# CONDITION DEPENDENCE OF SEXUAL DIMORPHISM IN THE ANTLER FLY, *PROTOPIOPHILA LITIGATA*

**MATHIEU OUDIN**

Thesis submitted to the

Faculty of Graduate and Postdoctoral Studies

University of Ottawa

In partial fulfillment of the requirements for the

M.Sc. degree in the

Ottawa-Carleton Institute of Biology

# ABSTRACT

In this thesis, I investigate the relationship between two variables for which persistent directional sexual selection is an evolutionary driver: condition dependence and sexual dimorphism. This joint dependence on sexual selection predicts that among traits within a given species, greater dimorphism should be associated with stronger condition dependence. Very few studies have tested this prediction, and those that have focus on species with highly exaggerated and strongly dimorphic traits between the sexes. Here, I quantified variation in a suite of morphological traits in a dipteran species – the antler fly, *Protopiophila litigata* – in which sexual dimorphism is less extensive. I manipulated condition via different larval diets and then quantified the effects on adult body size and shape in both sexes. Across traits, I found that the extent of sexual dimorphism was positively associated with the strength of condition dependence in males but not in females. These results suggest a shared developmental basis to condition dependence and sexual dimorphism in body shape, and suggest that this has arisen via sexual selection in males despite the absence of extremely dimorphic shared traits.

# RÉSUMÉ

Dans cette thèse, j'examine la relation entre deux variables qui partagent la sélection sexuelle directionnelle comme moteur évolutif : le dimorphisme sexuel et la condition-dépendance. L'effet similaire de la sélection sexuelle sur ces deux variables implique qu'à l'échelle intra-spécifique, les traits morphologiques les plus dimorphiques entre les sexes devraient être les plus condition-dépendants. Les quelques études qui ont testé cette prédiction se sont concentré sur des espèces présentant des caractères très exagérés et fortement dimorphiques entre les sexes. Dans mon étude, je me suis servi d'une espèce de diptère sans dimorphisme sexuel apparent – la mouche du bois, *Protopiophila litigata*. J'ai manipulé la « condition » des individus en les élevant avec différents traitements nutritionnels. Ensuite, j'ai quantifié les effets de ces traitements sur la taille et la forme des adultes des deux sexes. J'ai trouvé une corrélation positive entre l'ampleur du dimorphisme sexuel et l'intensité de la condition-dépendance chez les mâles, mais pas chez les femelles. Ces résultats suggèrent une base développementale partagée du dimorphisme sexuel et de la condition-dépendance entre mâles et femelles, et suggèrent que ceci est survenu via la sélection sexuelle chez les mâles malgré l'absence de traits extrêmement dimorphiques.

# TABLE OF CONTENTS

**Chapter 2** Condition dependence of sexual dimoprhism in the antler fly, *Protopiophila litigata*

# LIST OF TABLES

# LIST OF FIGURES

## ACKNOWLEDGEMENTS

First, I would like to sincerely thank my supervisor, Howard Rundle for his guidance, support and encouragement during the last two years. Howard was always tremendously patient when explaining concepts I had difficulty grasping. His enthusiasm for the study of evolutionary biology is contagious, and from this passion for his work, or whatever it is that motivates a person in life, I've learned more than what can be presented in this thesis.

Second, I would like to thank all past and present members of the Rundle lab for their support, advice, and contribution to creating a fun and friendly environment. These members include: Devin Arbuthnott, Brian Mautz, Maggie Stoeva, Kate Henbest, Nicolas Rode, Alison White, Corey Godfrey, and Julie Colpitts. A special thanks to Brian for showing me the ropes with the antler flies, in the lab and in the field; Maggie and Kate for their extensive help with my experiment; and Nicolas for spending an enormous amount of time explaining the relevance of various statistical analyses.

Third, I would like to thank Russell Bonduriansky from the University of New South Wales in Australia. His entomological background and empirical work directly related to my study was very helpful while carrying out the better part of my work. Russell was always hasty in helping with data analysis, result interpretation, or general experimental techniques. I want to thank my committee members: Dr. Gabriel Blouin-Demers from the University of Ottawa and Dr. Sue Bertram from Carleton University, their helpful feedback and input during committee meetings brought insight and a sense of comfort to my progress. My research, as well as conference funding, was supported by a Natural Sciences and Engineering Research Council of Canada (NSERC) grant to Howard, and by the University of Ottawa.

# CHAPTER 1- Introduction

## *i) Overview*

In this thesis, I use the antler fly, *Protopiophila litigata*, to provide an empirical test for condition-dependent sexual dimorphism, a prediction of sexual selection theory that has received limited attention. The results of my research are presented in Chapter 2, my main data chapter. Chapter 2 is written as a standalone manuscript for publication in a scientific journal, and the introduction is therefore necessarily rather brief, assuming fairly detailed background knowledge of the topic. In light of this, I have included an introductory Chapter 1 that provides additional background details on sexual selection, sexual dimorphism, condition dependence, and some general biology of the antler fly. This approach has resulted in some repetition of material, but I have attempted to keep this to a minimum.

The research in Chapter 2 is the result of a collaboration between myself, my supervisor (Howard Rundle), and Russell Bonduriansky at the University of New South Wales. Russell worked on the antler fly system as a graduate student at the University of Toronto, and his involvement was instrumental in providing background information on the collection and rearing of antler flies. While all three authors were involved in establishing the experimental design, analyzing and interpreting the data, and writing the manuscript (Chapter 2), I took the lead role in all of these stages. In addition, I was the sole contributor to data collection, including establishing the rearing environments, raising adults in these environments, and then preserving, dissecting and measuring them. The following manuscript from the work has been submitted for publication in a peer-reviewed scientific journal:

Oudin, M., Bonduriansky, R. & Rundle, H.D. 2014. Condition dependence of sexual dimorphism in the antler fly, *Protopiophila litigata*. Under review.

### *ii) Sexual selection*

Darwin described natural selection as a process that leads to the preservation of favourable variations and the removal of detrimental variations amongst individuals of a population due to the inherent ability to survive (Darwin, 1859). Darwin also introduces the concept of sexual selection in his work: "*On the Origin of S*pecies". This process differs from selection arising from variation in survival or fecundity (hereafter natural selection for simplicity) in that it arises from variation in reproductive success. Causes of natural selection can be directly linked to the biological or physical environment, and causes of sexual selection are the differential acquisition of mates via the social environment (i.e., mating success) and/or gaining access to their gametes (i.e., variation in fertilization success; Ghiselin, 1974). Individuals vary in traits that affect their ability to compete for mates and/or fertilization of their gametes, and it is this variation that causes differences in reproductive success that can lead to the evolution of exaggerated sexual displays and weapons of combat (Andersson, 1994). Sexual selection was first proposed by Darwin (1859) to explain the evolution of such elaborate secondary sexual traits as these often appear to decrease non-sexual fitness and therefore cannot be explained by natural selection alone.

Darwin (1859, 1871) distinguishes two main types of sexual selection: inter- and intrasexual selection. Intersexual selection occurs when members of one sex (usually females) have mating preferences for one or more characters (physical and/or behavioural) in individuals of the opposite sex (usually males), choosing non-randomly amongst them, hence determining which will bear offspring. A well-known example of intersexual selection (often termed female mate choice), is that of a peacocks train (its tail). Peahens prefer mating with males with elaborate tails (e.g., brighter eyespots; Loyau *et al.,* 2007) and this preference has apparently lead to the evolution of the stunning and elaborate tail that grace male peacocks and which are used to attract and court females. The size and number of eyespots on male tails is positively correlated with offspring survival and resistance to infection (Petrie, 1994; Loyau *et al.,* 2005), thus suggesting that they are indicative of a healthier mating partner for peahens.

In contrast, intrasexual selection arises when members of the same sex compete for access to members of the opposite sex for the purpose of reproduction. The male is the typical gender in which this occurs, although examples of female-female competition also exist (e.g., sexual role reversal; Gwyne, 1981). A classic example of a trait evolving via intrasexual selection is body size in the horned stag (fallow deer), where smaller males have decreased reproductive success because their reduced size makes them inferior competitors for access to females (Fiske *et al*., 1998). Traits involved in intrasexual selection are not always morphological, nor do they necessarily involve direct interactions among individuals of the competing sex. For example, male search ability during scramble competition is a common mode of intrasexual selection (Andersson, 1994).

With respect to intersexual selection, much attention has been given to the correlated evolution of mate preferences and signal traits that is referred to as the Fisherian runaway process. This correlated evolution arises because assortative mating creates a genetic association between alleles for the display trait in one sex and those for the preference for it in the opposite sex (Fisher, 1915). The genetic association arises because females with the preference tend to mate with males expressing the display, and their offspring inherit genes for both. The existence of the preference causes an advantage in reproduction for males carrying the display (i.e. sexual selection), and due to the genetic correlation of the display with the preference, both increase in frequency in a positive feedback loop. As long as there is heritable variation in the trait and preference, the process will continue until halted presumably by natural selection acting against the male trait (due to the non-sexual fitness costs of further exaggeration). A crucial assumption to the Fisherian runaway model is that there is no cost associated to female preference (i.e. viability and fecundity selection does not act against it): females with a strong preference for a trait share the same fecundity and survival as females lacking the preference. If selection acts against the female preference, this trait/preference reinforcing mechanism collapses. Some benefit to females must then exist for preferences to persist (Arnqvist & Rowe, 2005).

Sexual selection was initially suggested by Darwin to be less important than natural selection. However, empirical studies have suggested that the strength of sexual selection, on average, may be equal to or greater than other forms of selection arising from variation in non-sexual fitness (i.e. natural selection; Kingsolver *et al.*, 2001). Consistent with this, extravagant sexual displays and exaggerated weapons of combat are common in nature, and sexual selection is often implicated in the evolution of the extensive differences between females and males (i.e. sexual dimorphism) that characterize many species. Sexual selection is also likely to be persistent in that, while environments may vary within and among generations, irrespective of this males must compete and females may choose among potential mates. Much attention has been given to the consequences for trait expression of such persistent sexual selection, and life history theory suggests that it should result in the evolution of heightened condition dependence, a form of developmental plasticity that links trait expression to an individual's pool of accumulated resources (termed their 'condition'; Rowe and Houle, 1996). It is sexual selection's role in the evolution of sexual dimorphism and condition dependence on which my thesis focuses, and which I address in more detail in the following sections.

### *iii) Sexual dimorphism*

Sex-differences in the roles of males and females in reproduction mean that sexual selection is often sex-specific, acting on a trait in one sex but not on the homologous trait in the other sex. Sex-specific selection will cause different values of a shared trait to maximize fitness in males and females. If genes and the same loci affect trait expression in both sexes, then different alleles will be favoured in males and females and intralocus sexual conflict is said to occur (Bonduriansky & Chenoweth, 2009). Sexually antagonistic selection should favour the divergence of trait expression between the sexes, generating sexual dimorphism that allows males and females to more closely achieve their separate fitness optima and thereby partially or completely resolving the conflict. Sexual dimorphism is widespread in nature with males and females typically differing in body and other aspects of morphology, behaviour and ornamentation, suggesting a history of sexually antagonistic selection.

The magnitude of sexual dimorphism that evolves is determined by an interaction between the strength of sexually antagonistic selection and constraints arising from a genome that is largely shared between sexes (Lande, 1980; Rice, 1984; Bonduriansky and Chenoweth, 2009). In particular, for sexual dimorphism to evolve it is necessary that there be a certain level of independence in the genetic control of the trait in each gender, or in other words, the intersexual genetic correlation for the trait must be less than one (Lande, 1980; Bonduriansky & Chenoweth, 2009).  Experiments on various organisms have shown that when selection is imposed on a character of one sex, there is a generally a correlated effect on the homologous character of the opposite sex (Shaklee *et al.*, 1952; Harrison, 1953), consistent with the expectation of strong and positive intersex genetic correlations for shared traits that have no previous history of sexually antagonistic selection. Persistent sexually antagonistic selection should favour mechanisms that reduce the intersexual genetic correlation, however, including: 1) the evolution of sex linkage, 2) sex-specific epistasis, 3) duplication of sex-limited autosomal loci and 4) epigenetic processes such as genomic imprinting (Bonduriansky & Chenoweth, 2009). Consistent with this, traits tightly associated with sexual fitness have been observed to have intersexual genetic correlations that are often less than one (Jensen *et al.*, 2003).

### *iv) Condition dependence*

In addition to being involved in the evolution of sexual dimorphism, sexual selection also plays a central role in the evolution of condition dependence. An individual's condition is determined by the pool of metabolic resources they acquire and the efficiency with which they allocate these resources to the production and maintenance of competing traits (Rowe & Houle, 1996; Tompkins et al., 2004). Condition dependence itself is a form of developmental plasticity which relates condition to trait expression, allowing individuals to express key fitness-enhancing traits to the maximum degree they can afford. To some extent, the expression of every trait is likely to be condition-dependent, but life history theory suggests that traits that are subject to continued directional selection, including those targeted by persistent sexual selection, should evolve heightened condition dependence (Andersson, 1982; Nur & Hasson, 1984; Rowe & Houle, 1996). The reason is as follows. Persistent directional selection will drive the exaggeration of such traits to the point at which the benefit they bring to sexual fitness is offset by their costs to non-sexual fitness (Kirkpatrick & Ryan, 1991; Andersson, 1994), making the expression of the traits costly (Rowe & Houle, 1996). If the marginal costs of further trait exaggeration are lower for high than for low condition individuals, then high condition individuals can afford to allocate proportionally more resource to the trait, making its expression condition-dependent and the trait itself an honest signal of an individuals' condition (Getty, 1998; Proulx *et al*, 2002).

There are two major components that influence among-individual variation in condition (Bonduriansky, 2007a). First, the environment an individual experiences can constrain or otherwise determine the amount and quality of resources available to them, and in resource poor environments individuals may be prevented from achieving high condition because of this. Second, an individual's genetic quality, such as the load of deleterious mutations they carry, will determine the efficiency with which they can extract, process, and allocate these resources to competing traits. Both environmentally and genetically-induced variation in condition are assumed to similarly affect the expression of condition-dependent traits because the effects of both are mediated via their impact on an individual's condition. Although this assumption of concordance has received little attention, the alteration of environmental conditions (e.g., diet quality) is the standard experimental technique used to generate individuals of differing condition (reviewed in Cotton *et al*, 2004), likely because it is easier to manipulate than genetic quality.

### *v) Sexual dimorphism covaries with condition dependence*

As outlined above, for traits subject to persistent directional sexual selection, this selection should contribute to the evolution of both heightened condition dependence in these traits as well as their increased sexual dimorphism. In particular, male sexual traits are thought to be displaced from their viability optimum by sexual selection (assuming females are at the viability, or natural selection, optimum), and the degree of phenotypic divergence between males and females for a given trait is therefore expected to represent the magnitude of the viability costs associated with the expression of this trait (Bonduriansky, 2007a).  Theory also predicts that the extent of this viability cost has direct effect on the degree of condition dependence that evolves because it is differences in this marginal cost of trait expression that drives the evolution of condition dependence (Rowe and Houle, 1996). The joint dependence of both condition dependence and sexual dimorphism on the strength of sexual selection therefore predicts a positive association between them, or in other words, the evolution of condition-dependent sexual dimorphism. The predicted outcome is that, for a given trait, the extent of sexual dimorphism should be greater among high than among low condition individuals; a prediction that has reasonable empirical support (David *et al.,* 1994; Post *et al.,* 1999; David *et al.,* 2000; Karan *et al.,* 2000; Bonduriansky & Rowe, 2005; Weladji *et al.,* 2005; Bonduriansky, 2007a; Boughman, 2007; Punzalan *et al.,* 2008; Wyman *et al.,* 2009). In addition, among traits, it is also expected that there be a relationship between the extent to which traits are sexually dimorphic and the extent to which they are condition-dependent. This latter among-trait pattern has only been tested a few times, with both morphological studies utilizing species with obviously dimorphic features (Bonduriansky & Rowe, 2005; Bonduriansky, 2007a).

Despite the clear theoretical prediction of an association between condition dependence and sexual dimorphism, this subject has received little empirical attention. Bonduriansky (2007a) has previously investigated the effects of condition on sexual dimorphism in the fly *Telostylinus angusticollis*. For a suite of morphological traits, he showed that variation in the strength of condition dependence was positively correlated with the extent of sexual dimorphism. Remarkably, over 90% of the variation in the magnitude of sexual size dimorphism was explained by the variation in the strength of condition dependence for these traits in males, whereas no such relationship was seen in females (Bonduriansky, 2007a). This same pattern of condition-dependent sexual dimorphism was also detected across a suite of morphological traits in another obviously dimorphic fly species, *Prochyliza xanthostoma* (Bonduriansky & Rowe, 2005), where body shape was more condition-dependent in males than in females and was correlated with male trait exaggeration. Sexual dimorphism in gene expression has also been shown to increase in high condition individuals with high-condition populations of *Drosophila melanogaster* having more male-biased genes and more female-biased genes and greater sexually dimorphic expression of these genes (Wyman *et al.*, 2009).

My M.Sc. research will provide a direct test for condition-dependent sexual dimorphism in the antler fly, *Protopiophila litigata*, a new model system for the study of sexual selection. I test the predictions stated above, in a species that lacks obviously exaggerated male traits yet in which there is good reason to expect strong sexual selection given their natural history.

### *vi) General biology of the antler fly*

Belonging to the family Piophilidae (Bonduriansky, 1995), the antler fly, *Protopiophila litigata,* is an ideal model for the study of condition-dependent sexual dimorphism due to its aggressive mating behaviour and high sex-ratio biases during mating aggregations. The antler fly life cycle revolves around discarded cervid (moose and deer) antlers, and their distribution is therefore limited to areas where they have access to this resource (Bonduriansky, 1995). The species' known habitat therefore ranges from southern Ontario to eastern Canada (Nova Scotia), although it has not been mapped precisely. *P. litigata*, is easily distinguishable from other protopiophilids due to its pruinose body and light brown (as opposed to entirely black) femoras. Though their size is small, male antler flies are extremely aggressive and have been observed fighting with other fly species much larger than them. The antler fly follows the typical insect life cycle where eggs hatch into larvae that latter become pupae and finally adults emerge from the puparium.

The average size of wild-reared males ranges from 1.6 to 2.8 mm, whereas females measure 1.9 to 3.1 mm. Females lay eggs in cracks and pores of the discarded antler and the larvae develop inside, likely feeding on bacteria degrading the marrow. Larvae typically escape the antler and prepare to pupate during a rain event, perhaps triggered by the sound of water droplets hitting the antler. Larvae escape the porous bone tissue of the antler through a flicking behaviour. The larva curls itself in a vertical U shape relative to its substrate and grasps its posterior end with its mouth hooks.  After tightening its muscles, the larva releases its posterior end and propels itself off the discarded antler. This flicking behaviour can enable a larva to travel up to 0.5 m from the antler. Although this behaviour is not an autapomorphy for the antler fly, it likely offers several advantages. Moving quickly from the bone matrix to pupation sites, the maggot may be able to minimize chance of predation. Flicking may also reduce energetic costs by avoiding a slow crawl over a long distance (Bonduriansky, 2002).

There is a strong sex-ratio bias on antlers whereby males are present in very high densities relative to females. Females are typically only present on antlers when they are gravid and ready to mate. This may be an adaptation in females to avoid male harassment and forceful matings in situations when they are not sexually receptive. Copulation and oviposition behaviour of the antler fly has been previously studied in some detail. A complete copulation-oviposition cycle takes on average 2.3 hours, during which approximately 1.5 hours are spent copulating. Some males guard territories in leks where they must faceoff with other males using their forelegs as weapons. Other males aggregate near copulation and oviposition sites where they attack coupled pairs, attempting to separate them and gain access to the female (Bonduriansky, 1995).

Once a male has successfully mounted a female and begins copulating with her, the female carries the male to the underside of the antler where they remain until the oviposition period. The underside of an antler is not densely populated with flies, potentially allowing the pair to mate in a low harassment environment. During mating, males tap the abdomen of the female with their mid and hind legs. It is thought that this may allow the male to assess female egg load and thereby reject non-gravid females (Bonduriansky, 1996; Bonduriansky & Brooks, 1998a). This early courtship behaviour may also allow females to judge male size, and larger males appear to be more desired by female antler flies (Bonduriansky & Brooks, 1998a). Coupled males will also use their wings to push away other males when they try to attack the mating pair, and to defend females while they are ovipositing in cracks or pores in the antler.

My experiment required rearing flies across generations (i.e. establishing a laboratory stock population). This had never been done previously in this species (as far as I know), and I therefore provide specific details in Chapter 2 concerning the procedure for maintaining a multi-generation laboratory population (e.g., eggs collection, larval rearing, pupation, and adult collection and mating). My experiment also required a diet manipulation performed on the developing larvae, as a means of manipulating adult condition.  Developing a protocol to accomplish this diet required several attempts. At first, transferring eggs between oviposition dishes (see Chapter 2 for terminology) resulted in severe mould issues, possibly due to contamination while manipulating the oviposition dishes and/or due to having too few larvae present in the environment to out-compete mould growth.  I eventually developed a protocol for which mould problems were less severe (see Chapter 2). Having established techniques for the maintenance of outbred laboratory stocks, and for performing diet manipulations, should facilitate future work on this species.

# CHAPTER 2 - Condition dependence of sexual dimorphism in the antler fly, *Protopiophila litigata*

## INTRODUCTION

For traits subject to persistent directional selection, a component of fitness is an increasing function of investment in the trait, favouring individuals that allocate additional resources to expression of the trait (Andersson, 1982; Nur & Hasson, 1984). The evolutionary exaggeration of such traits should eventually be halted when any further benefit is offset by expression-dependent costs to other fitness components. Life history theory suggests that such costly, fitness-enhancing traits should evolve heightened condition dependence, a form of developmental plasticity that links trait expression to an individual's available pool of metabolic resources (i.e., their 'condition'; Rowe & Houle, 1996; Getty, 1998, 2006; Proulx *et al*., 2002), thereby allowing individuals to express the maximal trait value they can afford. Empirical studies of condition dependence have tended to focus on traits involved in mate acquisition, including sexual displays and armaments (see Cotton *et al*., 2004), because the evolution of condition dependence may provide a mechanism for the maintenance of genetic variance in the face of persistent and often strong directional sexual selection (Rowe & Houle, 1996).

In addition to being condition-dependent, traits that are targets of sexual selection are also often sexually dimorphic. Sexual dimorphism arises ultimately from differences in selection between the sexes on a shared trait. The divergent reproductive interests of males and females mean that sexual selection is usually present in one sex yet weak or absent in the other, and this sex-specificity can result in sexually antagonistic net selection that favours the evolution of dimorphism. While sexually antagonistic selection can also arise from differences in viability and/or fecundity selection alone (Darwin, 1871), the evolution of sexual dimorphism likely often involves natural and sexual selection, for example when costs to non-sexual fitness in females cause selection for the reduced expression of a costly mating display that is beneficial to sexual fitness in males.

Sexually dimorphic traits have a history of directional selection for trait exaggeration in one sex and the extent of dimorphism that evolves is a function of the strength of this selection together with constraints that arise from a genome that is largely shared between the sexes (Lande, 1980). When driven by sexual selection, the trait will be displaced from its viability optimum in males and the degree of phenotypic divergence between the sexes should reflect the magnitude of non-sexual fitness cost associated with the expression of the trait (Bonduriansky, 2007a). Costly traits that are the target of persistent directional selection are also expected to evolve condition dependence, the strength of which should reflect both the intensity of selection and the costs of trait expression (Rowe & Houle, 1996). The strength of condition dependence and the extent of sexual dimorphism are therefore both a function of the strength of sexual selection for exaggeration and the expression-dependent costs this generates, and this joint dependency predicts a positive association such that the extent of sexual dimorphism should itself be condition-dependent (Bonduriansky & Rowe, 2005; Bonduriansky, 2007a). This coevolution of condition dependence and sexual dimorphism predicts that within a trait, the extent of sexual dimorphism should be condition-dependent (i.e. greater among high as opposed to low condition individuals), and among traits, there should be a positive association between the extent of dimorphism and the strength of condition dependence. To the extent that sexual selection for trait exaggeration is male-specific, this pattern in among-trait variation should be stronger in males than in females.

It is well established that dimorphic sexual displays and armaments are condition-dependent in their expression (Cotton *et al.,* 2004). Several studies have gone further to show that the extent of sexual dimorphism for a given trait is sensitive to environmental factors that likely alter condition (David *et al.,* 1994; Post *et al.,* 1999; David *et al.,* 2000; Karan *et al.,* 2000; Bonduriansky & Rowe, 2005; Weladji *et al.,* 2005; Bonduriansky, 2007a; Boughman, 2007; Punzalan *et al.,* 2008), and sexual dimorphism in gene expression has also been shown to increase in high condition individuals (Wyman *et al.*, 2009). Few studies, however, have explored variation among phenotypic traits in condition dependence and its association with sexual dimorphism, and the two of which we are aware both use species exhibiting pronounced sexual dimorphism in body size and shape (Bonduriansky & Rowe, 2005; Bonduriansky, 2007a). In many insects, sexual dimorphism is more modest and obviously exaggerated traits are lacking. Despite the absence of highly dimorphic phenotypes, traits in such species still vary in both the extent of sexual dimorphism and in the strength of condition dependence, but whether the predicted covariation of these can be detected is an open question.

Here, we investigate the relationship between sexual dimorphism and condition dependence in the antler fly, *Protopiophila litigata.* Antler flies are small insects (1.5-3.2 mm body length) that breed on discarded cervid (i.e. moose and deer) antlers, and males exhibit a remarkable site fidelity that makes them an ideal system for longitudinal studies in the wild (e.g., Bonduriansky & Brassil, 2002). Males aggressively defend mating territories on the surface of an antler and large males are both more successful doing so, and also more attractive to females (Bonduriansky & Brooks, 1998a, 1999). In addition to fighting over territories, males guard females during oviposition and sometimes also attempt to dislodge other males that are mating, taking over the mating when successful (Bonduriansky & Brooks, 1998b). These aggressive intrasexual interactions, together with highly male-biased operational sex ratio on antlers, suggest that sexual selection may be strong despite the apparent lack of any highly dimorphic secondary sexual traits and similar body sizes. We investigated condition-dependent sexual dimorphism using a manipulation of larval diet quality to generate high and low condition individuals. While conventional studies of sexual dimorphism and trait allometry tend to focus on extravagant display traits or secondary sexual traits (Bonduriansky, 2007b), antler flies, like most species, lack such exaggerated traits so we analyze variation in a suite of morphological traits including thorax, head, leg, and wing dimensions.

Some traits are sex limited and cannot be included in an analysis of sexual dimorphism, but their condition dependence may still be of interest. The male intromittent organ, called the aedeagus in insects, is one such example. In antler flies, the aedeagus is highly elongated, suggesting a past history of sexual selection although the functional consequences of this elongation have not been investigated. All else being equal, theory predicts strong stabilizing selection on aedeagus length due to the adaptive advantage to males of being able to mate with the greatest range of females, and/or as a means of preventing interspecific matings that results in low fitness hybrids (Eberhard *et al.*, 1998; Eberhard, 2010). Such stabilizing selection should be reflected in a shallow allometric slope, indicating that the length of the aedeagus is relatively insensitive to male body size, and decreased sensitivity to a diet manipulation indicative of diminished condition dependence. We include male aedeagus length in our analyses to test this prediction.

## MATERIALS AND METHODS

### *i) Flies and culture techniques*

An outbred laboratory stock population of *P. litigata* (Fig. 1) was created from a large sample (>500) of adult flies collected in the spring early summer of 2012 at the Wildlife Research Station, Algonquin Park, Ontario, Canada. The population was maintained at the University of Ottawa via non-overlapping generations at 23°C, 60% relative humidity and under a 17:7 L:D photoperiod.

Each generation, 150-300 adults were housed in each of 3-5 acrylic cages 3.4x10$^{-3}$ m$^{3}$. Cages were supplied with abundant granulated sugar (in a small petri dish within each cage) and water (a filled vial with a porous stopper). To collect eggs for the next generation, a single oviposition dish was placed within a cage (two if the density was greater than approximately 200 flies). An oviposition dish was created from the base of a 3.8 cm diameter petri dish and contained a basal layer of approximately 2.5 g of 'regular' (i.e. non-lean) ground beef (Loblaws, Canada) which was covered by an 'egg pad' consisting of a foam sponge (Scrub Buddies, Greenbrier International, Inc.) cut to a 3.8 cm diameter to fit snuggly within the petri dish. The lid of the petri dish had a 1.9 cm hole in the center through which the sponge protruded, and it was taped or glued onto the base to seal the dish to prevent larval escape around the edges. Oviposition dishes remained in adult cages for one week during which females laid eggs into the sponge through the hole. After being removed from cages, up to six same-generation oviposition dishes were placed into a 2.5 L mason jar with a layer (approximately 3 cm deep) of coco peat (Nutri+, India) at the base and a mesh lid to allow air circulation. New oviposition dishes were added to the cages when the egg-saturated dishes were relocated to larval jars. Three times per week, a variable amount of ground beef solution was added to the oviposition pad to ensure that the sponge remained moist. This solution consisted of 20% (w/v) of ground beef that had been let sit in tap water for 24 h, after which the solids were strained from the liquid and discarded. Adults that emerged into the larval jars were collected and transferred to new or existing population cages of next generation flies. Adults of the previous generation were discarded once a large sample (>200) of next generation adults had been established.

Our experiment involved a manipulation of larval diets to create three treatments (high, mid and low quality) that differed in the ratio of ground beef to fibre within the oviposition dishes, as well as the concentration and quantity of ground beef solution applied during larval development. The high quality diet used only regular ground beef, as described for the stock population above, while the intermediate (mid) diet consisted of a 9:1 mixture of ground beef:fibre ('Inulin', Exact$^{TM}$, Canada). Both diets were prepared by homogenising the ground beef, with or without added fibre, using a standard household food blender. Preparations were stored in a freezer at -20°C prior to use. During larval development, high and mid quality diets also received 1.5 ml of a 10% ground beef solution (w/v) three times per week. A 10% solution was used in place of the 20% solution described above in an attempt to increase larval reliance on the ground beef/fibre below the egg pad. The low quality diet had no ground beef below the egg pad, and consisted of up to 3 ml (less if oviposition dish became fully saturated) of a 20% ground beef solution three times per week during larval development.

Our analyses were based on the offspring of generation six adults from the stock population when raised on these three diets. Offspring were collected by placing 50 randomly chosen adults of each sex in each of five population cages. Adult density was monitored daily, replacing any dead individuals with new stock flies and thereby ensuring 100 live individuals per cage and a 1:1 female:male sex ratio. These cages were housed under the same environmental conditions (temperature, humidity and photoperiod) as the stock population and received an abundant supply of granulated sugar and water. Eggs were collected by adding a single oviposition dish to each cage. A given oviposition dish remained in a cage for 48 h, after which it was replaced with a new one. Six sequential rounds of egg collection were performed, with no time between them, creating six temporal blocks of offspring. Diet treatments were not applied until after the egg pads were removed, meaning that during egg collection all pads were the same across all cages, preventing females from adjusting their egg laying in relation to diet treatment. All three diet treatments were applied within each of the six temporal blocks by adding ground beef (mid and high treatments), as well as the appropriate ground beef solution to the egg pad. After application of the diet treatment, oviposition dishes were individually relocated to separate 250 ml mason jars with 10 g of dry coco peat layering the base and a mesh cap to allow air circulation. Ground beef solution was applied during subsequent larval development, as previously described. Emerging adults were removed daily and held for 24 h in a vial to allow their cuticle to sclerotize, after which they were preserved in 99% ethanol and placed in a freezer at -20°C for subsequent morphological measurement.

### *ii) Morphometric data*

For each of the three diet treatments, 20 adults of each sex were measured. Developmental time differed among diets (see Results) and the measured individuals for each diet were therefore chosen to have a developmental time that was approximately average for all individuals on that diet while still including individuals from all six temporal blocks. For each fly, 10/11 (female/male) linear measurements were made (Fig. 1): thorax length (TL), head width (HW), head height (HH), fore-tibia length (FL), mid-tibia length (ML), hind-tibia length (HL), fore-tarsus length (FT), mid-tarsus length (MT), hind-tarsus length (HT), wing length (WL; from the inner r-m cross-vein to the distal end of the $R_{4+5}$ vein) and, in males only, aedeagus length (AD; from the proximal end where, the aedeagus attaches to the epandrium, to the distal tip). Prior to measurements, all appendages (legs, both wings, aedeagus and head) were severed at the point of attachment to the body using McPherson-Vannas micro iris scissors (Miltex/Integra LifeSciences, PA, USA). Dissected wings, legs and aedeagi were mounted on standard microscope slides using double sided tape, with a coverslip added to the aedeagi. The remaining head and thorax were placed in a droplet of glycerol on a microscope slide to avoid desiccation. Samples were photographed using A640 PowerShot Canon camera mounted on a Zeiss Discovery V.12 stereo microscope with an ocular micrometer. Images were captured using ZoomBrowser EX software (Canon Inc., Australia) and measurements were performed using ImageJ software 1.47v (National Institute of Health, Bathesda, Maryland). The measurement repeatability of each morphological trait was estimated by re-mounting, re-imaging and re-measuring 20 randomly selected individuals from either sex. Re-mounting traits involved delicately removing them from the tape with insect pins and re-positioning them in a different orientation. Repeatabilities were estimated as the ANOVA-based intraclass correlation coefficient (Lessells & Boag, 1987) and were $\geq 0.9$ for all traits (Table 1).

### *iii) Statistical analyses*

Traits covaried positively, as expected for morphological components that scale allometrically, and we therefore separately analysed variation in body size and size-corrected components of shape. As an index of body size, we used the first principal component (PC1) of the correlation matrix of all traits (except aedeagus length) across both sexes and all three diets. The traits all loaded positively and with similar magnitude on PC1, as expected for an index of body size (Table 2). The effects of sex, diet, and their interaction on body size (PC1) were tested using a general linear mixed model:

$$\text{PC1} = \text{Sex} + \text{Diet} + \text{Sex} \times \text{Diet} + \text{Block}, \qquad \text{Eqn. 1}$$

where Block is the random effect of temporal block and all other terms are fixed. Our analysis demonstrated a significant overall effect of diet treatment on body size, but post hoc comparisons revealed this to be due almost entirely to a significantly reduced size in the low quality diet with no difference between the mid and high treatments and no interaction with sex (see Results). Our subsequent analysis of the separate shape components therefore used the low and high diets only, reserving the mid quality diet to provide an independent estimate of sexual dimorphism for our across-trait examination of condition-dependent sexual dimorphism (see below).

Effects on body shape were tested in separate analyses of the head, legs, and wing measurements by including body size (PC1) as a covariate in Eqn. 1, thereby quantifying change in the relative size of these traits. For each trait, PC1 was re-calculated after excluding the focal trait (i.e. dependent variable) to avoid modelling a trait as a function of itself. There was no evidence of heterogeneity of the allometric slopes between diets treatments or the sexes for any of the traits (Table 3), so the interaction terms of these fixed effects with PC1 were excluded from the models. The only exception was a significant PC1 × Sex × Diet effect for wing length. However, this effect was not strong (Fig. 2) and we therefore proceeded with the analysis using a single, common slope. Results for this trait should nevertheless be interpreted with some caution. All models were fit using restricted maximum likelihood and significance of random effect terms (i.e., interactions with PC1) were determined via likelihood ratio tests that compared models that included and excluded each of these terms. Variation in aedeagus length, a trait which is absent in females, was tested using a simplified version of Eqn. 1 that included only the fixed diet and random block terms. This was done both including and excluding PC1 as a covariate, thereby testing for diet-induced variation in relative and absolute aedeagus size, respectively.

To explore the pattern of condition-dependent sexual dimorphism across traits, condition dependence was estimated as the difference in mean trait value between individuals raised on the high vs. low quality diets, separately by sex. To avoid autocorrelation of these values with the degree of sexual dimorphism (i.e. because some of the same data would contribute to estimates of both), dimorphism values were calculated for each trait as the difference in mean size between males and females using individuals from the mid quality diet. Body-size variation was removed from each trait prior to analysis by taking the residuals from a regression of each trait against PC1 of all other traits (using a single, common slope and excluding the dependent trait in the calculation of PC1 in each case). This was done separately for the condition dependence and sexual dimorphism data sets, combining the sexes in each case. The resulting values were standardized ($\sim N(0,1)$) across sexes prior to calculating sexual dimorphism (mid quality diet only), and across diets (low and high quality) prior to calculating condition dependence scores separately for males and for females. The relationship across traits between the degree of sexual dimorphism and the extent of condition dependence was visualized using reduced major axis regression, assuming equal error variances in both variables. Significance of the correlation was evaluated using a standard least-squares approach. All statistical analyses were performed using JMP version 10.0.0.

## RESULTS

Developmental time from oviposition to adult emergence increased significantly with decreasing larval diet quality (mean number of days ± SE; high quality: 32.6 ± 0.3; mid quality: 39.5 ± 0.5; low quality: 59.9 ± 2.6; ANOVA: $F_{2,9.97} = 19.5$, $P = 0.0004$). The average number of adults that emerged from each egg pad also decreased significantly with declining larval diet quality (mean number of individuals ± SE; high quality: 65.3 ± 16.1; mid quality: 40.2 ± 6.6; low quality: 9.8 ± 3.6; ANOVA: $F_{2,10} = 7.3$; $P = 0.0111$), indicating differences in hatching success and/or larval survival across treatments.

Adult body size (PC1) varied significantly with diet quality ($F_{2,93.34} = 51.5$, $P < 0.0001$) but did not differ between the sexes ($F_{1,96.94} = 2.2$, $P = 0.144$), nor was there any evidence of a sex × diet interaction ($F_{2,96.25} = 1.1$, $P = 0.345$). The diet effect was caused by a reduced average size of the flies reared on the low quality diet as compared to those reared on the mid and high quality diets, with these latter two groups differing little from each other (Fig. 3). Post hoc comparisons using Tukey's HSD confirmed that the only significant difference was between individuals reared on the low vs. mid and high quality diets. Given the non-significant difference in body size between the mid and high quality diet-reared individuals, our analysis of body shape focused on the low and high quality diets, reserving individuals from the mid quality diet for use in the among-trait analysis of condition-dependent sexual dimorphism.

Effects on body shape were examined by including body size (PC1) as a covariate when testing the effects of sex, diet and their interaction in separate analyses of head, legs and wing dimensions, thereby quantifying change in the relative size of these traits. Significant sexual dimorphism was detected for several shape traits (Table 4), with males having a larger relative size for some traits (i.e. fore-tibia length (FL), mid-tibia length (ML), hind-tibia length (HL)) and smaller for others (i.e. fore-tarsus length (FT), wing length (WL), head width (HW) and thorax length (TL)). Diet quality had a significant effect on three traits, with a high quality larval diet increasing relative hind-tibia length and relative head width and decreasing relative hind-tarsus length (Table 4). The sex × diet interaction was non-significant in all cases and, although it approached significance for relative mid-tarsus length ($P = 0.0605$), the pattern was opposite to that expected under condition-dependent sexual dimorphism with males and females differing more when raised on the low compared to the high quality diets. Despite the absence of a sex × diet interaction for any of the shape traits on their own, there was a pattern in multivariate trait space in which females were less sensitive to the effects of the diet manipulation than were males (Fig. 4). Finally, across these shape traits, there was a positive correlation between the strength of condition dependence in males and females (Fig. 5) that approached significance (Pearson correlation: $N = 10$, $r = 0.604$, $P = 0.064$).

To examine the relation between sexual dimorphism and condition dependence across traits, a condition dependence score was calculated for each trait, separately for males and females, as the difference in mean values between individuals raised on high vs. low quality diets. Sexual dimorphism for these traits was calculated as the difference in mean value (male – female) using individuals from the mid quality diet. In males, there was a significant positive association across traits between the degree of sexual dimorphism and the strength of condition dependence (Fig. 6a; Pearson correlation: $N = 10$, $r = 0.650$, $P = 0.042$). While a similar pattern was also present in females (Fig. 6b), there was less variation among traits in the strength of condition dependence and the correlation was not significant (Pearson correlation: $N = 10$, $r = 0.486$, $P = 0.154$). Results were qualitatively unchanged if sexual dimorphism was estimated using individuals from the high quality diet instead.

Variation in male genitalia was examined by modelling aedeagus length as a function of diet quality and body size (PC1). Heterogeneity of allometric slopes between diet treatments (i.e. the PC1 × Diet interaction) was non-significant using both a least-squares approach ($P = 0.283$, Table 3) and reduced major axis regression ($P = 0.484$; Fig. 7), and remained non-significant when excluding the single abnormally large-bodied, low diet-quality individual (least squares: $P = 0.065$). Combining individuals across diet treatments, the scaling of aedeagus length with body size was significant (Pearson correlation: $N = 38$, $r = 0.345$, $P = 0.034$), but it was weaker than for all the other morphological traits (Fig. 8; $r > 0.818$ for all other traits). Significance was also strongly influenced by a single low diet quality individual with an unusually short aedeagus relative to its small body size, and removal of this individual rendered the association non-significant ($N = 37$, $r = 0.208$, $P = 0.216$). Consistent with weak allometric scaling of this trait, absolute aedeagus length did not differ significantly between diet treatments (mean length in mm ± SE; high quality: 1.528 ± 0.015; low quality: 1.522 ± 0.011; $t_{40} = 0.34$; $P = 0.739$), unlike all other traits ($P < 0.0001$ in all cases). Because body size, but not aedeagus length, increased with higher diet quality, relative aedeagus length was significantly less in the high as compared to the low diet quality males (Table 4).

## DISCUSSION

Due to their shared dependence on persistent, directional sexual selection, theory predicts that the extent of sexual dimorphism and strength of condition dependence should coevolve such that within traits, the degree of dimorphism should increase with increasing condition, and among traits, the strength of condition dependence should covary with the extent of sexual dimorphism (Bonduriansky & Rowe, 2005; Bonduriansky, 2007a). Empirical support for these predictions comes from two morphological studies using species with pronounced sexual dimorphism (*Prochyliza xanthostoma* and *Telostylinus angusticollis*; Bonduriansky & Rowe, 2005; Bonduriansky, 2007a), and from a gene expression study in *D. melanogaster* (Wyman *et al.*, 2009). Here we tested these predictions in antler flies, a species in which sexual dimorphism is qualitatively weak and subtle, and males possess no ’exaggerated’ traits except a greatly elongated intromittent organ (aedeagus). We detected significant sexual dimorphism in the relative size of several traits and significant condition dependence of others. While there was little evidence that the extent of sexual dimorphism was condition-dependent for any particular trait, there was a significant association across traits in males but not females. Our results suggest that sexual dimorphism and condition dependence in body shape have coevolved in antler flies, and suggest that this is due, at least in part, to sexual selection on males. We discuss these results and their implications in more detail below.

### *i) Within-trait variation*

Body size was condition-dependent in that individuals raised on the mid and high quality diets were significantly larger than those raised on the low quality diet. In contrast, sexual dimorphism in body size was non-significant overall. The size of females relative to males did tend to increase across the three diet qualities, from near monomorphism on the low quality diet to noticeable dimorphism on the high quality diet (Fig. 3), but this was not sufficient to generate a diet × sex interaction and was driven largely by a slight reduction in male size on the high quality diet. Whether significant sexual size dimorphism may emerge among very high quality individuals is therefore an open question, but our current result demonstrating a similar pattern of condition dependence in both sexes suggests that body size has been the target of directional selection for exaggeration in both males and females. Sexual selection for larger males is likely as previous observational studies have detected an apparent female preference for larger-bodied males (Bonduriansky & Brooks, 1998a), and increased size also benefits males during intrasexual competition (Bonduriansky & Brooks, 1999). In females, fecundity selection may favour increased body size (Bonduriansky & Brooks, 1998a) and sexual selection may as well given that males have been suggested to be choosy (due to the high apparent costs of courtship, mating, and mate guarding; Bonduriansky & Brooks, 1998b) and may prefer larger females due to their increased fecundity.

With respect to body shape, male antler flies had relatively longer tibial segments than females on each of the three pairs of legs (i.e., fore, mid, and hind), suggesting these traits, or overall leg length, as potential targets of sexual selection. Mid and hind legs are involved in mating, likely serving to aid in both mounting and in actual copulation (Bonduriansky & Brooks, 1998b), and after mounting, males use their mid and hind legs to tap the females' abdomen, possibly as a means to assess gravidity (Bonduriansky & Brooks, 1998a, b). Males will also attempt to dislodge other mounted males and take-over mating with the female, sometimes resulting in prolonged wrestling bouts (Bonduriansky & Brooks, 1998b). Longer legs may therefore also function in resisting such attempts and/or preventing females escape. Hind-tibia length was the only one of these traits to exhibit significant condition dependence, with relatively greater values in males reared on high as compared to low quality food. However, there was no indication of stronger condition dependence of any of these traits in males compared to females (i.e., a sex × diet interaction).

Female antler flies had larger values than males for several shape traits including relative head width (HW), and thorax (TL), fore-tarsus (FT), and wing length (WL), with only relative head width increasingly significantly on the high quality diet. There was no indication that the strength of condition dependence varied between the sexes for any of these traits (i.e. a sex × diet interaction). Whether this female-biased dimorphism is the result of selection on males for smaller relative sizes of these traits, and/or selection on females for larger values, is unknown. Males do use their wings to fend off take-over attempts by other males during mating (Bonduriansky & Brooks, 1998b), so selection for smaller wings would seem unlikely. Whether and how sexual selection targets these traits in both sexes is an important topic for future study.

Condition-dependent sexual dimorphism requires non-parallel reaction norms to the diet treatments in males and females such that the sexes differ more when raised on a high as opposed to low quality diet. Neither body size nor any of our measured shape traits demonstrated such a pattern, despite significant sex and diet effects on some of these. The absence of this in antler fly body size and shape contrasts with past results in other species (including neriid and piophilid flies) in which dimorphism in body size and some (but not all) shape traits increased on a high condition diet (Bonduriansky & Rowe, 2005; Bonduriansky, 2007a). Insufficient power, resulting from the comparatively low sexual dimorphism that exists in this species compared to those used in the past studies, may have contributed to our inability to detect such an effect. Among-individual variation within a given trait may also be less than the among-trait variation, making the covariance of sexual dimorphism and condition dependence harder to detect in analyses of the former as compared to the latter.

The absence of condition-dependent sexual dimorphism within a trait could also arise if the genetic basis of condition dependence is shared between the sexes (Bonduriansky & Rowe, 2005). Such a shared genetic basis would constrain the developmental plasticity of the sexes to respond similarly to increased resources, reducing or even preventing any pattern of increased dimorphism. Covariation of condition dependence among homologous traits in males and females has been previously observed in carrion flies (Bonduriansky & Rowe, 2005) and was substantial and near-significant in our experiment ($r = 0.604$, $P = 0.064$; Fig. 5). Selection for heightened condition dependence in males may lead to the breakdown of the intersex genetic correlation for condition dependence, as suggested by the negative association between these in carrion flies (Bonduriansky & Rowe, 2005). In antler flies, our measured shape traits may only be subject to directional selection in one sex such that similar condition-dependent expression in both sexes may reflect, at least in part, an intersexual genetic correlation for condition dependence. In contrast, body size is likely subject to directional selection in males and females, as discussed above, producing a concordant pattern of condition dependence in both sexes.

### *ii) Among-trait variation*

In males, there was a significant association across traits between the strength of condition dependence and degree of sexual dimorphism, consistent with the evolution of condition-dependent sexual dimorphism. Although there was some evidence of a similar pattern in females, shape in this sex was less sensitive to the diet manipulation than it was in males (Fig. 4) and the association was non-significant. The weaker and non-significant association between condition dependence and sexual dimorphism among traits in females suggests that this pattern has evolved in response to directional sexual selection on males, favouring larger relative sizes of some morphological components and smaller relative size of others. The hypothesized effect of sexual selection on the evolution of condition dependence is also expected to result in a common genetic basis to sex-dependent and condition-dependent trait expression, and this prediction is supported by our results for the first time in a species lacking pronounced 'exaggerated' secondary sexual traits. In addition, we found some evidence of a correlation between male and female traits in the degree of condition dependence. This pattern suggests the presence of an intersexual genetic correlation for condition dependence, which may contribute to intralocus sexual conflict (Bonduriansky, 2007c).

While consistent with condition-dependent sexual dimorphism, the pattern in male antler flies differs somewhat from that previously observed in *Telostylinus angusticollis* and *Prochyliza xanthostoma*. In these species, male traits were always relatively larger in high as opposed to low condition individuals, with the magnitude of this effect being greater for traits that were more male biased (i.e., more sexually dimorphic, with males as the larger sex; Bonduriansky, 2007a; Bonduriansky & Rowe, 2005). In male antler flies, the strength of condition dependence tends to be greater for traits that are more sexually dimorphic, but this is not only because traits that are normally larger in males become even more so under high condition, but also because traits that are smaller in males tend to become relatively smaller in high condition males. In other words, although traits respond in different directions to the diet manipulation, those that are more dimorphic respond to a greater degree and the response tends to be in the direction of increasing dimorphism, just as in the other species where condition-dependent sexual dimorphism has been observed.

Theory predicts that traits that are more strongly dimorphic should also be more condition-dependent. In the current study, while the association among traits was significant, variation in condition dependence was not fully explained by variation in sexual dimorphism and the correlation we observed in antler flies was weaker than that seen in the neriid fly (Bonduriansky, 2007a). This is perhaps not surprising as limited dimorphism suggests relatively weak sexual selection for trait exaggeration in males, and hence only a moderate heightening of condition dependence of these traits. In such a situation, the relative importance of other factors affecting sexual dimorphism and/or condition dependence may be greater. For example, ecological and social conditions may mediate sex-specific resource acquisition and plasticity in its subsequent allocation (De Lisle & Rowe, 2014), and failure to account for these may weaken the observation association between sexual dimorphism and condition dependence. Direct estimates of sexual selection on male phenotypes will be key to testing this idea, as will additional data from a variety of species with differing degrees of dimorphism.

One caveat to the interpretation of our results stems from the significant decrease in the number of adults that emerged as diet quality declined. The diet treatments in our experiment were not applied until after eggs were laid, meaning that the number of eggs/replicate should not have differed and implying that the observed effects were due to differences across treatments in hatching success and/or subsequent larval survival. In theory, effects on trait means could therefore have arisen not from treatment-specific developmental reaction norms in response to diet, but rather from sampling effects caused by selective deaths in each treatment. The extent to which this may have occurred is difficult to assess, although such process would not be expected to produce the observed association between sexual dimorphism and condition dependence.

### *iii) Male genital trait expression*

Low phenotypic variation and reduced condition dependence relative to other traits is often observed in male genitalia of insects and spiders (Eberhard *et al.,* 1998; Bernstein & Bernstein, 2002; Vencl, 2004; House & Simmons, 2007; but see Arnqvist & Thornhill, 1998 and Green, 1999) and this has been suggested to be the product of stabilizing selection arising because intermediate sized genitalia may allow a male to mate with the greatest range of females, and/or reduce their risk of forming low fitness hybrids (Eberhard, 1985; Eberhard *et al.*, 1998; Eberhard, 2010). Male aedeagus size in antler flies demonstrated a similar pattern: allometric scaling of this trait with body size was weaker than for other body shape components and significance was sensitive to the inclusion of a single small bodied low quality diet individual. Weak or absent allometric scaling of the antler fly aedeagus length suggests that even greatly exaggerated genitalic traits can behave like typical ones, being fairly insensitive to body size variation.

Although aedeagus length does not appear to scale strongly with body size overall, it is also worth noting that heterogeneity of the allometric slopes (i.e., PC1 × diet interaction) ($P$ = 0.2825) approached significance if a single abnormally large-bodied, low diet-quality individual was excluded (Fig. 8; least squares: $P$ = 0.065). Fitting allometric slopes separately by diet quality reveals a much steeper relationship in the low diet quality individuals compared to the high diet quality, suggesting that aedeagus length may be sensitive to condition in low quality individuals but then plateau once a certain threshold is reached. The relationship between male aedeagus length and body size, in particular in low quality individuals, therefore remains an open question.

**Table 1.** Measurement repeatabilities for all morphological traits in *Protopiophila litigata*, estimated as the ANOVA-based intraclass correlation coefficient (N = 20 in all cases). The sex used for calculating the repeatability of each trait is included.

| Trait | Repeatability | Sex |
|---|---|---|
| Wing length (WL) | 0.99 | ♀ |
| Fore-tibia length (FL) | 0.95 | ♀ |
| Mid-tibia length (ML) | 0.96 | ♀ |
| Hind-tibia length (HL) | 0.96 | ♀ |
| Fore-tarsus length (FT) | 0.97 | ♂ |
| Mid-tarsus length (MT) | 0.95 | ♂ |
| Hind-tarsus length (HT) | 0.95 | ♂ |
| Head width (HW) | 0.95 | ♂ |
| Head height (HH) | 0.90 | ♂ |
| Thorax length (TL) | 0.94 | ♂ |
| Aedeagus length (AD) | 0.94 | ♂ |

**Table 2.** Eigenvectors of the first principal component (PC1) for all measured traits across males and females from the low, mid and high quality diets.

| Trait | PC1 loadings |
|---|---|
| Wing length (WL) | 0.274 |
| Fore-tibia length (FL) | 0.319 |
| Mid-tibia length (ML) | 0.327 |
| Hind-tibia length (HL) | 0.326 |
| Fore-tarsus length (FT) | 0.325 |
| Mid-tarsus length (MT) | 0.325 |
| Hind-tarsus length (HT) | 0.330 |
| Head width (HW) | 0.330 |
| Head height (HH) | 0.326 |
| Thorax length (TL) | 0.274 |

**Table 3.** Mixed model results testing for heterogeneity between the sexes and diet treatments in the allometric slopes representing the scaling of various traits with body size (PC1).

| | Model coefficient (*P*-value)* | | |
|---|---|---|---|
| Trait | PC1 × Sex | PC1 × Diet | PC1 × Sex × Diet |
| Fore-tibia length (FL) | -0.3 (0.752) | 0.4 (0.702) | 1.2 (0.187) |
| Fore-tarsus length (FT) | -0.7 (0.467) | -0.5 (0.696) | -0.5 (0.638) |
| Mid-tibia length (ML) | -1.2 (0.264) | -0.3 (0.790) | 0.2 (0.886) |
| Mid-tarsus length (MT) | 1.8 (0.149) | 0.3 (0.857) | 1.6 (0.189) |
| Hind-tibia length (HL) | -1.0 (0.355) | 0.6 (0.583) | 1.2 (0.265) |
| Hind-tarsus length (HT) | 1.2 (0.307) | 0.3 (0.849) | 0.4 (0.697) |
| Wing length (WL) | -0.6 (0.808) | -3.1 (0.308) | **-6.5 (0.007)** |
| Head width (HW) | 2.0 (0.195) | --1.8 (0.250) | 0.6 (0.713) |
| Head height (HH) | 0.3 (0.825) | -1.2 (0.506) | -1.2 (0.412) |
| Thorax length (TL) | 1.3 (0.770) | -3.1 (0.576) | 1.9 (0.645) |
| Aedeagus length (AD) | NA | -5.0 (0.283) | NA |

*For display purposes, coefficients have been multiplied by 1000 in all cases. Bold font denotes probabilities < 0.05.

**Table 4.** Least-squares means* of various shape traits for males and females when reared on low and high quality larval diets. Model coefficients and their significance are also shown for the fixed effects of sex, diet and their interaction (sex × diet) from the separate analysis of each trait.

| | Low quality diet | | High quality diet | | Model coefficient (*P*-value)‡ | | |
|---|---|---|---|---|---|---|---|
| Shape trait† | Males | Females | Males | Females | Sex | Diet | Sex × Diet |
| Fore-tibia (FL) | 0.467 | 0.437 | 0.473 | 0.448 | **-12.7 (<0.001)** | 3.0 (0.152) | 1.3 (0.400) |
| Fore-Tarsus (FT) | 0.479 | 0.496 | 0.469 | 0.488 | **9.0 (<0.001)** | -4.5 (0.130) | 0.6 (0.738) |
| Mid-tibia (ML) | 0.547 | 0.534 | 0.553 | 0.529 | **-0.9 (<0.001)** | 0.2 (0.922) | -2.6 (0.156) |
| Mid-tarsus (MT) | 0.553 | 0.540 | 0.533 | 0.536 | -2.4 (0.269) | -6.1 (0.118) | 4.2 (0.061) |
| Hind-tibia (HL) | 0.536 | 0.517 | 0.557 | 0.532 | **-10.7 (<0.001)** | **7.6 (0.003)** | -1.5 (0.422) |
| Hind-tarsus (HT) | 0.597 | 0.604 | 0.583 | 0.587 | 2.8 (0.124) | **-7.6 (0.014)** | -0.6 (0.730) |
| Wing (WL) | 1.030 | 1.058 | 1.043 | 1.079 | **15.9 (0.001)** | 8.3 (0.237) | 2.2 (0.619) |
| Head Width (HW) | 0.699 | 0.722 | 0.733 | 0.743 | **8.2 (0.004)** | **13.9 (0.001)** | -3.3 (0.223) |
| Head Height (HH) | 0.513 | 0.516 | 0.514 | 0.525 | 2.4 (0.316) | 1.2 (0.715) | 0.1 (0.953) |
| Thorax (TL) | 0.782 | 0.814 | 0.794 | 0.821 | **14.8 (0.045)** | 4.5 (0.716) | -1.2 (0.867) |
| Aedeagus (AD) | 1.554 | NA | 1.487 | NA | NA | **-33.7 (0.004)** | NA |

*Least squares means for the sex × diet interaction from Eqn. 1 with body size (PC1) also included.

†PC1 was included as a covariate for all traits and was highly significant in all cases ($P < 0.0001$) except aedeaugus length (see Results).

‡For display purposes, coefficients have been multiplied by 1000 in all cases. Bold font denotes probabilities < 0.05.

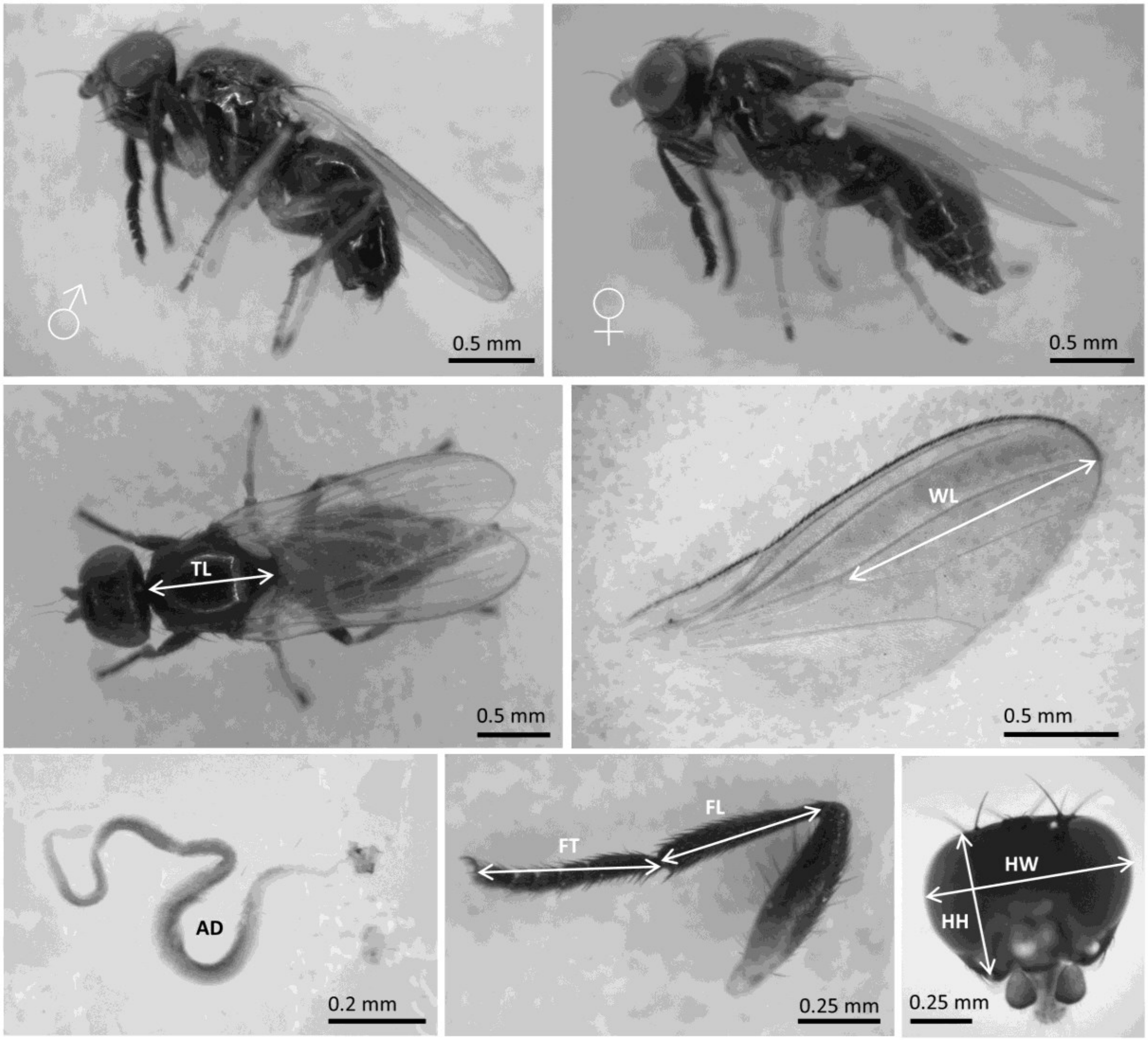

**Figure 1.** Morphological traits measured in the antler fly, *Protopiophila litigata.* Shown are: wing length (WL), fore-tarsus length (FT), fore-tibia length (FL), head height (HH), head width (HW), thorax length (TL), and male aedeagus length (AD). Mid-tibia length (ML), mid-tarsus length (MT), hind-tibia length (HL) and hind-tarsus length (HT) are not shown, but were measured analogous to that shown for the fore-leg.

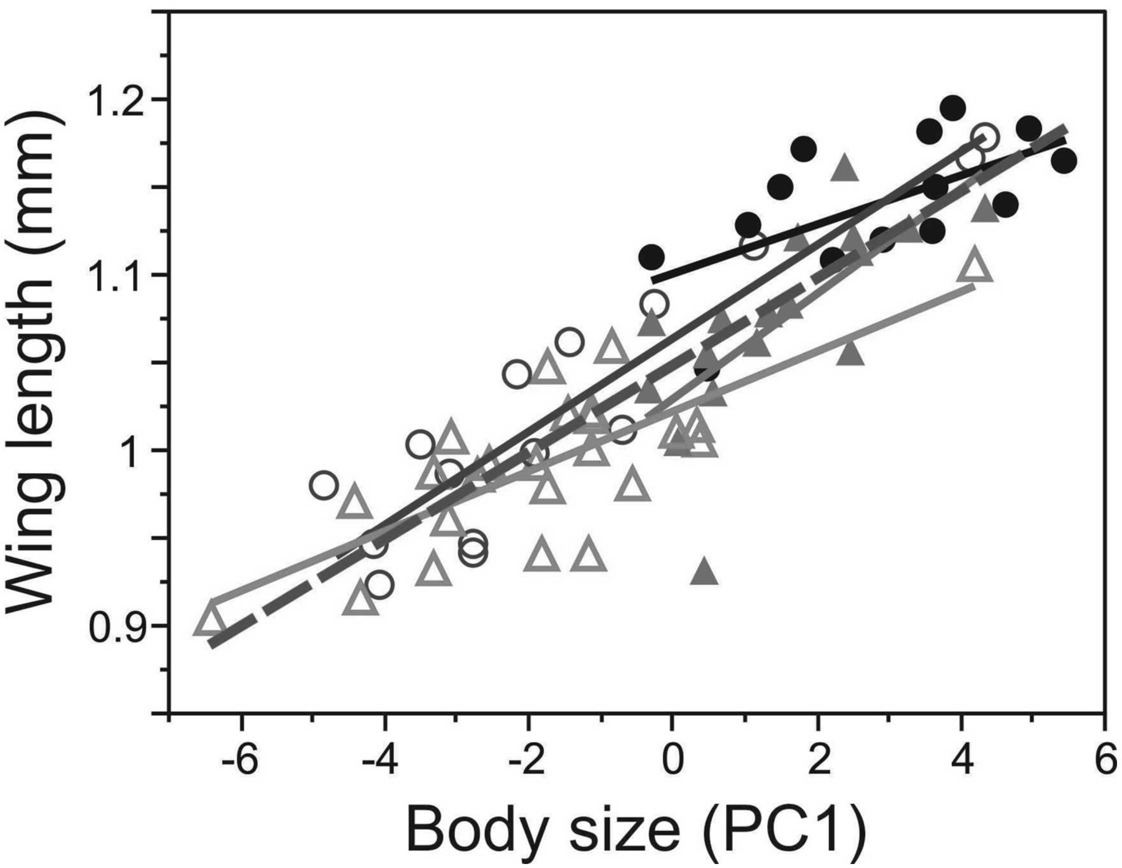

**Figure 2.** Allometric scaling of wing length with body size (PC1 of all other traits) for females (circles) and males (triangles) when raised on low (open symbols) or high (filled symbols) diet quality. Fitted lines are from ordinary least-squares regressions separately for each sex × diet combination (solid lines) or across both sexes and diets (dashed line).

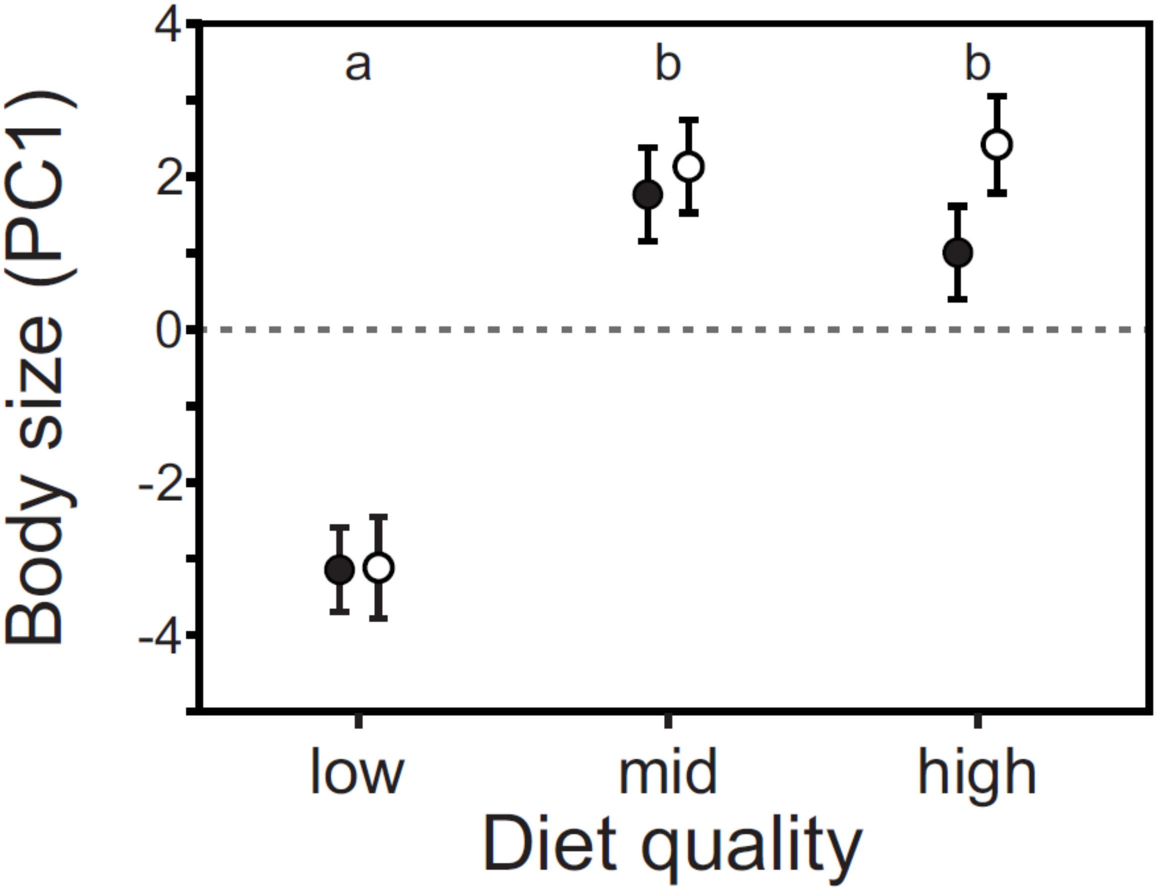

**Figure 3.** Body size comparisons across diets for males (closed circles) and females (open circles). Letters above diet treatments represent post hoc comparisons using Tukey's HSD test. Points are least squares means from Eqn. 1 ± 1 SE.

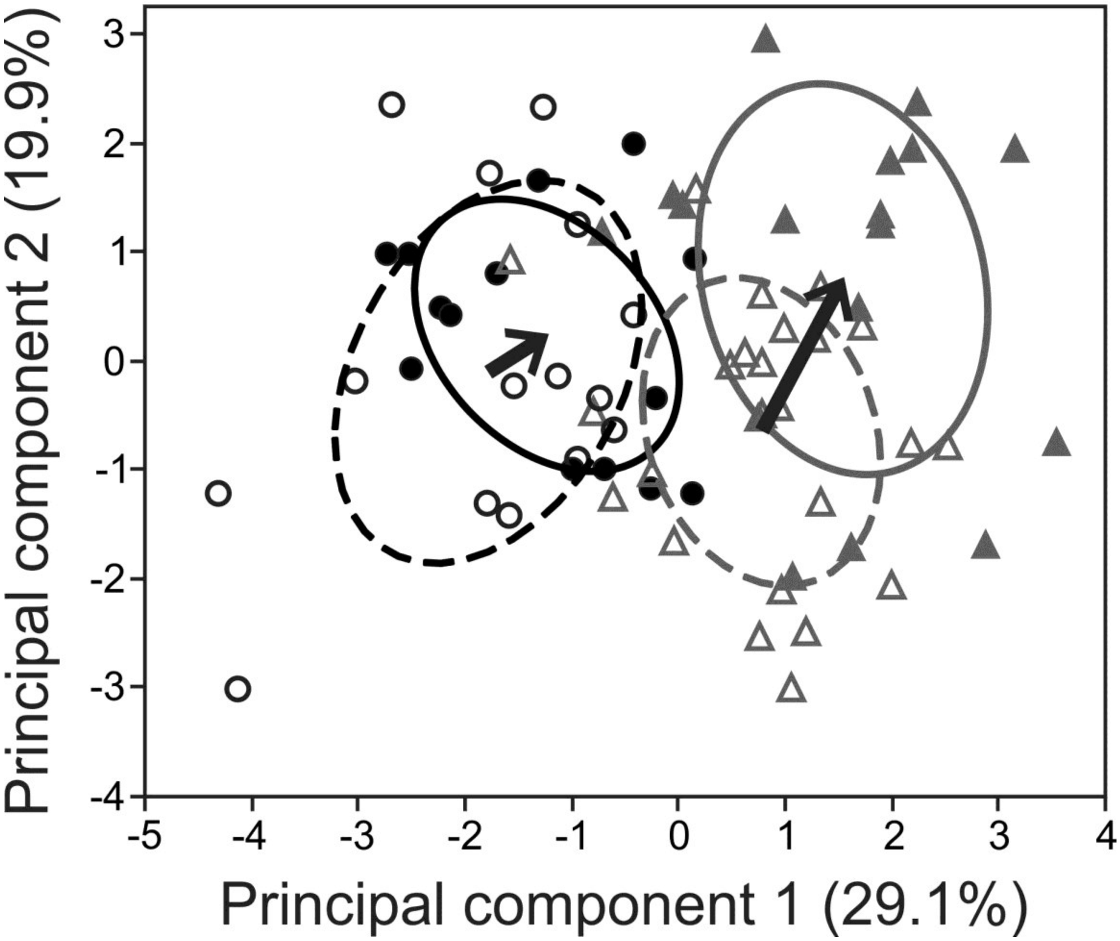

**Figure 4.** Multivariate variation in body shape in response to sex and diet. Points are individual values for males (grey triangles) and females (black circles), when raised on low (open symbols) and high (filled symbols) quality diets, for the first two principal components of the correlation matrix among the 10 morphological shape traits. Body-size variation was first removed by taking the residuals from a regression of each trait against PC1 (re-calculated each time after excluding the focal trait), employing a common slope across both sexes and diets. Large circles represent 50% bivariate normal density ellipses for low (dashed) and high (solid) diet quality individuals of each sex. The vectors depict the diet effect in each sex, joining the low diet quality centroid with the high diet quality centroid separately for males and females.

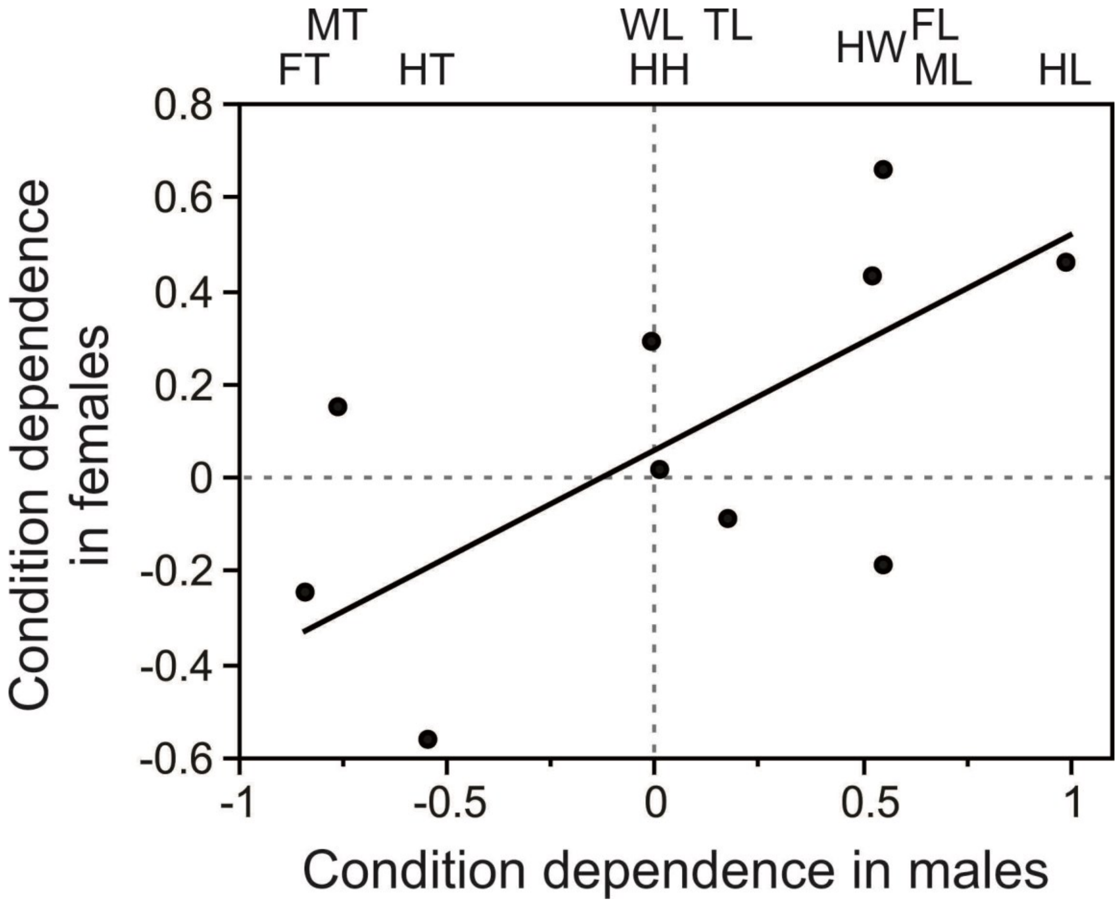

**Figure 5.** Covariation of degree of condition dependence (mean difference between diets: high – low quality) in males and females. Fitted line is from a reduced major axis regression (*r* = 0.604, *P* = 0.064). Trait labels (see Table 1) align vertically with their respective points.

Trait labels (see Table 1) align vertically with their respective points.

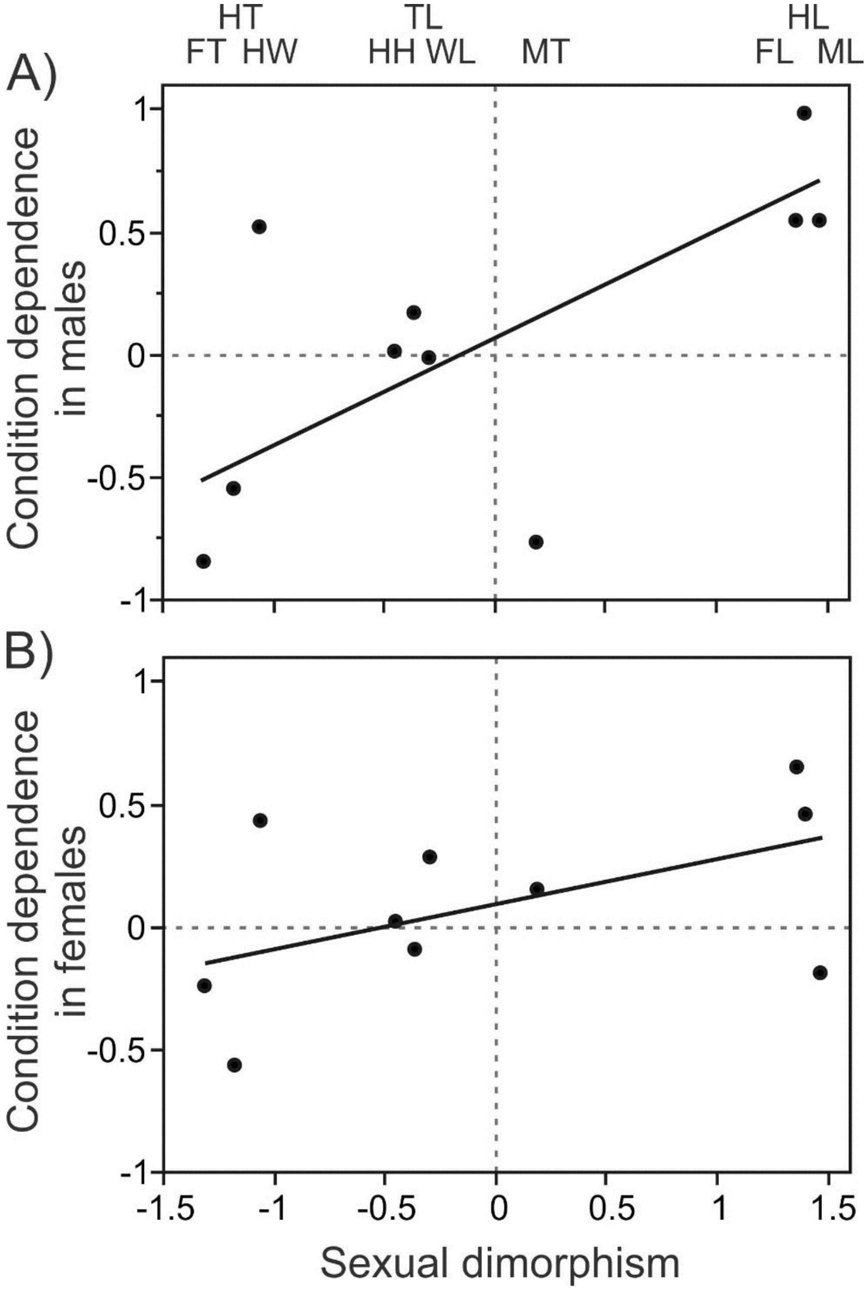

**Figure 6.** Covariation among traits in the strength of condition dependence (mean difference between high and low quality diet) and the extent of sexual dimorphism (mean difference between male and female) for (a) males and (b) females. Fitted lines are from a reduced major axis regression in each sex. Trait labels (see Table 1) align vertically with their respective points.

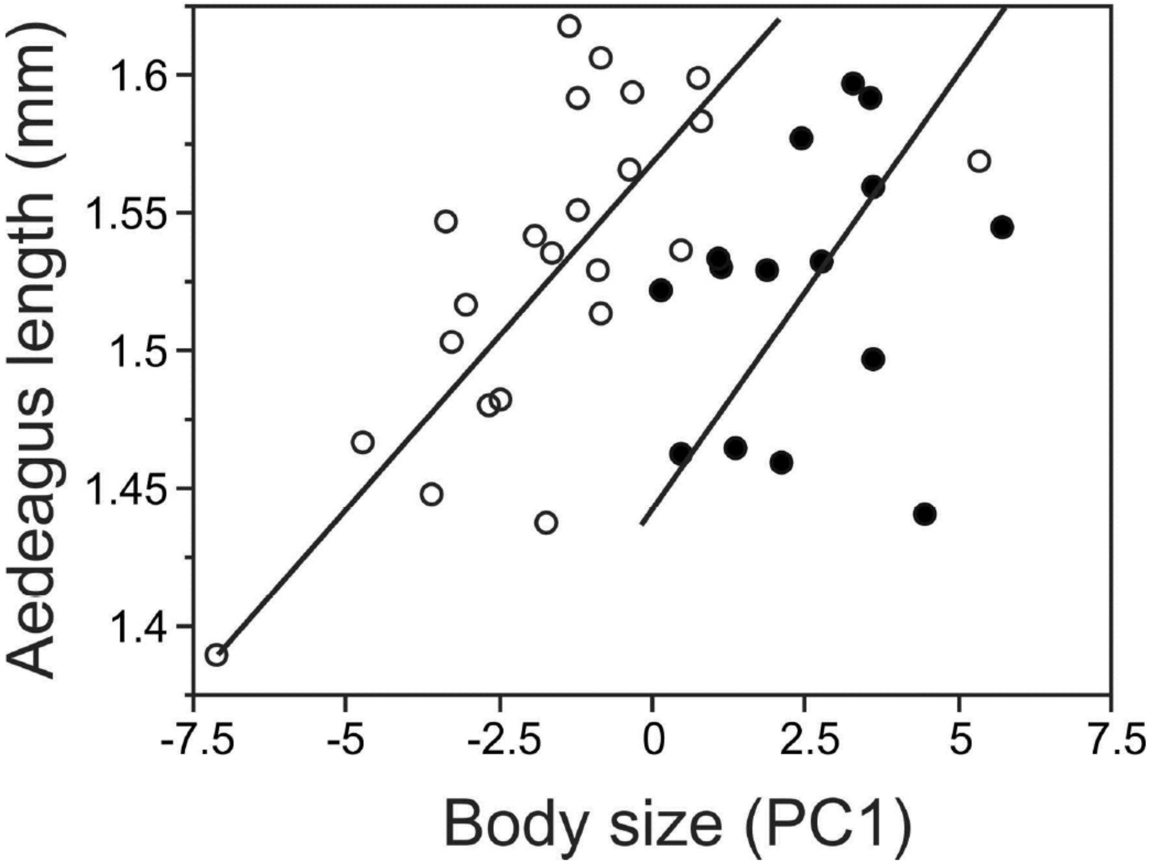

**Figure 7.** Allometric scaling of the length of the aedeagus (the male intromittent organ) with body size (PC1 of all other morphological traits) for males reared on a low (open circles) or high (filled circles) quality diets. Fitted lines are from separate reduced major axis regressions.

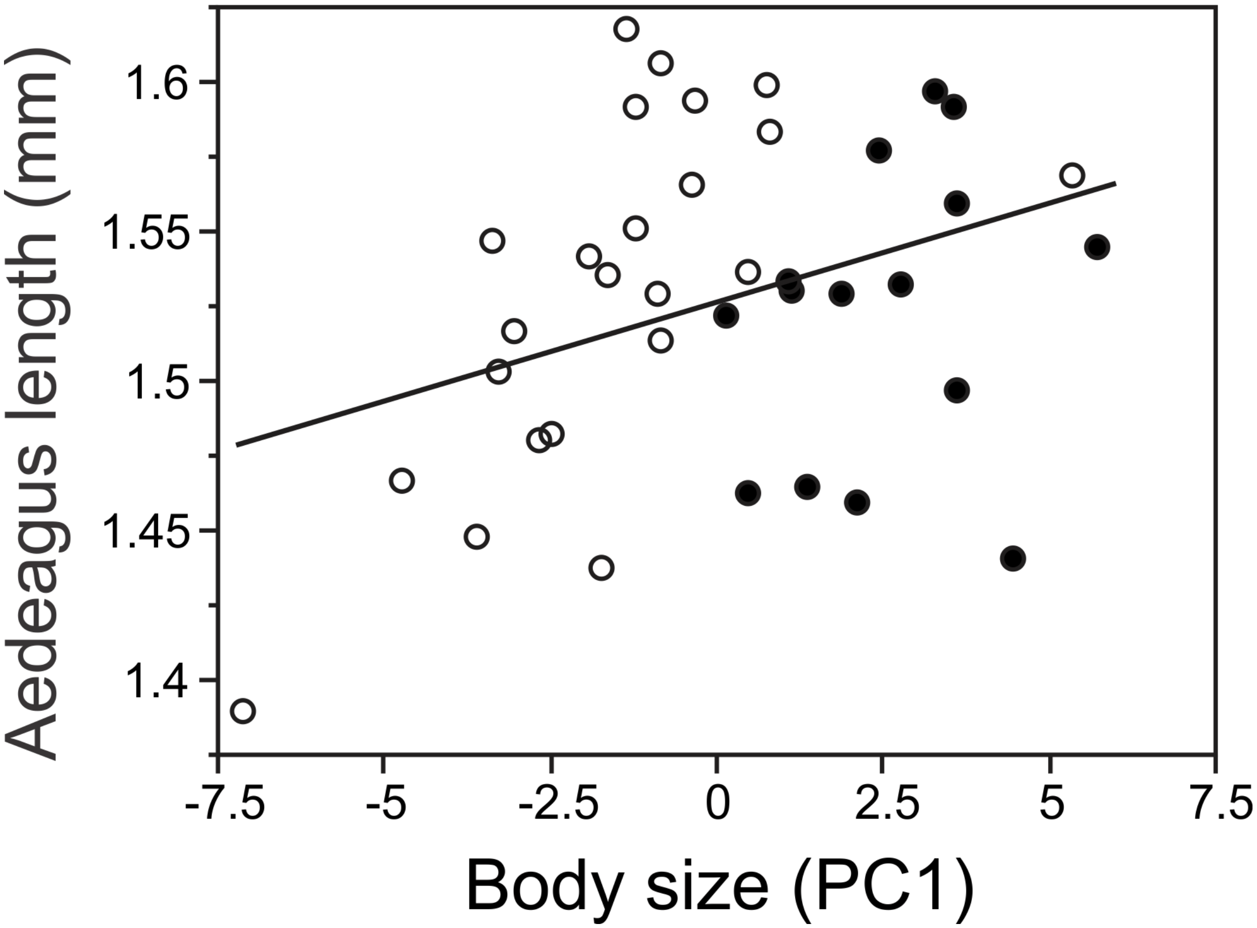

**Figure 8.** Aedeagus length (mm) as a function of body size (PC1) in *Protopiophila litigata* for low (open symbols) and high (closed symbols) diet quality males. Fitted line is from an ordinary least-squares regression across diet treatments.

# LITERATURE CITED

Andersson, M. 1982. Sexual selection, natural selection and quality advertisement. *Biol. J. Linn. Soc.* **17**: 375-393.

Andersson, M. 1986. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution* **40**: 804-816.

Andersson, M. 1994. *Sexual Selection*. Princeton Univ. Press, Princeton, New Jersey.

Arnqvist, G. & Thornhill, R. 1998. Evolution of animal genitalia: patterns of phenotypic and genotypic variation and condition-dependence of genital and non-genital morphology in water strider (*Heteroptera: Gerridae: Insecta*). *Genet. Res. Camb*. **71**: 193-212.

Arnqvist, G. & Rowe, L. 2005. *Sexual Conflict.* Princeton University Press, Princeton, NJ.

Bernstein, S. & Bernstein, R. 2002. Allometry of male genitalia in a species of soldier beetle: support for the one-size-fits-all hypothesis. *Evolution* **56**: 1707-1710.

Bonduriansky, R. 1995. A new Nearctic species of protophiophila dupa (Diptera: piophilidae), with notes on its behaviour and comparison with P. latipes (meigen). *The Canadian Entomologist* **127**: 859-863.

Bonduriansky, R. 2002. Leaping behaviour and responses to moisture and sound in larvae of piophillid carrion flies. *Department of Zoology* **134**: 647-656.

Bonduriansky, R. 2007a. The evolution of condition-dependent sexual dimorphism. *Am. Nat.* **169**: 9-19.

Bonduriansky, R. 2007b. Sexual selection and allometry: a reappraisal of the evidence and ideas. *Evolution* **61**: 838-849.

Bonduriansky, R. 2007c. The genetic architecture of sexual dimorphism: the potential roles of genomic imprinting and condition dependence. In: *Sex, Size And Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*. (D.J. Fairbairn, W.U. Blanckenhorn & T. Szekely, eds). pp. 176-184. Oxford University Press, Oxford, UK.

Bonduriansky, R. & Brooks, R.J. 1998a. Male antler flies (*Protopiophila litigata*; Diptera: Piophilidae) are more selective than females in mate choice. *Can. J. Zoolog.* **76**: 1277-1285.

Bonduriansky, R. & Brooks, R.J. 1998b. Copulation and oviposition behaviour of *Protopiophila litigata* (Diptera: Piophilidae). *Can. Entomol.* **130**: 399-405.

Bonduriansky, R. & Brooks, R.J. 1999. Why do male antler flies (*Protopiophila litigata*) fight? The role of male combat in the structure of mating aggregations on moose antlers. *Ethol. Ecol. Evol.* **11**: 287-301.

Bonduriansky, R. & Brassil, C.E. 2002. Rapid and costly ageing in wild male flies. *Nature* **420**: 377.

Bonduriansky, R. & Chenoweth, S.F. 2009. Intralocus sexual conflict. *Trends Ecol. Evol.* **24**: 280-288.

Bonduriansky, R. & Rowe, L. 2005. Sexual selection, genetic architecture, and the condition dependence of body shape in the sexually dimorphic fly *Prochyliza xanthostma* (Piophilidae). *Evolution* **59**: 138-151.

Boughman, J. W. 2007. Condition-dependent expression of red colour differs between stickleback species. *J. Evol. Biol*. **20**: 1577-1590.

Cotton, S., Fowler, K. & Pomiankowski, A. 2004. Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proc. R. Soc. Lond. B Biol. Sci.* **271**: 771-783.

Coyne, J. & Orr, H. 2004. *Speciation*. Sinauer Associates: Massachusetts.

Darwin, C. 1859. *The origin of species by means of natural selection*. J. Murray, London.

Darwin, C. R. 1871. *The Descent of Man and Selection in Relation to Sex*. J. Murray, London.

David, J.R., Moreteau, B., Gauthier, J.P., Pétavy, G., Stockel, A. & Imasheva, A.G. 1994. Reaction norms of size characters in relation to growth temperature in *Drosophila melanogaster*: an isofemale lines analysis. *Genet. Sel. Evol.* **26**: 229-251.

David, P., Bjorksten, T., Fowler, K. & Pomiankowski, A. 2000. Condition-dependent signalling of genetic variation in stalk-eyes flies. *Nature* **406**: 186-188.

De Lisle S.P. & Rowe, L. 2014. Interactive effects of competition and social environment on the expression of sexual dimorphism. *J. Evol. Biol.* **27**: 1069-1077.

Delcourt. M., Rundle, H. 2011. Condition dependence of a multicomponent sexual display trait in Drosophila serrata. *The American Naturalist* **177**: 812-823.

Eberhard, W.G. 1985. *Sexual selection and animal genitalia*. Harvard Univ. Press, Cambridge, Massachusetts.

Eberhard, W.G. 2010. Evolution of genitalia: theories, evidence, and new directions. *Genetica* **138**: 5-18.

Eberhard, W.G., Huber, B.A., Rodriguez, R.L., Briceño, R.D., Salas, I. & Rodriguez, V. 1998. One size fits all? Relationships between the size and degree of variation in genitalia and other body parts in twenty species of insects and spiders. *Evolution* **52**: 415-431.

Fisher. R. A. 1915. The evolution of sexual preferences. *Eugen. Rev.* **7**: 184-192.

Fisher, R. A. 1930. *The genetical theory of natural selection*. Clarendon: New York.

Fiske, P., Rintamaki & P., Karvonen, E. 1998. Mating success in lekking males: a meta-analysis. *Behavioral Ecology* **9**: 328-338.

Getty, T. 1998. Handicap signalling: when fecundity and viability do not add up. *Anim. Behav.* **56**: 127-130.

Getty, T. 2006. Sexually selected signals are not similar to sports handicaps. *Trends Ecol. Evol.* **21**: 83-88.

Ghiselin, M.T. 1974. *The Economy of Nature and the Evolution of Sex*. University of California Press: Berkeley.

Green, A.J. 1999. Allometry of genitalia in insects and spiders: one size does not fit all. *Evolution* **53**: 1621-1624.

Gwyne, T. D. 1981. Sexual difference theory: Mormon crickets show role reversal in mate choice. Science **213**: 779-780.

Harrison, 1953. Reversal of a secondary sex character by selection. *Heredity* **7**: 153-164.

House, C.M. & Simmons, L.W. 2007. No evidence for condition-dependent expression of male genitalia in the dung beetle *Onthophagus taurus*. *J. Evo. Biol*. **20**: 1322-1332.

Jensen , H., Saether, B., Ringsby, T., Tufto, J., Griffith, S. & Ellegren, H. 2003. Sexual variation in heritability and genetic correlations of morphological traits in house sparrow (Passer domesticus). *Journal of Evolutionary Biology* **16**: 1296-1307.

Karan, D., Morin, J.-P., Gilbert, P., Moreteau, B., Scheiner, S.M. & David, J.R. 2000. The genetics of phenotypic plasticity. IX. Genetic architecture, temperature, and sex differences in *Drosophila melanogaster*. *Evolution* **54**: 1035-1040.

Kingsolver, J.G., Hoekstra, H.E., Hoekstra, J.M., Berrigan, D., Vignieri, S.N., Hill. C.E., Hoang, A., Gibert, P. & Beerli, P. The strength of phenotypic selection in natural population. *The American naturalist* **157**: 245-261.

Kirkpatrick, M. & Ryan, M. 1991. The evolution of mating preferences and the paradox of the lek. *Nature* **350**: 33-38.

Lande, R. 1980. Sexual dimorphism, sexual selection and adaptation in polygenic characters. *Evolution* **34**: 292-305.

Lessells, C.M. & Boag, P.T. 1987. Unrepeatable repeatabilities: a common mistake. *Auk* **104**: 116-121.

Loyau. A., Saint Jalme, M., Cagniant, C. & Sorci, G. 2005. Multiple sexual advertisements honestly reflect health status in peacocks (*Pavo cristatus*). *Behav.Ecol. Sociobiol.* **58**: 552-557.

Loyau, A., Gomez, D., Moureau, B., Théry, M., Hart, N., Saint Jalme, M., Bennett, A. & Sorci, G. 2007. Iridescent structurally based coloration of eyespots correlates with mating success in the peacock. *Behav. Ecol.***18***:* 1123-1131.

Nur, N. & Hasson, O. 1984. Phenotypic plasticity and the handicap principle. *J. Theor. Biol.* **110**: 275-297.

Petrie. M. 1994. Improved growth and survival of offspring of peacocks with more elaborate trains. *Nature* **371**: 585-586.

Post. E., Langvatn, R., Forchhamer, M.C. & Stenseth, N. 1999. Environmental variation shapes sexual dimorphism in red deer. *Proc. Natl. Acad. Sci. USA* **96**: 4467-4471.

Proulx, S.R., Day, T. & Rowe, L. 2002. Older males signal more reliably. *P. Roy. Soc. B. Biol. Sci*. **269**: 2291-2299.

Punzalan, D., Cooray, M., Rodd, F.H. & Rowe, L. 2008. Condition dependence of sexually dimorphic colouration and longevity in the ambush bug *Phymata Americana*. *J. Evol. Biol.* **21**: 1297-1306.

Rice, W. R. 1984. Sex-chromosomes and the evolution of sexual dimorphism. *Evolution* **38**:735-742.

Rowe, L. & Houle, D. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. *Proc. Biol. Sci*. **263**: 1415-1421.

Shaklee, W., Knox, C. & Marsden, S. 1952. The inheritance of the sex difference of body weight in turkeys. *Poultry science* **31:** 822-825.

Vencl, F. V. 2004. Allometry and proximate mechanisms of sexual selection in *Photinus* fireflies, and some other beetles. *Integr. Comp. Biol*. **44**: 242-249.

Weladji, R.B., Holand, O., Steinheim, G., Colman, J.E., Gjostein, H. & Kosmo, A. 2005. Sexual dimorphism and intercorhort variation in reindeer calf antler length is associated with density and weather. *Oecologia* **145**: 549-555.

Wyman, M., Agrawal, A. & Rowe, L. 2009. Condition-dependence of the sexually dimorphic transcriptome in *Drosophila melanogaster*. *Evolution* **64**: 1836-1848.