeys (Overduin-de Vries *et al*. 2015), this behavior could also be widespread in FSC contexts of EPC. But at present there is little direct evidence for FSC-EPC in primates, or of the role of tactical deception in this. This is due mostly to a lack of studies. Hence, such mechanisms *could* often be utilized in an FSC-EPC context; and there remains a need for further studies on this issue to determine if this is the case.

Hierarchies and Defiant FSC

Another social context in which FSC might appear is that based on rank and social hierarchy seen in a variety of species, including cetaceans, elephants, corvids and primates (Overduin-de Vries *et al*. 2015). A diverse array of such hierarchical systems exists. But the most relevant in terms of the evolution of human psychological processes are the primates. Here, hierarchies are based on both male and female rank (Drea 2005). Higher ranked females often have higher reproductive success. And such females are also known to suppress the reproduction of lower ranked females (Drea 2005). Hence, another type of FSC that could be important is that of 'defiant FSC' – where a lower ranked

female mates with a male, in defiance of a higher ranked female. As in the case of tactical deception, there is little direct evidence about defiant FSC. Nonetheless, a number of studies have provided indirect evidence that this could be common. For instance, both Overduin-de Vries *et al*. (2015) in macaques and le Roux *et al*. (2013) in gelada monkeys found evidence of male rank related audience effects on EPC – females and lower rank males were less likely to copulate when a male of higher rank was nearby; and were often punished by these males if sighted. Female rank related audience effects were not observed in either case. However, these are the first studies of their kind and highlight the potential for such defiant FSC to arise in nature.

FSC in Humans – "Woman Beware Woman"

It has also been suggested that FSC is likely to be common and important in humans. Like other primates, humans have complex social structures, and form close pair bonds. Females (women) have flatter 'hierarchies' than males (men) (Sidanius *et al*. 1994), but are known to enforce these via subtle social, as well as physical means (Campbell 2013; Stockley & Campbell 2013). Women are also known to 'cheat' on men, and are known to punish this behavior in other women, especially if the target of the FSC-EPC was 'their' man. In street 'gangs' such punishment can even lead to the death of another woman (Campbell 2013). Hence FSC is likely to be important in humans, and could well involve – or have been involved in the evolution of – the complex psychological processes and cognitive systems that define humans. But once

again there is very little solid observational or experimental work that would establish human FSC beyond anecdotal reports; so more studies are needed.

Conclusion

Female sneak copulation (FSC) is likely to be common in nature. However, all female-female sexual competitive interactions remain relatively poorly investigated, and FSC is no exception. Nevertheless, the field is starting to move forward, and it is likely that within the next 5 – 10 years the mechanisms of FSC, its prevalence in nature, and its importance in human evolutionary psychology will be better known. The results are awaited with anticipation!

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Cross-References

(Inclusive Fitness, Reproductive Strategies, Multiple Mating, Extra-Pair Mating, Sneak Copulation, Intrasexual Competition, Female Dominance Hierarchies, Female-Perpetrated Violence, Female Deception, Evolution of *Homo sapiens*)

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APPENDIX 3

EVOLUTION: SEX OR SURVIVAL

Evolution: Sex or Survival

Goodbye to old survival,

A simple life I know,

It seems that sex is where it's at,

So that is where I'll go!

J.H. 2016