Condition-Dependent Sexual Dimorphism

Influence of environmental variation on sexual dimorphism in *Drosophila* morphology among adaptively diverged populations and in an inter-specific comparative context.

By Maria Pesevski,

A Thesis Submitted to the School of Graduate Studies in the Partial Fulfillment of the Requirements for the Degree PhD

McMaster University

PhD (2021)

Hamilton, Ontario (Department of Biology)

TITLE: Influence of environmental variation on sexual dimorphism in *Drosophila* morphology among adaptively diverged populations and in an inter-specific comparative context.

AUTHOR: Maria Pesevski (McMaster University)

SUPERVISOR: Dr. Ian Dworkin

NUMBER OF PAGES: xx, 241

Abstract

Environmental variation, an important source of phenotypic variation, can influence phenotypes, fitness and even rates of evolution. My thesis explores the effects of evolutionary change on the response to different types of environmental variation. In the first study, I examined the evolution of environmental canalization in adaptively diverged populations of *Drosophila melanogaster* that vary in degree of genetic canalization. I use these populations to test the congruence hypothesis which predicts that genetic canalization is a co-product of the evolution of environmental canalization and thus should be correlated. My results show that, despite adaptive evolutionary changes in morphology and genetic canalization, these populations have similar degree of variability due to environmental variation. In the second study, I explore how both variation in temperature and food quality during development influence patterns of sexual dimorphism in wing morphology in adaptively diverged populations of *Drosophila melanogaster*. I compare the relative contributions of adaptation, food availability and temperature on sexual size and shape dimorphism of the *Drosophila* wing. In particular, I focus on how these factors influence size-shape allometry both in general and in a sex-specific manner. My results show that despite the large adaptive divergence and a strong influence of environmental manipulation on wing size and shape, sex-specific patterns of condition dependence remain relatively consistent between the two populations. In the third study, I explore the evolutionary patterns of condition-dependent sexual size dimorphism among 27 different species from the *melanogaster* species group with varying degrees of sexual size dimorphism. Using food availability manipulations during development, I examine how sexual size dimorphism changes in response to condition at both the intra-specific and the inter-specific level. The results of this study suggest that, although we see a correlation between sexual size dimorphism and condition dependence among traits within most species, sexual dimorphism and condition dependence do not seem to have a correlated evolution among species of the *melanogaster* species group.

Acknowledgements

Historically, most achievements are attributed to either a single individual or a small group of individuals, which undermines the importance, contributions, collaboration and support of the community. Without the community and network of support that surrounded me during the years working on this degree, it would have been impossible for me to complete it. I would like to first and foremost express my deepest gratitude to the most wonderful, supportive, enthusiastic, kind, and knowledgeable supervisor Dr. Ian Dworkin. His guidance through the years has been the most inspiring, driving force for me. With every conversation, meeting and lesson, he sparked an undying passion and curiosity for scientific discovery within me. His support and friendship through both the high exciting times and the low long days is the reason why I am where I am today. I will be eternally grateful to him for everything he has taught me. I would also like to thank my supervisory committee, Dr. Jonathan Stone, Dr. Ben Bolker and Dr. Ben Evans, for their investment in my progress, continued support through the years, their wisdom and advice when I needed it the most. I would like to thank the Dworkin lab members, past and current graduate students, undergraduate students and volunteers, for shared moments, mutual support, science discussion, lab hangouts, and most of all, for all the help with the long hours of fly work, dissections, phenotyping and coding. Finally, I would like to thank my network of personal support that includes my family and friends. First, I would like to thank my parents Liljana and Bobi, for giving me a roof over my head, putting up with living with a graduate student for many years, and for their unconditional love and support. I would like to thank my sister Angelina for being my emotional and mental support and my loudest cheerleader. I would like to thank my sister's family, Kiril, Ole and especially my nephew Orce for their undying support and motivation especially in the final stretch. I would also like to thank my grandma Olga for always believing in me, as well as my extended family and all of my friends for all of their support and kind encouraging words through the years.

Declaration of Authorship

I, Maria Pesevski, declare that this thesis titled, "Influence of environmental variation on sexual dimorphism in *Drosophila* morphology among adaptively diverged populations and in an inter-specific comparative context." and the work presented in it are my own. For all the chapters, the experimental design was done by Maria Pesevski and Ian Dworkin. Chapter 2 is already a published peer reviewed paper. Chapter 3, is a manuscript that is ready for submission. For Chapter 2, the raising, collection, preservation and dissection of flies for a part of the experiments was performed by the Pool lab and previous lab members of the Dworkin lab. All the experiments with environmental manipulation for Chapters 2, 3 and 4, were performed by Maria Pesevski, with some dissection, imaging and phenotyping help of undergraduate students and volunteers. Analysis for Chapters 2, 3 and 4 was performed by Maria Pesevski and Ian Dworkin.

Contents

List of Figures

List of Tables

To my nephew Orce Korunoski

Chapter 1

Introduction

1.1 Evolution of sexual dimorphism

Sexual dimorphism, the phenotypic difference between females and males, is among the most captivating phenomena in biology. Since the dawn of evolutionary theory, interest in how and why sexual dimorphism evolves has been at the forefront of theoretical thought (Darwin 1874). The reason for this is two-fold. First, sexually dimorphic traits represent a major source of phenotypic variation and diversity, ranging from modest body size differences to extravagant phenotypes such as exaggerated weapons, colourful displays and even extreme disparity in body size (Fairbairn 2007a). Some examples of these include horns in beetles (Kawano 2006), peacock tails (Petrie et al. 1991), butterfly wing colouration (Oliver and Monteiro 2011) and body size in elephant seals (McCann et al. 1989). The most extreme example of sexual dimorphism is found in the blanket octopus where females are on average 100 times larger than males (Norman et al. 2002). Second, the evolution of sexual dimorphism often presents a dilemma to evolutionary biologists because it evolves readily and sometimes to extreme extents, despite expected theoretical constraints that would potentially restrict the degree to which sexual dimorphism can evolve.

1.1.1 Why does sexual dimorphism evolve: Overview of hypotheses

Several hypotheses attempt to explain how and why sexual dimorphism evolves in different taxa and traits. These hypotheses stem from the assumption of sex differences in fitness components such as viability/survival and reproductive success including fecundity and mating success (Bateman 1948a; Arnold 1994; Trivers 1996). These differences arise due to differences in reproductive investment of males and females, with females in most species investing more time and energy in the form of costly egg production (anisogamy) and/or parental care (Bateman 1948a; Arnold 1994; Trivers 1996). The most popular and most widely accepted hypothesis is the sexual selection hypothesis, first proposed by Darwin (1874). The sexual selection hypothesis states that sexual dimorphism evolves as a result of sex-specific selection on traits that provide a reproductive advantage during competition for mates (either via direct combat or mate choice) (Darwin 1874; Hedrick and Temeles 1989; Andersson 1994). The best strategy for females is to be choosy when selecting mates and to mate with fewer, better-quality males because they invest more time and energy to reproduction to ensure that they pass on the best genes to their offspring increasing their fitness (Bateman 1948a; Arnold 1994; Trivers 1996). Males, on the other hand, benefit most from mating with as many females as possible and would develop strategies to maximize the number of mates, especially ways to either attract more females in the form of elaborate displays or ways to out-compete other males in the form of weapons or increase in overall size (Bateman 1948a; Arnold 1994; Trivers 1996).

Many models of sexual selection predict how sexual selection can contribute to the evolution of weaponry, ornamentation and greater overall size in males leading to male biased sexual size dimorphism (SSD). These models are supported by a large body of evidence (Fisher 1958; Andersson 1982; Johnstone 1995; Iwasa et al. 1991; Iwasa and Pomiankowski 1994; Iwasa and Pomiankowski 1999; Pomiankowski and Iwasa 1993; Pomiankowski and Iwasa 1998; Cotton et al. 2004b; Rowe and Houle 1996). Some of

these include the Fisherian runaway model, good genes models, handicap models and condition dependence models (Fisher 1958; Andersson 1982; Johnstone 1995; Iwasa et al. 1991; Iwasa and Pomiankowski 1994; Iwasa and Pomiankowski 1999; Pomiankowski and Iwasa 1993; Pomiankowski and Iwasa 1998; Cotton et al. 2004b; Rowe and Houle 1996). The Fisherian runaway model proposes that the evolution of larger, brighter, more extreme ornaments in males is reinforced by co-evolution of female preference of more extreme phenotypes, leading to a positive feedback loop (Fisher 1958; Pomiankowski and Iwasa 1993; Pomiankowski and Iwasa 1998; Iwasa and Pomiankowski 1994; Iwasa and Pomiankowski 1995). Handicap and condition dependence models will be further explored below.

The fecundity selection hypothesis, initially proposed as the fecundity advantage hypothesis by Darwin (1874), is often invoked to explain the evolution of female-biased SSD (Shine 1988). The fecundity selection hypothesis proposes that traits that maximize fecundity are under directional selection (Darwin 1874; Shine 1988; Pincheira-Donoso and Hunt 2017). This hypothesis predicts that a larger body size in females (either directly or via pleiotropy, for example, due to increasing of abdomen volume) allows for a greater reproductive output (fecundity) (Darwin 1874; Shine 1988; Reeve and Fairbairn 1999; Preziosi and Fairbairn 1997; Pincheira-Donoso and Hunt 2017). A great deal of evidence supports the predictions of the fecundity selection hypothesis, especially in ectothermic oviparous taxa such as insects, fish and reptiles, all taxa with predominantly female biased SSD (Shine 1988; Pincheira-Donoso and Hunt 2017; Reeve and Fairbairn 1999; Preziosi and Fairbairn 1997; Cox et al. 2003). In a species of waterstriders (*Aquarius remigis*), an insect with female biased SSD, fecundity selection directly increases abdomen length causing greater overall body length in females, and it therefore drives overall female biased SSD in this species (Preziosi and Fairbairn 1997). Using an artificial selection experiment in *Drosophila melanogater*, Reeve and Fairbairn (1999) demonstrated that selection for greater fecundity caused an increase in SSD for thorax

width, length and abdomen length driven by an increase in female size, while selection for lower fecundity did not affect SSD compared to the control (Reeve and Fairbairn 1999).

An alternative hypothesis that considers ecological causes of sexual dimorphism is the dimorphic niche hypothesis (Hedrick and Temeles 1989; Selander 1966; Slatkin 1984). This hypothesis was also first proposed by Darwin (1874), but Selander (1966) presented a theoretical model under which the the dimorphic niche hypothesis would be expected to operate. This hypothesis states that sexual dimorphism can result because males and females occupy different ecological niches due to their different energetic needs that arise as a consequence of ansiogamy and differential parental care (Slatkin 1984; Hedrick and Temeles 1989). The sexes deal with inter-sexual food competition by occupying different ecological niches and therefore adapt to the different environments they are exposed to such as feeding on different types of food (Slatkin 1984). Although sexual and fecundity selection are considered to be the primary mechanisms driving sexual dimorphism, particularly for SSD, ecological causes of sexual dimorphism may contribute a significant amount of variation and should not be discounted (Hedrick and Temeles 1989; Slatkin 1984; Shine 1989; Temeles et al. 2000; Temeles et al. 2010; Herrel et al. 2010; Kuo et al. 2009; Vincent et al. 2004). These causes have played an important role in explaining sexual dimorphism in size and shape of structures associated with feeding like jaws, beaks, and even whole heads (Temeles et al. 2000; Temeles et al. 2010; Herrel et al. 2010; Kuo et al. 2009; Vincent et al. 2004; Kaliontzopoulou et al. 2015).

Selander (1966) was among the first to consider ecological causes of sexual dimorphism in bird beak morphology. He found an association between sexually dimorphic foraging behaviour and sexually dimorphic feeding apparatus in a species of woodpecker (Selander 1966). A comparative study in snakes found that the variation for sexual dimorphism in head morphology is better explained by differential feeding behaviours between males and

females than sexual selection (Shine 1991). Another study found that sexual dimorphism in beak size in hummingbirds is caused by floral specialization by males and females (Temeles et al. 2000). More recent studies have considered the role of ecologically derived sexual dimorphism in the shape of skull and other skeletal traits in hummingbirds, snakes, lizards and turtles (Temeles et al. 2010; Vincent et al. 2004; Kuo et al. 2009; Herrel et al. 2009). Although these studies show some degree of support for the dimorphic niche hypothesis and its role in the evolution of sexual size and shape dimorphism, they mostly fail to demonstrate a direct causative relationship between inter-sexual ecological niche and sexual dimorphism. In fact, inter-sexual niche differentiation may be a secondary process that only enhances the sexual dimorphism that evolves as result of other types of sex-specific selection selection.

1.1.2 Antagonistic selection, intralocus sexual conflict and how its resolution leads to sexual dimorphism

Theory suggests that sexual dimorphism evolves as a resolution to intralocus sexual conflict which results from sexually antagonistic selection (Lande 1980; Cox and Calsbeek 2009; Bonduriansky and Chenoweth 2009; Rice and Chippindale 2001). When the two sexes have different fitness optima, sexually antagonistic selection in the form of sex-specific selection (sexual selection, fecundity selection, sex-limited viability selection) operates in opposite directions in each sex (Lande 1980; Cox and Calsbeek 2009; Bonduriansky and Chenoweth 2009). Intralocus sexual conflict arises under these conditions because of high intersex genetic correlation (r_{MF}) (Lande 1980). Males and females share the same genome, with the exception of sex chromosomes (when present) (Rice 1992). Therefore, selection on a trait beneficial in one sex can cause a correlated response in the other sex. However, these alleles that are beneficial in one sex may be deleterious in the other sex. For example, bright colour in males as part of a sexual display may be beneficial to males, as the benefits to attracting a mate may outweigh any incurred

cost due to increased visibility by predators. However, the alleles contributing to the increased colouration would be deleterious in females as they incur increased predation risk without any reproductive benefits. As such, this allele would be selected against in females while being beneficial in males (Lande 1980; Bonduriansky and Chenoweth 2009; Rice 1984; Rice 1992). An empirical example of this has been observed in guppies, where males with more numerous and colourful spots have more reproductive success compared to less ornamented males, but strong natural selection occurs against ornamentation in the presence of predators (Endler 1980; Kemp et al. 2009).

In order for of sexual dimorphism to evolve, organisms need to overcome the constraint that r_{MF} imposes. Estimates of r_{MF} vary for different traits, populations and species (Poissant et al. 2010). Estimates of r_{MF} are highest for morphological traits and lowest for physiological traits (Poissant et al. 2010). There is a negative correlation between estimates of r_{MF} and SD (Fairbairn 2007b; Bonduriansky and Rowe 2005a; Poissant et al. 2010). Despite the high r_{MF} that is observed among traits for both univariate (Poissant et al. 2010) and multivariate (Wyman et al. 2013) traits, it is generally observed that SD evolves readily. This could occur because even though r_{MF} is high, r_{MF} is often slightly less than 1, sufficient for traits to evolve in a sex specific manner. Furthermore, r_{MF} can decrease as a result of different evolutionary and environmental processes. Resolution of intralocus sexual conflict can occur via many mechanisms some of which include: sex-limited mutations (Rhen 2000), sex-linkage (Rice 1984; Connallon and Clark 2010), sex-specific expression of autosomal genes in the sexes via sex-linked modifiers (in adults and during development) (Rice 1984; Connallon and Clark 2010; Williams and Carroll 2009; Badyaev 2002), sex-limited expression of duplicated genes (Gallach and Betran 2011), condition-dependence of sexually dimorphic traits and/or gene expression (sex-genotype-environment interaction) (Rowe and Houle 1996; Bonduriansky 2007c; Wyman et al. 2010) and genomic imprinting (Bonduriansky 2007c; Bonduriansky and Chenoweth 2009; Day and Bonduriansky 2004). These mechanisms

all decrease r_{MF} either directly or indirectly (via pleitropy and/or epistatic interactions). However, these mechanisms are slow to evolve compared to the rate at which antagonistic selection operates (Bonduriansky and Chenoweth 2009). In many cases intralocus sexual conflict is unlikely to be fully resolved and thus can persist to constrain the evolution of sexual dimorphism. Indeed, despite some examples where sexually dimorphic traits apparently evolves slow (Stewart and Rice 2018) under mass selection regimes or under family based selection procedures, evolutionary changes can occur quite rapidly (Bird and Schaffer 1972; Eisen and Hanrahan 1972; Delph et al. 2011; Collet et al. 2016). Additionally, but relatively rarely studied, is the effect of trait plasticity and environmental variation on r_{MF} (Cheng and Kirkpatrick 2017; Berger et al. 2014; Punzalan et al. 2014; Lyons et al. 1994; Simons and Roff 1996; Fox et al. 2004; Vieira et al. 2000; Leips and Mackay 2000). Some of these studies have observed variation of r_{MF} in a variety of traits including morphological, physiological and fitness traits as a result of environmental variation (Cheng and Kirkpatrick 2017; Berger et al. 2014; Punzalan et al. 2014; Lyons et al. 1994; Simons and Roff 1996; Fox et al. 2004; Vieira et al. 2000; Leips and Mackay 2000). In some cases, they observed a reversal in the sign of r_{MF} (Lyons et al. 1994). This suggests that r_{MF} may be less of a constraint on patterns of dimorphism that originally thought. Therefore, studying sexually dimorphic traits under variable environmental conditions can help us understand how sexual dimorphism evolves and how its evolution overcomes the major constraints it faces.

1.1.3 Sexual size and shape dimorphism

Sexual dimorphism comes in many forms, shapes, sizes, colours, behaviours, physiological function and etc. For the purposes of this thesis, I will focus primarily on sexual size dimorphism (SSD) and sexual shape dimorphism (SShD).

SSD

One of the most commonly studied types of sexual dimorphism throughout the animal kingdom is SSD, manifesting as either whole body or trait-specific SSD (Fairbairn 2007a). SSD can be either male-biased (larger males) or female-biased (larger females). As discussed in previous sections, the magnitude and direction of evolved variation in SSD results from the relative contribution of mating and reproductive strategies used by animals, types of selection (sexual and fecundity selection) operating in each sex, and the reproductive roles of each sex (Fairbairn 1997; Fairbairn 2007a; Stillwell et al. 2010; Stillwell and Davidowitz 2010). SSD can also vary as a result of environmental variation, and exhibits varying degrees of phenotypic plasticity (Bonduriansky 2007b; Cotton et al. 2004b; Rowe and Houle 1996; Stillwell and Davidowitz 2010; Stillwell et al. 2010). Plasticity of SSD due to environmental variation is one of the major themes of this thesis and I will discuss this in more detail in subsequent sections. In general, female-biased SSD is most common among invertebrates and ectothermic vertebrates (Berry and Shine 1980; Shine 1994; Monnet and Cherry 2002; Kupfer 2007; Cox et al. 2007; Blanckenhorn et al. 2007b; Teder and Tammaru 2005), while male-biased SSD is most common among endothermic (Fairbairn 1997; Fairbairn 2007a; Abouheif and Fairbairn 1997). In insects, more than 70% of taxa have female biased SSD, but male-biased SSD appears across a wide range of taxa especially in species that have very strong sexual selection in males (for example, defensive territorial males) (Stillwell et al. 2010; Teder and Tammaru 2005).

The direction and magnitude of SSD varies greatly both within and among taxa (Blanckenhorn et al. 2007b). Comparative analysis of SSD has revealed a correlated pattern of variation of SSD with body size, known as Rensch's rule (Rensch 1960; Abouheif and Fairbairn 1997; Blanckenhorn et al. 2007b). Rensch's rule states that SSD increases with increase in body size in species with male-biased SSD and decreases with size in species with female-biased SSD (Rensch 1960). Rensch's rule is thought to arise as a

byproduct of sexual selection in males that tends to produce hyperallometry in secondary sexual traits and/or overall body size in males (Rensch 1960; Fairbairn and Preziosi 1994; Abouheif and Fairbairn 1997). Rensch's rule was accepted as a general pattern until recently (Fairbairn and Preziosi 1994; Abouheif and Fairbairn 1997; Fairbairn 1997). More recent studies have demonstrated that Rensch's rule is observed among taxa with primarily male-biased SSD but is often violated in taxa with female-biased SSD. Webb and Freckleton (2007) discovered that in birds, Rensch's rule is violated in avian families with female biased and mixed SSD, but holds for families with primarily male biased SSD (Webb and Freckleton 2007). Similarly, in insects, Rensch's rule is also violated half the time, where orders Coleoptera, Hymenoptera, and Phasmatodea have allometry inconsistent with or even opposite of Rensch's rule while Diptera, Heteroptera and Lepidoptera have allometries consistent with Rensch's rule (Blanckenhorn et al. 2007a). Rensch's rule was also tested at different taxonomic levels, for example at family level and/or at intra-specific level, by considering clines and/or environmental variation (Blanckenhorn et al. 2007a; Teder and Tammaru 2005). These studies suggest that even if Rensch's rule is followed at the higher taxonomic levels (Order and/or Family) it may not be followed at the within lower taxonomic levels (Genus, species and/or population) (Blanckenhorn et al. 2007a; Teder and Tammaru 2005).

While there are some notable exceptions, for example, horned beetles or *Drosophila prolongata* — the only melanogaster species group species that exhibits male biased SSD— in most insect taxa SSD is female biased. It is thought that fecundity selection is the main driver of female-biased SSD in insects because larger size in females allows for greater fecundity (Fairbairn 1990; Head 1995; Masaki 1967; Blanckenhorn et al. 1995; Fairbairn 2007a; Foellmer and Moya-Larano 2007). There is evidence that selection for greater fecundity is associated with female-biased SSD and greater body size in females and that selection on fecundity can affect female size disproportionately (Preziosi et al. 1996; Roitberg et al. 2015; Reeve and Fairbairn 1999). Viability selection is thought

to operate in the opposite direction of fecundity selection operating in both males and females, but because fecundity selection is stronger in females, viability selection is the antagonistic selection that keeps males small (Blanckenhorn 2000). Viability selection is the selection acting on all non-sexual non non-reproductive aspects of fitness. In males, growing to a larger size may be costly because it will potentially reduce viability either at juvenile and/or adult stages (Blanckenhorn 2000; Blanckenhorn et al. 1995; Pincheira-Donoso and Hunt 2017; Foellmer and Moya-Larano 2007). Growing to a larger size can increase development time, can be more metabolically taxing or can reduce agility leading in each case to lower reproductive success (Blanckenhorn 2000). When large male size is not necessary for territory defence, competition for mates and sexual displays — mating behaviours associated with sexual selection — it may be costly to have large males. For example, in insects with scramble competition mating systems, it is more important to be sexually mature early and to be more agile in order to find more mates (Reiss 1989; Blanckenhorn et al. 1995). In such taxa, the Ghiselin–Reiss hypothesis predicts that small males are favoured, allowing them to maintain high levels of performance at lower metabolic cost (Reiss 1989; Blanckenhorn et al. 1995; Blanckenhorn 2000). Therefore, the combined effects of fecundity and viability selection create the two opposing forces that select for larger females and smaller males leading to the evolution of female-biased SSD.

In *D. melanogaster*, sexual and fecundity selection select for both larger males and females, yet males remain smaller (Partridge and Farquhar 1983; Wilkinson 1987; Lefranc and Bundgaard 2000). Viability selection on aspects of larval development are thought to play a role in limiting the size at which males grow (Wilkinson 1987). An equilibrium size is reached that is a product of sexual selection during adulthood and viability selection during larval development (Wilkinson 1987).

Quantification of SSD

Standard methods have been developed in order to measure and analyze SSD. These methods are important because it is necessary to have a consistent way of measuring SSD in order to be able to study SSD in a comparative framework, looking at inter-specific variation of SSD, and also to be able to compare measurements from different studies. A review by Lovich and Gibbons (1992) compared different sexual dimorphism indices (SDIs) and discussed what makes a good SDI. There are two types of SDIs, based on ratios or differences (Lovich and Gibbons 1992). Some of the ratio-based measures include male-female ratio, logarithm of the male-female ratio, difference between males and females divided by the sum of the males and females all multiplied by a constant (either 200 or 100) also known as Storer's Index, and variations of it (Lovich and Gibbons 1992). Lovich and Gibbons (1992) discussed that a good SDI should (1) have appropriate scaling, (2) have intuitive values, (3) consider direction of SSD and (4) be symmetrical around a central value. Among all of the SDI that are discussed by Lovich and Gibbons (1992) which mostly fail to meet the criteria above, the one that meets most criteria and the authors propose is $SDI = \frac{Mean\ size\ of\ larger\ sex}{Mean\ size\ of\ smaller\ sex} - 1$ when females are the larger sex and $SDI = -1 * \frac{Mean\ size\ of\ larger\ sex}{Mean\ size\ of\ smaller\ sex} + 1$ when males are the larger sex. Even though this SDI is the best among the rato-based SDIs, it is not without limitations. Although SDIs are useful for comparisons of SSD among species and across different studies, statistical tests on ratio-based SDIs can be challenging to interpret correctly (Smith 1999). This type of SDI also fails to consider the within-sex size variation (Smith 1999). An alternative approach to a ratio-based SDIs would be to use a regression of one sex onto the other (Smith 1999). Smith (1999) reanalyzed published data sets using both rato-based SDIs and regression approaches to evaluating SSD in order to compare the two methods. Smith (1999) found that the two most useful ratio-based SDIs are the natural logarithm of the ratio between males and females and the SDI proposed by Lovich and Gibbons (1992. Smith (1999) tested least squares (LS) and reduced major

axis (RMA) regression as an alternative to the ratio-based SDI. The benefits of these approaches are they avoid the non-normality issues of ratios and can account for within-sex size variation. He concluded that even though both ratio-based SDIs and regression approaches had their strengths and weaknesses, they are both very useful for studying SSD and one cannot replace the other (Smith 1999). For the purposes of this thesis, despite its limitations, I will be using the ratio-based SDI to measure SSD.

Sexual shape dimorphism (SShD)

Additionally, sex differences in shape (SShD) of traits and/or whole bodies contributes a significant proportion of the morphological variation observed for sexual dimorphism. Morphological shape, often thought of as the relative proportions of a trait, although related to size, has tremendous impact on overall biological function, such as feeding, locomotion, mating, parental care among others. For example, the shape of skeletal components facilitates locomotion as as much as their size in vertebrates (Martin-Serra et al. 2014; Biewener 1990). Additionally, the shape differences between males and females can evolve independently of size under sexually antagonistic selection and play an important role in mating behaviour, copulation, fecundity among others. For example, in species of wall lizards, head shape variation is associated with micro-habitat divergence when comparing species, but head shape and associated differences in bite force vary due to sex within species (Kaliontzopoulou et al. 2012; Crespo et al. 2002). In a water skink without any overall body size SSD, Schwarzkopf (2005) found substantial SShD differences in body shape, where head width was greater in males (used in male-male combat) and abdominal region was greater in females (used to produce and store eggs) (Schwarzkopf 2005a). Although overall body size differences between sexes are minimal in these lizards, the relative proportions of the different body parts differ in males and females (Schwarzkopf 2005a). In some Dipteran species, convergent evolution of whole body SShD has been observed (Bonduriansky 2006). In species that are not closely

related, certain traits in males evolve in a similar way causing the evolution of convergent body shape with elongated bodies, eye stalks, head width increase and pointy extensions in exoskeleton, increase in leg length and width (Bonduriansky 2006). Male flies in these species independently evolve this similar body shape because it provides an advantage when competing for mates via male-male combat, suggesting that body shape and SShD are very important for reproductive success (Bonduriansky 2006). *Drosophila* often display subtle but significant differences in wing shape between males and females. This SShD of the wing is both due to size-dependent (allometric) and size-independent (non-allometric) variation as a result of sexually antagonistic selection on wing shape directly (Gidaszewski et al. 2009; Abbott et al. 2010). In fact, intralocus sexual conflict has been observed for both wing size and wing shape in *Drosophila* (Abbott et al. 2010). In hominid species including humans, males and females differ in cranofacial shape both due to allometric effects due to size and non-allometric SShD as a result of independent sex-specific selection on cranofacial shape (Schaefer et al. 2004; Weston et al. 2007; OHiggins and Dryden 1993). These examples highlight the importance of SShD both of specific traits traits and overall bodies.

SShD has been less well studied than SSD both theoretically and empirically primarily because of the additional complexities of dealing with the multivariate nature of shape and associated statistical methods (Berns 2013). With the development of modern geometric morphometrics techniques, the study of SShD has been gaining interest among evolutionary biologists studying sexual dimorphism (Berns 2013).

In geometric morphometrics, shape can be defined as the geometric properties of a form that remain after size, position and scaling have been removed (Dryden and Mardia 1998; Kendall 1977; Zelditch et al. 2012). Historically, shape was measured using linear measurements, ratios of linear measurements and truss measurements (linear measurements between homologous landmarks) (Zelditch et al. 2012). However these traditional

morphometrics approaches have serious limitations. First, they use all measurements of size in order to evaluate shape; therefore separating size and shape is extremely hard, if not impossible (Zelditch et al. 2012). Second, the shape information is contained within the ratios of the linear measurements, but ratios are problematic for statistical analysis (Zelditch et al. 2012). Using linear measurements can also be problematic because it is hard to define what exact part of the trait is being consistently measured (Zelditch et al. 2012). This issue is resolved using truss measurements, by establishing homologous landmarks and taking measurements between those landmarks (Zelditch et al. 2012). However, the problem with this approach is that there are too many possible measurements that can be taken but not all of them are necessary as they contain highly correlated information (Zelditch et al. 2012). Finally, the traditional morphometrics approaches discussed above can help us determine the relative distances of points within a structure but do not actually convey any information about the geometric structure of the form that is measured, such as the position of the landmark points, or outline curvature and/or surface (Zelditch et al. 2012).

Geometric morphometrics, on the other hand, uses the coordinates of homologous landmarks, and in some cases curvature and surface data, to quantify shape (Zelditch et al. 2012; Adams et al. 2013; Klingenberg 2016; Mardia and Dryden 1989; Goodall 1991; Bookstein 1991). The raw Cartesian coordinates of the landmarks are put through a process that eliminates non-shape variation — scale, position, rotation — called generalized Procrustes analysis (GPA) superimposition (Mardia and Dryden 1989; Goodall 1991; Bookstein 1991; Adams et al. 2013). This process involves a least squares process that minimizes the distance between specimens (Mardia and Dryden 1989; Goodall 1991; Bookstein 1991; Adams et al. 2013) . The data generated from a GPA can then be analyzed using a MANOVA to calculate differences between groups such as males and females, which represents SShD (Mardia and Dryden 1989; Goodall 1991; Bookstein 1991; Adams et al. 2013). A standard measure of SShD has not been established in the

literature. Traditionally studies report p values for the sex terms from their models in order to show that there is SShD. Additionally, they visualize the SShD by either using a PCA plot showing variation due to sex in PC1 and PC2 or landmark outline plot of the mean male and mean female shapes (Fernández-Montraveta and Marugán-Lobón 2017; Laporte et al. 2018; Franklin et al. 2007; Valenzuela et al. 2004). These quantitative measures are often accompanied by a qualitative description of the shape differences between males and females (Fernández-Montraveta and Marugán-Lobón 2017; Laporte et al. 2018; Franklin et al. 2007; Valenzuela et al. 2004). However, quantitative measures of SShD akin to the SDI index that is used to estimate SSD is rarely used (Gidaszewski et al. 2009; Testa and Dworkin 2016; Adams et al. 2020). One such measure estimates the magnitude of shape difference males and females is the Procrustes distance (Euclidean distance) between mean male and mean female shape (Gidaszewski et al. 2009; Testa and Dworkin 2016). Although this approach gives us an idea of the difference in magnitude between males and females, it ignores within-sex variation because it is calculated from the mean shapes of the sexes. However, this approach provides us with a quantitative way to measure differences in SShD so that we can compare these differences among studies. One way to overcome this limitation is to use multiple lineages derived from a single population in order to get multiple estimates of SShD for the population which can then be tested by using some kind of bootstrap or permutation test. We take this approach in Chapter 3 to measure SShD in the wing of in two adaptively diverged African populations of *Drosophila melanogaster*. Since shape is a multidimensional trait, it can vary both in magnitude and direction. Therefore, another way that we can measure the differences in SShD is to calculate the correlation between the mean male and female shape vectors. However, these correlations are often very high between the sexes and hard to interpret. A better, more standard method for quantifying and analyzing SShD is necessary in order to get more consistency across studies. Adams et al. (2020) proposes a multivariate extension of the SDI approach for SSD, similar to the

one proposed by Lovich and Gibbons (1992) discussed above. They propose using the Euclidean distances obtained from size-standardized log-trait values between males and females and multiplying the distance by $+1$ or -1 depending on the direction of sexual dimorphism (Adams et al. 2020). This approach can be used when measuring shape using linear measurements (Adams et al. 2020). When using landmark coordinates as data, Adams et al. (2020) propose using the Procrustes distance between mean male and mean female shape as an SShD index.

With the advancement of the geometric morphometrics field, studying SShD has become easier allowing us to study sexual dimorphism in a multivariate framework (Adams et al. 2013). Taking this approach can resolve some of the theoretical issues that arise regarding the evolutionary constraints of sexual dimorphism (Wyman et al. 2013). Organisms evolve as whole, integrated, correlated systems and studying shape and SShD considers this (Lande 1979; Lande and Arnold 1983b; Wyman et al. 2013; Adams et al. 2020). A multivariate framework takes into account not just patterns of variation, but, explicitly patterns of covariation (Lande 1979). In the context of evolutionary studies, this multivariate perspective has demonstrated that while heritable variation for individual traits may exist, the genetic correlations of trait combinations under the strongest selection may be prevalent (Blows and Mcguigan 2014; Blows 2007; Hansen and Houle 2008; Lande and Arnold 1983a; Agrawal and Stinchcombe 2009). With respect to sexual dimorphism, researchers study the properties of the $\mathbf{B}$ matrix, the intersex genetic covariance matrix, partitioning between-sex genetic covariance (Lande 1980; Gosden et al. 2012; Wyman et al. 2013; Cheng and Houle 2020; Ingleby et al. 2014; Poissant et al. 2016). While such studies have demonstrated evidence of genetic constraints relating to sexual dimorphism, in some cases the most dimorphic traits appear to have the least evidence of high genetic covariances (Ingleby et al. 2014), and theoretical work suggests that such relationships do not substantially impede adaptation (Matthews et al. 2019a). In this framework, high r_{MF} is less of a constraint to the evolution of sexual dimorphism

because **G**, the genetic variance-covaraiance matrix, varies in both magnitude and direction allowing for more ways for the sexes vary genetically, and **B** can be asymmetric allowing selection to affect one sex more than the other (Wyman et al. 2013; Poissant et al. 2016). In fact, sex differences in **G** have been observed for many species for morphological, life history and other traits (Poissant et al. 2016; Lewis et al. 2011; Jensen et al. 2003; Rolff et al. 2005; Sztepanacz and Houle 2019).

Just like SSD, SShD can result from direct sex-specific selection (sexual, fecundity and sex-limited natural selection) on either whole body or specific traits (Berns 2013; Kaliontzopoulou et al. 2015). A limited number of studies have attempted to test the direct causes of SShD. Some of them include testing whether SShD is a result of sexual selection, fecundity selection and/or sex-limited natural selection due to divergent ecological niches of the sexes (Berns 2013; Herrel et al. 2010; Navarro et al. 2009; Kuo et al. 2009; Schwarzkopf 2005b; Olsson et al. 2002). These studies have found that all types of sex-specific selection play an important part in the evolution of SShD with most support for sexual and fecundity selection and mixed support for the ecological hypotheses (Berns 2013; Herrel et al. 2010; Navarro et al. 2009; Kuo et al. 2009; Schwarzkopf 2005b; Olsson et al. 2002). Most of these studies are limited to reptilian taxa, some in birds. It is not clear if they are applicable to other systems.

On the other hand, when shape-size allometry occurs, SShD can result as a correlated response to the sex-specific selection for different size in the sexes that leads to SSD. This is known as allometric SShD. In fact, allometry plays a significant role in shape variation for many traits, in general, and it can contribute a significant amount of variation in SShD (Klingenberg 2016). I provide a detailed discussion of shape-size allometry and its contribution to SShD in Chapter 3. It is often of great interest to partition total SShD into its allometric and non-allometric components, in order to examine whether or not SShD evolves simply as a consequence of SSD and shape-size allometry or whether it is

under the influence of direct selective forces on shape itself and evolves independently (Gidaszewski et al. 2009; Kaliontzopoulou et al. 2008a; Schaefer et al. 2004; Kleisner et al. 2021). A recent study tested the influence of male-male competition of both SSD and SShD of femur and wings in two *Sepsis* (Diptera) species (Baur et al. 2020). They found that fore-femur size and allometric portion of shape in males were strongly associated with mating success, but non-allometric shape was not (Baur et al. 2020). This study is an important demonstration of the effect of the allometric relationship between SSD and SShD (Baur et al. 2020). The study highlights the importance of studying allometric and non-allometric causes of SShD. Considerably more work is required to better understand these direct and indirect causes of SShD and how to distinguish between the two. We discuss this in more detail in chapter 3, as we attempt to estimate the allometric and non-allometric variation in wing shape sexual dimorphism in altitudinally diverged *Drosophiila melanogaster* populations raised in different environmental conditions. As we discuss in Chapter 3, disentangling allometric from non-allometric effects can be surprisingly tricky.

1.2 SD and environmental variation - Condition dependence and phenotypic plasticity

1.2.1 Overview - phenotypic plasticity of SSD and SShD

As discussed in previous sections, sexual dimorphism can contribute a considerable portion of overall phenotypic variation observed among populations and species. Sexually dimorphic traits tend to exhibit an increased sensitivity to environmental variation as a result of sex-specific developmental plasticity (Fairbairn 2005; Stillwell et al. 2010; Cotton et al. 2004b). Sex-specific plasticity has been observed in taxa with varying degrees and directions of sexual dimorphism under numerous ecological and environmental variables including seasonal variation (Miller et al. 2016), temperature (Stillwell

and Davidowitz 2010; Stillwell and Fox 2007; Starostova et al. 2010; Fischer and Fiedler 2002; Fischer and Fiedler 2000; Morin et al. 1996; Block and Stoks 2003; David et al. 1994; Karl and Fischer 2008; Rohde et al. 2015; Rohner et al. 2017), diet (Stillwell and Davidowitz 2010; Bonduriansky 2007b; Bonneaud et al. 2016; Cassidy et al. 2014; Ceballos and Valenzuela 2011; Fernandez-Montraveta and Moya-Larano 2007; Gebhardt and Stearns 1988; Oudin et al. 2015; Rohner et al. 2017; Rohner and Blanckenhorn 2018), density (Bitner–Mathe and Klaczko 1999; Rohde et al. 2015), photoperiod (Block and Stoks 2003), pathogen exposure (Cotton et al. 2004b) and among others (Stillwell and Davidowitz 2010; Stillwell et al. 2007; Stillwell and Fox 2007; Teder and Tammaru 2005; Cotton et al. 2004b; David et al. 1994). These studies include a variety of traits such as morphological traits that exhibit SSD and SShD including either whole body and/or specific structures (Stillwell and Davidowitz 2010; Stillwell and Fox 2007; Starostova et al. 2010; Morin et al. 1996; Bonduriansky 2007b; Bonneaud et al. 2016; Cassidy et al. 2014; Ceballos and Valenzuela 2011; Fernandez-Montraveta and Moya-Larano 2007; Gebhardt and Stearns 1988; Oudin et al. 2015; Rohde et al. 2015; Rohner et al. 2017; Rohner and Blanckenhorn 2018); pigmentation (Punzalan et al. 2008; Gibert et al. 1999); life-history traits such as development time, development rate and life-span (Stillwell and Davidowitz 2010; Stillwell and Fox 2007; Fischer and Fiedler 2002; Fischer and Fiedler 2000; Block and Stoks 2003; Adler et al. 2013; Fernandez-Montraveta and Moya-Larano 2007; Gebhardt and Stearns 1988). In general, the more sexually dimorphic a trait the more plastic it is, in particular, to environmental variation that strongly affects organismal condition such as diet (Bonduriansky 2007b; Stillwell et al. 2010). In species where SSD is female-biased, females are more plastic than males, while in species where SSD is male-biased, males are more sensitive to environmental variation (Stillwell et al. 2010; Teder and Tammaru 2005; Rohner et al. 2018c). Most insect species (>70% female biased SSD) females are more plastic, with some notable examples (Stillwell et al. 2010; Teder and Tammaru 2005). Horn structures in dung beetle species are some of

the most interesting and extreme cases of sex-specific plasticity of highly exaggerated sexually dimorphic traits used as weapons or ornaments. The size of head structures in males of these species is almost entirely dependent on developmental nutrition (Emlen 1994; Emlen 1997). The mechanisms by which this type of plasticity is facilitated is well studied (Koyama et al. 2013; Gotoh et al. 2014; Lavine et al. 2015; Emlen and Nijhout 1999). Nutritional quality and quantity during development alter the hormonal response via the juvenile hormone pathway that is influenced by both sex-determination genes and insulin-like growth factor pathway determining the extent to which dung beetles grow their horns or whether they grow the horns at all (Koyama et al. 2013; Gotoh et al. 2014; Lavine et al. 2015; Emlen and Nijhout 1999; Moczek and Emlen 1999). Similar mechanisms are at play in mediating the sex-specific plasticity to nutrition in other insects, for example *Drosophila*, although not to the same degree as in dung beetles (Millington et al. 2021). Different body parts of males and females in *Drosophila melanogaster* respond differently to both diet quality and quantity, with female traits being more sensitive to diet variation (Shingleton et al. 2017). This sex-specific plasticity in *Drosophila melanogaster* is mediated by the sex-determination pathway gene *tra* and its influence on the insulin-like growth factor pathway, where it causes greater plasticity to nutrition variation in females (Millington et al. 2021).

How and why sex-specific plasticity to environmental variation evolves has gained more attention in the past 20 years. Two hypotheses have been proposed that attempt to explain the evolution of sex-specific plasticity (Stillwell et al. 2010). The adaptive canalization hypothesis predicts that we should observe decreased plasticity in traits under strong selection, particularly traits closely associated with fitness (Stillwell et al. 2010; Fairbairn 2005; Rohner et al. 2018a). On the other hand, the condition dependence hypothesis predicts that we should observe the greatest plasticity in traits that are under strong selection as it would allow for maximizing resource usage efficiency (Stillwell et al. 2010; Bonduriansky 2007c; Bonduriansky 2007b; Rohner et al. 2018a). The differences

in phenotypic plasticity can arise via both processes. According to the the adaptive canalization hypothesis, sex-specific plasticity can occur when traits in one sex become more canalized (less plastic) due to stabilizing selection acting on the trait (Stillwell et al. 2010; Fairbairn 2005; Rohner et al. 2018a). On the other hand, sex-specific plasticity can evolve due to greater direction selection on a trait, as per the condition dependence hypothesis (Stillwell et al. 2010; Bonduriansky 2007c; Bonduriansky 2007b; Rohner et al. 2018a). Distinguishing between these two hypotheses can be hard. Explicit tests require (1) knowledge of the type of selection acting in each sex, (2) testing which sex is more plastic and how that relates to the type of selection the sex is under and (3) testing multiple traits within each sex in order to determine a baseline for plasticity in order to see what direction plasticity changed in the trait of interest (Stillwell et al. 2010). Such explicit tests are rare, especially for the adaptive canalization hypothesis, and studies attempting to test this hypothesis often fail to meet all the criteria (Stillwell et al. 2010; Fairbairn 2005; Walzer and Schausberger 2014; Hallsson and Bjorklund 2012; Fernandez-Montraveta and Moya-Larano 2007). The condition dependence hypothesis, on the other hand, has received both more attention and more support (Bonduriansky 2007c; Bonduriansky 2007b; Cotton et al. 2004b; Stillwell et al. 2010). In particular, condition dependence of exaggerated sex ornaments and weapons in males has a large body of empirical and theoretical support (Andersson 1986; Houle and Kondrashov 2002; Rowe and Houle 1996; Bonduriansky 2007c; Bonduriansky 2007b; Cotton et al. 2004b; Stillwell et al. 2010). This theory and evidence is a central theme of this thesis and I will discuss these in more detail in sections below.

1.2.2 What is condition?

Organismal *condition* is both a quintessential concept in evolutionary biology, and also a concept that has been defined in many different ways. Many authors define condition as the nutritional and energy reserve status of the organism (Andersson 1982; Bonduriansky

2007b; Moya-Laraño et al. 2008). Another common definition of condition relates the pool of resources available to the organism, with their ability to allocate those resources efficiently to traits affecting performance and ultimately fitness (Bonduriansky 2007b; Cotton et al. 2004b; Rowe and Houle 1996). This definition relates the genetic capacity and its interaction with the environment to create a singular trait that is condition (Bonduriansky 2007b; Cotton et al. 2004b; Rowe and Houle 1996). The most inclusive definition is provided by Hill (2011), "the relative capacity of an organism to maintain optimal functionality of essential cellular processes" (Hill 2011). Hill (2011) states that condition is determined by the somatic, genetic and epigenetic state of an organism. The somatic state of the organism is the current state of the organism determined by the influence of the internal and external environments such as exposure to parasites and toxins, energy resources, oxidative stress, age, social status, past and present damage or injury and many more factors (Hill 2011). The genetic state of condition includes the genetic variation for organism's health and disease resistance, resource acquisition, usage and storage, stress resistance and other life history processes (Hill 2011). Finally, the epigenetic state of the organism is the lifetime genetic effects on the phenotype such as transcriptional and translational regulation of gene expression that are influenced by the interaction between genotype and environment (Hill 2011).

Although Hill's definition of condition is extensive, manipulating condition defined in this way is complicated. One cannot account for all the somatic, genetic and epigenetic factors influencing condition. The most common way to manipulate condition is to vary nutritional quality (Bonduriansky 2007b; Cotton et al. 2004b; Hill 2011). Other manipulations of condition include exposure to different types of stress using parasites, temperature, genetic stress via inbreeding, exercise and more (Cotton et al. 2004b). As complex as it is to manipulate condition, measuring it is an even greater challenge, especially non-destructively (Cotton et al. 2004b). Overall body size is often considered to be a standard proxy of condition, as it is often most reflective of it (Cotton et al. 2004b;

Jakob et al. 1996; Moya-Laraño et al. 2008). However, using body size as a measure of condition is complicated because (1) there are different ways to measure body size, (2) larger organisms are affected by poor environmental conditions disproportionately because they requre more resources and (3) there are both intra- and inter-specific variation in the degree to which condition affects body size complicating comparative analysis of condition (Cotton et al. 2004b; Jakob et al. 1996; Moya-Laraño et al. 2008). As I will discuss further in chapter 4, this is particularly problematic for studies of sexual dimorphism. In the literature, size is measured using linear measurements of the whole organism; linear measurements of a trait as a proxy of overall size; volume of the organism; volume of a trait as a for proxy overall size or mass of the organism (Cotton et al. 2004b; Jakob et al. 1996; Moya-Laraño et al. 2008). Often whole body measurements are unavailable, or are difficult to measure in some species and proxy traits have to be used as estimates of body size (Cotton et al. 2004b; Jakob et al. 1996; Moya-Laraño et al. 2008). Difficulties arise because these proxy traits are affected differently by condition and often scale allometrically with body size but with different relationships (Cotton et al. 2004b; Jakob et al. 1996; Moya-Laraño et al. 2008). Some common metrics of condition include the size ratio index, slope adjusted ratio index and a residual index (Jakob et al. 1996). The size ratio index is a ratio between mass and linear measure of body (Jakob et al. 1996). The slope adjusted ratio index is measured by using an independent standard population and generating the slope of the regression of ln(body mass) against ln(length of body part) (Jakob et al. 1996). This slope is then used to calculate an index for each organism of interest (Jakob et al. 1996). The residual index is calculated by first regressing body mass onto body size after data is transformed (Jakob et al. 1996). The residual distance from each point to the regression line is used as an estimate of condition (Jakob et al. 1996). Issues with these three indices are that they often produce different results (Cotton et al. 2004b; Jakob et al. 1996). Additionally, each of these measures imposes different assumptions that are not statistically or biologically

justified because the true relationships of the variables being compared are not known (Cotton et al. 2004b; Jakob et al. 1996). Another measure of condition that is often used in the literature is measurement of macronutrient composition, for example fat, because energy reserves may be reflective of condition (Cotton et al. 2004b; Jakob et al. 1996; Wilder et al. 2016). However, getting accurate body composition measurements can be difficult without invasive procedures, may often require sacrifice of specimens, may not be reflective of conditon in some species and may require expensive reagents and machinery (Cotton et al. 2004b; Jakob et al. 1996; Wilder et al. 2016; Moya-Laraño et al. 2008). The best approach for measuring condition is to use several of the methods mentioned above in order to minimize limitations of each individual method.

1.2.3 How does condition relate to sexual dimorphism - theory and evidence

Large body of theoretical and empirical work has hypothesized and supported a positive correlation between condition and the degree of sexual dimorphism (Andersson 1982; Johnstone 1995; Bonduriansky 2007b; Cotton et al. 2004b; Emlen 1994; Rowe and Houle 1996). This pattern has been observed both within and among populations and inter-specifically. Many theoretical and empirical studies of the relationship between sexual dimorphism and condition dependence are limited to exaggerated male-biased traits that have evolved to extraordinary sizes due to sexual selection via either direct male-male competition or female choice. Below I explore the different hypotheses that have been proposed in order to explain this greater condition dependence in sexually dimorphic traits.

The handicap principle hypothesis

Several hypotheses have been proposed in order to explain the relationship between condition dependence and sexual dimorphism. One such hypothesis is the handicap hypothesis (Zahavi 1975; Zahavi 1977; Andersson 1982; Andersson 1986; Rowe and Houle 1996; Iwasa et al. 1991). This hypothesis states that exaggerated ornaments in males evolve or are maintained because the best quality males that are most capable of surviving are the ones that can do it with large handicapping ornaments (Zahavi 1975; Zahavi 1977; Andersson 1982; Andersson 1986; Iwasa et al. 1991). The ornaments serve as a signal of good genes to females (Zahavi 1975; Zahavi 1977; Andersson 1982; Andersson 1986; Iwasa et al. 1991). The hypothesis predicts that the handicap more strongly affects males of lower quality than males of higher quality because low quality males cannot afford to produce the handicapping trait (Zahavi 1975; Zahavi 1977; Andersson 1982; Andersson 1986; Iwasa et al. 1991). Using a simple model that includes Fisher's runaway process and principles from the handicap mechanism, Andersson (1982) showed that ornaments should evolve to be condition dependent (Andersson 1982; Andersson 1986). Acceptance of the the handicap hypothesis has been mixed and it has been strongly criticized; thoroughly explored in Penn and Szamado (2020)(Kirkpatrick 1986; Smith 1976; Penn and Szamado 2020).

The genic capture model

Rowe and Houle (1996) proposed the genic capture model which states that high genetic variance is maintained in sexually selected traits through capture of the high genetic variance of condition through the evolution of condition dependence of secondary sexual traits (Rowe and Houle 1996). As discussed in previous sections, under strong sexual selection, constraints arise that would theoretically impede the evolution of sexual dimorphism (Rowe and Houle 1996). One such constraint, often referred to as the lek

paradox, is the expectation of diminishing genetic variance due to directional sexual selection that would lead to a halt in the evolution of exaggerated traits once the genetic variation is exhausted (Rowe and Houle 1996). Despite these theoretical predictions, it has been observed that sexually selected traits have greater genetic variance than expected, and their genetic variance was similar to that of life-history traits (Pomiankowski and Moller 1995). The genic capure model allows the secondary sexual traits in males to reflect the genetic variation not only in the genes that directly affect the trait but also the in loci that affect acquisition and allocation of resources, the genetic component of condition (Rowe and Houle 1996; Bonduriansky 2007c; Chandler et al. 2013). This genic capture may in fact reduce the genetic correlation between the sexes and may lift some of the constraints of intralocus sexual conflict (Bonduriansky 2007c). Under this model, it is expected that secondary sexual traits in males signal both good genes and good environment and this may drive the co-evolution of female choice (Bonduriansky 2007c; Johnstone 1995; Rowe and Houle 1996). One empirical study tested the genic capture hypothesis by looking at the genetic variance of sexually dimorphic traits under different conditions and found mixed results in two populations of black scavenger flies (Dmitriew and Blanckenhorn 2014). They found that under poor condition male genetic variance increased more than female genetic variance for the fore femur, a male biased highly dimorphic trait, as it would be predicted by the genic capture model (Dmitriew and Blanckenhorn 2014). However, they observed this only in one of the populations that they tested (Dmitriew and Blanckenhorn 2014). Further tests of this hypothesis in different populations and species with varying types, magnitudes and directions of sexual dimorphism are necessary in order to determine if genic capture is in fact used by organisms to overcome the evolutionary constraints of sexual dimorphism. Despite often acrimonious debates about the relative contributions of these processes, these mechanisms represent a continuum (Kokko et al. 2002); runaway processes can evolve into good genes or condition-dependent like processes (Chandler et al. 2013).

Specific examples of condition dependent sexual dimorphism

Condition-dependence of sexual traits has been observed widely across many taxa (Cotton et al. 2004b). For the purposes of this report, only examples in arthropods will be reviewed because they are most relevant to the studies outlined in chapters 2, 3 and 4. One of the most interesting cases of condition dependence of a sexually dimorphic trait is in the polyphonic horned beetles (Emlen 1994). The manifestation and size of the horn, a head structure present only in large males that is used in territory defense, depends primarily on diet quality and quantity during development (Emlen 1994; Emlen et al. 2012). In the cactus bug *Narnia femorata*, seasonal variation in food availability produced greater variation in the size of the highly exaggerated hind leg femurs in males than in other non-sexually dimorphic traits (Miller et al. 2016). Male cactus bugs use their hind legs to engage in combat for access to females (Miller et al. 2016). Condition dependence of sexual dimorphism has also been observed in several different dipteran species (flies) including stalk-eyed flies, neriid flies and waltzing flies (Bonduriansky 2007b; Bonduriansky and Rowe 2005b; David et al. 1998). In all species, it was discovered that the traits that were most sexually dimorphic, the eye-stalk span in stalk-eyed flies, the antenna length in neriid flies and the head and antenna length in waltzing flies, were the most condition dependent (Bonduriansky 2007b; Bonduriansky and Rowe 2005b; David et al. 1998). In neriid flies and waltzing flies, level of sexual dimorphism and condition dependence are strongly correlated (Bonduriansky 2007b; Bonduriansky and Rowe 2005b). Oudin et al.(2015) tested the condition dependence of sexual dimorphism in moderately sexually dimorphic organisms was performed using the antler fly, a species that lacks highly exaggerated secondary sexual traits in males despite male territorial mating behaviour (Oudin et al. 2015). They observed minor condition dependence of sexual dimorphism for body size and for different traits with varying but moderate sexual dimorphism including traits with both male- and female-biased sexual dimorphism but this was not statistically significant (Oudin et al. 2015). However, they

did observe a correlation among traits between the extent of sexual dimorphism and condition dependence in males only (Oudin et al. 2015). They concluded that coevolution of condition dependence and sexual dimorphism is widespread even in moderately dimorphic traits (Oudin et al. 2015). Rohner et al. (2018) studied two *Sepsis* species which exhibit change in direction of SSD in the European and North American populations. They tested whether the sex-specific reproductive roles or selection for greater size were the drivers of greater plasticity in these flies, as well as performing a meta analysis of other holometabolous insects (Rohner et al. 2018c). In general, they found that the larger sex tends to be more plastic, suggesting that sex-specific plasticity may be more associated with selection for greater size rather than sex-specific reproductive roles (Rohner et al. 2018c). However, they also observed that in the sepesid flies they tested experimentally, there was a asymmetrical response in the sexes to environmental variation with males being more plastic when there was male-biased SSD than females when there was female-biased SSD (Rohner et al. 2018c).

All of these examples, with the exception of Oudin et al. (2015) and Rohner et al. (2018) systems, represent species with exaggerated secondary sexual traits in males. These systems are over-represented in the literature, because the theoretical predictions for the evolution of condition dependence are generally made for traits under sexual selection which tend to be exaggerated and male-biased. However, most insect taxa exhibit female biased SSD of moderate magnitude which are under more complex selective circumstances than just under strong sexual selection (Bonduriansky 2007a; Fairbairn 2007a; Teder and Tammaru 2005; Stillwell et al. 2010). SShD has rarely been studied in the context of condition dependence in insects (Bonduriansky and Rowe 2005b), particularly using modern geometric morphometrics. It is important to explore these concepts in more complex systems in order to fully how and why sexual dimorphism and condition dependence co-evolve.

1.2.4 Other sources of environmental variation affect SD

Beyond variation in diet, other types of environmental variation that can differently affect each sex leading to sex-specific plasticity. As discussed above, temperature is one of the most important environmental variables with potentially drastic consequences on size, growth rate, development time, gene expression, fecundity, and in some cases environmental sex-determination, especially in ectothermic taxa (Atkinson 1994; Stillwell and Fox 2005; Partridge et al. 1994; Atkinson 2010; Have and Jong 1996; Lang and Andrews 1994; Bull and Vogt 1979; Baroiller et al. 2009). However, except within the context of stressful values, temperature variation is not considered to be directly linked to organismal condition (Cotton et al. 2004b). Yet, some taxa show evidence of sex-specific temperature plasticity (Block and Stoks 2003; Hirst et al. 2015; Stillwell et al. 2010; Stillwell et al. 2007; Stillwell and Fox 2007; Stillwell and Davidowitz 2010; Fairbairn 2005; Teder and Tammaru 2005; Hu et al. 2010). The causes of sex-specific plasticity to temperature and other environmental variation are not fully understood. The differential adaptive canalization hypothesis may provide some insight. This hypothesis predicts that sexually dimorphic traits under strong selection may become more canalized leading to sex-sepcific plasticity. However, as discussed in previous sections, this hypothesis has received mixed support from experimental evidence (Fairbairn 2005; Teder and Tammaru 2005; Hu et al. 2010; Stillwell et al. 2010; Rohner et al. 2018c).

Environmental variation in other contexts - environmental vs genetic canalization

As previously discussed, phenotypic plasticity and phenotypic variation due to environment occur not just in the context of sexual dimorphism, but also more generally. Organisms are constantly exposed to environmental variation that may result in deviations from phenotypic optima with deleterious consequences. Thus, organisms evolve mechanisms that reduce the impact of such variation on phenotype; referred to as canalization

(Waddington 1942; Waddington 1957; Wagner et al. 1997). The reduction in phenotypic variation in the face of environmental variation is called environmental canalization (Eshel and Matessi 1998; Wagner et al. 1997). Theoretically, when a trait is near its optimum and under stabilizing selection, organisms can evolve increased canalization (Waddington 1957; Flatt 2005; Wagner et al. 1997; Stearns and Kawecki 1994). In contrast, at least under some models, while under strong directional selection canalization may be reduced or lost (Layzer 1980; Kawecki 2000; Hayden et al. 2012; Lack et al. 2016a; Groth et al. 2018). This loss of canalization under directional selection may occur for a variety of reasons, including exposure to new environments that are beyond the capacity of the buffering mechanism and/or rapid shift in allele frequencies that may result in loss of canalization. Additionally, antagonistic pleiotropy can result in alleles contributing to changes in the population mean, which may also result in changes in canalization (thus influencing trait variance) (Lack et al. 2016a; Groth et al. 2018; Clarke and McKenzie 1987; Clarke et al. 2000; McKenzie and Clarke 1988). This release in variation (both genetic and environmental components) can potentially be deleterious. However, decanalization is often accompanied by a release in cryptic genetic variation that may contribute to increased response to selection (Paaby and Rockman 2014; Gibson and Helden 1997; Ledon-Rettig et al. 2014; Lande 2009).

Not only do canalizing mechanisms evolve as a result of persistent environmental variation (environmental canalization) but they also might evolve as a way to buffer against new mutations (genetic canalization). Population genetic models suggest that genetic canalization may evolve only in a limited set of conditions because deleterious alleles tend to be purged by natural selection (Bagheri-Chaichian et al. 2003; Wagner et al. 1997). Only in organisms with extremely high mutation rates (eg. RNA viruses), in cases of high pleiotropy, or under epistasis with complete masking, is it theoretically possible to accumulate deleterious mutations and evolve such genetic canalization mechanisms (Bagheri-Chaichian et al. 2003; Wagner et al. 1997). Yet, we observe genetic

canalization in many taxa beyond what is predicted (Dun and Fraser 1959; Dworkin 2005c; Dworkin 2005a; Lack et al. 2016a; Groth et al. 2018). One potential solution that was proposed to solve this problem was the congruence hypothesis (Wagner et al. 1997). This hypothesis suggests that genetic canalization evolves as a by-product of the evolution of environmental canalization mechanisms (Wagner et al. 1997). Therefore, the hypothesis predicts that environmental and genetic canalization should be correlated (Wagner et al. 1997). Empirical studies have provided mixed support for this hypothesis, although the many of them have several limitations including ambiguous and indirect estimation of genetic canalization and usage of lab induced mutations that may not be representative of mutations occurring in nature (Lehner 2010; Stearns and Kawecki 1994; Stearns et al. 1995; Szollhosi and Derenyi 2009; Dworkin 2005a; Dworkin 2005c; Borenstein and Ruppin 2006). In fact, the most explicit tests of the congruence hypothesis have been performed by our lab group (Dworkin 2005a; Dworkin 2005c; Pesevski and Dworkin 2020), one of which is outlined in Chapter 2. The main theme of Chapter 2 is testing the congruence hypothesis in a *Drosophila melanogaster* population that has undergone genetic decanalization as a result of directional selection for greater size due to life at high altitude. I explore these concepts of genetic and environmental canalization and their relationship in more detail in the introduction and discussion sections of chapter 2.

1.3 Adaptive divergence and how it may affect SD and environmental variation

Sexual dimorphism is widespread, evolves readily in many different taxa and is the cause of some of the most captivating phenotypic variation in nature. However, sexual dimorphism does not evolve in isolation and selective forces that lead to its evolution often interact with other types of selective pressures (Connallon 2015; Lasne et al. 2018; Blanckenhorn et al. 2006; Svensson et al. 2018). Organisms often have to adapt to

novel environments and the strength and direction of both sexual and adaptive selection and their interaction can have tremendous consequences on the genomic architecture (Connallon 2015; Lasne et al. 2018; Svensson et al. 2018; Blanckenhorn et al. 2006). It is therefore important to consider how sexual dimorphism responds and is maintained under rapid adaptation. Some theoretical work has explored the interaction between sex-specific selection selection and adaptive evolution (Connallon 2015). Using a theoretical model, Connallon (2015) found that, before adaptive evolution, sex-specific selection acts antagonistically to produce and reinforce sexual dimorphism, while during adaptive evolution, sex-concordant selection drives adaptation to the new environment, reducing intersex conflict and allowing both sexes to change in parallel (Connallon 2015). Empirically, very few studies have examined the interaction between sex-specific and adaptive selective forces (Svensson et al. 2018; Lasne et al. 2018). Examining variation in sexual dimorphism as a result of clinal variation is one way to study the interaction of sex-specific selection and natural selection in a natural setting (Connallon 2015; Lasne et al. 2018; Blanckenhorn et al. 2006). A meta analysis that examined interaction between SSD and clinal body size patterns in both vertebrate and invertebrate species discovered a possible relationship between sexual dimorphism allometry and clinal variation, where slopes are steeper in males (Blanckenhorn et al. 2006). However this pattern was observed in only a subset of the species and was not considered to be a general pattern (Blanckenhorn et al. 2006). Lasne et al. (2018) looked at clinal variation due to latitude of sexual dimorphism in body size (trait with high r_{MF}, as well as heat, cold, desiccation and starvation resistance (traits with intermediate r_{MF}) in Australian *Drosophila melanogaster* populations. Studying traits with a lower r_{MF} can provide insight into how sexes adapt differently to novel environments when intralocus sexual conflict is lower, leading to less sex-specific constraint (Lasne et al. 2018). Only starvation resistance had slightly different slopes as a result of latitudinal clines in the sexes but this was primerly driven by only one of the populations they studied. For the other traits they observed

parallel slopes for males and females to latitudinal cline (Lasne et al. 2018). This suggests that spatially variable selection was sex-concordant and that r_{MF} may facilitate this parallel clinal adaptation in the sexes (Lasne et al. 2018).

Even rarer are studies examining the interaction between sexual dimorphism, local adaptation and sex-specific plasticity (Svensson et al. 2018). Pitchers et al. (2013), measured wing size and shape of African *Drosophila melanogaster* populations from altiudinal clines and examined both sexual dimorphism and differences in phenotypic plasticity to temperature due to clinal variation. Although they do not observe any differences in sexual dimorphism due to altitude, they do observe some differences in sex-specific slopes to altitude at 18°C compared to 24°C (Pitchers et al. 2013). Another study looked at the interaction between sex, adaptive divergence and plasticity in two populations of copepod *Acartia tonsa* from Florida and Connecticut (Sasaki et al. 2019). They observed population specific differences in plasticity to temperature, with both females and males from the Connecticut population having a greater reduction in size due to increase in rearing temperature. They also observed a slight difference in SSD between the two populations (Sasaki et al. 2019). I reanalysed of their publicly available data and found that there were significant differences in size due to the interaction of sex, plasticity and population of origin (Sasaki et al. 2019). This highlights the importance of studying the interplay between sexual dimorphism and local adaptive divergence in the context of phenotypic plasticity because it can reveal unforeseen patterns of interaction between different selective forces as well as plasticity when exposed to novel environments. I explore this complex interaction between sexual dimorphism, local adaptation and phenotypic plasticity in Chapter 3, where I use two adaptively diverged *Drosophila melanogaster* populations from two different altitudes to assess whether sexual dimorphism and sex-specific plasticity of wing size and shape has changed as a result of adaptation to high altitudes. I use modern geometric morphometric methods to assess how sexual dimorphism, adaptive divergence and phenotypic

plasticity due to temperature and diet quality as well as their interplay affect allometric relationship between wing size and shape.

1.4 Comparative study of SD and CD

As highlighted above, it is very important to study sexual dimorphism and its condition dependence (and other sex-specific plasticity) under different evolutionary contexts. This includes the evolution of condition dependent sexual dimorphism at the macro-evolutionary scale. Comparative analysis of condition dependent sexual dimorphism can help us discover new patterns and understand conditions under which sexual dimorphism and condition dependence are likely to co-evolve. Rensch's rule is one of the most widely accepted and studied inter-specific pattern of SSD variation (Rensch 1960; Abouheif and Fairbairn 1997; Blanckenhorn et al. 2007b). As discussed in Section 1.2, it is both theoretically predicted and empirically established that the condition dependence of a sexually dimorphic trait is positively correlated with the degree of sexual dimorphism (within the same organism, intra-specifically and inter-specifically). According to Rensch's rule, inter-specific variation of SSD is positively correlated with size. Therefore, we would expect that condition dependence of sexually dimorphic traits to be positively correlated with size, at least at the inter-specific level. Although there are many studies examining condition dependent sexual dimorphism and sex-specific plasticity in numerous species (Cotton et al. 2004b; Stillwell et al. 2010; Teder and Tammaru 2005), to date, only one study has considered studying condition dependence of sexual dimorphism in a comparative framework, using modern phylogenetic comparative methods (Rohner and Blanckenhorn 2018). Rohner and Blanckenhorn (2018) examined the condition dependence of sexual dimorphism in several (sub)species of sepsid flies with varying degrees of SSD including both male- and female-biased SSD (SDI $-0.08 - 0.12$) (Rohner and Blanckenhorn 2018). Some of the species from the European continent had counterparts in North America with complete SSD reversal that went in either direction (male-biased

to female biased and female-biased to male-biased) (Rohner and Blanckenhorn 2018). Rohner and Blanckenhorn (2018) raised the flies under a range of nutritional qualities and measured sizes in traits that vary in sexual dimorphism in these flies. They found consistent phylogenetic patterns of sex-specific condition dependence and sexual dimorphism across the species, with species that are more sexually dimorphic being more condition dependent (Rohner and Blanckenhorn 2018). The traits with greater degree of sexually dimorphism, in both male-biased and female-biased species, had heightened condition dependence than the less dimorphic traits (Rohner and Blanckenhorn 2018). They concluded that there may be common genetic and developmental basis for sexual dimorphism and its condition dependence (Rohner and Blanckenhorn 2018). More studies like these are necessary in order to get a greater understanding of the co-evolution of condition dependence and sexual dimorphism at the inter-specific level. In our lab, we observe that intra-specific plasticity-induced variation in wing size and shape in *Drosophila melanogaster* can exceed inter-specific variation among the whole melanogaster species subgroup (unpublished data). In chapter 4, I outline my comparative study of condition dependent sexual dimorphism in species from the *melanogaster* species subgroup, where I have raised these species under limited food availability during development and then measured wing size, thorax size, and size of different components of the leg.

1.5 Using Drosophila to study sexual dimorphism, condition dependence and environmental variation in both the context of adaptive divergence and in a comparative framework

Drosophila is one of the most well studied organisms and has been an insect model for genetics, evolution, physiology and behaviour for decades. As in the majority of insect species, *Drosophila* exhibits moderate female biased SSD where most traits and

its overall size are 15-20% larger in females than in males (David et al. 2003; Teder and Tammaru 2005). *Drosophila* is a great model organism because it is are easy to rear and manipulate in the lab and there are numerous lineages and species are readily available. The *Drosophila* wing is particularly useful as a model for studying sexual size and shape dimorphism because it is practically two-dimensional. There are many geometric morphometrics tools that have been developed in order to study the size and shape of the wing (Adams et al. 2013). The *Drosophila* wing exhibits both SSD and sexual shape dimorphism (SShD) and evidence suggests that they are both under sexual and natural selection (David et al. 2003; Ewing 1964; Gidaszewski et al. 2009; Abbott et al. 2010; Menezes et al. 2013). Drosophila males use the wing in courtship to produce a visual and auditory display (Ewing and Bennet-Clark 1968). This courtship display varies among different *Drosophila* species and these differences in courtship behaviour may be used as species recognition mechanism (Ewing and Bennet-Clark 1968). Wing size and shape may be important for both the visual and auditory courtship display. In a classical study by Ewing (1964) wing size manipulations via rearing at different temperatures, selection for greater wing-thorax ratio and artificial wing amputations were done. For all manipulations, Ewing (1964) discovered that larger wing area increased copulation success. The influence of wing shape on mating success was also assessed by measuring mating success in flies that have undergone manipulation of wing shape by selection for either elongated or rounded wings (Menezes et al. 2013). This study found that males with elongated wings were more successful at mating than males with rounded wings (Menezes et al. 2013). An analysis of wing SSD and SShD among the *melanogaster* species subgroup revealed that there is significant divergence among the different species in both SSD and SShD (Gidaszewski et al. 2009). However, with regards to SShD, the observed divergence did not show any phylogenetic signal (Gidaszewski et al. 2009). Both the allometric and non-allometric components of shape showed variable SShD among species suggesting that allometry may not be the primary

driver of SShD evolution (Gidaszewski et al. 2009). Sexual conflict has been detected for both wing shape and size (Abbott et al. 2010). A recently discovered trait, the wing interference pattern (WIP), has also been shown to be under sexual selection (Katayama et al. 2014). Greater saturation and intermediate hues in the magenta range were shown to be most attractive (Katayama et al. 2014). In fact, intra-sexual selection has been observed among *Drosophila* males in many species (Bateman 1948b). Most *Drosophila* species use scramble competition as a mating system (Partridge et al. 1987). Males often perform an elaborate courtship display that can include specific locomotor, auditory and tactile cues such as hopping and dancing, wing vibrations, wing displays, licking, touching (Ewing 1983). Interestingly, despite the observed sexual selection on males for greater size (Partridge et al. 1987), we still observe female biased SSD in *Drosophila*. This suggests that there may be opposing natural selection or other constraints that do not allow for the exaggeration of the *Drosophila* traits in males, such as the wing. Using a system like the *Drosophila* wing to study condition dependence of sexual dimorphism can help us understand how these processes co-evolve in a moderate female-biased SSD (in most species) under complex selective forces. Other than SSD and SShD, additional secondary sexual characters in *Drosophila* include sex combs, wing spots, body colouration (Kopp et al. 2000; Kopp and True 2002). The *Drosophila* phylogeny has been pretty well resolved and can be readily used in phylogenetic comparative analysis (O'Grady and DeSalle 2018; Linde and Houle 2008). Among *Drosophila* species, we generally observe female biased SSD for overall body size, with the exception of *D. prolongata* (Rohner et al. 2018b). Unlike most insects, Drosophilids follow Rensch's rule (Blanckenhorn et al. 2007b). The development of *Drosophila* has been studied extensively allowing us to examine the developmental mechanisms that lead to the production of conditionally-dependent sexually dimorphic traits. Proximate causes of SSD in *Drosophila* include varied development time, with males taking longer to develop compared to females, as well as different sex-specific weight loss during third instar and pupation stages (Testa

et al. 2013; Butler and Losos 2002). The differences in SSD are genetically mediated through sex determination pathway genes such as *transformer* and their influence on the insulin-like growth factor pathway (Millington et al. 2021; Rideout et al. 2015; Mathews et al. 2017). Using *Drosophila*, we can ask questions many questions about the evolution of condition dependence of sexual dimorphism under different evolutionary scenarios. Therefore, *Drosophila* is an attractive model for studying the dynamics of the evolution of condition-dependent sexual dimorphism because of the large variety of lines and species that are available with both known and unknown evolutionary histories.

1.6 Quick overview of thesis and rationale

In this thesis, I address major themes and questions that have been central in the field of evolutionary biology. These include questions about evolution of sexual dimorphism, phenotypic plasticity, canalization, adaptive evolution, macro-evolutionary patterns as well as their interplay. Studying these questions in such an integrated way is important because organisms in nature evolve in complex environments and are often the target of multiple influences including their evolutionary history, their genomes, their development, the environment that they develop in and live as adults, and the interaction of their external and internal (genomic and epigenomic) environments. All of these factors can have a huge impact on the phenotypic expression of traits which can determine how future evolution occurs.

In chapter 2, I study the influence of adaptive evolution on the breakdown of environmental canalization. In this study, I use two adaptively diverged African populations of *Drosophila melangaster*; one population that has adapted to life at high altitude by increasing body size and wing loading and the other population from a low altitude near the ancestral range of *Drosophila melangaster*. Previous studies have determined that

the high altitude population has undergone a breakdown of genetic canalization as a result of the adaptation to high altitude with evidence of greater sensitivity to mutagenesis (Lack et al. 2016a). In my study, I test the congruence hypothesis (Wagner et al. 1997), which predicts that environmental and genetic canalization should be correlated because genetic canalization evolves as byproduct of the evolution of environmental canalization. The theoretical background behind this hypothesis is summarized in section 1.2.4 and the introduction and discussion of chapter 2. I examined the environmental canalization using wing size and shape, at three different levels. I quantified micro-environmental canalization by studying within-individual variation (fluctuating asymmetry of wing size and shape) and among-individual within-line variation, and I studied macro-environmental variation by examining reaction norms to temperature treatments strains derived from the high- and low-altitude populations. I assessed whether these measures of micro- and macro-environmental canalization for wing size and shape are correlated with proportion of wing defects in order to examine whether environmental and genetic canalization are correlated. I did not find any differences in micro- and macro-environmental canalization and there were no significant correlations between these measures and proportion of wing defects. These findings suggest that, in these populations of *Drosophila melanogaster*, environmental and genetic decanalization are not in fact associated (Pesevski and Dworkin 2020). This study is one of the most explicit studies testing the congruence hypothesis since its proposal. It is the first study to use a naturally existing population in which direct evidence of genetic canalization have been observed. Previous studies have either used indirect measures of genetic canalization or have used lab induced mutations in lab adapted populations (Lehner 2010; Stearns and Kawecki 1994; Stearns et al. 1995; Szollhosi and Derenyi 2009; Dworkin 2005a; Dworkin 2005c; Borenstein and Ruppin 2006). However, this study tests the congruence hypothesis in only a single case where there is naturally occurring genetic decanalziation. More studies are necessary in order to establish whether the congruence hypothesis can be fully dismissed. It is important

to understand the evolutionary mechanisms by which both environmental and genetic canalization evolve or break down because they can have tremendous impact on how organisms express their phenotype and that in turn can affect how they evolve in the future.

In chapter 3, I use the same altitudinally diverged African populations from chapter 2, in order to examine the effect of adaptive divergence on wing size and shape sexual dimorphism and its condition dependence. I raised strains from the two population under different diet qualities and developmental temperatures and measured wing size and shape using geometric morphometrics methods. I discovered that, despite the drastic differences in phenotype of wing size and shape between the high- and low-altitude populations, both SSD and SShD and their response to nutrition were relatively similar in the two populations. The low-altitude population had a slightly greater reduction in SSD but not SShD as a result of low developmental temperature. Since wing size varied strongly due to all the different factors: adaptive divergence, sex, nutrition and temperature, I wanted to examine the influence of these factors on the allometric vectors of wing shape. I calculated the size-shape allometric vector distances and correlations between comparable groups and found that allometric vectors differed least for sex, moderately for adaptive divergence and diet and the most for temperature. Interestingly, the largest differences were observed between allometric vectors from the high and low altitude populations at 18°C and 28°C. Finally, I wanted to determine whether allometry played an important role to the SShD that we observed in these populations at the different diets and temperatures. I partitioned allometric and non-allometric SShD in all the different groups and discovered that both contributed to overall SShD. However, to do this I assumed common allometry in the sexes and discovered that this assumption is violated despite the high allometric vector correlations, leading to the allometric and non-allometric SShD not adding up to the total SShD. This study demonstrated that, at least within these populations of *Drosophila melanogaster*, adaptation does not have

an influence on sexual dimorphism of the wing and its condition dependence. However, more systems like these need to be studied in order to fully understand the interplay between adaptation, sexual dimorphism and condition in the future.

In chapter 4, I examine whether sexual dimorphism and condition dependence have correlated evolution at the inter-specific level using *Drosophila* species from the *melanogaster* species group with varied degrees of SSD. I raised strains from 27 different species under unrestricted larval diet (high condition) and restricted larval diet (low condition) and measured the thorax, leg segments and wing area. One of the species examined in this study, *Drosophila prolongata*, has male-biased SSD. I found that within each species, the more dimorphic a trait is the more condition dependent it is. In most species, the wing had the highest degree of both SSD and condition dependence, while the leg segments were the least dimorphic and condition dependent. However, this pattern did not hold in *Drosophila prolongata*, in which tibia length and width were the most dimorphic, but the wing had the greatest condition dependence. Additionally, I observed relatively strong phylogenetic signal for all traits and SSD of all the traits but not for condition dependence. At the inter-specific level, evolutionary patterns of SSD and condition dependence did not correspond, suggesting that in the *melanogaster* species group sexual dimorphism and condition dependence do not have correlated evolution.

Chapter 2

Genetic and environmental canalization are not associated among altitudinally varying populations of *Drosophila melanogaster*

2.1 Abstract

Organisms are exposed to environmental and mutational effects influencing both mean and variance of phenotypes. Potentially deleterious effects arising from this variation can be reduced by the evolution of buffering (canalizing) mechanisms, ultimately reducing phenotypic variability. There has been interest regarding the conditions enabling the evolution of canalization. Under some models, the circumstances under which genetic canalization evolves is limited, despite apparent empirical evidence for it. It has been argued that genetic canalization evolves as a correlated response to environmental

canalization (congruence model). Yet, empirical evidence has not consistently supported predictions of a correlation between genetic and environmental canalization. In a recent study, a population of *Drosophila* adapted to high altitude showed evidence of genetic decanalization relative to those from low altitudes. Using strains derived from these populations, we tested if they varied for multiple aspects of environmental canalization We observed the expected differences in wing size, shape, cell (trichome) density and mutational defects between high- and low-altitude populations. However, we observed little evidence for a relationship between measures of environmental canalization with population or with defect frequency. Our results do not support the predicted association between genetic and environmental canalization.

Key Words

Canalization, *Drosophila melanogaster*, Adaptation, high altitude, Wing shape, Body size, Phenotypic Integration, Geometric morphometrics, Cell size

2.2 Introduction

In addition to differences in trait means, there can be considerable variation in how much variation is observed among individuals of a given genotype (Waddington 1942; Rendel 1963; Felix and Barkoulas 2015; Flatt 2005; Visser et al. 2003; Siegal and Leu 2014; Klingenberg 2019; Mayer and Hansen 2017; Pelabon et al. 2010; Gibson and Wagner 2000). Theoretical work has examined this propensity to vary with respect to the evolution of phenotypic robustness or canalization. Such evolved properties are important to examine, as environmental and mutational variation influence trait variance, ultimately influencing organismal performance and fitness (Arnold 2003; Arnold 1983). The sensitivity of a given genotype in its response to mutational or environmental influences can vary *among* genotypes. It has been empirically demonstrated that under mutational or environmental perturbation, there is often the expression of cryptic genetic variation, which has previously been used as evidence for genetic canalization (Paaby et al. 2015; Paaby and Rockman 2014; Scharloo 1991; Gibson and Helden 1997; Dworkin 2005a). Theory suggests that robustness to environmental variation — environmental canalization — can readily evolve as organisms are constantly exposed to the influence of environmental effects (Eshel and Matessi 1998; Wagner et al. 1997). Yet, as deleterious mutations are often purged by natural selection, this can result in weak selection for genetic canalization (assuming stabilizing selection on the trait), making it potentially less likely to evolve (Wagner et al. 1997; Proulx and Phillips 2005; Visser et al. 2003; Gibson and Wagner 2000).

The congruence hypothesis was proposed as a solution to the apparent inconsistency between theoretical and empirical work regarding the evolution of genetic canalization (Wagner et al. 1997). This hypothesis predicts that genetic canalization evolves as a correlated response during selection for environmental canalization (Wagner et al. 1997;

Gibson and Wagner 2000; Visser et al. 2003). Empirical evidence for associations between genetic and environmental canalization is mixed. Some studies provide supporting evidence both from simulations (Ancel and Fontana 2000; Siegal and Bergman 2002; Shu et al. 2007) and empirical work (Lehner 2010; Stearns and Kawecki 1994; Stearns et al. 1995; Szollhosi and Derenyi 2009). However, some explicit tests for the congruence model did not find support for it (Dworkin 2005a; Dworkin 2005c; Borenstein and Ruppin 2006). The most likely explanation is that the evolution of genetic and environmental canalization are not homogeneous, given the complex interplay of selection, mutation rates, genetic architecture, and evolutionary history.

There are a number of important methodological and conceptual issues that influence the debate on the congruence hypothesis, and the study of the evolution of canalization more generally. First, the conditions in which the release of cryptic genetic variation can be used to infer genetic canalization may be more limited than once thought (Hermisson and Wagner 2004; Geiler-Samerotte et al. 2019). Rather, mutation accumulation or mutagenesis experiments are likely to be more fruitful for investigating genetic canalization. Second, environmental canalization is often measured using multiple approaches that differ with respect to what aspects of environmental robustness they seek to capture. Within-individual variation (fluctuating asymmetry), among-individual within-genotype variation, and reaction norm of trait means under common or different environmental treatments (Dworkin 2005b) have all been employed. Using these methods to study genetic and environmental canalization, some studies have seen modest evidence of association between degree of sensitivity to genetic perturbation (changes in trait means) and within- or among-individual variance within a genotype (Dworkin 2005a; Dworkin 2005c; Chari and Dworkin 2013; Camara and Pigliucci 1999; Chandler et al. 2017). However, a number of other studies do not show a consistent relationship between magnitude of perturbation and among-individual within-line or within-individual variance (Haber and Dworkin 2017; Debat et al. 2009; Levy and Siegal 2008). This suggests that

there are multiple, partially distinct properties when considering robustness for a given genotype.

Surprisingly, one issue that has not been broadly considered in the literature regarding the evolution of canalization is the influence of both lab adaptation (domestication) and the use of lab induced mutations. Most studies of canalization and robustness use lineages that have likely undergone some degree of adaption to lab environments. Furthermore, many studies often use lab-induced mutations as a source for genetic perturbations (Paaby et al. 2015; Gibson and Helden 1997; Dworkin 2005a; Dworkin 2005c; Haber and Dworkin 2017; Hallgrimsson et al. 2006; Debat et al. 2011; Levy and Siegal 2008). However, lab domestication and induced mutations may be unrepresentative of natural populations (Dittmar et al. 2016; Rockman 2008; Orgogozo et al. 2015). Lab-induced mutations may not reflect the spectrum of mutational effects experienced by natural populations. This may bias inferences regarding the ability of genotypes to buffer the effects of mutations that organisms are exposed to during their evolutionary history. Furthermore, when considering the evolution of canalization, the evolutionary history of the experimental populations matters. In some experimental studies, collections of natural lineages or families that have heterogeneous geographical origins, and/or have been maintained in the lab for long periods of time are used (i.e. Dworkin(2005), Dworkin(2005)). Thus, explicit tests for the correlated evolution of genetic and environmental canalization (*sensu* Wagner et al. (1997)) is difficult without knowledge of the evolutionary history of such populations.

Another shortcoming of many empirical which examine properties of phenotypic robustness and canalization is that they have examined variation with a univariate perspective (Dworkin 2005a; Dworkin 2005c), even when examining many traits (Levy and Siegal 2008; Takahashi et al. 2011; Takahashi et al. 2010; Takahashi 2017). Considerable evidence and theory have demonstrated that a multivariate perspective on evolutionary

change ($\Delta\bar{\mathbf{z}} = \mathbf{G}\beta$) improves predictions and understanding of evolutionary responses to selection (Lande and Arnold 1983a; Lande 1979; Schluter 1996; Walsh and Blows 2009; Houle et al. 2017a; Blows and Mcguigan 2014; Mcguigan and Blows 2007; Pitchers et al. 2014; Agrawal and Stinchcombe 2009; Hansen and Houle 2008). Yet, this perspective has only been considered in a modest number of studies examining variational properties of phenotypes (Debat et al. 2009; Debat et al. 2011; Hallgrimsson et al. 2009; Hallgrimsson et al. 2006; Green et al. 2017; Debat et al. 2006; Breuker et al. 2006; Cheverud et al. 1983; Pavlicev et al. 2009). When considering properties of trait (co)variation in this perspective, it is not just the magnitude of variation (matrix size), but direction (of major axes of variation) and the shape of the variance-covariance matrix (a proxy for trait integration) that need to be considered as well. In a recent study examining variation in $\mathbf{E}$ (from $\mathbf{P} = \mathbf{G} + \mathbf{E}$) across naturally derived strains and lab induced mutations, it was demonstrated that changes to trait means and relative orientation (directions of major axes of variation) of phenotypic (co)variances matrices were more variable than phenotypic integration (Haber and Dworkin 2017). This suggests that a multivariate perspective needs to be consistently applied to studies examining trait (co)variation (Klingenberg 2019).

Thus, what has been lacking for empirical studies testing evolutionary models of canalization is a system with the relevant natural history that can be studied with a multivariate approach. Lack et al.(2016), among other recent studies, demonstrated that populations of *Drosophila melanogaster* from sub-Saharan Africa have recently adapted to a high-altitude environment. As is common for small insects evolving to high altitude environments (Dillon et al. 2006b), the high-altitude African population has evolved increase in cold-tolerance (Pool et al. 2016) and melanism (Bastide et al. 2016). They have also adapted via increased body size, wing size and shape (Pitchers et al. 2013; Lack et al. 2016a; Lack et al. 2016b; Bastide et al. 2016; Fabian et al. 2015; Klepsatel et al. 2014), likely to deal with changes in flight response in cold, thin air (reviewed

in Dillon et al. 2006b). Intriguingly, there is a substantial increase in the frequency of qualitative mutational defects of wing morphology in the high altitude population (Lack et al. 2016a). Partially inbred Strains derived from a high-altitude Ethiopian population have defect frequencies as high as 40-50% (Lack et al. 2016a). This increase in frequency was not simply a result of hitchhiking of deleterious alleles, or a strong bottleneck, (Pool et al. 2012a) but due to reduced mutational robustness, as assessed using mutagenesis experiments (Lack et al. 2016a). Importantly, the population-specific mutational sensitivities are pleiotropically linked to variants that influence the increase in wing size. This appears to be the case whether considering the variants among the high- and low-altitude populations (Lack et al. 2016a), or even in putative ancestral lowland populations (Groth et al. 2018) that have been artificially selected for larger wing size. Currently, it is inferred that the increase in mutational sensitivity in the high-altitude population may have been a result of strong directional selection on size, leading to rapid adaptation, with negative pleiotropic consequences. Compared with the ancestral low-altitude population that likely experienced a long history of stabilizing selection (and thus potentially promoting the evolution of canalization), the increase in size due to adaptation to conditions at high altitude, or due to strong artificial selection (Groth et al. 2018) have resulted in the evolutionary loss of canalization. This may represent a situation similar to that envisioned by Waddington (1942), where the population has not yet re-evolved its canalization mechanism after a long bout of strong directional selection for larger body size, wing size and shape.

The evolutionary history of the high- and low-altitude populations provides an ideal opportunity to test the relationship between genetic and environmental canalization. While there is considerable genetic variation within populations, strains derived from a high-altitude population in Ethiopia from an elevation of ∼3000 m are much larger in body and wing size, have distinct wing shapes and have a greater frequency of qualitative 'mutant' phenotypes than low-altitude populations from Zambia from an elevation

of ∼500m (Lack et al. 2016a). Wing size and shape in *D. melanogaster* is a model system for studies of plasticity, sensitivity to mutational perturbation and within- and among-individual variability using both natural and lab-induced variation (Haber and Dworkin 2017; Debat et al. 2011; Breuker et al. 2006; Debat et al. 2006; Soto et al. 2008; Klingenberg and Zaklan 2000; Pelabon et al. 2006). In this study, we compared environmental canalization between the high and low-altitude populations. We used different measures of environmental canalization: within-line, among-individual variation (micro-environmental variation), within-individual variation (fluctuating asymmetry) and phenotypic plasticity across a temperature gradient (macro-environmental variation). Further, we examined associations between different measures of environmental canalization and mutational perturbation to test the congruence hypothesis and determine whether strains with greater proportion of defects are also more variable (aka more decanalized). Despite demonstrating substantial population and environmental differences in wing size, shape, cell density and penetrance of mutational perturbation consistent with previous studies, we observed no consistent differences in measures of micro-environmental and macro-environmental canalization among populations. These results are discussed within the context of our ongoing understanding of the evolutionary mechanisms that influence trait variability.

2.3 Materials and Methods

2.3.1 Fly strains and Growth conditions

Drosophila melanogaster strains used in the current study represent a subset of those from Lack et al. (2016) and Lack et al. (2016). The high-altitude inbred strains were derived from flies collected in Fiche, Ethiopia at an altitude of 3070 m in December 2011. The low-altitude strains were collected in Siavonga, Zambia at an altitude of 530 m, and a 3125 km linear distance away from the high-altitude Ethiopian population in July 2010. These strains underwent inbreeding in the lab, which is expected to substantially

reduce the effects of lab adaptation (because of the small N_e within each strain), but this does result in substantial genetic drift within lines (but should not substantially alter allele frequencies among lines).

The flies for the micro-environmental variation experiments were raised as per Lack et al. (2016). Flies were raised at 25°C, in 70% humidity, with 12:12 hour light/dark chamber, on standard cornmeal molasses food at a low larval density. This experiment was performed in June 2013.

A subset of the strains described above were used for the temperature plasticity (macro-environmental variation) and fluctuating asymmetry experiments. These strains were raised on a 1:1.5 protein to sugar ratio diet; recipe outlined in Table A-S3 at 24°C for two generations prior to the experiment. Newly emerged adults (10-20 males and females), were collected and placed in egg collection chambers with apple agar plates with yeast. Eggs were collected, 50 at a time, and placed into vials with food. Flies were raised at 18°C, 24°C, and 28°C in 12:12 hour light/dark chambers until emergence. Adults were collected within 2 days of emergence and preserved in 70% ethanol. This experiment was performed in 2017. While these strains are partially inbred, as a check, we confirmed that the phenotypic effects of the size related traits from these lineages remained correlated with the low altitude populations (where variation for size is considerable), and also showed the same overall patterns (for mean size, shape and defect frequencies).

2.3.2 Phenotyping

Wing size and shape - Micro-environmental canalization

The right wing of each fly was dissected and imaged using an Olympus DP30B camera mounted on an Olympus BX51 microscope (Olympus software V.3,1,1208) using a 2x objective (20X total magnification). Landmark and semi-landmark data was captured using a modified version of the "WINGMACHINE" pipeline (Houle et al. 2003; Pitchers

et al. 2019a). Coordinates of two starting landmarks were recorded using tpsDig2 software (V2.16). These coordinates are the humeral break on the leading edge of the wing and the alula notch on the trailing edge of the wing. B-splines were fit to veins and wing margin for each image using Wings (V3.7), reviewed and manually adjusted, if necessary. Landmark and semi-landmark positions were extracted and the shape information after adjusting for size, position, and rotation information using CPReader software (V1.12r). This produced data composed of 12 landmarks and 36 semi-landmarks (Figure 2.1B) as well as centroid size of for each specimen. The strains used in this experiment are outlined in Table A-S1.

Measuring trichome (cell) density

A subset of strains used for the the initial size and shape analysis were re-imaged with a higher-resolution camera. We chose 15 strains from each population as follows: five strains each were chosen with the highest and lowest within-line coefficient of variation (CV) for wing size. Additionally, five strains were chosen at random from each population. This allowed us to maximize the variation we examined within each population. We phenotyped 15-20 males and females from each strain. Wings were imaged using an Olympus DP80 camera mounted on an Olympus BX43 microscope, using a 4X objective (total 40X magnification). Images were captured with cellSens Standard (V1.14) software. Cell density was quantified by counting trichomes on the surface of the wing using the ImageJ FijiWings macro (V2.2) (Dobens and Dobens 2013). Each trichome represents a single cell (Dobzhansky 1929). We used a $0.0065mm^2$ (75x75 px) measurement area in each of 16 different locations in the wing (Figure 2.4A).

Wing size and shape - Macro-environmental canalization

The right wing of each fly was imaged using the same microscope settings as the cell density experiments. Wing size and shape were quantified using the same pipeline as

the micro-environmental canalization experiment. We used 3 replicate vials per strain per rearing temperature. Strains used for this experiment are outlined in Table A-S2.

Fluctuating Asymmetry of wing size and shape

Left and right wings were phenotyped for two lines from each population (E39 and E73 from high-altitude, Z254 and Z311 from low-altitude, total number of individuals: 509) to assess fluctuating asymmetry. Duplicate measurements were taken of the left and right wings from 77 individuals chosen randomly from different populations, sexes and rearing temperatures to estimate measurement error. The same phenotyping methods were used as the micro-environmental canalization experiment.

Quantification of wing defects

Each wing image was manually scored for venation defects. For the micro-environmental canalization experiment, proportion of defects was calculated as the ratio of the number of wings with defects to total wings for each line. For the macro-environmental canalization experiment, each individual wing was scored based on whether they have a defect or not, using a binary scale (1 for defect observed, 0 for defect not observed). The proportion of defects for each line was calculated by averaging the scores for all individuals within line and experimental treatment.

2.3.3 Analysis

Data was analyzed using R (v3.5.1) (R Core Team 2018) in RStudio (v1.1.456) on a MacBook Pro, running macOS Mojave (V10.14.2). Mixed models were run using *lmer* and *glmer* from the package *lme4* (V1.1.19) (Bates et al. 2015), glmmTMB (Brooks et al. 2017) and *procD.lm* from the package *geomorph* (V3.0.7) (Adams et al. 2018). Generalized linear models were run using *glm* from the *stats* package (V3.5.1) (R Core Team 2018).

Modeling wing size, shape, cell density and wing defects

Linear mixed models were fit with the wing size, and cell density data, and generalized linear mixed model (binomial distribution, with a logit link) was fit with the wing defects data using population, sex, rearing temperature (for macro-environmental canalization experiment) and their interactions as fixed effects. Where possible, the intercept and sex effects were allowed to vary as random effects of line (strain). Additionally, for the cell density data, wing region was included as a fixed effect to test whether there is variation in cell density across the wing, and an individual level random effect was included to account for the multiple measures per wing.

For the temperature plasticity experiment, we used a model similar to the one described above, but allowing temperature effects to vary according to linear and quadratic effects (using 2nd degree orthogonal polynomials), including interaction effects with temperature and within the random effects of line nested within population.

For wing shape, we fit a multivariate linear model using *procD.lm* estimating the contributing effects of centroid size, population and sex and their interactions as fixed effects, and line nested within population as a random effect. Statistical inference was performed using a randomized residual permutation procedure in geomorph using 1000-2000 permutations for each effect.

Estimating among-individual, within-line variation of wing size, shape and cell density

For wing size, among-individual, within-line variation was estimated in two ways for each strain, using the coefficient of variation ($CV = \frac{\sigma}{\mu}$) and the median form of Levene's deviates (used for all formal statistical inference) (Van Valen 2005; Dworkin 2005b). For the macro-environmental canalization experiment, sex effects were first modeled out and then CV and Levene's deviates were calculated for each line at each temperature.

To capture some of the multivariate variational properties for wing shape, we focused on two measures estimated for each strain. First we used matrix size (total variance), which is the trace of variance-covariance matrix for the strain. This is meant to capture overall variation. This is equivalent to the sum of the eigenvalues (Van Valen 2005). Total variance estimates were multiplied by a factor of 1000.

We also examined trait integration of wing shape using two measures derived from the within-line covariance matrix. Specifically we used matrix eccentricity as well as the standard deviation of its eigenvalues (scaled), (Jones et al. 2003; Kirkpatrick 2009; Haber 2011; Pavlicev et al. 2009; Van Valen 1974). The standard deviation of eigenvalues of the covariance matrix has been used extensively as a proxy for integration (Pavlicev et al. 2009; Cheverud et al. 1983; Haber 2011). We calculated the relative standard deviation of eigenvalues (rSDE) and the relative standard deviation of the eigenvalues scaled by total variance (rSDE2) (Pavlicev et al. 2009; Van Valen 1974; Haber 2011). rSDE estimates were multiplied by a factor of 10000 and rSDE2 estimates were multiplied by a factor of 10. The shape of the VCV matrix can also be quantified using matrix eccentricity. While typically defined as the ratio between the first two eigenvalues (Jones et al. 2003; Kirkpatrick 2009), we used a generalization which was the ratio between the largest eigenvalue and the total variance (Haber and Dworkin 2017), which has been shown to be proportional to rSDE2. Variation due to sex and size was modeled out prior to estimating total variance, eccentricity and rSDE for each strain.

The registration process (Procrustes superimposition) influences covariation within and among landmarks. As such, the use of Procrustes residuals for analysis is of potential concern. However, in our previous study, we demonstrated using a variety of approaches, that at least for *Drosophila* wing shape, results from Procrustes superimposition were extremely similar to those generated via spatial interpolation of the data to generate multivariate variables (Haber and Dworkin 2017). As such, for this study we used the

Procrustes residuals for simplicity.

CV and Levene's Deviates, were also calculated for trichome (cell) density for each strain. CV was calculated in two ways. First by averaging the cell density across the wing for each individual and calculating the within-individual CV, and then averaging CV for line. Alternatively, cell density CV was calculated by averaging cell density for each line first and then calculating CV. These two approaches of measuring CV produced similar results and only the first one is used in this paper. Associations between the measures calculated above across strains was performed using a Pearson correlation coefficient.

We examined whether any of the variation measures (CV and Levene's deviates for size, total variance, eccentricity, rSDE and rSDE2, as well as cell density CV and Levene's deviates, as response variables) varied due to the effects of population and sex. Generalized linear mixed models were fit, sex (excluded for for macro-environmental experiment), population, temperature (for macro-environmental canalization experiment) and their interactions as fixed effects. The intercept and where possible sex were allowed to vary as random effects by line (nested within population). Given that all of these responses can only take on continuous positive values we assumed a Gamma distribution with an inverse link function.

Fluctuating Asymmetry of wing size and shape

To quantify measurement error, duplicate measures were taken for left and right wing shape for 77 individuals. Wing size measurement error was estimated using an analysis of variance (ANOVA) and wing shape measurement error estimated using a Procrustes ANOVA. For both wing size and shape, individual, side and their interaction were used as effects. The side effect represents directional asymmetry, the individual effect represents variation among individuals and the side:individual interaction term represents fluctuating asymmetry The residual variance in this model estimates measurement error

(Klingenberg and McIntyre 1998; Debat et al. 2009; Palmer and Strobeck 1986; Palmer and Strobeck 2003; Palmer 1994). We compared the variation of the side:individual interaction term with the residual variation in order to determine whether the measurement error was negligible with respect to fluctuating asymmetry (Supplementary Tables A-S29 and A-S30).

Fluctuating asymmetry of wing size was calculated using standard FA indices: FA1 ($FA1 = |R - L|$) and FA8a ($FA8a = |ln(\frac{R}{L})|$) (Palmer and Strobeck 1986; Palmer and Strobeck 2003; Palmer 1994).

In order to estimate and assess differences in developmental stability (based on FA), we fit a generalized linear mixed model using the FA indices for wing size $FA1$ and $FA8a$ as response variables, temperature, sex, population and their interactions as fixed effects. Random effects of the intercept, sex and temperature were allowed to vary according to line nested within population. A Gamma distribution and an inverse link function for the response were used. The FA component for shape was extracted for each specimen using the *bilat.symmetry* function from *geomorph* to remove directional asymmetry. As a confirmation of this analysis, wing shape FA was calculated as the Procrustes distance between the left and right wing for each individual PD_{RL}. We fit a generalized linear mixed model using PD_{RL} as response variables, temperature, sex, population and their interactions as fixed effects and line as random effect assuming a Gamma distribution and an inverse link function. As a confirmation of the FA analysis, morphological disparity analysis was performed to compare the difference in FA among groups. Each analysis provided largely similar results and only the first two are shown.

2.4 Results

2.4.1 Wing size, shape and wing defects vary between high- and low-altitude populations

We first confirmed differences in trait means across populations. Consistent with previous findings (Lack et al. 2016a; Pitchers et al. 2013; Fabian et al. 2015), the high-altitude population has substantially larger wing size compared to the low-altitude population (Figure 2.1A, Table A-S4). Wing shape also varies in a manner consistent with previous studies (Procrustes distance of 0.013 between populations) (Pitchers et al. 2013) shown in Figure 2.1C and Table A-S5.

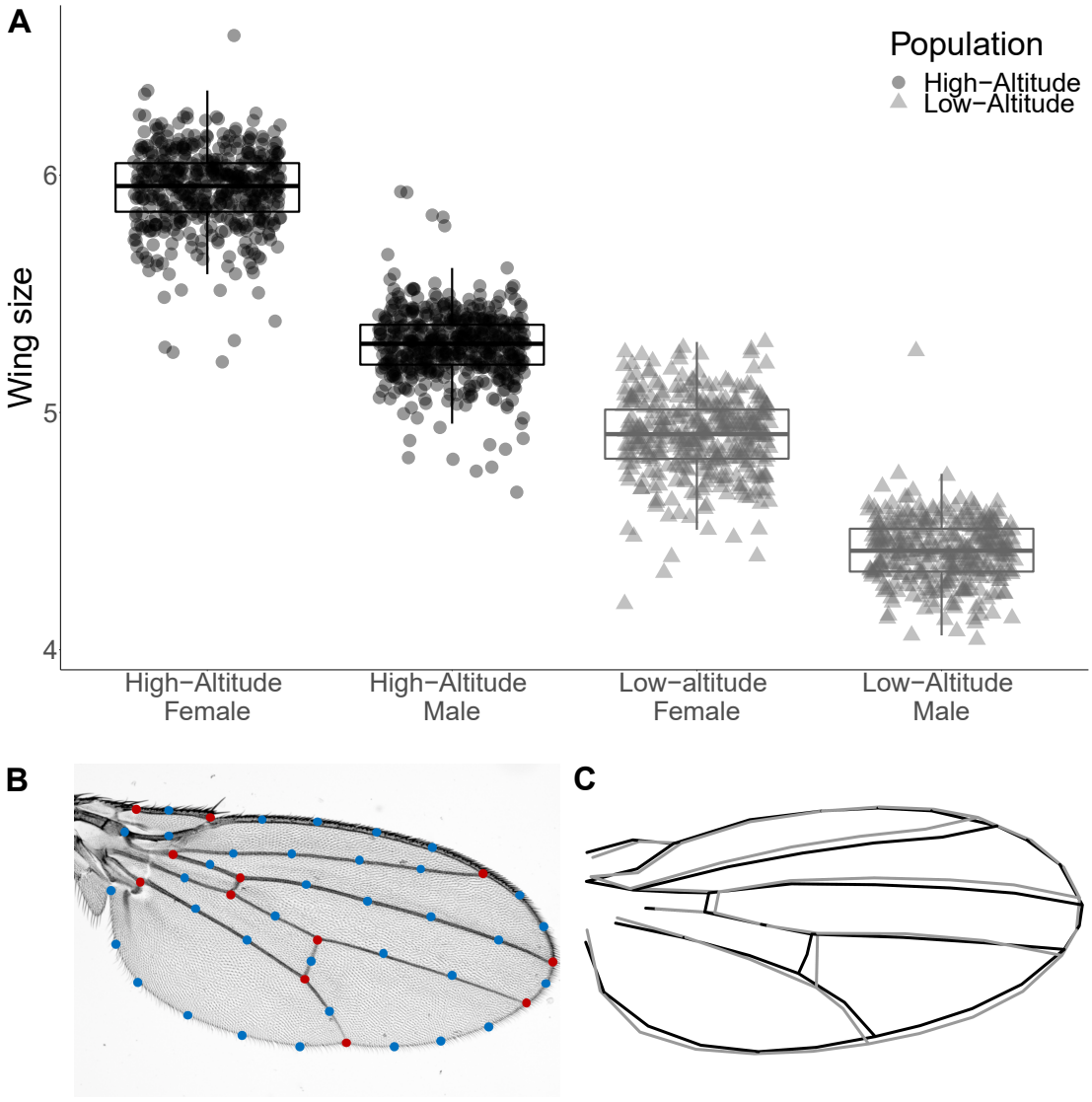

Figure 2.1: Wing size (centroid size) and shape variation within and among high- and low-altitude populations. (A) High-altitude population has larger wings than the low-altitude population (B) Landmarks (red) and semi-landmarks (blue) used in the analysis of wing shape. (C) Mean difference in wing shape between the high- and low-altitude population, scaled 2x. Procrustes distance between mean shapes of the two populations is 0.013

Consistent with Lack et al. (2016), flies from the high-altitude population have a greater proportion of wing defects compared to the low-altitude population (Figure A-S1 , Table A-S8). On average, 26.7% (CI 22.8% - 29.9%) of high-altitude females and

22.0% (CI 18.6% - 25.8%) of males show such defects. In contrast, the average for the low-altitude females is 10.6% (CI 7.53% - 14.6%) and 12.2% (CIs 8.22% - 17.6%) for males.

2.4.2 Micro-environmental variation for wing size is similar between high- and low-altitude populations

While it was previously demonstrated that the high-altitude population is genetically decanalized (Lack et al. 2016a), it is unclear whether this is also associated with any form of environmental decanalization. To enable comparisons with previous studies we used both the coefficient of variation (CV) and Levene's deviates to measure among individual, within-line variation. CV was plotted for ease of interpretation, but all statistical analyses were performed using Levene's deviates (Van Valen 2005; Dworkin 2005b). However, Levene's deviates and CV are highly correlated (high-altitude r = 0.89 CIs 0.81 - 0.94; low-altitude r = 0.98 CIs 0.94 - 0.99) (Figure A-S3 B). As shown in Figure 2.2A, Figure A-S3 A and Tables A-S6 and A-S7 , measures of among individual, within-strain variability are similar between the high-altitude and low-altitude populations.

We examined the relationship between CV and proportion of defects, which showed a weak negative correlation, in the high-altitude population $r = -0.28$ (CIs -0.55 - 0.045), and a correlation close to zero in the low-altitude population $r = -7.45 \times 10^{-3}$ (CIs -0.46 - 0.45) (Figure 2.2B, Figure A-S4 A) although confidence intervals included zero for both populations. Similar results were observed when comparing within-line Levene's Deviates with proportion of defects (Figure A-S4 A).

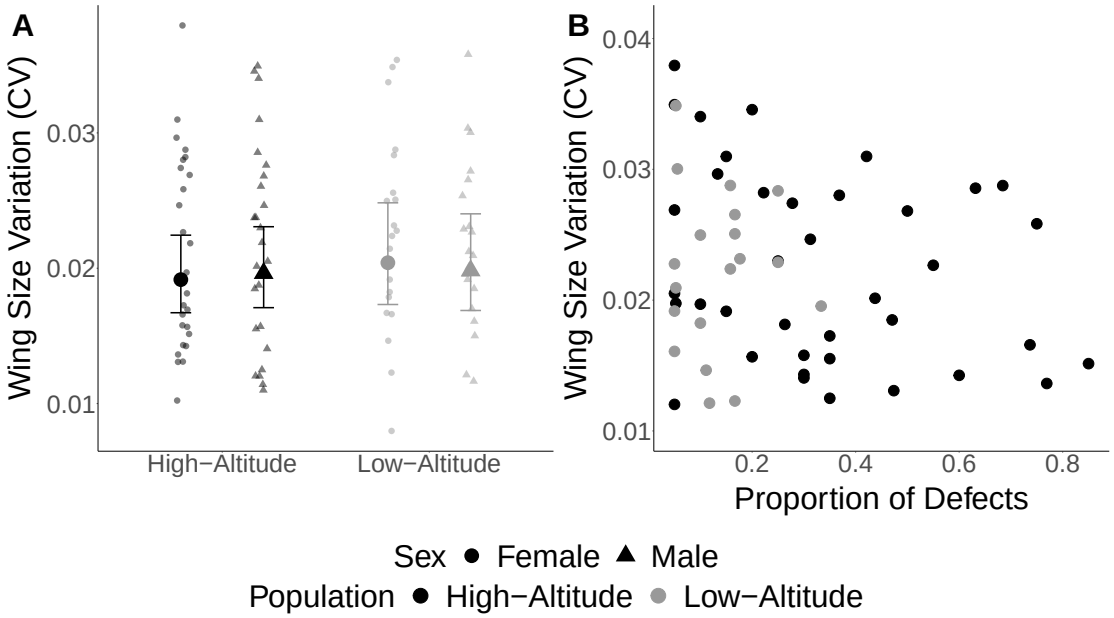

Figure 2.2: Within-line, among individual variation for wing size is similar across high- and low-altitude populations. (A) Within-line coefficient of variation for wing size is similar in high- and low-altitude populations. Large symbols represent fitted values, small symbols represent coefficient of variation by line. Error bars are 95% CI (B) Within-line variation for wing size is not correlated with proportion of defects in both the high-altitude population $r = -0.28$ (CIs -0.55 - 0.045) or the low-altitude population $r = -7.45 \times 10^{-3}$ (CIs -0.46 - 0.45)

2.4.3 Micro-environmental variation for wing shape is similar between high- and low-altitude populations

Strains derived from high- and low-altitude populations have similar levels of wing shape variation measured as the total variance (matrix size). This is also true for measures of integration (Figure 2.3 and Tables A-S9, A-S10, A-S11, A-S12). We observed this using both the relative standard deviation of eigenvalues and eccentricity of the covariance matrices (Figure A-S6). Similar to the patterns for wing shape among populations, there is little evidence that total variance, eccentricity, rSDE and rSDE2 are correlated with frequency of wing defects (Figures A-S2 and A-S5) in either the high-altitude (Total variance: r = 0.16 CI -0.26 - 0.53; eccentricity: r = -0.24 CI -0.59 - 0.18; rSDE:

r = 7.6×10^{-3} 95% CI −0.40 - 0.41; rSDE2: r = −0.29 95% CI −0.62 - 0.12) or low-altitude populations (total variance: r = 0.34 CI −0.24 - 0.74; eccentricity: r = −0.33 CI −0.73 - 0.24; rSDE: r = 0.12 95% CI −0.44 - 0.61; rSDE2: r = −0.17 95% CI −0.65 - 0.39).

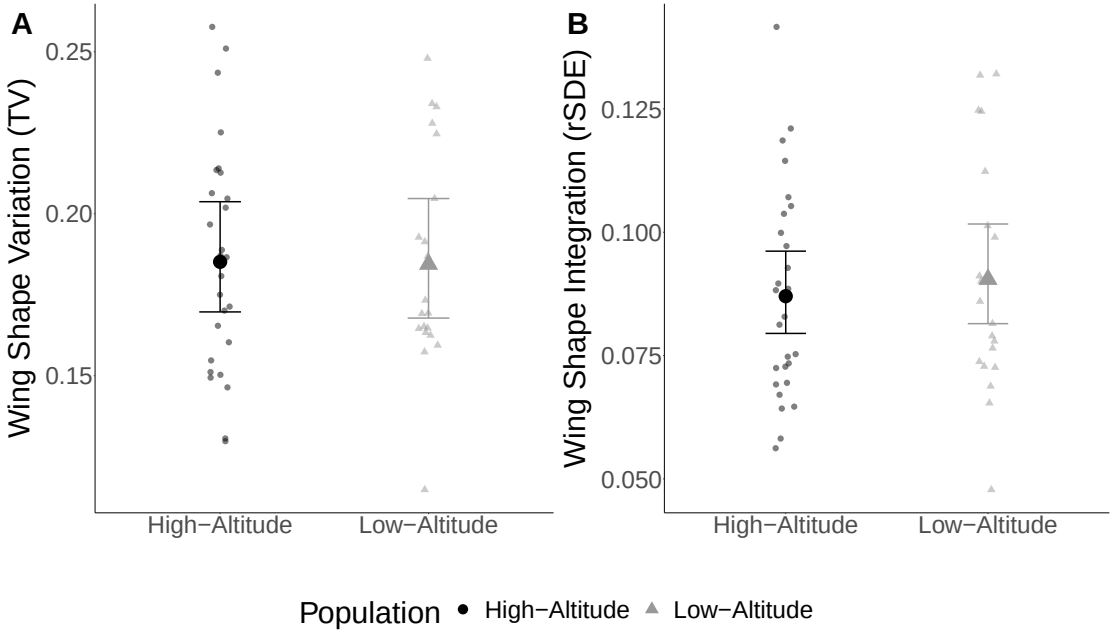

Figure 2.3: Similar patterns of within-line measures of variability for wing shape between high- and low-altitude populations using (A) a measure of wing shape variation, total variance of the VCV matrix (values multiplied by 1000), and (B) measure of wing shape integration using relative standard deviation of the eigenvalues of the VCV matrix (rSDE; values multiplied by 10000). Error bars are 95% CIs

2.4.4 Cell Density varies across the wing, between population and sexes

Consistent with previous work, we observed that average cell density is lower (cell size is greater) in the high-altitude population relative to the low-altitude population (Figure 2.4B) (Fabian et al. 2015; Lack et al. 2016b) as well as for females versus males (Alpatov 1930; Dobzhansky 1929). Most studies count trichomes (cells) in a single small region of the wing. Yet, cell sizes are known to vary in different regions of the wing from

less than 7.9μm to greater than 11.3μm in diameter (Gonzalez-Gaitan et al. 1994). To account for local effects, we measured cell density in 16 regions across the wing (Figure 2.4A, Figure A-S7). While cell density varies considerably across the wing, with some intriguing interactions between sex, region of the wing and population, the overall pattern as observed in previous work (Lack et al. 2016b; Fabian et al. 2015) remains (FigureA-S7 ; TableA-S13).

2.4.5 Variation within and among individuals for cell density is not associated with among-individual variability in size or shape

After we confirmed and expanded upon the previously demonstrated association between cell density and wing size with respect to population and sex, we asked whether variation in cell density, within the wing was directly associated with variation among individuals in wing size and shape. That is, do lines that show the greatest degree of among-individual, within-line variation for wing size and shape also show the greatest variation for cell density within and between individuals? We calculated the within-line CV for cell density across the wing in order to determine if there are any differences in within-line variation for cell density between the high- and low-altitude populations. As shown in Figure 2.4, we did not observe substantial differences in cell density CV between populations but observed an effect of sex that was consistent across both populations (Figure 2.4C; Table A-S14). Similarly, we did not observe differences in within-line cell density Levene's deviates between the high- and low-altitude populations (Figure A-S8 A; Table A-S15).

Additionally, we did not observe a strong association between within-line cell density CV and within-line wing size CV for either the high-altitude population ($r = 0.076$ CIs -0.31 - 0.44) or the low-altitude population ($r = 0.25$ CIs -0.12 - 0.56)(Figure 2.4D). We observed a weak negative correlation between within-line cell density CV and within-line total variance, in the low-altitude population ($r = -0.41$ CIs -0.67 - -0.060;), but

we did not observe any correlation between within-line cell density CV and within-line total variance for the high-altitude population (high-altitude $r = 0.062$ CIs -0.32 - 0.42). Similarly, we did not observe any association between within-line cell density CV and within-line eccentricity in either the high- or the low-altitude population (high-altitude $r = 0.28$ CIs -0.10 - 0.59, low-altitude $r = -0.30$ CIs -0.60 - 0.063) (Figure A-S8 C, D). Further, we did not observe any evidence for associations between within-line cell density CV and proportion of wing defects for both the high- and low-altitude populations(high-altitude $r = -0.043$ CIs -0.44 - 0.37, low-altitude $r = 0.36$ CIs -0.076 - 0.68) (Figure A-S8 B)

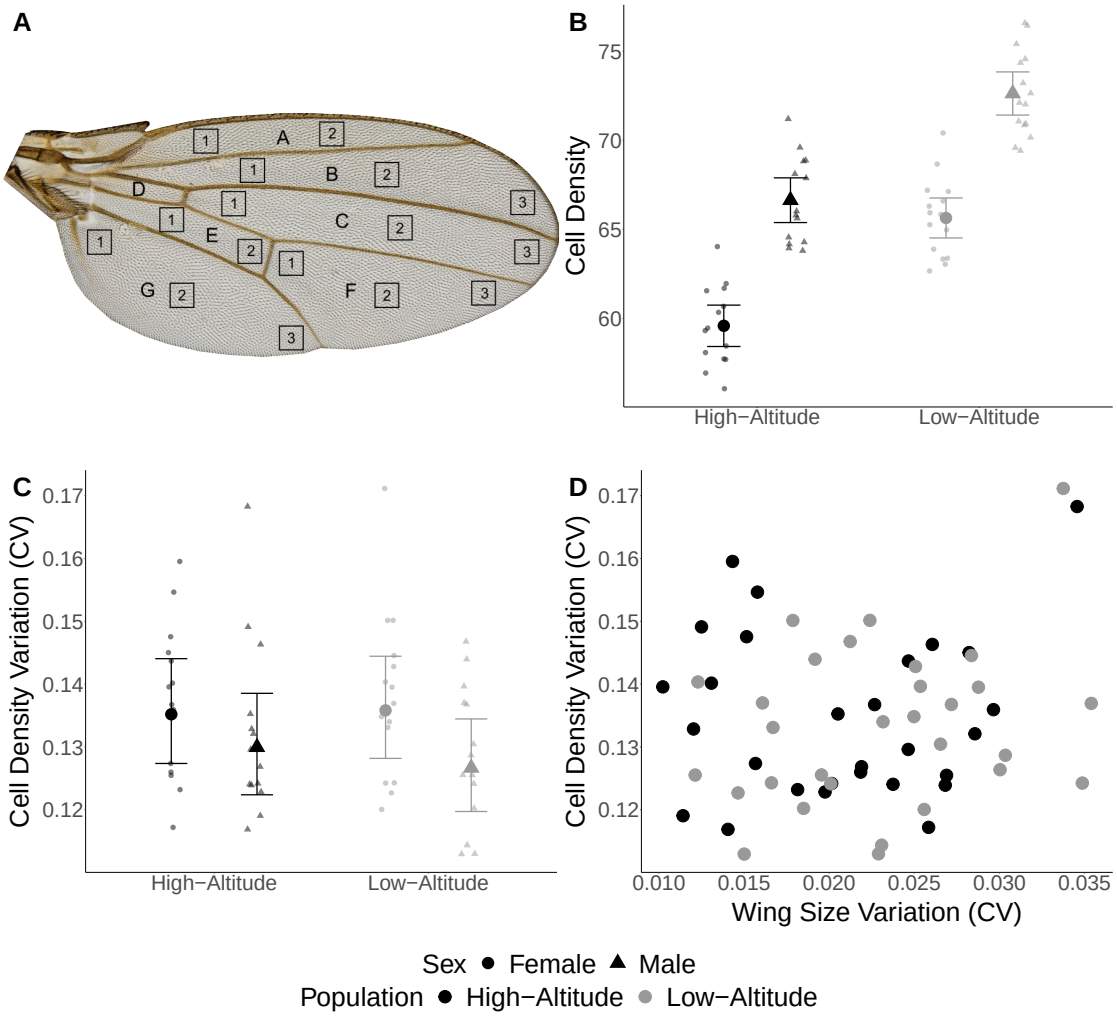

Figure 2.4: Mean cell density show population and sex differences, but no differences in variability. (A) Wing regions used for cell density measurement. Squares represent a $0.0065mm^2$ measurement area. (B) Cell density varies between the sexes and between the high- and low-altitude populations. Error bars are 95% CIs (C) Among-individual, within-line variation measured as CV for cell density is similar between high- and low-altitude populations. (D) Within-line cell density variation and within-line wing size variation (CV) are not strongly correlated in either the high-altitude population ($r = 0.076$ CI -0.31 - 0.44), and the low-altitude population ($r = 0.25$ CI -0.12 - 0.56)

2.4.6 Temperature induced plasticity

To assess whether patterns of phenotypic plasticity varied among populations (macro-environmental canalization) we reared strains derived from both populations at three temperatures. Consistent with previous studies, wing size is larger for flies raised at lower temperature and the reaction norms showed modest evidence of non-linearity (David et al. 1994; Partridge et al. 1994; James et al. 1997). Our data suggest that mean wing size of the high-altitude population may be more plastic compared to the low-altitude population (Figure 2.5A, Table A-S16, Table A-S17). We observed an increase in the Procrustes distance between mean shapes of the high- and low-altitude populations as temperature increases (Figure A-S11).

We quantified the proportion of wing defects for the high- and low-altitude populations at the three different rearing temperatures. Consistent with our previous results, the high-altitude population has a greater proportion of defects than the low-altitude population, however, we did not observe substantial differences in proportion of defects due to temperature (Figure A-S9 A, Table A-S24).

In general, we did not observe any differences in the high- and low-altitude populations for among individual, within-line measures of variability at each temperature treatment. For the within-line CV for wing size, we observed an increase at both 18°C and 28°C for high-altitude females and an increase in CV at 18°C but not at 28°C for high-altitude males. Within-line CV for low-altitude males and females is consistent across temperatures (Figure 2.5B; Table A-S18). We observed a similar pattern when using Levene's deviates as we did for CV (Figure A-S9 B; Table A-S18), with a modest effect of rearing temperature but little evidence for population level differences. For within-line wing shape total variance, we observe a consistent increase with temperature for both high- and low-altitude populations (Figure 2.5C; Table A-S20). Degree of integration of wing shape is similar across populations and temperatures (Figure 2.5D; Table

A-S21, A-S22, A-S23). This pattern holds whether examining rSDE or eccentricity of the covariance matrix (Figure A-S9 C, D).

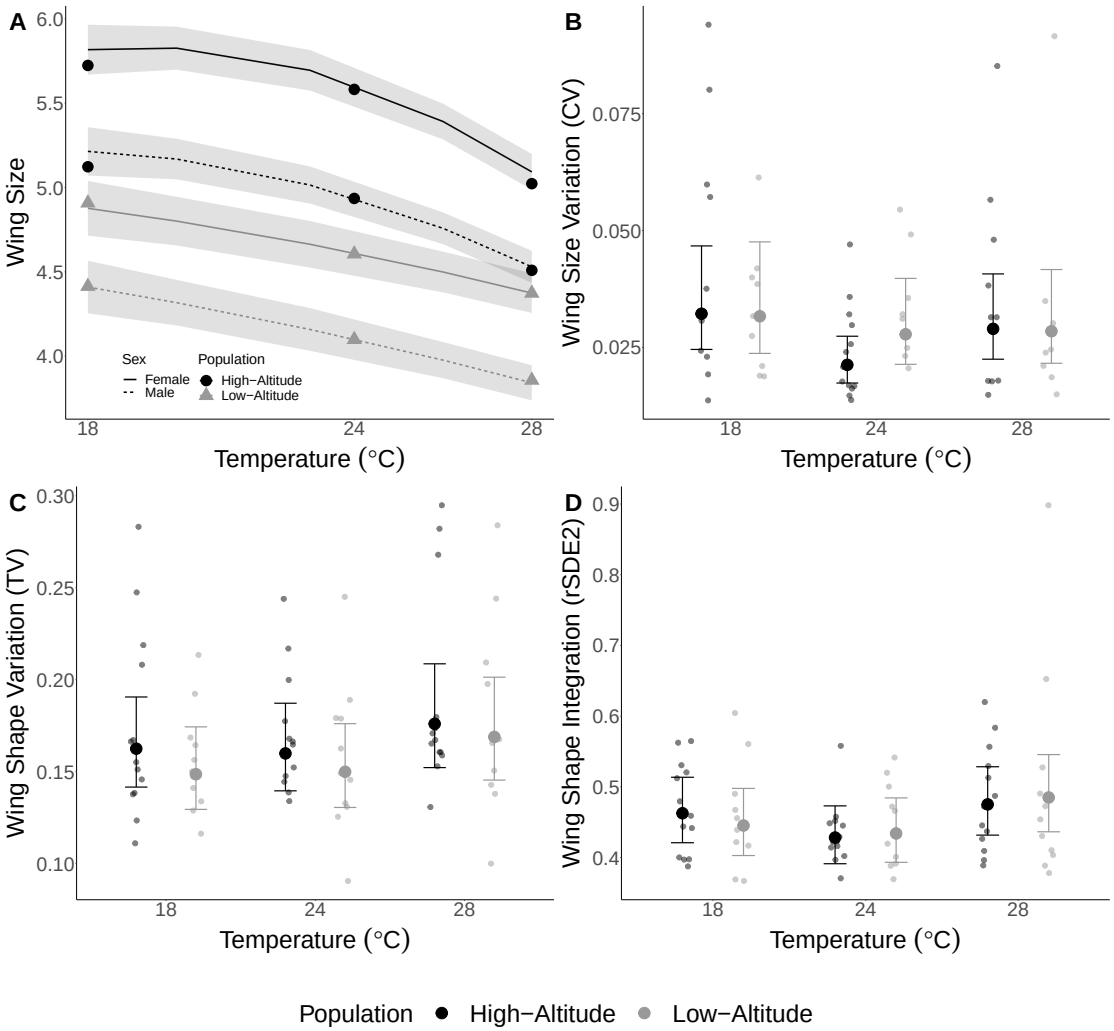

FIGURE 2.5: Considerable plasticity for mean wing size under different temperature rearing environments, but with minimal differences in trait variation (A) Mean differences observed in wing size (centroid size) at different temperatures in males and females in the high- and low-altitude populations (B) Within-line variation for wing size is similar across sexes and populations within each temperature treatment. (C) Within-line variation for wing shape measured as the total variance of the VCV matrix (multiplied by a factor of 1000) and (D) Within-line wing shape integration measured as rSDE2 (relative standard deviation of the eigenvalues scaled by the total variance) are similar between the high- and low-altitude populations across the different rearing temperatures. Gray shading and error bars are 95% CIs

2.4.7 Fluctuating asymmetry for wing size and shape is similar between high- and low-altitude populations

While among-individual, within-genotype variation and within-individual (among-sides) variation might be expected to capture similar aspects of developmental stability, empirical work shows that these measures do not always agree, with correlations ranging from $\sim 0.07 - 0.6$ for wing size and $\sim 0.35 - 0.48$ for shape (Debat et al. 2006; Debat et al. 2009; Breuker et al. 2006). Thus we measured asymmetry for both wing size and shape in a subset high- and low-altitude lines (2 strains for each population). We first estimated measurement error for wing size and shape and determined that measurement error was negligible with respect to FA for size (Table A-S29) although had a larger impact on shape (Table A-S30). Using FA8 as an index for wing size FA, we compared developmental instability for high- and low-altitude populations at three temperatures (18°C, 24°C and 28°C). We did not see clear evidence of differences in FA8 between high- and low-altitude populations or across the different rearing temperatures, except for consistently lower FA8 in the high-altitude males at all temperatures (Figure 2.6A; Table A-S26). For comparison we also report FA1 as a measure of developmental instability (Figure A-S12 ; Table A-S25), but importantly FA1 does not account for mean trait size and should be interpreted with caution.

We measured FA for wing shape in two different ways. First, we calculated FA by removing directional asymmetry (DA) and then calculating the Procrustes distance (PD) for the FA component for each individual. The high-altitude females had a slight, but consistent increase in FA across all three temperatures while the other groups had similar FA to each other and across the different temperatures (Figure 2.6B Table A-S28). We also calculated Procrustes distance between left and right wings (PD_{LR}). We observed a similar pattern as we did using the first method to calculate wing shape FA, where there is consistently greater PD_{LR} in the high-altitude females across temperatures (Figure A-S12 B; Table A-S27).

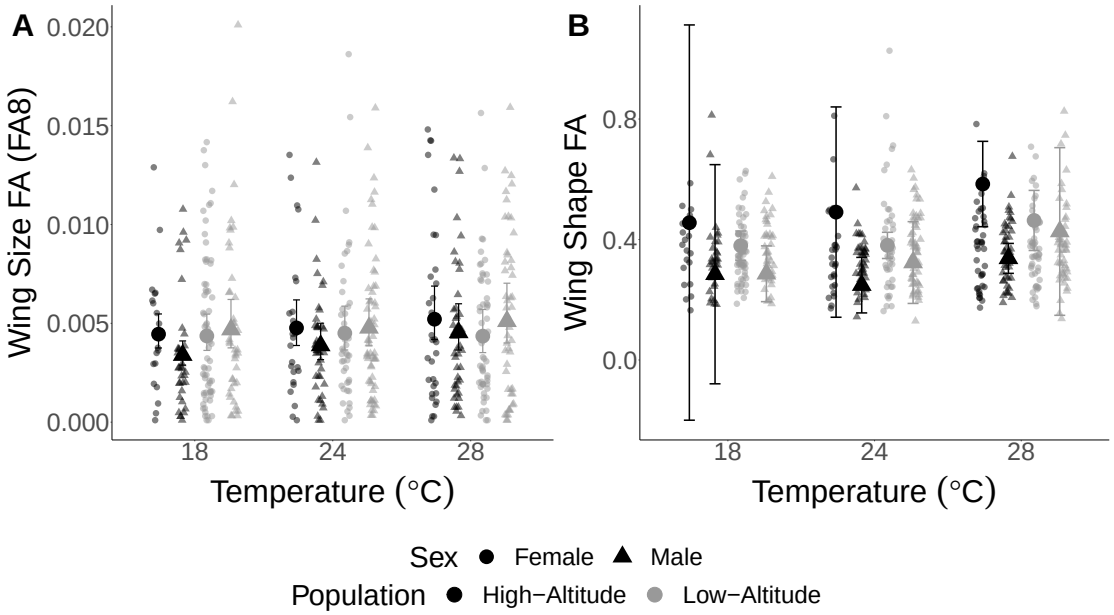

Figure 2.6: Inconsistent differences in fluctuating asymmetry among the high- and low-altitude populations for (A) wing size fluctuating asymmetry measured as $FA8$ and (B) wing shape fluctuating asymmetry between the high- and low-altitude populations across temperatures. High-altitude males have consistently lower fluctuating asymmetry and high-altitude females have consistently greater wing shape fluctuating asymmetry across temperatures. Large symbols represent population means, small symbols represent individuals, error bars represent 95% CIs

2.5 Discussion

Since the proposal of the congruence hypothesis, researchers have tested for associations between genetic and environmental canalization. To date, empirical evidence has been equivocal. The majority of studies that support the congruence hypothesis were conducted in RNA viruses and microRNAs *in vivo* and *in silico*, which may not necessarily representative of multi-cellular organisms (Szollhosi and Derenyi 2009). Studies in other systems have not provided evidence to support the congruence hypothesis (Dworkin 2005a; Dworkin 2005c). However, most empirical studies were conducted using lab domesticated lineages, laboratory-induced mutations, and with arbitrary measures of

genetic canalization which may not be representative of naturally occurring phenomena that would lead to congruent evolution of genetic and environmental canalization.

In this study, we address these issues using a naturally occurring system across adaptively diverged populations. The high-altitude population was previously demonstrated to have reduced mutational robustness (via mutagenesis), and these effects were pleiotropically linked to variants influencing changes in wing and body size that appear to be targets of selection (Lack et al. 2016a). Using strains derived from both high- and low-altitude populations, we examined multiple measures of environmental canalization. Despite recapitulating previously observed divergence in wing size & shape (Pitchers et al. 2013; Lack et al. 2016a; Lack et al. 2016b) (Figures 2.1, 2.5A, A-S11), cell size (Figure 2.4, Figure A-S7) and frequencies of mutational defects (Figure 2.2B, Figure A-S1), we did not observe any evidence for associations between genetic and micro-environmental canalization (Figures 2.2, 2.3, 2.4C-D, 2.5B-D). Additionally, measures of among individual, within-line variance for wing size and shape were not correlated with the proportion of mutational defects both in the high- and low-altitude populations (Figures 2.2B, 2.4D, Figures A-S2 , A-S4 , A-S8 B, A-S10). We did observe greater temperature induced plasticity of mean wing size in the high-altitude population (Figure 2.5A). We observed a subtle increase in within-line variation for wing size at 18°C and 28°C compared to 24°C in both the high- and low-altitude population for wing size, and this increase was greater in the high-altitude population (Figure 2.5B, Figure A-S9 B), although there is at best, marginal evidence for a significant treatment effect of population or its interaction with rearing temperature (Table A-S16). Intriguingly, we observed a decrease in developmental stability for wing size (measured using fluctuating asymmetry) for high-altitude females across all temperatures (Figure 2.6). Yet, we did not observe this same pattern in the males derived from the same population, nor did we see any increase in qualitative wing defects at varying temperatures and the measures of among-individual, within-line of variation were not correlated with the proportion of

defects at any of the developmental temperatures (A-S10). Therefore, our results are largely inconsistent with congruent evolution of genetic and environmental canalization and that with respect to adaptation to life at high altitudes driving changes in both trait means and variances, they are likely to evolve via separate underlying mechanisms.

Our study is one of the few to test of the congruence hypothesis using strains derived from natural populations with known evolutionary histories. However, we are aware of several important caveats. Our study compares a single high-altitude population to a single low-altitude population. Replication of our experiments with additional populations from independent altitudinal clines would provide stronger support of our findings. The strains used in this study were collected approximately 2 years prior to the first experiment and seven years prior to the temperature manipulation experiment. As such both drift (due to the initial inbreeding process) and some degree of lab adaptation may have occurred. As we used multiple strains from each population, the impact of drift with respect to allele frequencies should be modest. Additionally lab domestication should be weak as N_e is extremely small within each strain. Although the two populations have modest genetic differentiation ($F_{ST} = 0.15$), lines derived from African populations tend to have have high residual heterozygosity even after 8 generations of inbreeding (Lack et al. 2016a). However, given that our results for mean size and shape of the wings and cell densities recapitulate previous findings, the impact of both drift and lab domestication appear to be minor.

While the variants contributing to divergence in size both between the high- and low-altitude population (Lack et al. 2016a) and under artificial selection derived from the low-altitude population (Groth et al. 2018) appear to be pleiotropically linked to the mechanism influencing sensitivity to mutational perturbation, these are not in fact the same traits. The penetrance of wing abnormalities among lines derived from high altitude, and the increased sensitivity under mutagenesis may reflect one aspect of genetic

canalization (that is linked to variants influencing mean size and shape), but they do not necessarily influence variance for these traits. Indeed, under high temperature stress (31°C) one of three replicates of lineages artificially selected for increased size (from a low-altitude ancestral population) showed a substantial increase in penetrance of wing abnormalities (Groth et al. 2018). Interestingly we observed no increase in such abnormalities at our high temperature rearing (28°C) for the high-altitude strains. Whether this reflects insufficient stress or a difference in response is unclear. However, it is clear that the degree of genetic correlation between trait means and variances for wing size, shape and penetrance of abnormalities is complex.

While the work of Lack et al. (2016), Lack et al. (2016) and Groth et al. (2018) clearly demonstrate evolutionary changes in genetic canalization associated with adaptive trait evolution (body size and wing morphology), the most likely evolutionary scenario is one of the loss of genetic canalization associated with the pleiotropic effects of variants that have contributed to the evolution of mean size at high altitudes. This is reminiscent of the evolution of insecticide resistance in blowflies which showed a pleiotropic increase in fluctuating asymmetry due to the resistance locus or in sticklebacks with the effects of the *Eda* locus on both the expression of armour plates, and on fluctuating asymmetry of plates (McKenzie and Clarke 1988; Morris et al. 2019). Consistent with Waddington's model for the evolution of canalization, it could be that modifiers that increase the mutational robustness of wing morphology in *Drosophila* have not yet risen to appreciable frequency in the high-altitude population, as has occurred with the modifier variants influencing asymmetry in the blowflies (Davies et al. 1996). Indeed it is not yet clear whether wing size is near its optima for the high-altitude population, and whether that is necessary for the evolution of canalization.

Based on what is known about the genetic architecture of body size, wing size and wing shape the fact that genetic de-canalization occurred is surprising. The mutational

target size of body size, wing size and wing shape are quite large (Weber et al. 2005; Houle and Fierst 2012; Carreira et al. 2008; Carreira et al. 2011). Similarly these traits harbour extensive standing genetic variation in populations and are polygenic in nature (Weber 1990b; Weber 1990a; Weber et al. 1999; Mezey et al. 2005; Mezey and Houle 2005b). As such the expected modest changes in individual allele frequencies would seem to be unlikely to result in changes in canalization. Yet, that is exactly what has been observed in the high-altitude population which increased its wing (and body) size, and also from the low-altitude population artificially selected for large wing size (Lack et al. 2016a; Groth et al. 2018). Body and wing size has been a frequent subject of study in *Drosophila*, but until now this pattern has not been previously observed. As such future work both examining additional populations that vary for size and also on on identifying variants influencing the adaptive divergence in wing size and morphology and how they shape mutational robustness are necessary.

Where does this leave the congruence scenario? While the results from the current study, and some previous studies (Dworkin 2005a; Dworkin 2005c; Borenstein and Ruppin 2006) are not consistent with the congruence hypothesis, it is perhaps best to consider under what conditions the direct evolution of genetic canalization or its evolution as a correlated response are probable. Under this model, if deleterious alleles are purged efficiently enough, adaptive genetic canalization will not have the opportunity to evolve. However, this does not consider that deleterious alleles are not always purged efficiently for numerous reasons (pleiotropy, linkage with beneficial mutations, drift, GxE, fluctuating selection etc). Indeed, with the system we investigated in this study, the reduction in mutational robustness appears to be a direct pleiotropic consequence of the allelic effects on organismal size (Lack et al. 2016a; Groth et al. 2018). In such instances, genetic canalization may evolve to suppress deleterious mutational effects (ie. alter patterns of pleiotropy). As has been demonstrated previously, the likelihood of evolving either genetic or environmental canalization is in part a function of the fitness load imposed by

the frequency and magnitude of environmental and genetic perturbations (Proulx and Phillips 2005; Hermisson et al. 2003). However, our knowledge of the distribution of this fitness load and the frequency of relevant environmental perturbations is limited. While studies of mutational load give us some idea of the distribution of fitness effects of new mutations, this is less clear in natural environments. Indeed, it has been argued that new allelic combinations produced due to the normal processes of mating and recombination may act as a "genetic perturbation" to the input of new mutations (Stearns et al. 1995), as genetic backgrounds are constantly shuffled. Alternatively, it may be that genetic canalization may be beneficial when it occurs, but rarely the result of persistent and direct selection (Wagner et al. 1997; Gibson and Wagner 2000; Visser et al. 2003; Siegal and Bergman 2002; Proulx and Phillips 2005). Experimental evolution and artificial selection may continue to be the strongest framework to test the theory and understand under what conditions, both genetic and environmental canalization, are direct targets of selection. This approach should be coupled with studies of adaptively diverged natural populations that are likely to share the appropriate evolutionary history to address these questions (i.e. Morris et al. (2019), McKenzie and Clarke (1988)). Finally, this work suggests that trying to clearly delineate between selection for 'environmental' and 'genetic' canalization may be difficult given the interplay between genotypic and environmental effects in terms of trait expression and variation.

Chapter 3

The influence of adaptation to life at high altitude on condition dependent sexual shape and size dimorphism in *Drosophila melanogaster*

3.1 Abstract

Sexual dimorphism is common despite high inter-sex genetic correlations and intralocus sexual conflict that might limit its evolution. Sexual dimorphism can be phenotypically plastic and condition dependent. The degree of plasticity and condition dependence of sexual dimorphism may be a target of selection. It remains unclear how sexual dimorphism and its condition dependence evolve under circumstances of rapid adaptation to a new environment. Using sub-Saharan populations of *Drosophila melanogaster* that

vary for size and shape as a result of adaptation to high-altitude environments, we examined sex specific patterns of developmental plasticity. We raised strains of *Drosophila* from low (Zambia) and high (Ethiopia) altitude populations at different food quality or rearing temperature during development. We observed expected differences in wing size and shape due to population, sex and plasticity. While larval mass showed substantial evolved changes for sex-specific condition dependence, effects of diet and temperature on sexual size and shape dimorphism for wing size and shape were similar in the two populations. We examined shape-size allometric effects for the sexes, populations, diet and temperature. Allometric effects were generally similar across sexes, but differed substantially due to population of origin and plasticity. We discuss findings within the context of the evolution of plasticity for sexual dimorphism, condition dependence and allometric relationships.

3.2 Introduction

Sexual dimorphism is prevalent among animals and can sometimes lead to the evolution of extreme phenotypes. While many studies examine species with male-biased sexual size dimorphism, particularly with conspicuous exaggerated ornaments or weapons, female-biased sexual size dimorphism (SSD) is more common, and with some exceptions (e.g. angler fish), differences between the sexes are often subtle (Fairbairn 2007a). Sexual selection is frequently invoked to explain male-biased SSD, while fecundity selection seems to be a common explanation for female-biased SSD (Darwin 1874; Shine 1988; Pincheira-Donoso and Hunt 2017) along with other ecological mechanisms (Darwin 1874; Selander 1966; Shine 1989; Hedrick and Temeles 1989). The study of the evolutionary origins and maintenance of sexual dimorphism remains an active area of interest for at least two reasons. First, despite potential antagonistic effects due to intra-locus sexual conflict and constraints due to high inter-sex genetic correlations (r_{MF}) (Poissant et al. 2010), sexual dimorphism occurs widely and may represent the resolution of conflict

(Lande 1980; Bonduriansky and Chenoweth 2009; Cox and Calsbeek 2009). Despite high r_{MF}, sexual dimorphism evolves rapidly in some contexts (Reeve and Fairbairn 1999; Bird and Schaffer 1972; Eisen and Hanrahan 1972; Alicchio and Palenzona 1971) though not all (Stewart and Rice 2018; Tigreros and Lewis 2011; Reeve and Fairbairn 1996). Second, some sexually dimorphic traits, for example, water strider legs, are multi-functional, reflecting the balance between sex-specific and shared functions that are targets of selection, potentially influencing multiple fitness components (Lande 1980; Lasne et al. 2018; Matthews et al. 2019b; Connallon 2010; Connallon and Hall 2016; Connallon et al. 2018).

It is important to consider how adaptive evolution of such multi-functional traits influences sexual dimorphism (Cox and Calsbeek 2009) and how sexual dimorphism and high r_{fm} in turn influence trait evolution towards new adaptive optima (Lande 1980; Connallon 2015). Adaptation to a new environment may be influenced by sexually antagonistic selection and r_{fm} (Lande 1980; Cox and Calsbeek 2009). When selection is consistent in the sexes in the new environment, a generally high r_{fm} may facilitate the adaptive process, though the process may be limited in the presence of sexually antagonistic selection (Connallon 2015; Connallon and Hall 2016). If selection is discordant between the sexes (Cox and Calsbeek 2009), with respect to the new environment, this can constrain adaptive responses. For instance, the gender load imposed by sex specific effects on rate of adaptation has been estimated to be on the order of 50% in a wild population of *Parus major* (Poissant et al. 2016). However, the degree to which selection is generally discordant is unclear (Singh and Punzalan 2018), and issues such as changes in r_{fm} across environments (Punzalan et al. 2014) and imprecision in estimates of selection (Morrissey 2016) makes studying the interaction between sex-specific and adaptive evolutionary forces a challenging empirical and theoretical undertaking.

Studies of sexual dimorphism for morphology often focus on SSD, examining a single

trait at a time. However, a multivariate perspective in general, and sexual shape dimorphism (SShD) in particular, is increasingly recognized as both pervasive and important (Gidaszewski et al. 2009; Abbott et al. 2010; Sztepanacz and Houle 2019; Sztepanacz and Houle 2021). Multivariate (Butler and Losos 2002) and geometric morphometric approaches not only have increased sensitivity to detect subtle changes, but SShD itself appears to be a target of selection in some instances (Gidaszewski et al. 2009; Abbott et al. 2010; Menezes et al. 2013). Sex-specific patterns of selection in multivariate traits and the influence of the inter-sex covariance matrix **B** can result in complex patterns of response to selection (Wyman et al. 2013; Sztepanacz and Houle 2019; Gosden et al. 2012; Poissant et al. 2016). Despite this, sexual shape dimorphism is clearly evolving in numerous systems (Houle et al. 2017b; Gidaszewski et al. 2009; Sztepanacz and Houle 2021; Sanger et al. 2013; Evans et al. 2019; Chazot et al. 2016). Additionally, allometric relationships between shape and size can contribute substantially to SShD (Butler and Losos 2002; Kaliontzopoulou et al. 2008b; Gidaszewski et al. 2009; Sztepanacz and Houle 2021), making it difficult to disentangle direct and indirect effects of sex-specific selection on shape. When both shape-size allometry and SSD occur, both of these will contribute towards SShD. SShD can also be generated by sex differences in covariance patterns for the structures contributing to shape. Partitioning the relative contributions of allometric and non-allometric factors and the degree to which they are evolving independently remains an important and ongoing research area into the evolution of SShD (Fernandez-Montraveta and Marugan-Lobon 2017; Kaliontzopoulou et al. 2008b; Butler and Losos 2002).

Phenotypic plasticity size and shape of traits further complicates the study of SSD and SShD (David et al. 1994; Bitner–Mathe and Klaczko 1999; Imasheva et al. 1999; Karan et al. 2000; Bubliy et al. 2001; Bubliy and Loeschcke 2002; Debat et al. 2003; Debat et al. 2009; Shingleton et al. 2009). Patterns of plasticity are often dependent on the particular traits under consideration and the environmental variables generating

the plastic response (Shingleton et al. 2009). Plasticity for size may in turn influence patterns of trait covariation and allometry with shape. Nutritional plasticity is a particularly interesting case with regards to sexual dimorphism, as it is directly linked to organismal condition (Andersson 1986; Iwasa et al. 1991; Rowe and Houle 1996; Cotton et al. 2004b; Bonduriansky 2007c; Bonduriansky 2007b). Condition reflects the contribution of both environmental factors via access to resources and genetic factors via efficiency of resource utilization (Cotton et al. 2004b; Hill 2011). The most common type of experimental manipulation of condition used by researchers is to vary access to resources (Cotton et al. 2004b). While there are several hypotheses regarding the evolution of sex-specific plasticity (adaptive canalization and condition dependence chief amongst them), considerable evidence has demonstrated that the most sexually dimorphic traits tend to be the most condition dependent (Andersson 1986; Rowe and Houle 1996; Bonduriansky 2007c; Bonduriansky 2007b), a pattern that also holds for sex-biased gene expression (Wyman et al. 2010; Zinna et al. 2018). The majority of evidence in the literature supports the correlation between sexual dimiprhism and its condition dependence in male-biased SSD systems with highly exaggerated secondary sexual traits (Bonduriansky and Rowe 2005b; Bonduriansky 2007c; Bonduriansky 2009; Cotton et al. 2004a; Cotton et al. 2004b; Rohner and Blanckenhorn 2018; Rohner et al. 2018c; Stillwell et al. 2007). However, there is considerably less theoretical and empirical work that explores the interplay between sexual dimorphism and condition in systems with moderate female biased SSD, the most common type of sexual dimorphism in animals (Rohner and Blanckenhorn 2018; Oudin et al. 2015). With some notable exceptions (Ceballos and Valenzuela 2011), we still know relatively little about sex-specific plasticity for shape and SShD.

With many factors influencing trait variation, it is important to consider how strong directional selection on a trait resulting in local adaptation may result in correlated effects on sex-specific trait plasticity generally, and more specifically condition dependence

. The evolution of *Drosophila* wing morphology is an excellent system to explore these questions. While there is extensive genetic and mutational variation for wing size and shape within and between populations (Mezey and Houle 2005a; Pitchers et al. 2013), they generally have very high r_{fm} (Sztepanacz and Houle 2019). Comparative studies suggest that *Drosophila* wing shape has evolved relatively slowly (Gidaszewski et al. 2009; Houle et al. 2017b; Sztepanacz and Houle 2021), despite substantial variation for SSD (Rohner et al. 2018b; Huey et al. 2006; Blanckenhorn et al. 2007b; Gidaszewski et al. 2009) and SShD (Gidaszewski et al. 2009). Allometric effects may account for close to 50% of the variation in SShD among species (Gidaszewski et al. 2009; Sztepanacz and Houle 2021), although the consequences of sex-specific allometry have not been investigated thoroughly. In this study, we examine developmental plasticity in wing morphology in response to temperature and food quality in order to specifically assess condition dependence in a pair of populations of *Drosophila melanogaster* originating from different altitudes. Populations of *Drosophila melanogaster*, diverge considerably along altitudinal and latitudinal clines in both its native and more recently colonized contients (clinal variation in Americas, Australia) (Gibert et al. 2004; James et al. 1997; David and Bocquet 1975; Gilchrist et al. 2000; Hoffmann and Weeks 2007; Azevedo et al. 1998). Wing morphology shows considerable plasticity to nutrition, rearing temperature and oxygen partial pressure (David et al. 1994; Bitner–Mathe and Klaczko 1999; Imasheva et al. 1999; Karan et al. 2000; Bubliy et al. 2001; Bubliy and Loeschcke 2002; Debat et al. 2003; Debat et al. 2009; Shingleton et al. 2009; Peck and Maddrell 2005). Previous work has demonstrated that some of the plastic response is aligned with clinal variation for a number of traits, including wing size and shape (Gilchrist et al. 2000; Pitchers et al. 2013; Klepsatel et al. 2014; Fabian et al. 2015). In addition to the wing's role in flight performance (Ray et al. 2016), various aspects of wing morphology including size (Ewing 1964; Abbott et al. 2010), shape (Menezes et al. 2013; Abbott et al. 2010), wing interference pattern (Katayama et al. 2014) and even wing musculature

(Tracy et al. 2020) appear to be direct or indirect targets of sexual selection.

Drosophila melanogaster likely originates from the Miombo and Mopane forests in Zambia and Zimbabwe (Pool et al. 2012b; Sprengelmeyer et al. 2020; Mansourian et al. 2018) and expanded its ancestral range in the past 13000 years, colonizing highland environments in Ethiopia between 2340 - 3060 years ago (Sprengelmeyer et al. 2020). In this study, we examine two populations from these altitudinally varying ranges. The low altitude (LA) population comes from Zambia and is assumed to be representative of ancestral *Drosophila* variation. The high altitude (HA) population comes from the highlands of Ethiopia. As is common with small insects adapting to life at high altitudes (reviewed in Hodkinson, 2005), a number of morphological, physiological and life history traits have evolved in the HA population (Pitchers et al. 2013; Pool et al. 2012b; Lack et al. 2016a; Lack et al. 2016b; Klepsatel et al. 2014; Fabian et al. 2015; Klepsatel et al. 2013). In particular, body size, wing size and, in particular, wing loading (body mass/wing area) have increased ∼20% relative to the LA counterparts (Pitchers et al. 2013; Pool et al. 2012b; Lack et al. 2016a; Lack et al. 2016b; Klepsatel et al. 2014; Fabian et al. 2015; Klepsatel et al. 2013; Pesevski and Dworkin 2020). There is evidence of concordance between the direction of temperature-induced plasticity and wing shape between lowland and highland populations (Pitchers et al. 2013). In this study, our goals were to (1) investigate how selection on wing form during adaptation to life at high altitude impacted plasticity (and more specifically, condition dependence) for sexual size and shape dimorphism, and (2) determine the relative contribution of shape-size allometries on the observed shape changes. We discuss our results within the context of the evolution of sexual shape dimorphism and the influence of allometry.

3.3 Materials and Methods

3.3.1 *Drosophila* husbandry

Drosophila melanogaster strains used in these experiments are a subset previously used in Lack et al. (2016), Lack et al. (2016) and Pesevski and Dworkin (2020). Strains from the high altitude (HA) population were collected in Fiche, Ethiopia (3070m asl) in December 2011, while strains from the low altitude (LA) population were collected in Siavonga, Zambia (530m asl) (Supplementary Tables B-S1 and B-S2). There is no evidence of recent gene flow between these populations (Lack et al. 2016a; Lack et al. 2016b). Prior to experimental treatments described in the following section, flies were raised on high protein food (HP; 1.5:1 protein to carbohydrate ratio) for two generations at room temperature to minimize maternal effects. Given that the strains derived from each population were maintained in the lab for a relatively long time, we checked for lab adaptation effects by comparing the results from this study with results from our previous study and found that the phenotypes were consistent Pesevski and Dworkin (2020).

3.3.2 Experimental Design

Egg collections and experimental treatments

Adults were placed in egg laying chambers with apple juice agar plates with killed yeast patches (to avoid yeast growth and control for amount of food). After 12h egg laying windows, eggs were collected and placed in vials with high protein (HP) food, 50 eggs per vial (food is 2:1 carbohydrate to protein ratio). We performed two different treatments (1) varying food quality and (2) varying rearing temperature. Based on previous unpublished experiments in the lab, food treatments were done by raising flies on high quality (100% HP food), low quality (dilution to 15% of HP food) and very low quality food (dilution to 5% of HP food). While the viability for the 15% food was

∼15%, for the 5% treatment it was very low (∼1%). As such, we decided to compare the 100% and 15% food treatments only. Flies under varying food treatments were reared at 24°C. Flies for temperature treatments were raised only on high quality food (100%), in incubators at 18°C, 24°C and 28°C with a 12:12 light/dark cycles. Some results of the temperature manipulation were included in a previous study (citation masked so as not to identify authors), but were done concurrently with the food manipulation study. For all temperature and 100% food treatments, 3 replicate vials of 50 eggs each were collected for each strain. For the 15% food treatment, 6 replicate vials were collected for each strain. Adults were collected after cuticle scleratization and stored in 70% ethanol. This experiment was repeated twice (in January 2017 and June 2017), once with all of the lines and a second time with a subset of the lines to increase sample sizes for strains with low viability, and additional lines to assess block effects. The two replicate experiments are considered as two blocks in the analysis. Sample sizes for each strain and population are outlined in Supplementary Tables B-S1 and B-S2. We measured larval mass at the 3rd instar wandering stage to confirm that the nutritional manipulation reduced size and condition by repeating the egg laying experiment with a subset of strains. We added 25 eggs onto small petri dishes (30mm x 15mm) filled with either 100% or 15% food. We let the flies develop to the 3rd instar larval stage, determined their sex, washed, dried and weighed them, pooling 5 individuals of the same sex and nutritional manipulation together for each independent biological sample.

Dissections and Imaging

Adult flies were collected from each treatment/strain/replicate (samples sizes B-S1 and B-S2). The right wing of each fly was mounted on glass slides in 70% glycerol in PBS solution. Each wing was imaged using an Olympus DP30B camera mounted on an Olympus BX51 microscope (Olympus software version 3,1,1208) using a 4X objective (total of 40X magnification) and images were taken using cellSens Standard (version

1.14) software at 4060 x 3072 resolution.

Phenotyping

Wing size and shape were phenotyped using a modified version of the *"WINGMACHINE"* pipeline (Houle et al. 2003; Pitchers et al. 2019b). Initial landmarks were placed at the humeral break on the leading edge of the wing and the alula notch on the trailing edge of the wing using *tpsDig2* software (version 2.16). *"Wings"* software (version 3.7) was used to fit B-splines to wing veins and wing margins and the splines were manually adjusted when necessary. The x and y coordinates of 12 landmarks and 36 semi-landmarks were extracted after Procrustes superimposition (removing position, orientation and scale) as well as the centroid size of each wing using *CPReader* software (version 1.12r). Approximate positions of landmarks and semi-landmarks are represented in Figure 3.2A.

3.3.3 Analysis

Analyses were performed in *R* (v4.0.3) (R Core Team 2018) using *R Studio* (v1.4.1103) on a *MacBook Pro*, running *macOS Big Sur* (v11.2.1). Linear mixed models were run using *lmer* from the *lme4* package (v1.1.26)(Bates et al. 2015), *glmmTMB* from the *glmmTMB* package (v1.0.2.1) (Magnusson et al. 2017) and *procD.lm* from the *geomorph* package (v3.3.2) (Adams et al. 2018). Contrasts and effects were compared using *emmeans* from the *emmeans* package (1.5.4) (Russell et al. 2018). We used *pairwise* from the *RRPP* package (v0.6.2) to perform pairwise comparisons of distance and directions of mean shapes between different groups (Collyer and Adams 2020)

Modeling wing size and shape

Linear mixed models were fit with population, sex, environmental manipulation (either nutrition or temperature), and their interactions as fixed effects (up to 3rd order). We

allowed intercept, sex effects and environmental treatment to vary as random effects of strain (line). We fited these models with both *lmer* and *glmmTMB* and confirmed that estimates for parameters of interest were similar with both unconstrained and diagonal covariance structures for random effects.

Given the high-dimensional nature of our wing shape data, we used an alternative strategy for model fitting. Using *procD.lm* we fit models with population, sex and environmental manipulation and their interactions as fixed effects. To account for random effects of strain, we nested strain within population, and updated calculations of relevant F-ratios accordingly for terms in the model. ANOVA tables for both wing size and wing shape analyses are included in the supplementary data (Supplementary Tables B-S3, B-S4, B-S5 and B-S6)

Calculating and comparing SSD and SShD

We used a standard size dimorphism index (SDI) to calculate sexual size dimorphism (SSD), $SDI = \frac{Size\ of\ larger\ sex}{Size\ of\ smaller\ sex} - 1$. This SDI is generally used in species with female-biased SSD (Lovich and Gibbons 1992; Fairbairn 2007a). Mean female and mean male size were estimated for each strain and used to compare changes in SSD within and between populations and environmental manipulations (nutrition and temperature).

We assessed both the magnitude and direction of sexual shape dimorphism vectors. We calculated Procrustes distances between mean female and mean male shape vectors, and examined the direction of sexual shape change by calculating vector correlations (r) between female-male difference vectors among populations and environmental treatments (food and temperature). We generated confidence intervals for these statistics using non-parametric bootstraps (percentile intervals), sampling within groups.

Comparing allometric vectors

In our study design there are multiple sources contributing to size variation, which in turn influence shape-size allometric relationships. There is variation in size due to sex, genetic effects due to both population of origin, and strain (within population), plastic effects due to the experimental manipulations of temperature and food during development, and finally, any residual size variation among individuals. Given that the focus of this study is on the evolutionary changes in sexual shape dimorphism, we explored only a subset of these contributions in this study. We estimated shape-size allometric relationships per treatment group (i.e. partitioned by population, sex and either nutrition or temperature manipulation). Using *pairwise()* in *RRPP* we calculated vector correlations between allometry vectors, and the magnitudes of allometry vectors (i.e. shape change per unit change in size). To assess differences among groups for allometry, we fit reduced models assuming common allometry, and used *RRPP* to generate permutations of estimates under the full (group specific allometries and reduced (common allometry) models. An important limitation of our approach is that we did not account for strain specific allometries (strains within each population) in these models as we observed computational difficulties in model fitting when trying to account for these effects in *geomorph/RRPP*. As such, the population effect represents a complete pooling with respect to strain within the focal groups under consideration. This is an important caveat as the work presented here, and in previous studies (Pitchers et al. 2013) have demonstrated strain-level variation in SShD.

Partitioning allometric, non-allometric and total SShD

To assess the relative contribution of changes in total SShD ($SShD_T$) due to allometric ($SShD_A$) and non-allometric ($SShD_{NA}$) effects we used an approach related (but not identical) to Gidaszewski et al. 2009, assuming common allometry for males and females, and partitioned the relevant components. We estimated non-allometric SShD by

computing Procrustes distance between the sex-specific intercepts in common allometry models. For allometric component of SShD we estimated the Procrustes distance between predicted vectors of shape variables at mean sizes of males and females respectively (but using the same "intercept" for both sexes) from this model. This approach assumes a common allometric relationship between males and females. While these vectors are often similar in direction and magnitude (Tables 3.1 and 3.2), they are not identical, which will result in the partitioned values being difficult to interpret (see Gidaszewski et al. 2009 for further examples and discussion below). However, as an approximation and with appropriate caution, these still may provide some context to the relative contribution of allometric and non-allometric components to SShD. The approach we took to estimate $SShD_{NA}$ and $SShD_A$ differ from some published studies (Sztepanacz and Houle 2021; Gidaszewski et al. 2009) which computes one of the two components, and then uses the difference between these estimates and $SShD_T$ to compute the other value. As we show in the results and elaborate on in the discussion, when the assumption of common allometry between the sexes is violated, this may produce estimates that are difficult to reconcile.

3.4 Results

Wing size varies due to adaptive divergence and environmental plasticity, but SSD remains similar between populations

Consistent with previous observations (Pitchers et al. 2013; Klepsatel et al. 2014; Lack et al. 2016a; Lack et al. 2016b; Pesevski and Dworkin 2020), individuals from the high-altitude (HA) population have wings larger than those from the low-altitude (LA) population (Figure 3.1C). As expected, female-biased SSD was observed, with female wings being ∼18% larger than males in both populations. Food and temperature treatments also affected wing size. Wing size in HA females raised on 100% food is ∼17% larger than wing size in HA females raised at 15% food, while in HA males raised at 100%

food it is 12% larger than in HA males raised at 15% food. Wing size in LA females raised at 100% food is 13% larger than wing size in LA females raised at 15% food, and in LA males raised at 100% food it is 9% larger than in LA males raised at 15% food (Figure 3.1A, Supplementary Table B-S3). Despite these substantial differences in wing size, SSD in the two populations is not significantly different (Supplementary Table B-S3). Consistent with expectations of condition dependence, SSD is reduced by the reduction in food quality (significant sex-by-diet term, Supplementary Table B-S3), but the reduction remains unchanged due to adaptive divergence between the HA and LA populations (sex-by-diet-by-population term is not significant, Supplementary Table B-S3, Figure 3.3C). In the HA population SSD is 0.136 in flies raised at 100% food, and it is reduced to 0.098 in flies raised at 15% food. Similarly, in the low altitude population, SSD is 0.134 in flies raised at 100% food and it is reduced to 0.106 in flies raised at 15% food (Supplementary Table B-S3).

We measured larval mass at the wandering 3rd instar larval stage to determine if condition is being affected by food quality (Figure 3.2). We observed similar patterns for larval mass compared to patterns for wing size due to population, sex and nutrition (Figure 3.2). Importantly, we observed population and sex specific reduction in larva mass due to nutrition (Figure 3.2). The HA female larva were most sensitive to size reductions when reared on 15% food compared to other groups (Figure 3.2). This hints at strong population specific condition dependence of SSD for larval weight and potentially in overall body size.

Consistent with previous observations (Bitner–Mathe and Klaczko 1999; David et al. 1994; Debat et al. 2003; Pesevski and Dworkin 2020; Pitchers et al. 2013), an inverse relationship is seen between wing size and temperature (Figure 3.1B), and an overall increase in SSD with increasing temperature (Figure 3.3D). We observed evidence of sex-specific temperature plasticity as well as evidence for population-specific plasticity

(significant sex-by-temperature and population-by-temperature interaction effects, Supplementary table B-S4, Figure 3.1B, Figure 3.3D). Modest population-specific changes in SSD in response to temperature (significant sex-by-population-by-temperature interaction effect, Supplementary Table B-S4, Figure 3.3D) was observed. In the HA population SSD is 0.117 at 18°C, 0.136 at 24°C and 0.138 at 28°C, while in the LA population SSD is 0.118 at 18°C, 0.134 at 24°C and 0.138 at 28°C (Figure 3.3D).

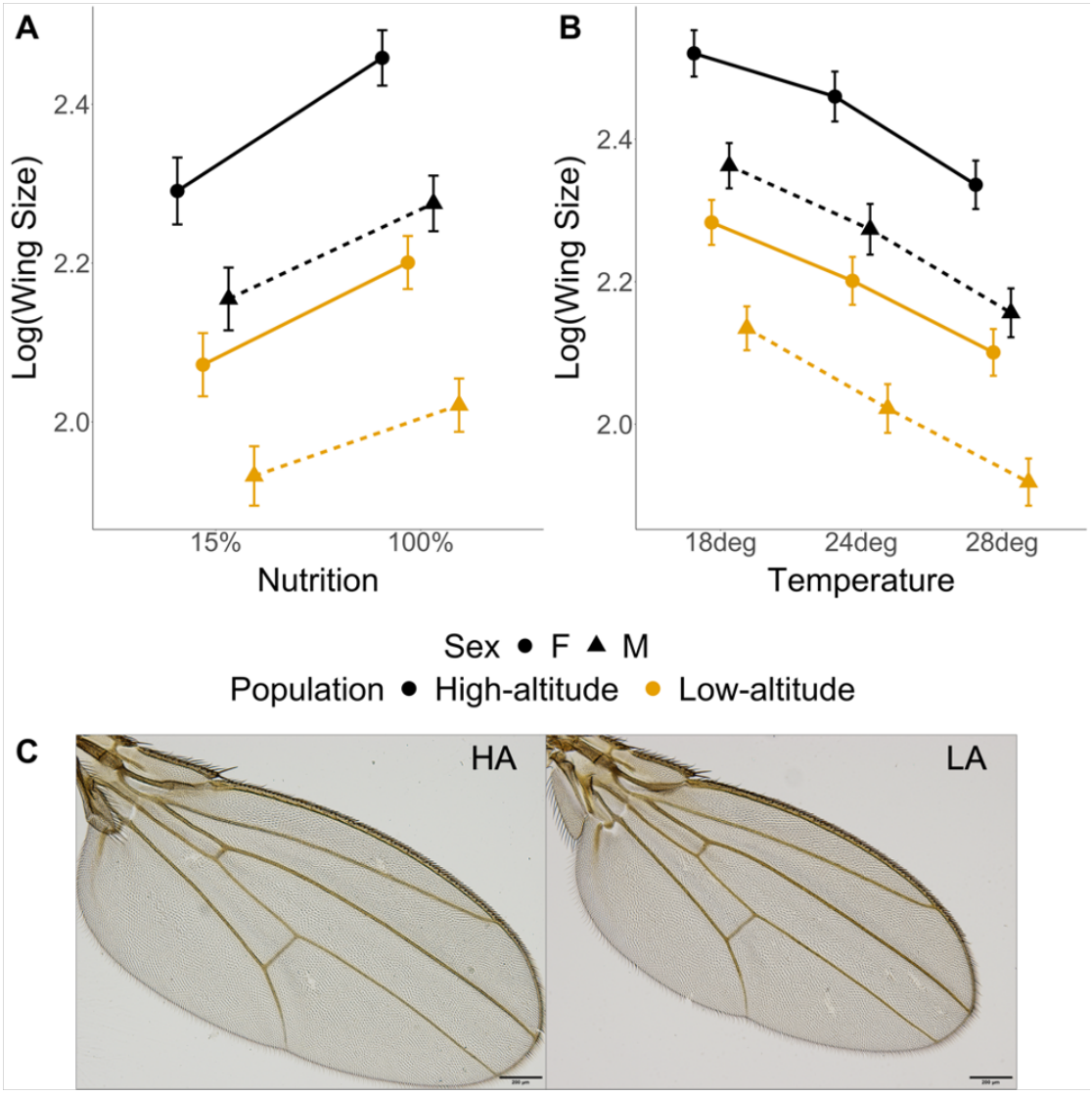

Figure 3.1: Wing size plasticity in high-altitude (HA) and low-altitude (LA) populations. A) Wing size plasticity in response to food quality is similar the HA and LA populations, despite substantial differences in overall sizes across both sex and population of origin. B) Wing size plasticity in response to rearing temperature in the HA and LA populations is similar, with an overall decrease in wing size with increase in temperature. C) Representative wings from the HA and LA populations. In both A and B, estimates are averaged across strains within each population. Error bars represent 95% CIs

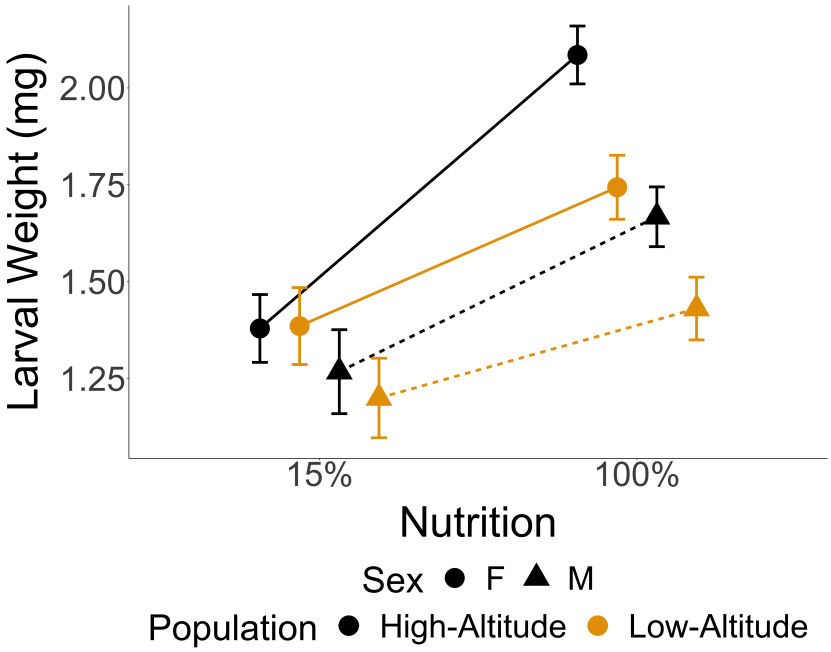

Figure 3.2: Larval weight plasticity in response to food quality in the high-altitude (HA) and low altitude (LA) populations. Larval weight in the HA females is the most sensitive to food quality reduction compared to the other groups.

3.4.1 Wing shape and total SShD vary due to environmental plasticity in a population-specific manner

Patterns of SShD between HA and LA populations were consistent with previous observations (Pitchers et al. 2013; Pesevski and Dworkin 2020) (Figure 3.3, Supplementary Tables B-S5 and B-S6). We did not observe substantial difference in SShD between the two populations, despite evidence for genetic variation in shape and sexual dimorphism among strains within populations (Supplementary Tables B-S5 and B-S6). Food quality had significant effects on wing shape, with some evidence of sex and population-specific differences (significant sex-by-diet and population-by-diet effects, Supplementary Table B-S5). SShD decreased at low food quality, with a slightly greater effect in the HA population compared to the LA population (Figure 3.3C, Supplementary Table B-S5). In the HA population, SShD is 0.016 on 100% food which was reduced to ∼0.013 on

15% food while in the LA population SShD is 0.016 on 100% food and it was reduced to ∼0.014.

We observed consistent evidence of shape plasticity due to rearing temperature, including significant population-by-temperature and sex-by-temperature interactions (Supplementary Table B-S6). We observe slightly different patterns of SShD in response to temperature in HA and LA populations, where the greatest SShD is at 24°C in both populations, but the two populations differ greatly in the amount of SShD at 18°C (Figure 3.3D). For the HA population SShD is 0.015 at 18°C, 0.016 at 24°C and 0.015 at 28°C, and in the LA population SShD is 0.013 at 18°C, 0.016 at 24°C and 0.016 at 28°C.

Despite observed differences in magnitude of SShD due to food quality and rearing temperature in HA and LA populations, we see similar changes in direction of SShD across populations and environmental treatments. Correlations among SShD vectors (across populations and environmental treatments) are all greater than 0.8, with many above 0.9 (Figure 3.4A,B). In particular, correlations of SShD vectors across populations are quite high, with somewhat lower values due to the food quality and temperature treatments (Figure 3.4A,B). This is consistent with much of the change in SShD being due to changes in magnitude rather than direction.

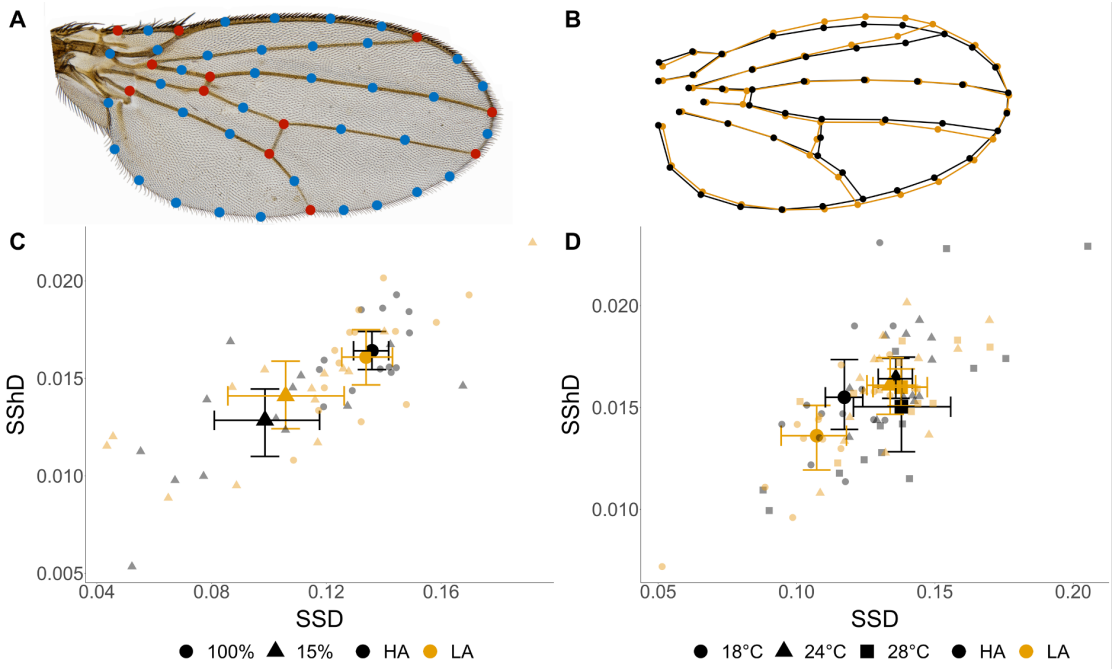

Figure 3.3: SShD variation in high-altitude (HA) and low-altitude (LA) populations. A) Landmarks (red) and semi-landmarks (blue) used in geometric morphometrics analysis to evaluate wing shape. B) Overlaid mean wing shapes of males (black) and females (orange) wings in the HA population at 24°C raised on 100% food quality as an example of SShD. C) Variation in SSD and SShD due to population and food quality. There is a greater reduction for SShD, but not for SSD, in the HA population compared to the LA population D) Variation in SSD and SShD due to population and rearing temperature. SShD is greatest at 24°in both HA and LA populations, but there is a greater reduction for SShD at 18°in the HA population compared to the LA population. For C and D, large opaque dots represent population means and small translucent dots represent means for each strain. Error bars represent 95% CIs

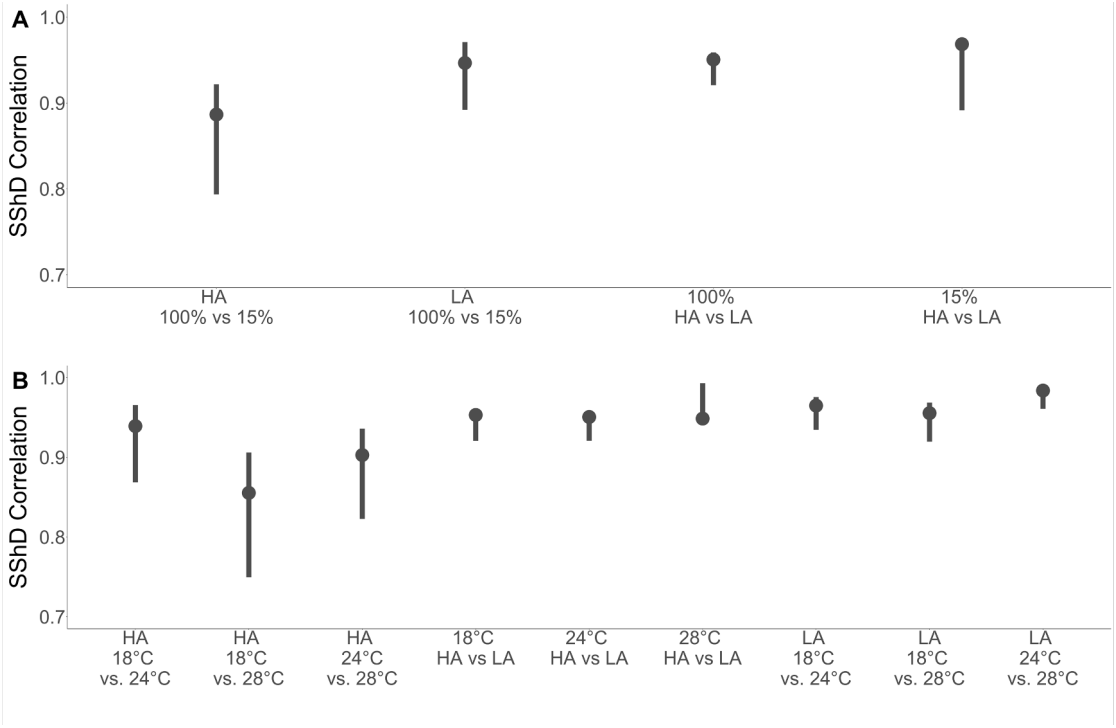

FIGURE 3.4: Correlations between SShD vectors in the high-altitude (HA) and low-altitude (LA) populations due to (A) food quality and (B) temperature plasticity. SShD vector correlations are very high, demonstrating that SShD varies is primarily in magnitude rather than direction. Error bars are bootstrap 95% CIs

3.4.2 Differences in shape-size allometry are modest for SShD and environmental plasticity, but greater among diverged populations

We compared allometric vectors between populations, sex and environmental treatments, examining differences in magnitude (absolute difference) and direction (r) for allometric vectors between all groups (Tables 3.1 and 3.2). Overall, there are both sex-by-size and sex-by-population-by-size interactions (Supplementary tables B-S5 and B-S6) resulting from changes in magnitude, direction or both. Magnitude of shape change (per unit change in size) is modest, but consistently greater in females in both populations and across food quality treatments. However, in direct paired comparisons across the sexes, differences are rarely significantly different from one another (Table 3.1A, Figure 3.5).

The impact of food quality on magnitude of shape change is idiosyncratic, with modest evidence of changes only in LA females (Table 3.1A). This can be contrasted with the allometric effects across populations, where differences in magnitudes occur, but with the sign of differences reversing across sexes (Table 3.1A, Figure 3.5).

Significant differences are observed for the direction of allometric effects across sexes in both HA and LA populations, with the exception of the 15% food treatment in the LA population (Table 3.1B). In all of these cases, correlations among allometric vectors are high (Table 3.1B). While somewhat lower in the 15% food treatments, the direction of allometric effects remain similar (albeit significantly different), all with correlations of 0.84 and greater (Table 3.1B). This can be contrasted with greater differences between the allometric vectors in the two populations, with correlations closer to 0.5 in the 100% food treatments, but higher correlations (near 0.8) in the 15% food treatment (Table 3.1C). When comparing allometry vectors between food treatments (100% vs. 15%), we observe modest differences in both absolute distance and correlations, these differences being intermediate between the effects for sex and population (Table 3.1D).

The shape-size allometric patterns with respect to temperature treatments were distinct and more variable than for the food quality treatments (Table 3.2, Figure 3.6). Significant interactions between size and other factors in the model are observed (Supplemental table B-S6). Sex differences in magnitudes of allometry vectors due to rearing temperature remain modest, with little change to direction of allometric effects (Table 3.2A). There is a notable exception at 28°C (Table 3.2A). When comparing temperature effects within population and sex, we see a much more variable distribution of allometric effects, but consistent effects among populations (as observed for the population effects with varying food quality) in direction and magnitude (Table 3.2B-D, Figure 3.5). Allometric relationships also vary more across temperature manipulations within sex and population (Figure 3.6), contrasting with the allometric relationships under varying food

quality treatments (Figure 3.5, Table 3.1).

Table 3.1: Pairwise comparisons of allometric vectors between populations and sexes for the food quality treatments. (A) Magnitudes (L2 norm) of allometry vectors for each group, and comparisons of allometric vectors between (B) sexes, (C) populations and (D) food treatments. Comparisons for distance are calculated as absolute difference between the magnitudes of allometric vectors and comparisons for direction are calculated as vector correlation (r) between the allometric vectors for each group.

A	Females	Males
HA 100%	0.075	0.054
HA 15%	0.059	0.056
LA 100%	0.063	0.065
LA 15%	0.077	0.065

	Absolute Difference	**Z**	**p**	**Direction (r)**	**p**
B					
HA 100% F - HA 100% M	2.20×10^{-2}	2.9	0.01	0.84	0.02
HA 15% F - HA 15% M	3.00×10^{-3}	-0.4	0.55	0.93	0.02
LA 100% F - LA 100% M	2.00×10^{-3}	-0.6	0.66	0.92	0.03
LA 15% F - LA 15% M	1.10×10^{-2}	3.0	0.03	0.96	0.13
C					
HA 100% F - LA 100% F	1.20×10^{-2}	1.4	0.01	0.42	0.01
HA 100% M - LA 100% M	1.20×10^{-2}	1.5	0.01	0.65	0.01
HA 15% F - LA 15% F	1.70×10^{-2}	4.5	0.01	0.81	0.01
HA 15% M - LA 15% M	9.00×10^{-3}	1.7	0.06	0.86	0.01
D					
HA 100% F - HA 15% F	1.60×10^{-2}	2.0	0.06	0.83	0.01
LA 100% F - LA 15% F	1.30×10^{-2}	3.7	0.01	0.88	0.01
HA 100% M - HA 15% M	2.00×10^{-3}	-0.7	0.71	0.75	0.01
LA 100% M - LA 15% M	2.00×10^{-4}	-1.2	0.98	0.87	0.01

Table 3.2: Pairwise comparisons of allometric vectors between populations and sexes for the temperature treatments. (A) Magnitudes (L2 norm) of allometric vectors for each group and comparisons of allomeric vectors between (B) sexes, (C) populations and (D) temperature treatments. Comparisons for distance are calculated as absolute difference between the magnitudes of allometric vectors and comparisons for direction are calculated as correlations (r) between allometric vectors for each group. (Values for 24°C are equivalent to 100% treatments in Table 1 and are therefore not represented here to avoid redundancy)

A	Females	Males			
HA 18°C	0.055	0.044			
HA 28°C	0.056	0.057			
LA 18°C	0.075	0.063			
LA 28°C	0.070	0.072			
	Absolute Difference	**Z**	**p**	**Direction (r)**	**p**
B					
HA 18°C F - HA 18°C M	1.11×10^{-2}	2.05	0.05	0.90	0.22
HA 28°C F - HA 28°C M	1.76×10^{-3}	−0.96	0.80	0.51	0.01
LA 18°C F - LA 18°C M	1.15×10^{-2}	2.09	0.04	0.93	0.60
LA 28°C F - LA 28°C M	2.20×10^{-3}	−0.49	0.57	0.96	0.92
C					
HA 18°C F - LA 18°C F	1.93×10^{-2}	3.80	0.01	−0.18	0.01
HA 18°C M - LA 18°C M	1.89×10^{-2}	3.90	0.01	0.17	0.01
HA 28°C F - LA 28°C F	1.46×10^{-2}	2.94	0.02	0.46	0.01
HA 28°C M - LA 28°C M	1.51×10^{-2}	2.21	0.05	−0.04	0.01
D					
HA 18°C F - HA 24°C F	2.00×10^{-2}	2.97	0.01	0.52	0.01
HA 18°C M - HA 24°C M	9.22×10^{-3}	0.78	0.17	0.50	0.01
HA 18°C F - HA 28°C F	1.62×10^{-4}	−1.43	1.00	0.43	0.01
HA 18°C M - LA 28°C M	1.29×10^{-2}	1.39	0.13	0.85	0.17
HA 24°C F - HA 28°C F	1.95×10^{-2}	2.44	0.04	0.71	0.04
HA 24°C M - HA 28°C M	7.98×10^{-3}	0.46	0.22	0.51	0.02
LA 18°C F - LA 24°C F	1.15×10^{-2}	2.09	0.03	0.76	0.01
LA 18°C M - LA 24°C M	2.07×10^{-3}	−0.8	0.75	0.76	0.01
LA 18°C F - LA 28°C F	4.55×10^{-3}	0.03	0.43	0.89	0.01
LA 18°C M - LA 28°C M	9.20×10^{-3}	1.02	0.15	0.90	0.33
LA 24°C F - LA 28°C F	7.00×10^{-3}	0.85	0.20	0.86	0.03
LA 24°C M - LA 28°C M	7.12×10^{-3}	0.61	0.23	0.85	0.14

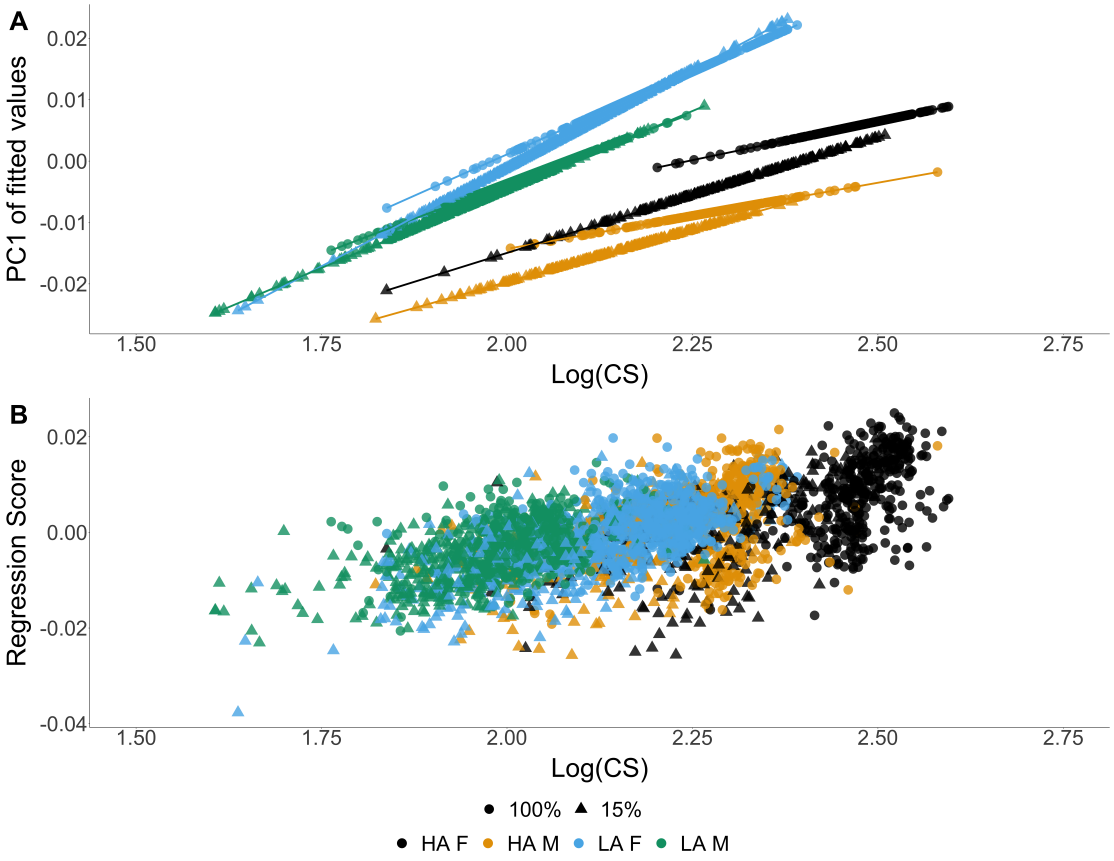

Figure 3.5: Size - shape allometry of wings in the high-altitude (HA) and low-altitude (LA) populations under different food treatments. (A) PC1 of fitted values plotted against wing size to visually represent the allometric vectors. (B) Regression shape scores plotted against wing size to visually represent variation within groups for allometry

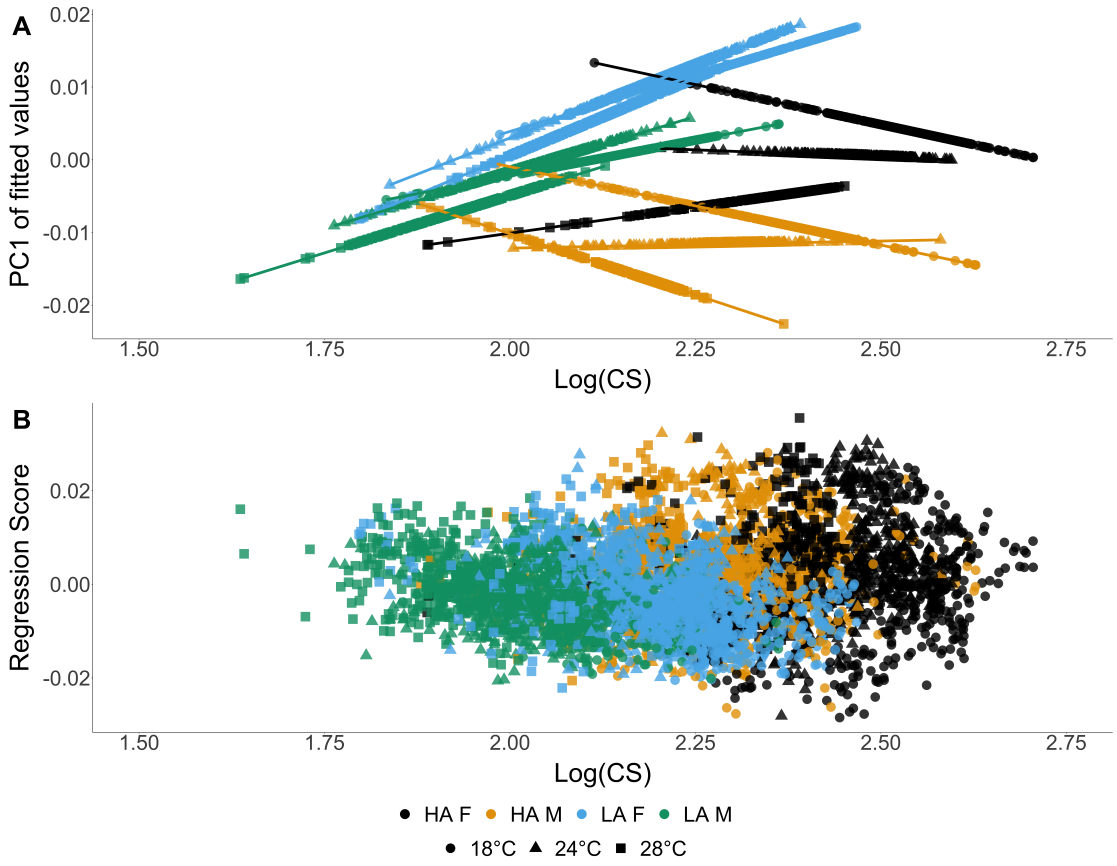

Figure 3.6: Size-shape allometry of wings in the high-altitude (HA) and low-altitude (LA) under different temperature treatments. (A) PC1 of fitted values plotted against wing size to visually represent the allometric vectors. (B) Regression shape scores plotted against wing size to visually represent variation within groups for allometry

3.4.3 Both allometric and non-allometric effects contribute to SShD

When considering the influence of size on shape, a common goal is to assess contributions of allometric and non-allometric effects. This is especially useful when studying SShD, because when there is SSD this may result in allometric SShD ($SShD_A$) that can contribute a significant portion to total SShD ($SShD_T$). The portion of $SShD_T$ that is not due to allometry is called non-allometric SShD ($SShD_{NA}$) Most current approaches require the assumption of common allometry be at least approximately met

(see Klingenberg (2016)). However, even when vector correlations are high, subtle violations of this assumption can result in difficult to interpret patterns (Gidaszewski et al. 2009). Despite these caveats, we assumed that since allometric vectors between the sexes had high correlations, we could assume common allometry and partitioned $SShD_T$ into $SShD_A$ and $SShD_{NA}$ components. Similar to previous results (Gidaszewski et al. 2009; Sztepanacz and Houle 2021), we observed that $SShD_A$ and $SShD_{NA}$ both contributed substantially to $SShD_T$ (Figure 3.7). However, we observe evidence that the assumption of common allometry is violated as values for $SShD_A$ and $SShD_{NA}$ do not sum to $SShD_T$, and result in different estimates depending on how the components are calculated (Supplementary Table B-S10).

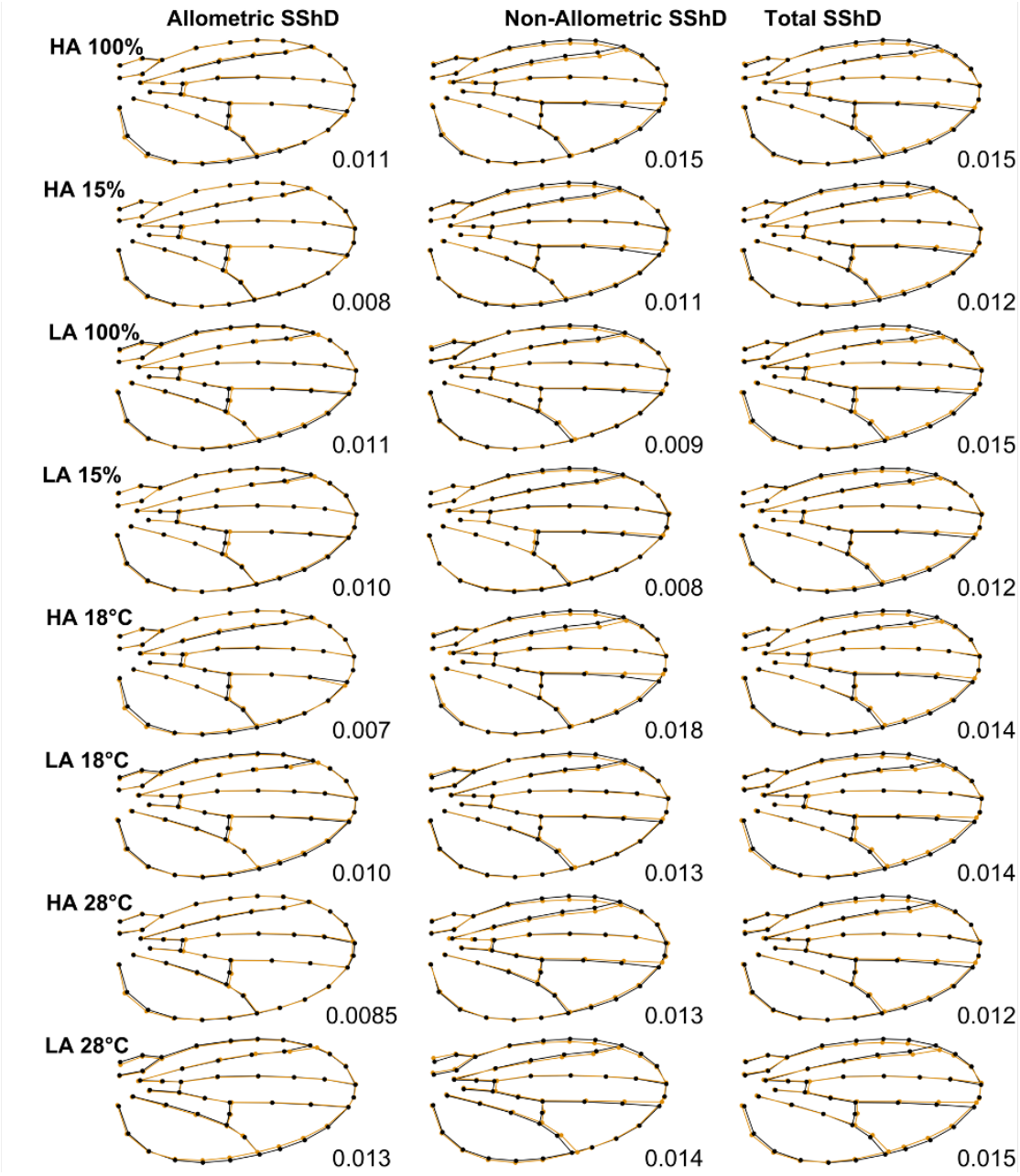

FIGURE 3.7: Allometric ($SShD_A$), non-allometric ($SShD_{NA}$) and total SShD ($SShD_T$) within each population and environmental treatment. SShD values are calculated as the Procrustes distance (PD) between mean female and mean male shape. Both $SShD_A$ and $SShD_{NA}$ contribute towards $SShD_T$, despite evidence that common allometry assumption between the sexes is being violated

3.5 Discussion

3.5.1 Influence of rapid adaptation on sex-specific plasticity

One of the main goals of this study was to examine how adaptive evolution influences sex-specific plasticity and condition dependence. As is typical for small insects (Hodkinson 2005; Dillon et al. 2006a), a number of traits contribute to the adaptive response to life at high altitudes, including body size, wing size and shape (Hodkinson 2005; Pitchers et al. 2013; Pool et al. 2012b; Lack et al. 2016a; Lack et al. 2016b; Klepsatel et al. 2014; Klepsatel et al. 2013; Fabian et al. 2015). The HA population used in this study has experienced strong directional selection for larger body size and disproportionately larger wing size (Pitchers et al. 2013; Pool et al. 2012b; Lack et al. 2016a; Lack et al. 2016b; Klepsatel et al. 2014; Fabian et al. 2015; Klepsatel et al. 2013). Wing shape has also evolved compared to the LA population, although the degree to which it is a direct target of selection is unclear (Pitchers et al. 2013). Aspects of wing morphology, including size and shape, are targets of sexual selection in *D. melanogaster* (Ewing 1964; Abbott et al. 2010; Menezes et al. 2013); show sexual dimorphism within *D. melanogaster* and among other *Drosophila* species (Gidaszewski et al. 2009); and demonstrate sex-specific condition dependence. As such, we expected that the selection on size and shape contributing to the adaptive changes observed in the HA population may have altered genomic architecture such that it would influence sex-specific response to environmental variation (Connallon 2015; Connallon et al. 2018). Contrary to these predictions, and despite substantial population differences in wing size and shape (Figures 3.1 and 3.3, Supplementary Tables B-S3,B-S4, B-S5, B-S6), changes in SSD and SShD are modest between populations (Figure 3.3C and D). As expected, we observed a reduction in SSD and SShD as a result of poor food quality (Figures 3.1A, 3.3C , Supplementary tables B-S3, B-S5). However, despite the evolved increase in overall size in the HA population, we did not observe a substantial increased sensitivity to food quality for SSD of wing size, and only a modest increase in sensitivity for SShD in the HA population (Figure

3.3C). We observed a much stronger change in sex specific plasticity for larval mass, a proxy measure for body size. Larval mass showed a much greater reduction in SSD (sex specific condition dependence) in the high altitude population where females had a stronger response to food availability compared to the other groups (Figure 3.2). While SSD and SShD increased as a result of increasing rearing temperature, the sensitivity to temperature of SSD and SShD in both populations was similar ((Figures 3.1B, 3.3D, Supplementary tables B-S4, B-S6)). This is similar to what has been observed for r_{fm} number of traits for *Drosophila* populations along a latitudinal cline (Lasne et al. 2018). Additional selective forces may be maintaining the relative plastic response for wing morphology (Pesevski and Dworkin 2020), but not for overall body size. However, wing loading is likely to be a major target of selection in the HA population (Klepsatel et al. 2014), and variation in wing shape can influence flight performance in lab environments for *Drosophila* species (Ray et al. 2016; Fraimout et al. 2018). Thus, wing size and shape may be under strong stabilizing selection in each environment resulting in greater environmental canalization.

Evolution, plasticity and sexual dimorphism for shape-size allometry

The second goal of this study was to evaluate what portion of the observed shape changes across sex, population and rearing environments was a consequence of allometry with size. This is a required step to ultimately identify whether wing shape was a direct target of selection resulting in the evolutionary wing shape changes observed in the HA population, or whether these changes are simply a correlated response due to allometry with size. Mean wing shape not only differs due to adaptive differences in the two populations, but there are significant mean shape differences due to sex, as well as due to nutritional and temperature plasticity (Supplementary Tables B-S5 and B-S6). However, as with most shape changes, shape-size allometry contributes significantly to shape variation (Klingenberg 2016), including in *Drosophila* (Gilchrist et al. 2000;

Debat et al. 2003; Gidaszewski et al. 2009; Bolstad et al. 2015). Thus, we wanted to assess the relative contribution of allometric and non-allometric effects to changes in wing shape. Given that wing size varied substantially due to population, sex and environmental plasticity (diet and rearing temperature), partitioning allometric effects can be challenging (Figure 3.1, Supplementary tables B-S3, B-S4, B-S5, B-S6). We used multiple approaches to assess the contribution of allometric and non-allometric effects on wing shape. In addition to discussing this in the context of our results, we also briefly discuss the effectiveness of each approach in the context of understanding allometric effects. As is often the case, partitioning the effects using a multivariate linear model (and allowing size effects to vary according to sex and other predictors) suggests that we observe significant variation in allometric relationships across groups (Supplementary Tables B-S5 and B-S6). Given the relatively large sample sizes used in this study, it is not surprising we detect statistically significant effects. More importantly, we examined the relative contribution of changes in direction and magnitude of shape change associated with changes in size, as summarized in Tables 3.1 and 3.2, and Figures 3.5 and 3.6. Despite significant interactions between sex and size, changes in magnitude of allometry (i.e magnitude of shape change per unit change in size) were generally modest as were changes in direction of allometric effects (Table 3.1, Figure 3.5). This pattern seems to largely hold with respect to temperature mediated effects on sexual dimorphism for allometry (Table 3.2, Figure 3.6). On the other hand, differences in allometric effects among populations show evidence of divergence for direction and magnitude and have a surprising lack of concordance in direction. Whether this reflects that shape itself or shape-size allometry has been a direct target of selection is unclear. One clear finding from our study is how different environmental mediators of change in size (and shape) like diet and rearing temperature, elicit different patterns of allometry in terms of population of origin and sexual dimorphism (contrast Figure 3.5 and 3.6). In particular, while allometric effects in response to food quality elicit parallel changes in shape across sexes

and populations, influence of rearing temperature differs substantially. An analogous pattern has been observed for *D. melanogaster* for multivariate allometry of size when both rearing temperature and food availability were varied (Shingleton et al. 2009). The degree to which these differences reflect potentially adaptive temperature plasticity is unclear, but previous work demonstrated alignment between shape changes between HA and LA populations and temperature plasticity (Pitchers et al. 2013).

In this study, we attempted to infer relative contributions of allometric and non-allometric effects on SShD. Current approaches to partitioning allometric and non-allometric SShD require an assumption of common allometry between the sexes within each population and rearing condition (Sztepanacz and Houle 2021), and violations of that assumption can result in misleading inferences. While this assumption was clearly violated across populations, allometric patterns across the sexes were generally the most similar with respect to both direction and magnitude (Tables 3.1B,3.2B, Figures 3.5A, 3.6A), as has been observed previously (Gidaszewski et al. 2009; Testa and Dworkin 2016). Given the relatively modest violation of the assumption of common allometry, we explored this approach to determine the degree to which it might be informative. Despite vector correlations among allometry vectors mostly greater than 0.9 between males and females, we still observe problematic effects where summed contribution of $SShD_A$ and $SShD_{NA}$ exceed $SShD_T$ (Figure 3.7). To the extent that any meaningful inference can be made, our evidence suggests that the $SShD_A$ and $SShD_{NA}$ components are contributing to $SShD_T$ relatively evenly (Figure 3.6). Very similar effects and likely similar problems have been observed when partitioning SShD among *Drosophila* species (Gidaszewski et al. 2009; Sztepanacz and Houle 2021). Overall, this suggests that even when the direction of allometric effects appears very similar, partitioning allometric and non-allometric SShD under an assumption of common allometry may be misleading. Thus the approach used for partitioning such effects can result in substantially different answers (Supplementary Table B-S10). Care must still be taken when there is no

significant interaction effect between sex and size, as this often is a function of studies with modest sample sizes (or where size variation is small) (Klingenberg 2016). In such cases, allometric relationships can be estimated with high uncertainty, suggesting an apparent (but potentially incorrect) similarity in relationship. Developing approaches to partition allometric effects that are robust to such assumptions remain an important open problem in geometric morphometrics.

Despite being among the first studies examining the influence of rapid adaptation on condition dependent sexual dimorphism (and sex-specific plasticity in general) in a moderately female biased SSD system, our study has some limitations. First, we are studying the interplay of adaptation, sexual dimorphism and plasticity within a single pair of adaptively diverged populations. Even though we did not observe evidence of changes in patterns of sex-specific plasticity as a consequence of rapid adaptive evolution, such changes may occur in other populations and/or species, particularly in systems where adaptive and sexual selection forces are misaligned. While sexual selection generally operates on body size (Testa et al. 2013), wing size (Ewing 1964; Abbott et al. 2010), shape (Menezes et al. 2013; Abbott et al. 2010) and other aspects of wing morphology (Katayama et al. 2014) of *D. melanogaster*, we do not know the strength or direction of sexual selection in the populations we examined. Natural selection for greater body and wing size in the HA population could have altered the strength of sexual selection in the wings of HA population. We observed that the degree of SSD for the wing is similar in the two populations hinting at potentially unchanged patterns of sexual selection in the two populations, but this remains to be confirmed experimentally.

As with any manipulative experiment, differential viability among treatments may result in biased sampling of individuals used for phenotyping, despite efforts to sample randomly. In this study, we measured egg-to-adult viability (Figures B-S1 and B-S2) observing more than a ∼65% reduction in viability for individuals raised on 15% food

(survivorship at about 11–16%) relative to flies raised on the 100% food (survivorship at about 45–46%). Viability in the low-altitude population was significantly higher than for the HA population for the 15% food treatment (supported by a significant nutrition:population interaction, Supplementary Table B-S8). This difference in viability suggests in the HA population, the poor quality treatment may decrease fitness more than in than the LA population. This 'invisible fraction' may also represent a non-random subset of phenotypes. We did not observe any differences in viability between populations at different rearing temperatures (Supplementary Table B-S9).

This study focused on the evolutionary changes in wing morphology. Other than larval mass, we did not take measurements of other body parts. Previous work has demonstrated that the evolved increase in size was observed among other traits (Pitchers et al. 2013; Pool et al. 2012b; Lack et al. 2016a; Lack et al. 2016b; Klepsatel et al. 2014; Klepsatel et al. 2013; Fabian et al. 2015), but that the increase in wing size was disproportionately large. However, it is known that different organs of *Drosophila* respond differently to nutritional and temperature manipulations(Shingleton et al. 2009). In retrospect, these measurements would have been useful to explore the differences in plastic response for different traits and overall body size, and potentially scaling effects of overall body size and the wing. Despite this, the strength of our study is the exploration of wing shape and SShD, as well as, the allometric and non-allometric contributions of shape variation in these two populations. The results from the examination of larval mass suggest that we may be underestimating the condition dependence of SSD by focusing only on the wing because we observe a heightened condition dependence of SSD for larval weight in the HA population, particularly in the HA females (Supplementary Figure 3.1, supported by significant sex:population:nutrition effect, Supplementary table B-S7).

Finally, strains used in this study were established in 2011, while the experimental manipulations (performed simultaneously) were done in 2018. As such, potential issues

due to inbreeding, genetic drift and to a lesser extent lab domestication can occur. Since multiple strains were used from each population (Supplementary tables B-S1 and B-S2), the impact of drift on allelic frequencies across all strains within a population will be generally modest. Lab domestication does not appear to have affected morphological changes substantially. In a previous study, we examined phenotypic variation of these same strains at two different time points ($\sim$5 years apart) and observed that wing size and shape were highly correlated, and remained consistent with previous studies (Pesevski and Dworkin 2020). This suggests minimal influence of lab domestication on traits under study. This is perhaps not surprising as when established as strains from single females N_e is small for each strain, and thus selection is unlikely to be particularly efficient except for variants with large effects on fitness.

In summary, although we observed sex-specific plasticity for wing size, we did not observe major changes in these patterns as a result of adaptive divergence. We observed slight increase in sex-specific plasticity for wing shape. Differences in wing shape due to sex, adaptive divergence and plasticity are a product of both allometric and non-allometric components. Despite the limitations of the method for partitioning allometric and non-allometric SShD, both allometric and non-allometric components of SShD contribute to overall SShD. These findings are the beginning of the exploration of the interplay between adaptive evolution, sexual dimorphism and sex-specific plasticity (Ceballos and Valenzuela 2011). However, our understating of these patterns remains limited and further theoretical and empirical work is needed to explore these relationships further. Although there has recently been a greater interest in examining the co-evolution of sexual dimorphism and condition dependence (Bonduriansky 2007b; Rohner and Blanckenhorn 2018; Cotton et al. 2004a; Stillwell et al. 2007), there is still a gap in understanding of the underlying mechanisms that govern the relationships between sexual dimorphism and condition dependence. Further examination of influence

of local adaptation on the relationship between sexual dimorphism and condition dependence is also needed because natural populations experience simultaneous selective pressures. Although the HA population is an example of natural system that allowed us to examine the interplay between condition dependent sexual dimorphism and local adaptation, exploring the relationship between condition and sexual dimorphism in different populations and species with different evolutionary histories, including a range of traits that vary in the magnitude and direction of SSD (and SShD), would be most welcome in order to fully understand these processes and their interaction.

Chapter 4

Comparative analysis of sexual dimoprhism and its condition dependence in the *melanogaster* species group

4.1 Introduction

Sexual dimorphism and its condition dependence have been studied extensively in many different taxa, mostly in species with exaggerated male biased-SSD and, to a limited extent, in species with moderate female-biased SSD. However, inter-specific patterns of the correlated evolution of sexual dimorphism and condition dependence have rarely been studied at the macro-evolutionary level (Cotton et al. 2004b; Stillwell et al. 2010; Teder and Tammaru 2005; Rohner and Blanckenhorn 2018). As discussed in the introductory chapter and in the introduction of chapter 3, theoretical and empirical studies have demonstrated a strong association between sexual dimorphism and condition dependence (Bonduriansky 2007c; Bonduriansky 2007b; Stillwell et al. 2010; Cotton et al. 2004b).

Yet, it is unknown whether similar correlations of condition dependence and sexual dimorphism exist at the inter-specific level and whether they follow similar evolutionary patterns: is the evolution of greater SSD associated with the evolution of greater condition dependence? Previous studies have examined at most 1-3 closely related species at a time and have focused mainly on intra-specific co-variation of SSD and condition dependence, often examining a variety of traits with varying degrees of SSD among individuals, strains and populations within the same species (Bonduriansky 2007c; Siomava et al. 2016; Cassidy et al. 2014; Stillwell and Fox 2007; Fernandez-Montraveta and Moya-Larano 2007). Patterns of inter-specific co-variation of SSD and condition dependence have been inferred mainly from meta-analyses and systematic reviews that have not taken a comparative-methods approach (Cotton et al. 2004b; Stillwell et al. 2010; Teder and Tammaru 2005). These studies have concluded that there is an association between degree of SSD and condition dependence among species. However, none of these studies ever accounted for phylogenentic relatedness among species or studied these patterns within a closely related taxonomic group in order to assess the evolutionary dynamics of SSD and condition dependence in a comparative framework. To date, a single study has attempted to study condition dependence of sexual dimorphism in a phylogenetic context (Rohner and Blanckenhorn 2018). In species of *Sepsis* flies with varying degree and direction of SSD, Rohner and Blanckenhorn (2018) demonstrated that SSD and sex-specific condition dependence co-vary at the inter-specific level, especially in traits with greater SSD. The relationship between magnitude of SSD and degree of condition dependence was stronger in species with male-biased SSD, supporting the theoretical predictions that sexual selection acting in males may be the main driver of heightened condition dependence in these traits (Rohner and Blanckenhorn 2018). Condition dependence was also positively correlated with SSD in species with female-biased SSD, but to a lesser degree (Rohner and Blanckenhorn 2018). In order to understand how these

two important sources of phenotypic variation co-evolve under different evolutionary circumstances, we must study the evolutionary patterns of SSD and condition dependence in more than one taxonomic group.

In our study outlined in Chapter 3, I examine the condition dependence of sexual dimorphism in two *Drosophila melanogaster* populations from different altitudes that vary drastically in size due to adaptive divergence. Within each population, we observed a decrease in both SSD and SShD when flies were raised at low quality food compared to high quality food, but the two populations did not differ in their condition dependence for SSD and only slightly for SShD. In Chapter 3, my goal was to examine the effects of micro-evolutionary adaptive forces on the interplay between sexual dimorphism and condition dependence. In chapter 4, my goal is to examine the co-evolution of sexual dimorphism and condition at the macro-evolutionary scale within the *melanogaster* species group. In doing so, I would like test the theoretical predictions of the condition dependence family of hypotheses that predict that the greater degree of sexual dimorphism is associated with greater degree of condition dependence. The predictions of these hypotheses have a number of potential evolutionary causes, two of which are: (1) strong directional sexual selection causing release of phenotypic variation as a result of decanalization and (2) genic capture of genetic variation of condition by sexually dimorphic traits via sex-specific modifiers allowing for the relaxation evolutionary constraints brought on by high r_{MF} and depleted genetic variance in the face of strong selection (Bonduriansky 2007c; Rowe and Houle 1996; Bonduriansky 2007b). These hypotheses are explored in more detail in section 1.2.3 of Chapter 1. These hypotheses have yet to be tested in species with primarily female-biased SSD and, as mentioned above, have been tested in comparative framework in a single study within one taxonomic group. Among *Drosophila* species, there is evidence of sexual selection in males, particularly for overall body size, wing size, wing shape, leg size as well as other sex-specific secondary sexual traits such as sex-combs, body coloration, wing spots, among others (Bateman

1948a; Ewing 1964; Abbott et al. 2010; Markow et al. 1996; Kopp and True 2002; Morimoto et al. 2016). Yet, in most *Drosophila* species, except *Drosophila prolongata* which shows strong male-biased SSD, most traits display female-biased SSD. *Drosophila* has been an insect model for the study of genetics, evolution, development and behaviour, it is very easy to rear and manipulate in the lab and many lab and wild strains are readily available. Previous studies have demonstrated that different traits of *Drosophila* species exhibit some level of plasticity due to food, temperature, larval density and other environmental manipulations (Morimoto et al. 2016; David et al. 1994; Bitner–Mathe and Klaczko 1999). Therefore, examining patterns of sexual size dimorphism and condition dependence, within and among *Drosophila* species can help us better understand the dynamics of these two important sources of phenotypic variation among species with mostly female-biased SSD.

The first aim of this study is to examine the relationship of sexual dimorphism and condition within each species. To do this, I examine whether sexual dimorphism and condition dependence are correlated among different traits within each species. The second aim is focused on inter-specific patterns of sexual dimorphism and condition dependence, and I intend to examine whether evolutionary patterns of SSD at the comparative level are corresponding to evolutionary patterns of condition dependence in order to examine if sexual dimorphism and condition dependence co-evolve.

For this chapter, I have chosen 27 *Drosophila* species that have a common ancestor about 15 million years and have varying degrees of SSD, and in some cases direction of SSD (male-biased SSD for all traits in *D. prolongata*, and leg segments in some other species). I raised these species under varying starvation periods during development to manipulate condition. I measured thorax size, length and width of the tibia and the length of the first tarsal segment of the frontal right leg, and wing area for each fly. In general, although I observe correlation of sexual dimorphism and condition dependence

within each species, I do not observe the expected corresponding co-evolution at the inter-specific level for condition dependence and sexual dimorphism among the species from the *melangoaster* species group.

4.2 Materials and Methods

4.2.1 Species and Growth conditions

Wild and lab strains from 27 *melanogaster* species group were used in this experiment. Supplementary Table C-S1 lists the all the strains and species and their origin. The strains were maintained on standard cornmeal molasses food (for recipe click here) at room temperature. Strains of *D. sechellia* were raised on layered Carolina potato medium on top of standard cornmeal molasses food. Prior to experimental treatments, the flies were raised on high protein (HP) food (2:1 carbohydrate to protein ratio, recipe outlined in Supplementary table C-S2) for one generation to minimize maternal effects. Before the experiment, *D. sechellia* was raised on the HP food supplemented with octanoic (0.7%), hexanoic acid (0.7%) and L-DOPA (0.375mg/L), for one generation.

4.2.2 Experimental design

Adult flies from each species were placed in egg laying chambers with apple-agar plates. Dead yeast patches were used for most species, but were not included for species that do not lay eggs in the presence of yeast (determined by observation of egg laying performance). The adults were left to lay eggs for 12h windows in an incubator at 24°C (12h light/dark cycle). Eggs were collected, 50 at a time, and placed in vials with HP food, and the supplemented HP food for *D. sechellia*. As the eggs were collected, they were placed in distinct cohorts, so that eggs that were collected on the first day were in cohort 1, and eggs collected on subsequent days were in cohorts 2 and 3. In species that have longer development times I collected up to cohort 5. When cohort 1 reached the wandering larval stage, larva from all cohorts for each species were collected by floating

them in 5% sucrose solution. Thus, cohort 1 flies were fully fed, cohort 2 underwent 24 hours of starvation at the end of larval period, cohort 3 underwent 48 hours, and so on. The collected larva were washed and placed in vials with a moist cotton ball to prevent desiccation. The flies were kept at 24°C until eclosion and wing sclerization. The emerged flies were then collected in tubes with 70% ethanol to preserve them. The whole experiment was split into 3 blocks of 12-13 species each to allow for more manageable handling and collection. The blocks were all performed on the same batch of food within 2 weeks of each other to minimize variation among blocks.

The right wing and right frontal leg of 30 adult males and 30 adult females were dissected for each strain/species and mounted on glass slides in 70% glycerol solution in the same order of dissection. At the time of dissection, digital images of the thorax were taken in the same order of wing and leg mounting. Taking photos of the thorax at the time of wing and leg dissections allowed us to assign the same label to the thorax, wing and leg images so that we have corresponding measurements for each individual fly. The thorax images were taken using a Leica IC90 E camera mounted on Leica MZ75 microscope at 5X magnification (total of 50x magnification) using the LAS X 3.0 imaging software at 1024 x 768 resolution. The wing and leg images were taken using an Olympus DP30B camera mounted on an Olympus BX51 microscope (Olympus software version 3.1.1208) using a 4X objective (total of 40X magnification) and images were taken using cellSens Standard (version1.14) software at 4060 x 3072 resolution.

Linear measurements were taken of the thorax, length and width of the tibia, and length of the first tarsal segment of the leg using the measure tool from the ImageJ software, and area measurements were taken of the wing were taken using a custom macro written in ImageJ (not publicly available).

I originally took a different approach to alter condition by altering food quality for

each species. I used the HP food and created specific diets for species that had special dietary requirements (supplemented food for *D. sechellia*, and sugary food for D. suzukii). I created 100%, 50%, 35% 25% 15% dilutions of the HP food and raised the flies at 24°C.I measured wing area, leg segments and thorax length for a subset of these species and did some preliminary analysis. Surprisingly, this had very modest impact on adult size in most species and I decided to perform the starvation protocol outlined above.

4.2.3 Analysis

Statistical analysis was performed in R(v4.0.3) (R Core Team 2018) using R Studio(v1.4.1103) on a MacBook Pro, running macOS Big Sur(v11.2.1). Linear models were run using *lm* from the *stats* package (v4.0.3) and *lmer* from the *lme4* package (v1.1.26). Contrasts and effects were compared using emmeans from the emmeans package. Comparative analysis was performed using functions from the *ape* package (v5.4.1), *caper* package (v1.0.1), *geiger* package (v2.0.7), *phytools* package (v0.7.70) and *nlme* package (v3.1.152), for general phylogeny input and manipulation, phylogenetic general least squares (PGLS) analysis, ancestral state estimation, and estimating parameters for different evolutionary models (Brownian Motion, Ornstein–Uhlenbeck, and Pagel's lambda). Trait evolution on phylogenetic trees was visualized using functions from the *ggtree* package (v2.4.1).

To simplify our analysis, we converted the cohorts for each species into a condition variable. We coded cohort 1 as high condition (HC), because this cohort did not undergo any starvation, and we coded the highest cohort within each species (3 for most species; 4 or 5 for species with longer development times) as low condition (LC), because those cohorts underwent the longest duration of starvation. The new condition variable allowed us to have species-specific condition treatments that are similar across species and are standardized based on development time, as it is hard to consistently alter condition

in so many species due to inter-specific differences in response to nutritional needs and resistance to stress.

Before analysis we transformed the measurements of the traits to ease with the analysis. We log_2 transformed the linear measurements (in micrometers) to standardize the measurements across traits and species. For wing we first took the square root of the area measurement so that it is on a common scale as the other linear measurements.

Intra-specific patterns of sexual dimorphism and condition dependence

We first examined intra-specific patterns of sexual dimorphism and its condition dependence for each trait. We fitted linear models by using each trait measurement as the dependent variable, and condition, sex and their interaction as the independent variables. We fitted regular linear models with the *lm* function by including species as an independent variable in order to get species-specific estimates for each trait. We fitted a mixed model with block and species as a random effects allowing the effect of condition and sex to vary among species.

We calculated a sexual dimorphism index (SDI) as a measure of SSD for each trait at HC and LC. We used the sex contrasts generated by the *emmeans* function and back-transformed from the log scale to get the ratio between females and males. Then we subtracted 1 from the ratio in order to generate the SDI described in Lovich and Gibbons (1992). To check the validity of this SDI index, we calculated SDI by taking the male and female means for each species at HC and LC, calculating the female-male ratio and subtracting 1. We concluded that these two ways to calculate SDI were identical and continued with the first approach. We took a similar approach to calculate a condition dependence degree (CDD) for each sex within each species, we used the contrasts generated by the *emmeans* function, as a measure of condition dependence.

Because our data is log transformed, if we back-transform the CDD it will represent the ratio between HC and LC measurements.

In order to quantify the covariation of condition dependence and SSD we fit a linear model with CDD as a dependent variable for each trait and SDI at the HC as an independent variable. We did this for each sex, within each species in order to examine whether the traits that are most dimorphic are most condition dependent.

Inter-specific patterns of sexual dimorphism and condition dependence

We used a modified version of the tree produced by Suvorov et al. (2021). We removed the species on the tree that were missing from our analysis and added 3 species (*D. santomea*, *D. pseudotakahashi* and *D. prolongata*)that were not in the original tree. We used additional trees from the literature to determine the correct position of these three species in the tree that we use for our analysis (O'Grady and DeSalle 2018; Linde and Houle 2008; Rohner et al. 2018b; Sessegolo et al. 2016). The branch lengths for the three added species were approximated at halfway between the existing tip and the most common ancestor node to the next closest node/tip. The phylogeny is shown in Figure 4.1

We estimated the evolutionary rate (σ^2) under Brownian motion (BM), Ornstein – Uhlenbeck (OU), and maximum likelihood Pagel's λ evolutionary models for each trait within each sex at HC and LC and compared the model fits using AICc using the *fitContinuous* function from the *geiger* package. We also did estimated σ^2 for SDI at HC and LC, and for CDD within females and males, to estimate the rate of evolution of SSD and condition dependence and to compare which evolutionary model best models their evolution.

We performed ancestral state reconstruction using the *fastAnc* function from the *phytools* package for each trait at HC and LC in order to estimate the evolutionary

changes for each trait on the phylogeny. We also performed ancestral state reconstruction for SDI at HC and LC and CDD within females and males to estimate the evolutionary changes in SSD and condition dependence. We plotted the ancestral state estimations for each trait, SSD and condition dependence on phylogenetic trees using *ggtree*.

We performed a PGLS analysis using the *pgls* function from the *caper* package, by fitting CDD for each sex as the dependent variable and SDI at HC as the independent variable in order to see whether there is evidence of phylogeny corrected co-variation for condition dependence and SSD.

4.3 Results

4.3.1 Intra-specific patterns of sexual dimorphism and condition dependence

At the intra-specific level we looked at whether there is an association between sexual dimorphism and condition dependence among traits within each species. For each trait that we measured we observed varying degrees of SSD and varying degrees of sensitivity to the condition manipulation (Figures 4.2, 4.3, 4.4, 4.5, 4.6). For thorax length, most species exhibit female-biased SSD and most exhibit a decrease in thorax size under low condition (LC) compared to high condition (HC). Notable exceptions to this pattern include monomorphism (non-significant differences between males and females at HC) in *D. elegans* and *D. fuyamai*, and male-biased SSD in *D. prolongata*, and lack of reduction in thorax length due to condition in *D. elegans* and *D. rufa*, and females of *D. fuyamai*. Tibia length and width are mostly monomorphic across species and are in general less sensitive to condition than thorax size. Notable exception to this pattern include male-biased SSD in *D. elegans*, *D. ficusphila*, *D. fuyamai*, *D. prolongata* and *D. suzukii* for tibia length, and male-biased SSD in *D. ficusphila*, *D. fuyamai*, *D. kikkawai*, *D. prolongata* and female biased SSD for *D. malerkotliana* for tibia width. Tarsus length has

primarily female-biased SSD among the species. Exceptions include monomorphism in *D. ficusphila*, *D. fuyamai*, *kikkawai* and *D. santomea*, and male biased SSD in *D. elegans* and *D. prolongata*. Reduction in tarsus length due to condition is more variable than for the other leg segment measurements, but in general, it is lower than thorax length reduction. Finally, for wing area, we observe similar patterns as with thorax length: female-biased SSD in almost all species, with the exception of *D. elegans*, *D. fuyamai* which are monomorphic and *D. prolongata* which exhibits male-biased SSD. Wing area decreases under low condition in most species except for *D. elegans* and *D. rufa* and the reduction in wing area is in general stronger compared to thorax.

To examine intra-specific co-variation of the degree of SSD and condition dependence, we looked at the relationship between the absolute value of SDI and CDD within each species among all the traits that we measured(Figure 4.7). We used this approach largely for consistency with some of the previous studies examining the relationship between SSD and condition dependence (Bonduriansky 2007b; Rohner and Blanckenhorn 2018). In general, wing size is the most dimorphic and the most condition dependent trait while the legs are the least dimorphic and the least condition dependent, with the thorax having intermediate degree of SSD and condition dependence. This pattern is broken in *D. elegans*, *D. fuyamai*, *D. prolongata* and *D. rufa*. In most species, females are more condition dependent among most traits.

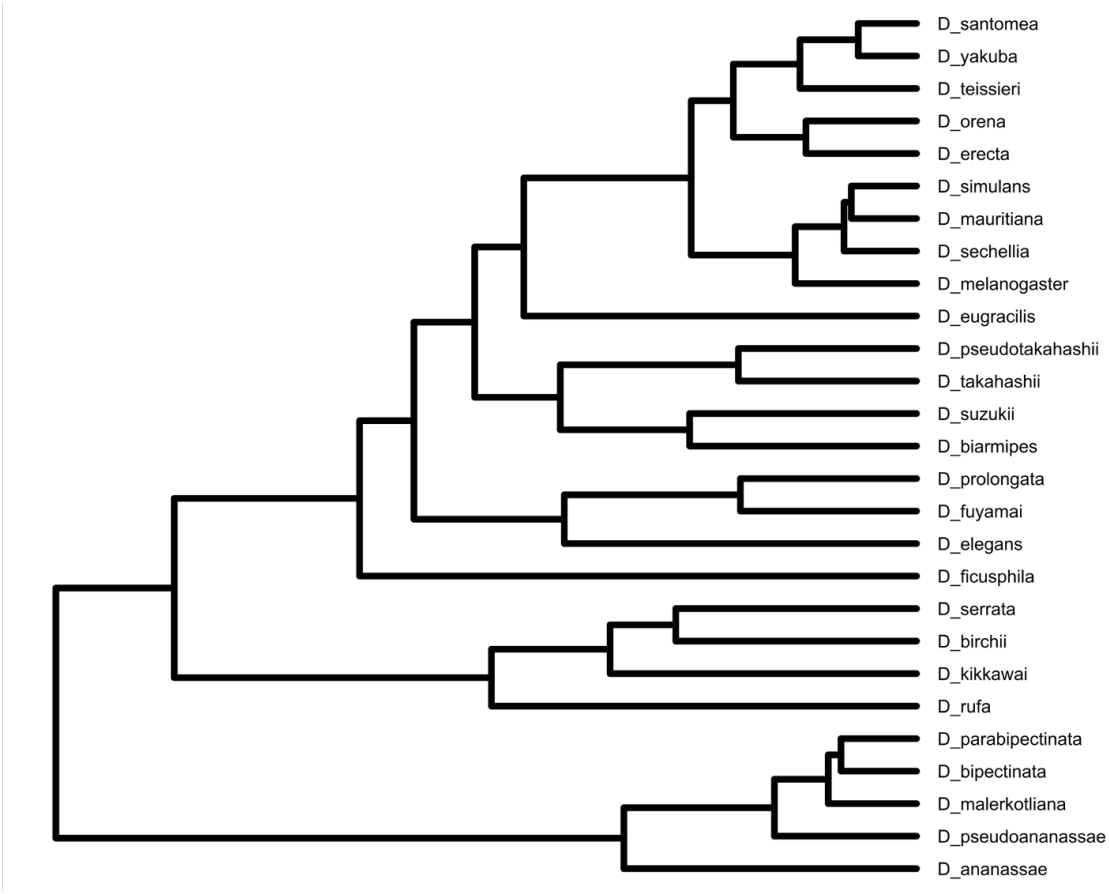

Figure 4.1: Phylogeny of *melanogaster* species group (Suvorov et al. 2021; O'Grady and DeSalle 2018; Linde and Houle 2008; Rohner et al. 2018b)

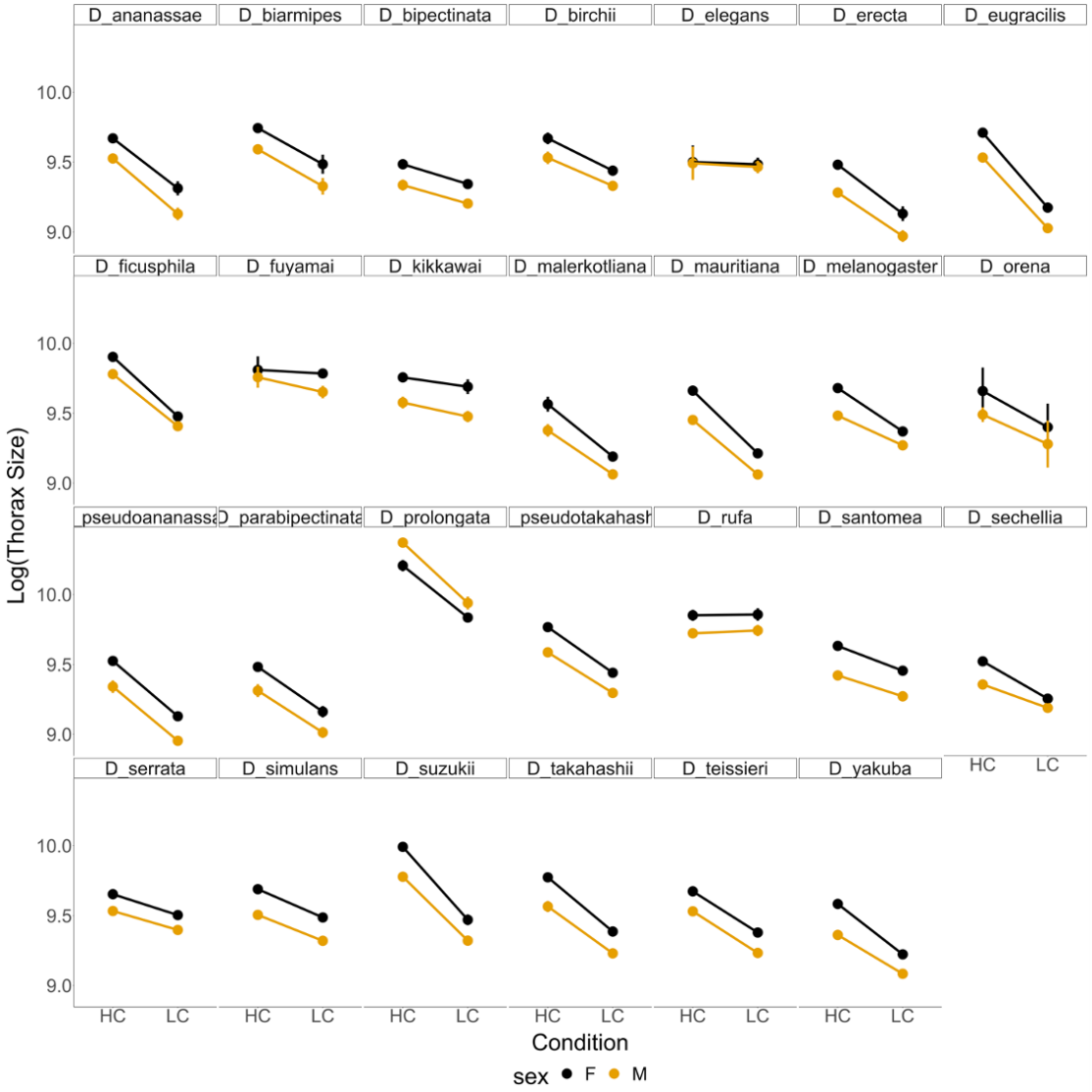

Figure 4.2: Thorax length within species, for males and females at high condition (HC) and low condition (LC). Error bars represent 95% CIs

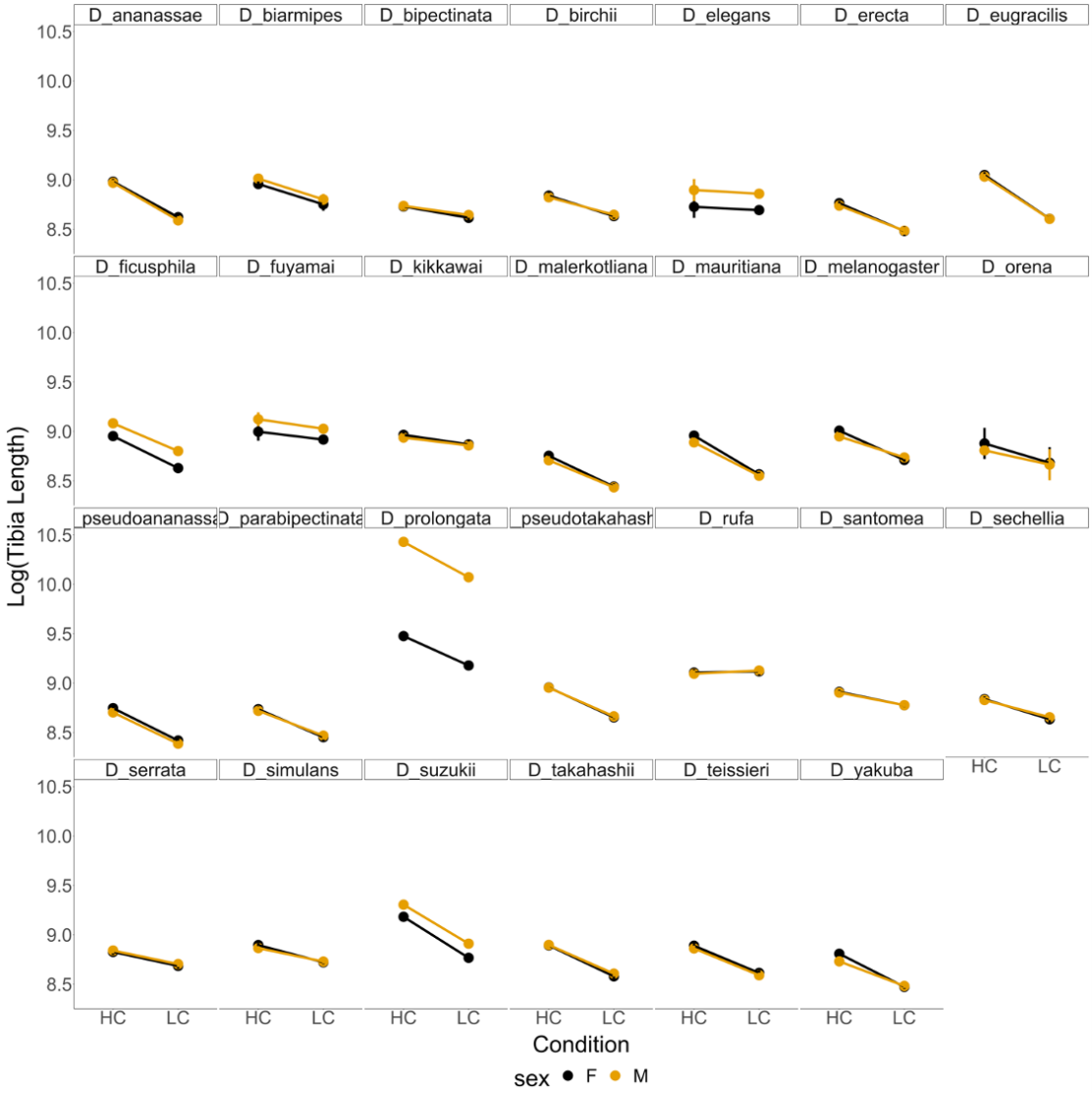

FIGURE 4.3: Tibia length within species, for males and females at high condition (HC) and low condition (LC). Error bars represent 95% CIs

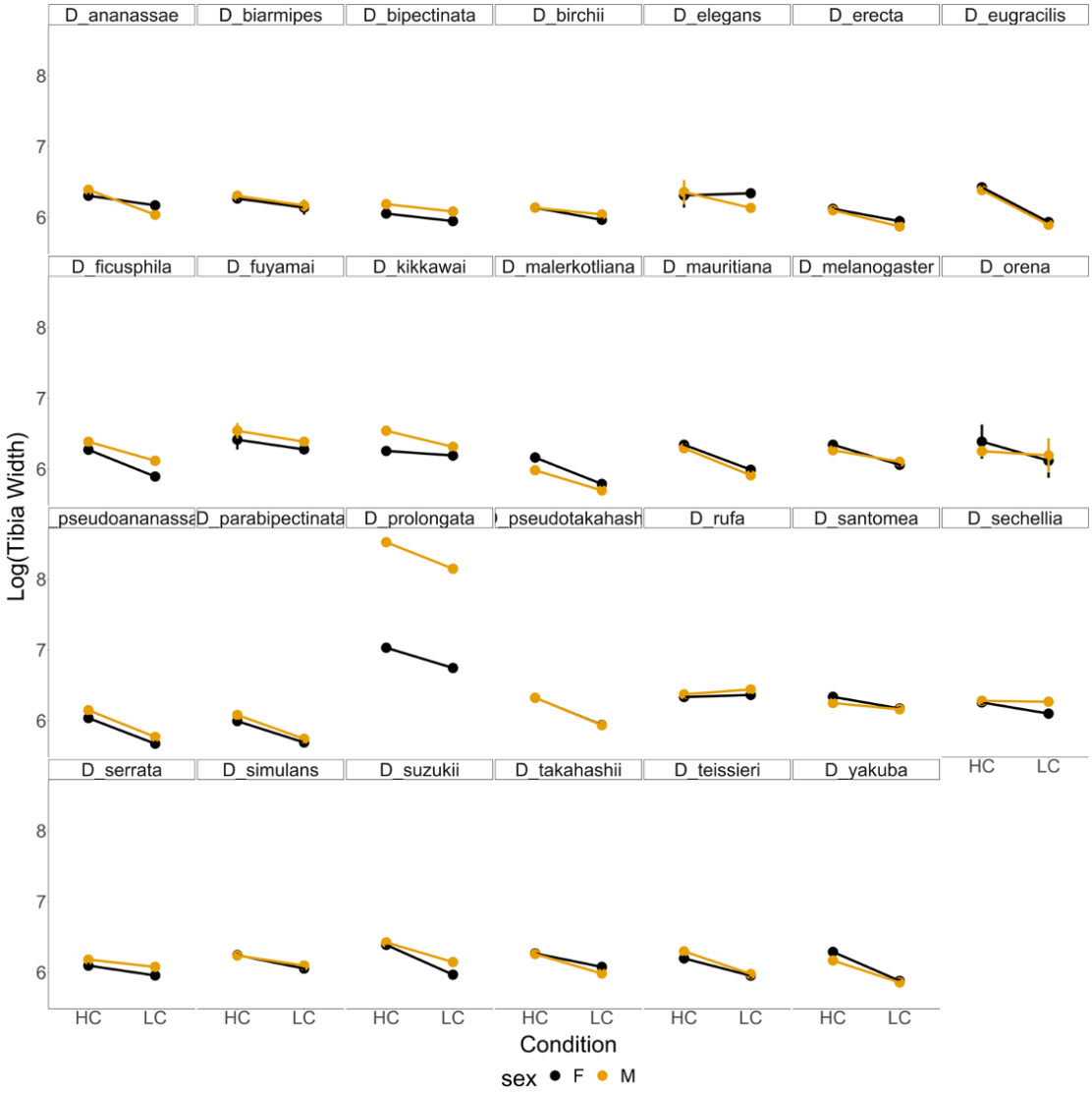

FIGURE 4.4: Tibia width within species, for males and females at high condition (HC) and low condition (LC). Error bars represent 95% CIs

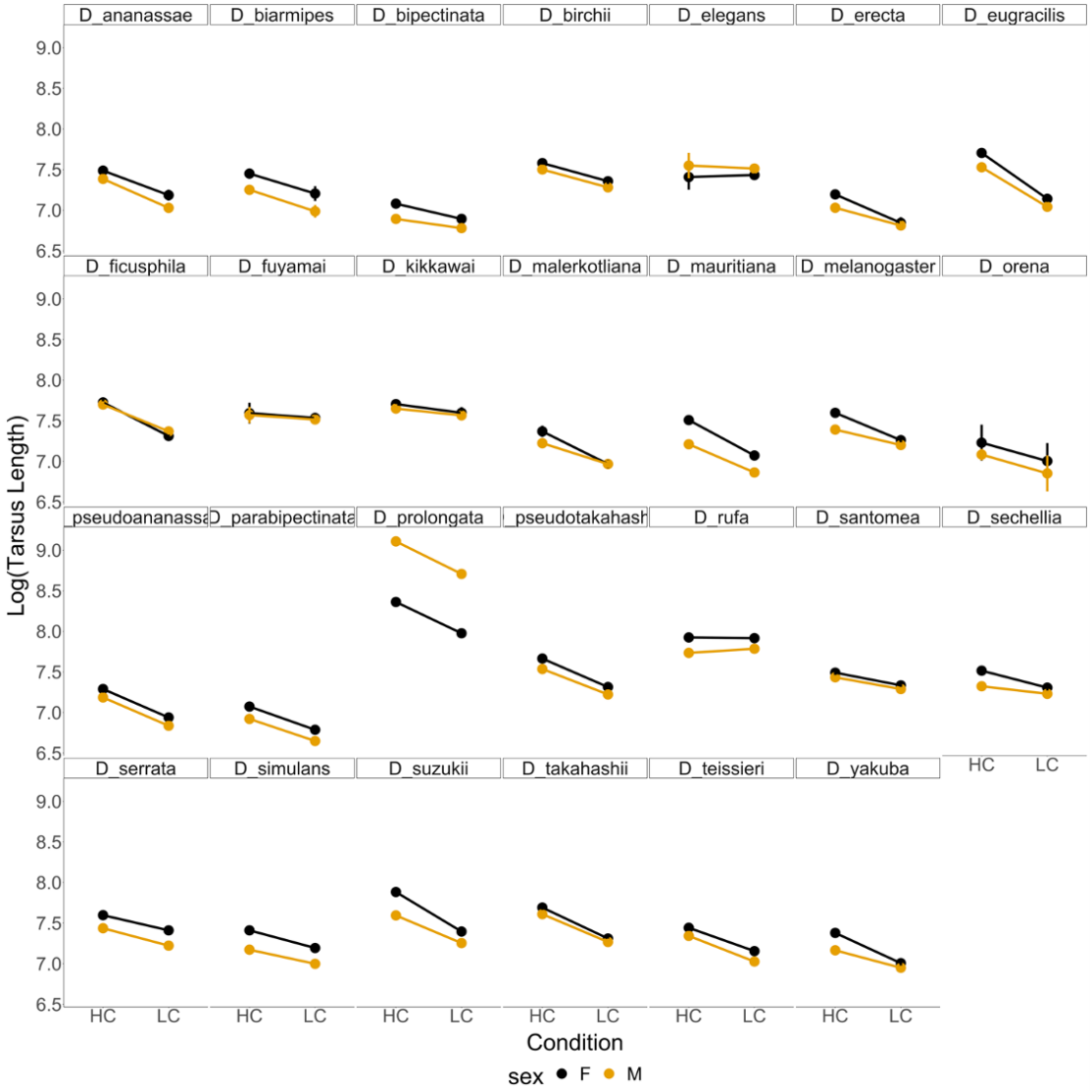

Figure 4.5: Tarsus length within species, for males and females at high condition (HC) and low condition (LC). Error bars represent 95% CIs

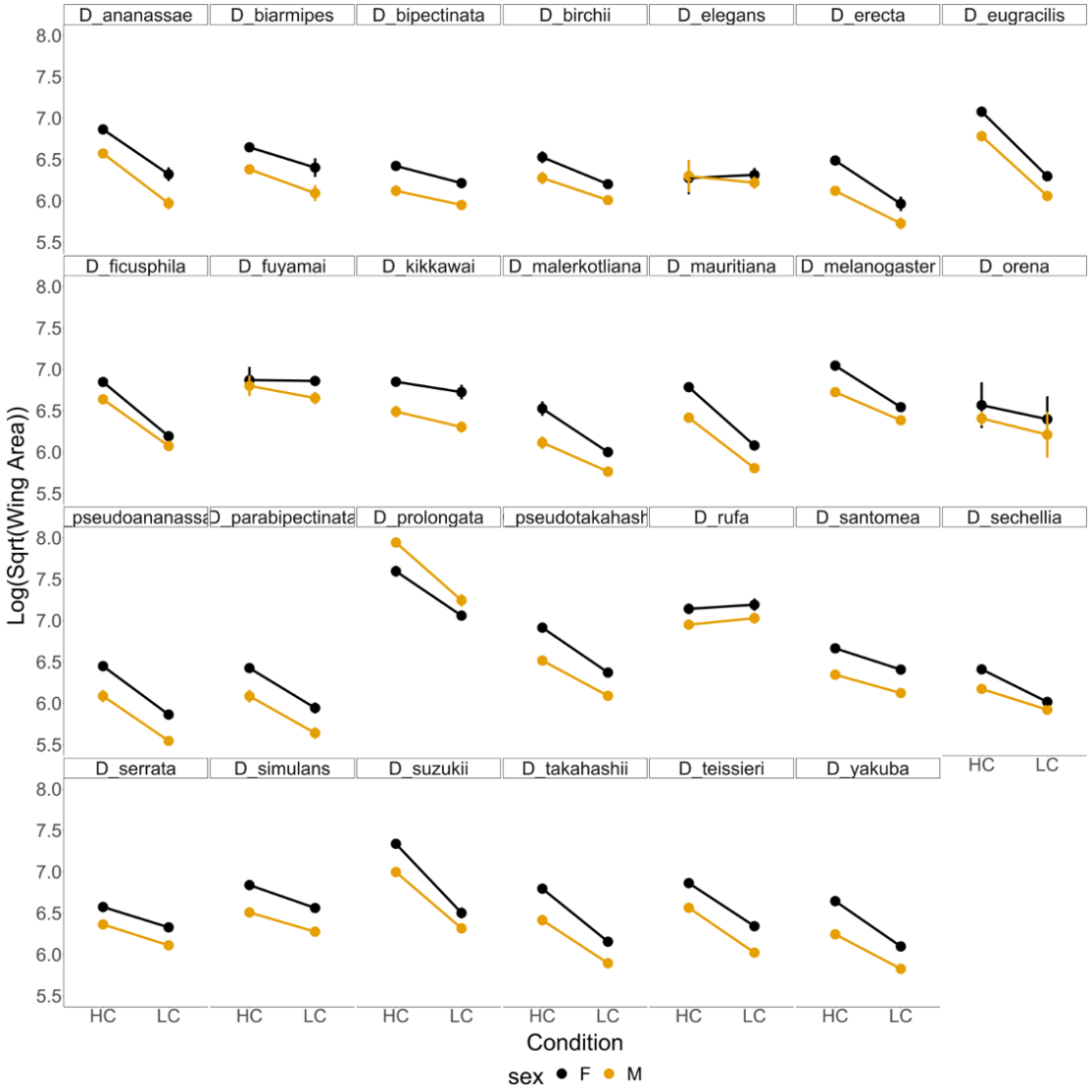

FIGURE 4.6: Wing area within species, for males and females at high condition (HC) and low condition (LC). Error bars represent 95% CIs

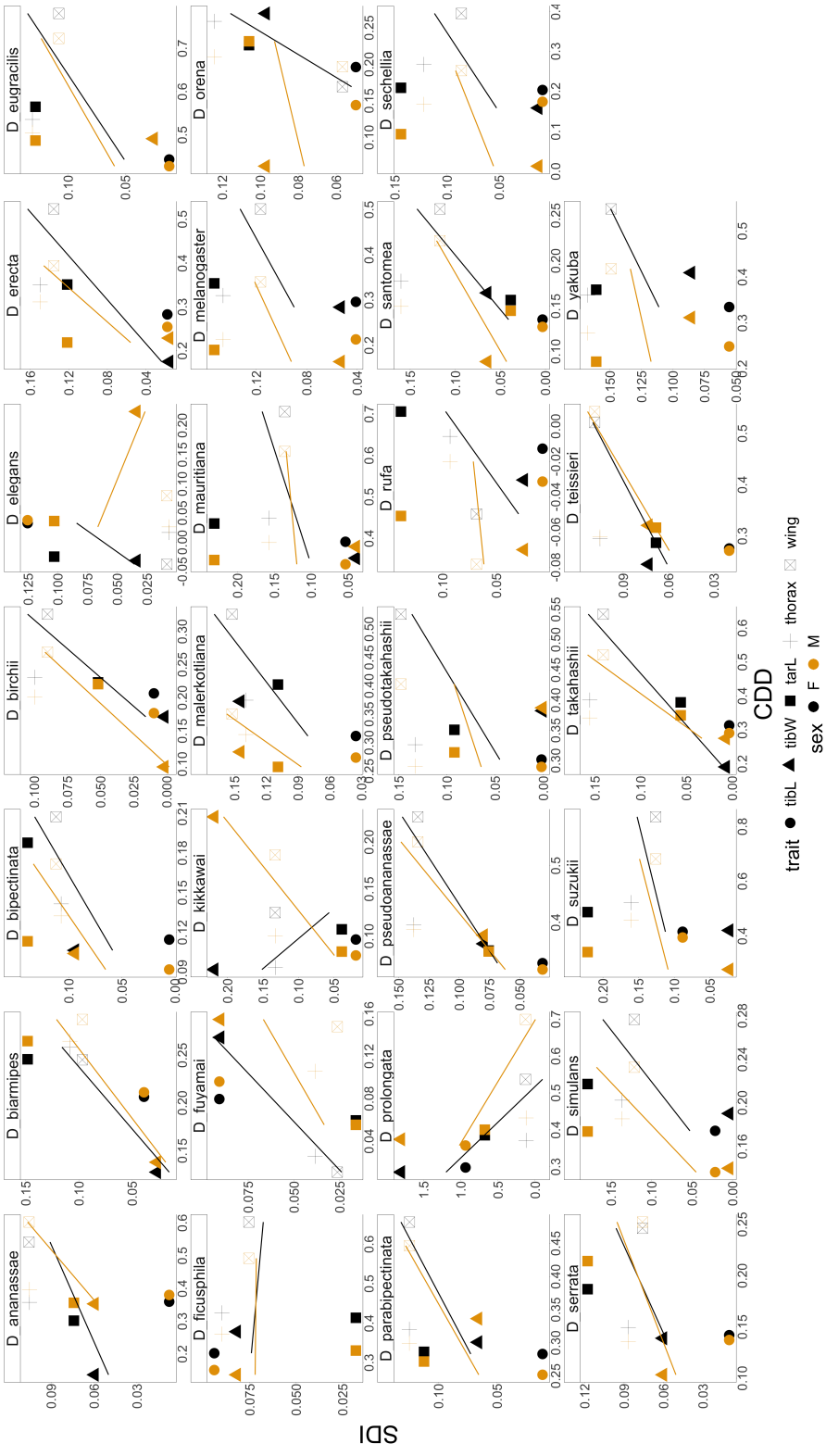

FIGURE 4.7: Intraspecific patterns of covariation between sexual dimorphism and condition dependence among traits. Wing area is generally both the most dimorphic and most condition dependent in most species, while leg segments are generally least dimorphic and condition dependent. For SDI the absolute values were plotted to avoid variation in direction

4.3.2 Inter-specific patterns of sexual dimorphism and condition dependence

In order to evaluate the evolutionary patterns among the traits we measured in the 27 *Drosophila species* (Figure 4.1), we first examined which evolutionary models best describe evolution of each trait at HC and LC within females and males. We also estimated Pagel's λ quantify the phylogenetic signal for each of these traits at different conditions within each sex (Table 4.1 and 4.2). In general, all the models fitted equally well. Leg segments have higher evolutionary rates in males compared to females and in thorax and wing. The evolutionary rate is moderately reduced in some cases at low condition (consistent under all evolutionary models). Most traits had high phylogenetic signal among both treatments and sexes with the exception of wing area in high condition females. SSD of each trait evolves more slowly than the traits themselves. For SSD, phylogenetic signal is detected in most traits at high condition and low condition, except for tibia width. Similarly, evoutionary rates for CDD for each trait within each ses are much slower compared the trait evolutionary rates. We detect relatively low to no phylogenetic signal for CDD among the traits.

From the ancestral reconstruction trees for SSD within each trait, we can see that more closely related species have more similar SSD (Figures 4.8, 4.9, 4.10, 4.11, 4.12,) . For example, the species most closely related to *D. prolongata* have SDI that is closer to monomorphism and/or male-biased SSD dependent on the trait. These patterns hold at both HC and LC.

Condition dependence, on the other hand, had much less evolutionary signal. Although females were in general more condition dependent, condition dependence patterns were very similar between males and females among all the species. In fact, patterns of condition dependence evolution among species were consistent for all traits. The species that exhibited the greatest condition dependence for all traits is *D. eugracilis*. Most of

the other species have intermediate levels of condition dependence. *D. rufa* exhibited the lowest condition dependence (Figures 4.13, 4.14, 4.15, 4.16, 4.17).

For each trait, we tested whether condition dependence and SSD have correlated evolution by fitting a PGLS model with CDD within each sex as the dependent variable and SDI at high condition as the independent variable (Table 4.5). The results from the models confirm that condition dependence and SSD are not associated for any of the traits within each sex.

Table 4.1: Model fit comparisons and evolutionary rates for wing and thorax for Brownian motion (BM), Ornstein – Uhlenbeck (OU) and maximum likelihood Pagel's λ evolutionary models as well as the maximum likelihood Pagel's λ estimates for each trait within each sex (females: F; males: M) at high condition (HC) and low condition (LC)

Cond	sex	$\Delta AICc_{BM}$	$\Delta AICc_{OU}$	$\Delta AICc_{\lambda}$	σ^2_{BM}	σ^2_{OU}	σ^2_{λ}	Pagel's λ
Thorax								
HC	F	0.00	0.23	2.31	2.0×10^{-3}	4.0×10^{-3}	2.0×10^{-3}	0.93
LC	F	2.42	2.61	0.00	3.0×10^{-3}	5.0×10^{-3}	2.0×10^{-3}	0.80
HC	M	0.00	1.09	2.54	3.0×10^{-3}	5.0×10^{-3}	3.0×10^{-3}	1.00
LC	M	0.00	1.56	0.03	4.0×10^{-3}	5.0×10^{-3}	2.0×10^{-3}	0.87
WingA								
HC	F	2.97	0.00	2.88	3.0×10^{-3}	6.0×10^{-3}	1.0×10^{-3}	0.12
LC	F	2.75	0.77	0.00	3.0×10^{-3}	6.0×10^{-3}	1.0×10^{-3}	0.70
HC	M	0.62	0.00	3.16	3.0×10^{-3}	6.0×10^{-3}	3.0×10^{-3}	1.00
LC	M	0.35	0.35	0.00	3.0×10^{-3}	5.0×10^{-3}	2.0×10^{-3}	0.81

Table 4.2: Model fit comparisons and evolutionary rates for leg traits for Brownian motion (BM), Ornstein – Uhlenbeck (OU) and maximum likelihood Pagel's λ evolutionary models as well as the maximum likelihood Pagel's λ estimates for each trait within each sex (females: F; males: M) at high condition (HC) and low condition (LC)

Cond	sex	$\Delta AICc_{BM}$	$\Delta AICc_{OU}$	$\Delta AICc_{\lambda}$	σ^2_{BM}	σ^2_{OU}	σ^2_{λ}	Pagel's λ
				TibL				
HC	F	0.77	0.00	3.31	2.0×10^{-3}	5.0×10^{-3}	2.0×10^{-3}	1.00
LC	F	2.56	1.52	0.00	3.0×10^{-3}	6.0×10^{-3}	2.0×10^{-3}	0.77
HC	M	0.33	0.00	2.88	9.0×10^{-3}	1.7×10^{-2}	9.0×10^{-3}	1.00
LC	M	0.00	0.40	1.57	8.0×10^{-3}	1.4×10^{-2}	6.0×10^{-3}	0.86
				TibW				
HC	F	0.63	0.00	1.83	3.0×10^{-3}	5.0×10^{-3}	2.0×10^{-3}	0.71
LC	F	0.38	0.00	0.04	4.0×10^{-3}	8.0×10^{-3}	3.0×10^{-3}	0.82
HC	M	0.94	0.00	3.49	2.0×10^{-2}	3.0×10^{-2}	2.0×10^{-2}	1.00
LC	M	1.72	0.00	2.17	2.0×10^{-2}	4.1×10^{-2}	1.0×10^{-2}	0.62
				TarL				
HC	F	0.26	0.00	2.63	6.0×10^{-3}	1.0×10^{-2}	4.0×10^{-3}	0.88
LC	F	0.00	1.76	0.92	5.0×10^{-3}	7.0×10^{-3}	4.0×10^{-3}	0.91
HC	M	0.92	0.00	2.60	1.4×10^{-2}	2.7×10^{-2}	8.0×10^{-3}	0.75
LC	M	0.00	0.79	1.36	1.2×10^{-2}	1.9×10^{-2}	9.0×10^{-3}	0.87

Table 4.3: Model fit comparisons and evolutionary rates for Brownian motion (BM), Ornstein – Uhlenbeck (OU) and maximum likelihood Pagel's λ evolutionary models as well as the maximum likelihood Pagel's λ estimates for SSD of each trait at high condition (HC) and low condition (LC)

Cond	$\Delta AICc_{BM}$	$\Delta AICc_{OU}$	$\Delta AICc_{\lambda}$	σ^2_{BM}	σ^2_{OU}	σ^2_{λ}	Pagel's λ
Thorax							
HC	0.00	2.28	2.54	2.00×10^{-4}	2.00×10^{-4}	2.00×10^{-4}	1.00
LC	2.54	0.00	0.52	2.00×10^{-4}	5.00×10^{-4}	5.00×10^{-4}	0.47
TibL							
HC	0.66	0.00	3.20	3.20×10^{-3}	6.20×10^{-3}	3.30×10^{-3}	1.00
LC	0.82	0.00	3.36	2.70×10^{-3}	5.20×10^{-3}	2.70×10^{-3}	1.00
TibW							
HC	2.87	0.00	2.11	1.38×10^{-2}	3.26×10^{-2}	4.60×10^{-3}	0.00
LC	3.31	0.00	1.44	1.15×10^{-2}	2.92×10^{-2}	3.70×10^{-3}	0.00
TarL							
HC	0.48	0.00	2.37	2.50×10^{-3}	4.70×10^{-3}	1.50×10^{-3}	0.78
LC	1.51	0.00	2.48	2.30×10^{-3}	4.80×10^{-3}	1.10×10^{-3}	0.53
WingA							
HC	0.00	1.58	2.01	2.00×10^{-4}	4.00×10^{-4}	2.00×10^{-4}	0.92
LC	2.09	0.00	0.76	2.00×10^{-4}	4.00×10^{-4}	1.00×10^{-4}	0.48

Table 4.4: Model fit comparisons and evolutionary rates for Brownian motion (BM), Ornstein – Uhlenbeck (OU) and maximum likelihood Pagel's λ evolutionary models as well as the maximum likelihood Pagel's λ estimates for condition dependence (CDD) of each trait within each sex (females: F; males: M)

sex	$\Delta AICc_{BM}$	$\Delta AICc_{OU}$	$\Delta AICc_{\lambda}$	σ^2_{BM}	σ^2_{OU}	σ^2_{λ}	Pagel's λ
Thorax							
F	5.56	0.73	0.00	3.00×10^{-3}	1.12×10^{-1}	1.00×10^{-3}	0.42
M	5.08	0.08	0.00	2.00×10^{-3}	9.20×10^{-2}	1.00×10^{-3}	0.27
TibL							
F	9.22	0.22	0.00	2.00×10^{-3}	7.20×10^{-2}	1.00×10^{-3}	0.27
M	6.32	0.00	0.00	2.00×10^{-3}	6.90×10^{-2}	5.00×10^{-4}	0.27
TibW							
F	8.77	0.00	0.00	3.00×10^{-3}	9.70×10^{-2}	1.00×10^{-3}	0.00
M	1.46	0.00	0.00	3.00×10^{-3}	9.50×10^{-2}	1.00×10^{-3}	0.00
TarL							
F	6.10	0.00	0.00	3.00×10^{-3}	1.05×10^{-1}	1.00×10^{-3}	0.00
M	5.70	0.00	0.00	2.00×10^{-3}	8.20×10^{-2}	1.00×10^{-3}	0.00
WingA							
F	6.81	0.00	0.00	8.00×10^{-3}	3.07×10^{-1}	2.00×10^{-3}	0.00
M	8.52	0.00	0.00	6.00×10^{-3}	2.21×10^{-1}	2.00×10^{-3}	0.00

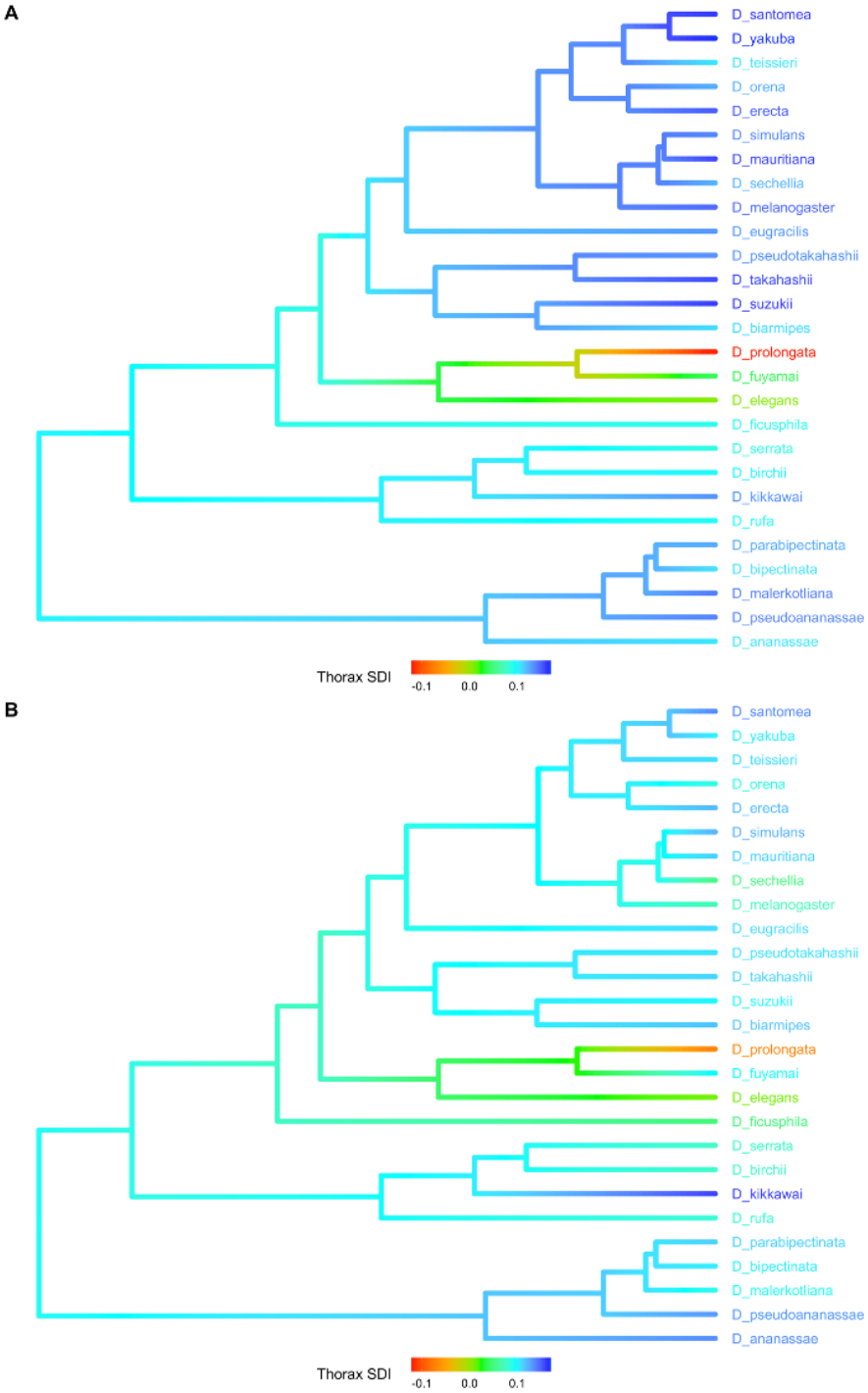

Figure 4.8: Ancestral state reconstruction of SSD at (A) HC and (B) LC for thorax

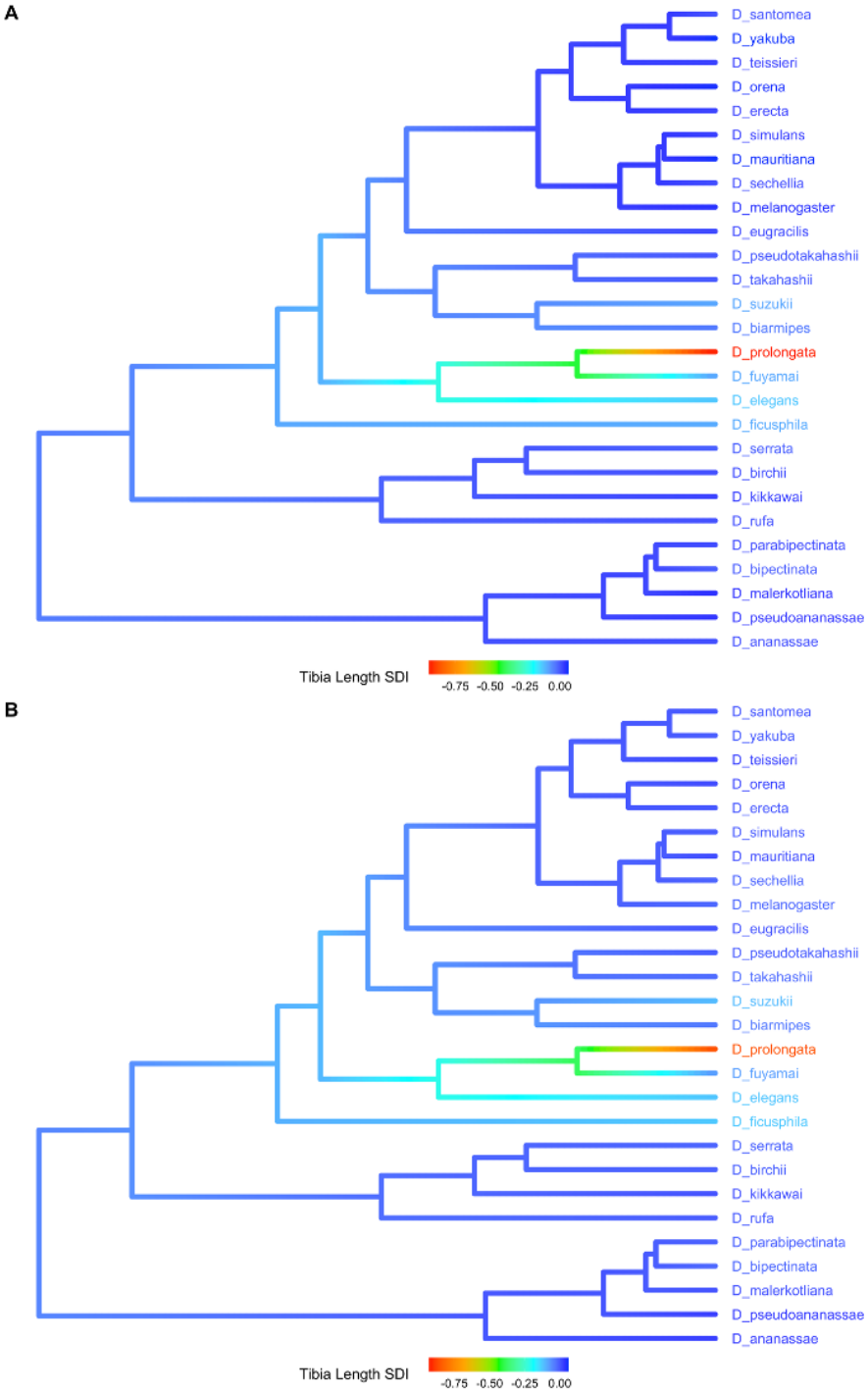

Figure 4.9: Ancestral state reconstruction of SSD at (A) HC and (B) LC for tibia length

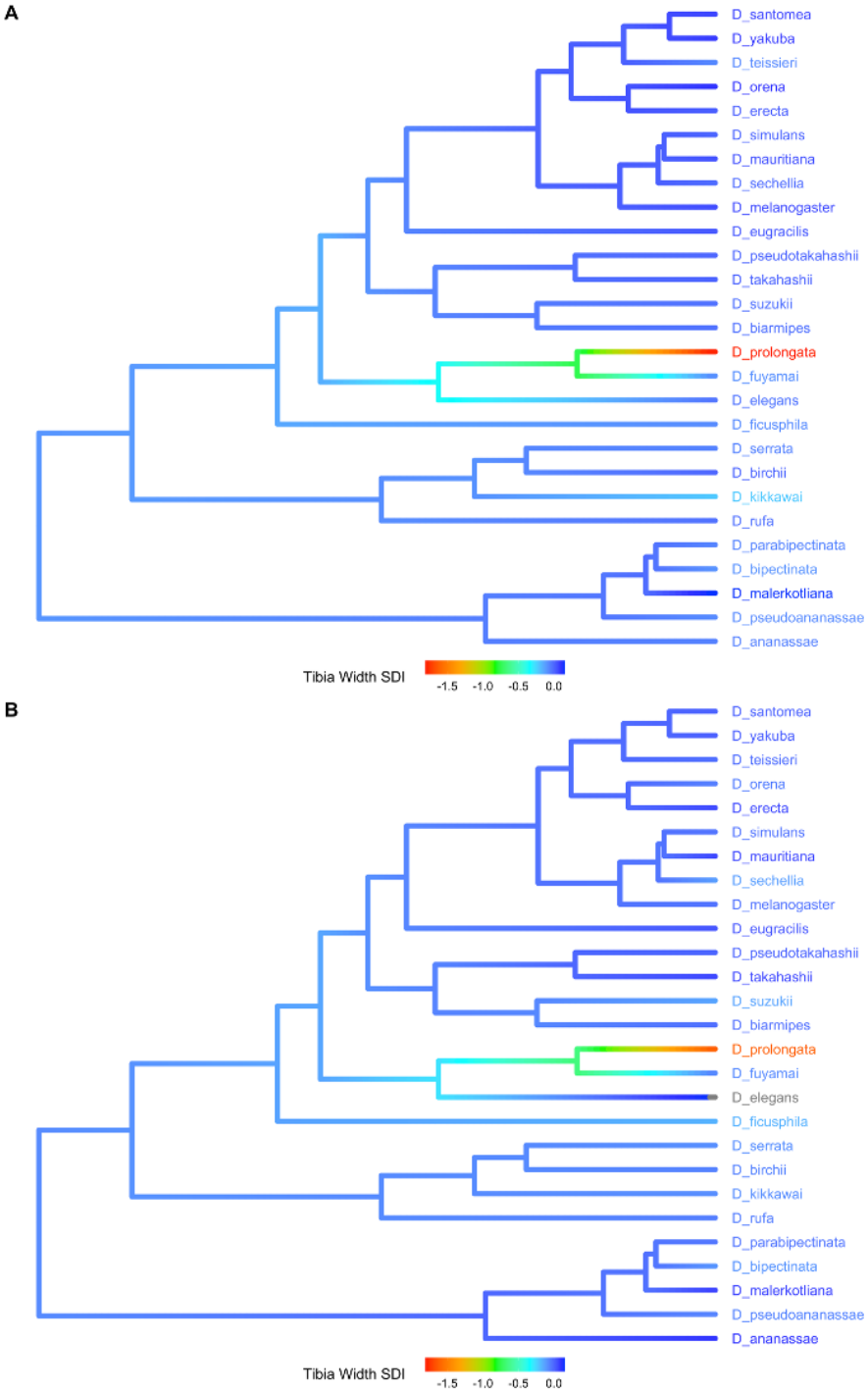

Figure 4.10: Ancestral state reconstruction of SSD at (A) HC and (B) LC for tibia width

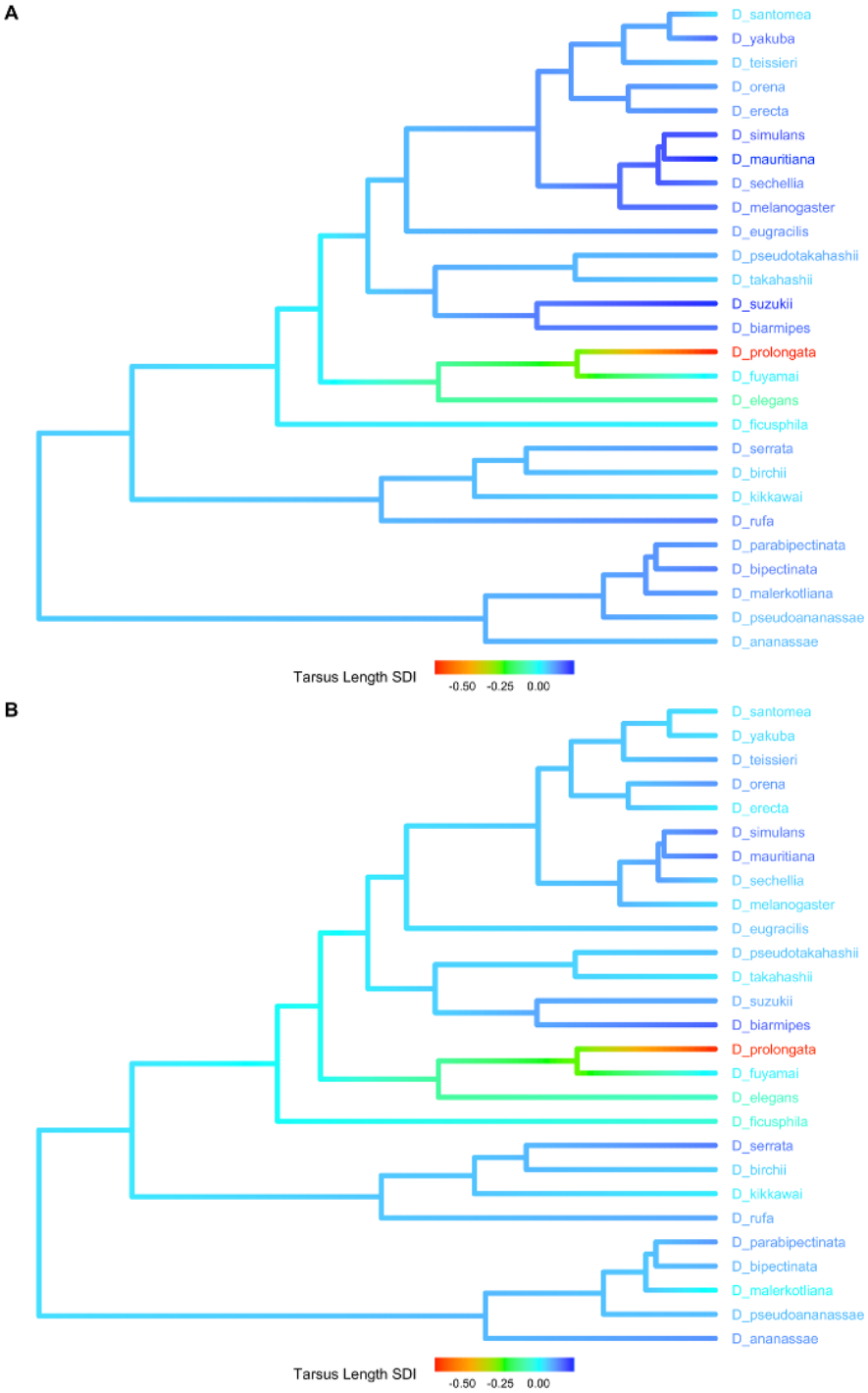

Figure 4.11: Ancestral state reconstruction of SSD at (A) HC and (B) LC for tarsus length

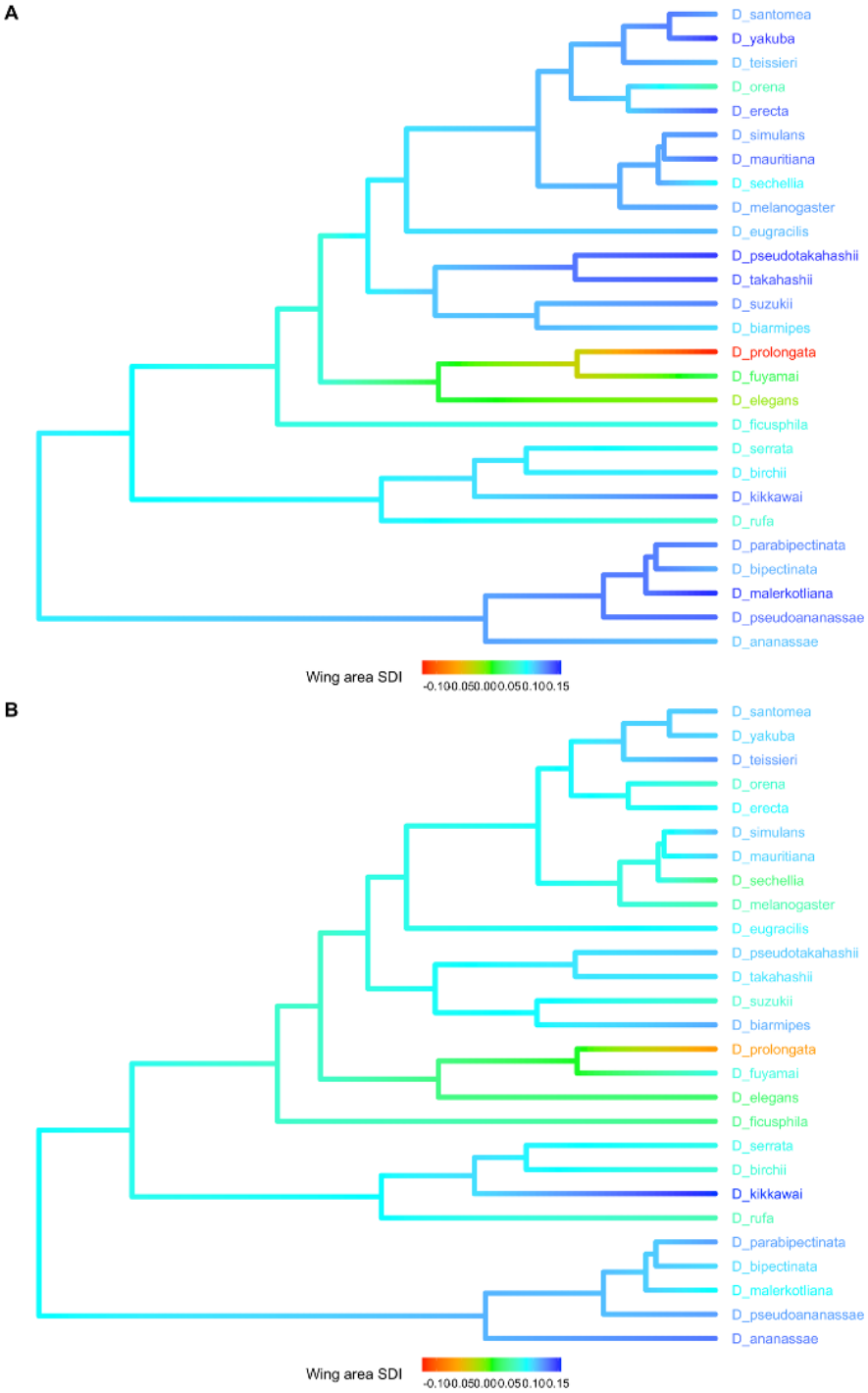

Figure 4.12: Ancestral state reconstruction of SSD at (A) HC and (B) LC for wing area

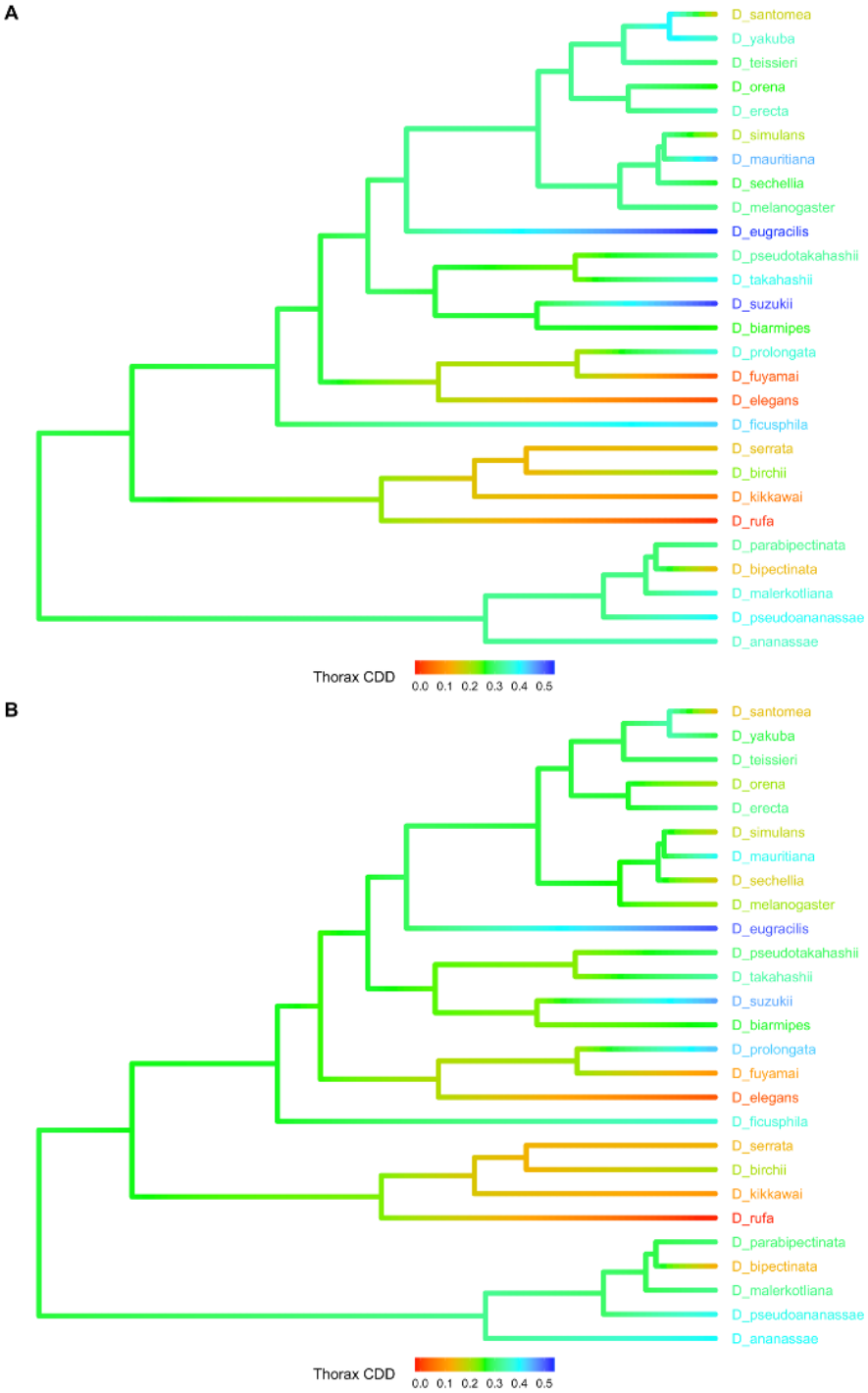

Figure 4.13: Ancestral state reconstruction of CDD in (A)females and (B) males for thorax length

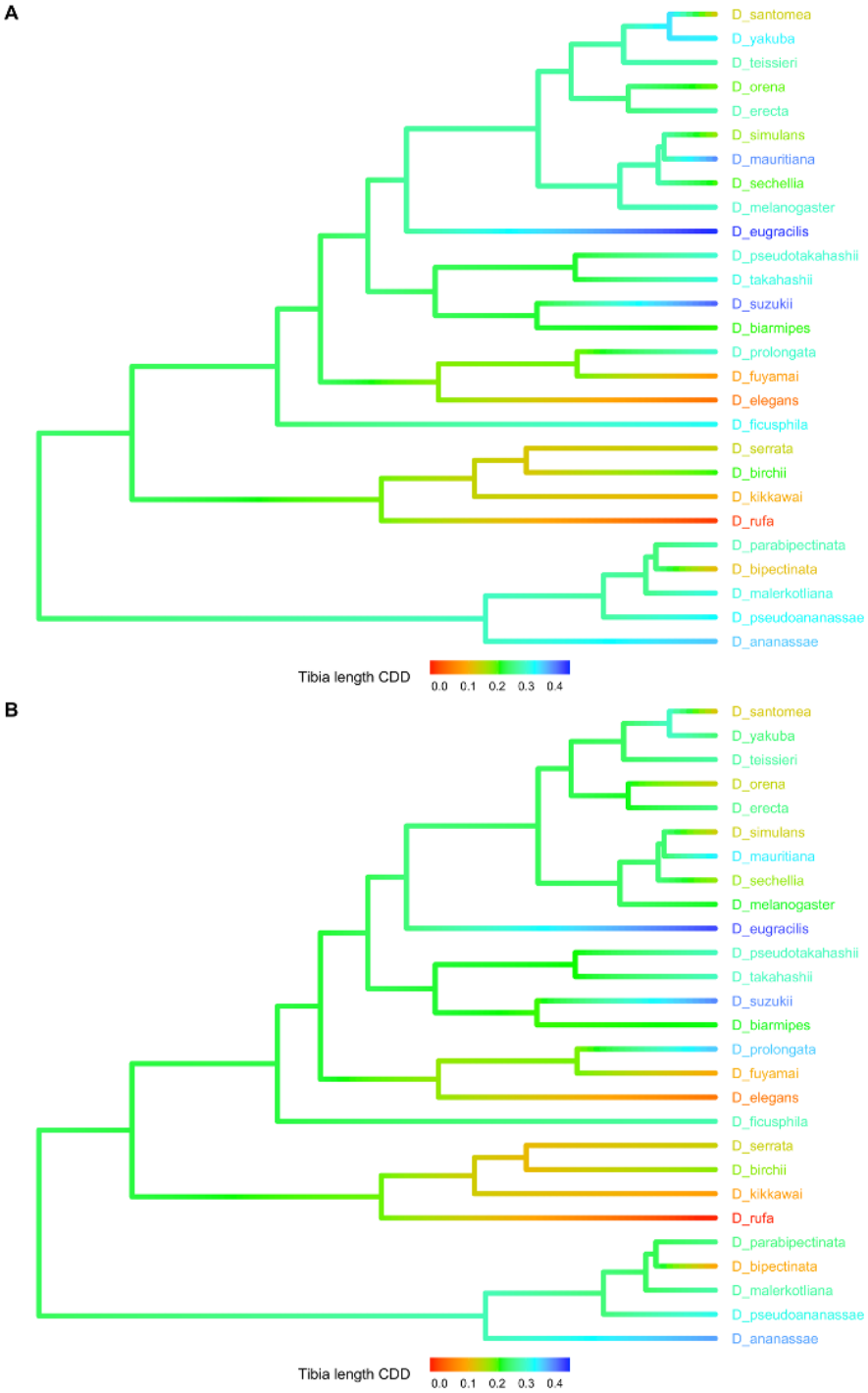

Figure 4.14: Ancestral state reconstruction of CDD in (A) females and (B) males for tibia length

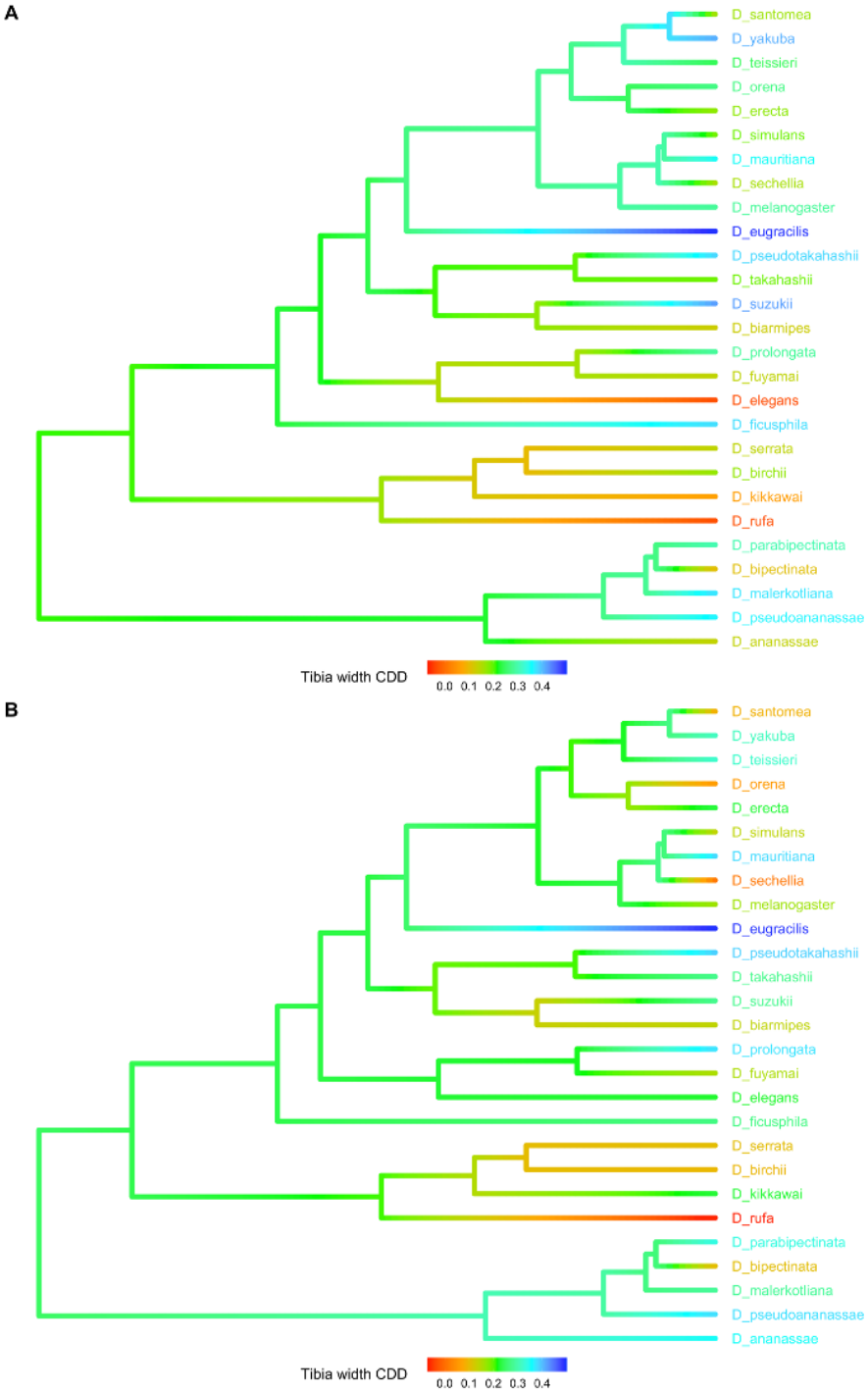

Figure 4.15: Ancestral state reconstruction of CDD in (A) females and (B) males for tibia width

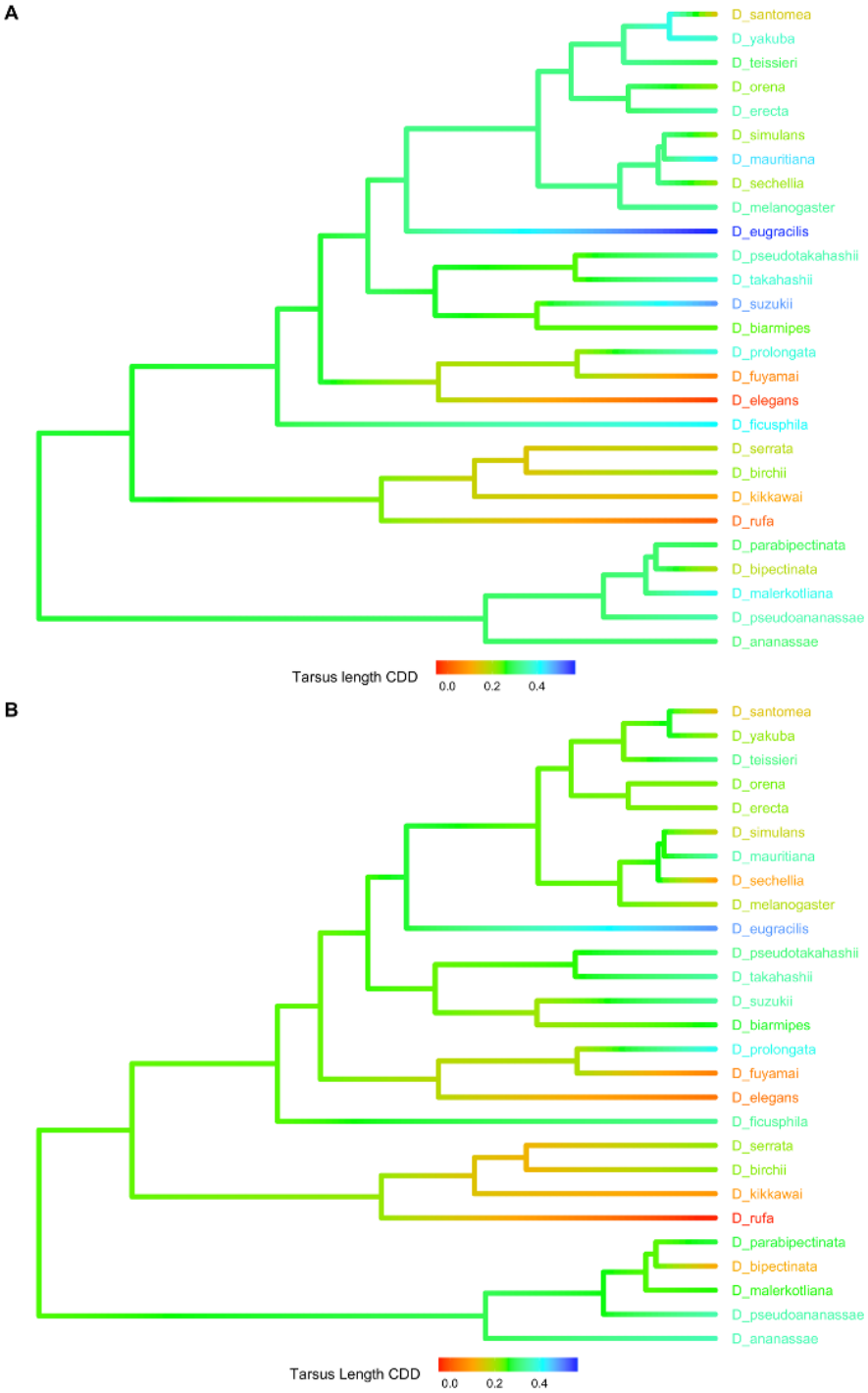

Figure 4.16: Ancestral state reconstruction of CDD in (A) females and (B) males for tarsus length

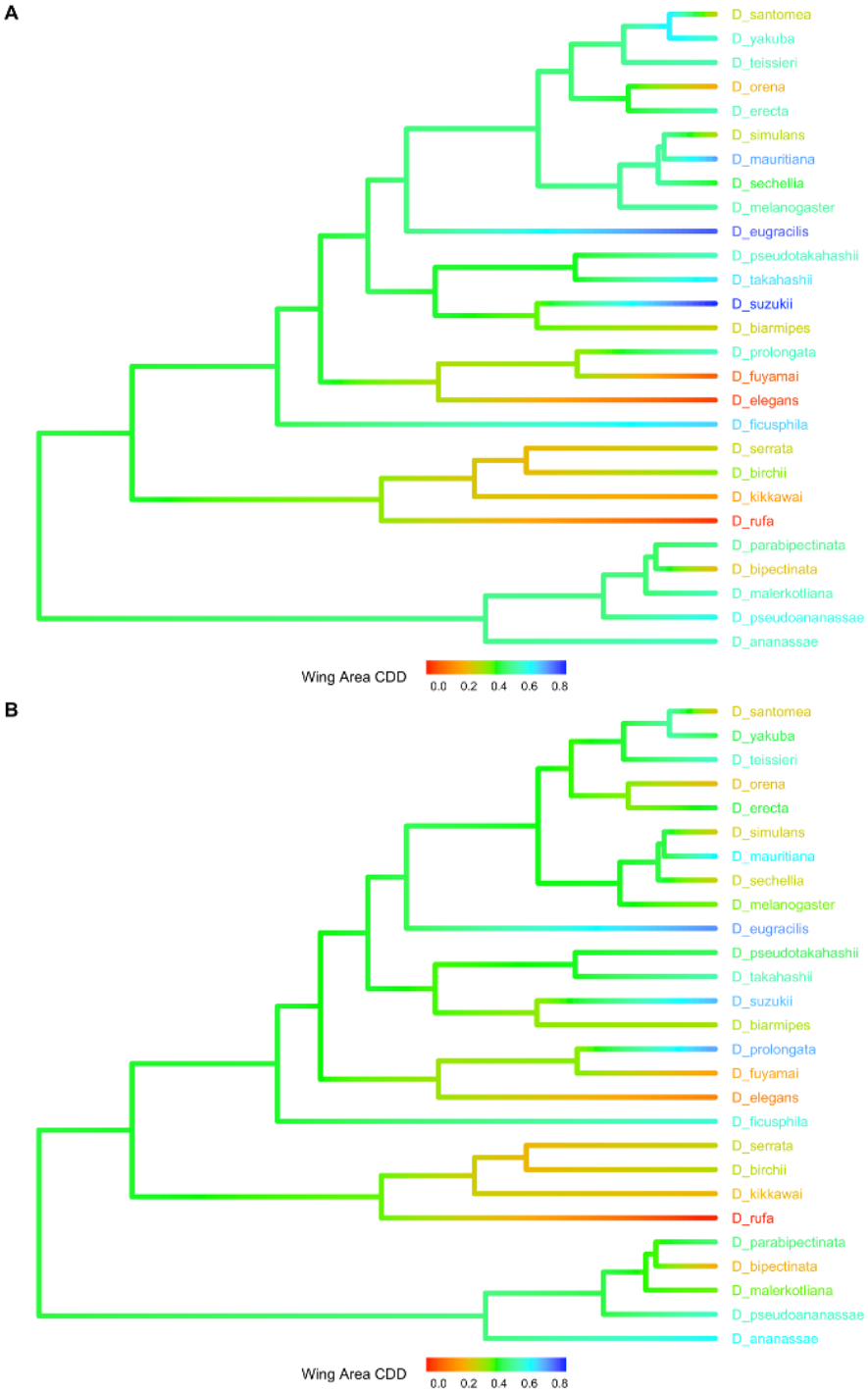

Figure 4.17: Ancestral state reconstruction of CDD in (A) females and (B) males for wing area

Table 4.5: Anova tables for PGLS models for each trait within each sex, with condition dependence as the dependent variable and SSD as the independent variable

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
			Thorax Length Females		
estimate.SSD	1	2.0×10^{-3}	2.0×10^{-3}	2.0	0.15
Residuals	25	2.0×10^{-2}	1.0×10^{-3}		
			Thorax Length Males		
estimate.SSD	1	2.0×10^{-4}	2.0×10^{-4}	0.25	0.62
Residuals	25	1.8×10^{-2}	1.0×10^{-3}		
			Tibia Length Females		
estimate.SSD	1	1.0×10^{-4}	1.0×10^{-4}	0.24	0.63
Residuals	25	1.6×10^{-2}	1.0×10^{-3}		
			Tibia Length Males		
estimate.SSD	1	1.0×10^{-3}	1.0×10^{-3}	1.8	0.19
Residuals	25	1.5×10^{-2}	1.0×10^{-3}		
			Tibia Width Females		
estimate.SSD	1	4.0×10^{-7}	4.0×10^{-7}	5.0×10^{-4}	0.98
Residuals	25	$1.9 \times 10^{-2}0.019$	1.0×10^{-3}		
			Tibia Width Males		
estimate.SSD	1	1.0×10^{-3}	1.0×10^{-3}	1.2	0.27
Residuals	25	$1.7 \times 10^{-2}0.017$	1.0×10^{-3}		
			Tarsus Length Females		
estimate.SSD	1	5.0×10^{-8}	4.8×10^{-8}	4.0×10^{-4}	0.98
Residuals	25	3.0×10^{-3}	1.0×10^{-4}		
			Tarsus Length Males		
estimate.SSD	1	9.0×10^{-4}	9.0×10^{-4}	1.2	0.28
Residuals	25	1.8×10^{-2}	1.0×10^{-3}		
			Wing area Females		
estimate.SSD	1	7.0×10^{-3}	7.0×10^{-3}	3.1	0.09
Residuals	25	5.3×10^{-2}	2.0×10^{-3}		
			Wing area Males		
estimate.SSD	1	2.0×10^{-4}	2.0×10^{-4}	0.12	0.73
Residuals	25	4.3×10^{-2}	2.0×10^{-3}		

4.4 Discussion

The main goal of this study was to examine the evolutionary patterns of SSD and condition dependence in the *melanogaster* species group and to see whether these two major sources of phenotypic variation demonstrate correlated evolution as it is predicted by the different condition dependence hypotheses. In order to achieve this goal we examined both intra-specific and inter-specific patterns of correlation of SSD and condition dependence. In general, within each species, we observed that the traits with greater degree of SSD exhibit greater degree of condition dependence. However, this pattern was not universal and was violated in the species in which we expected this pattern to hold the most, *D. prolongata*. According to the different condition dependence hypotheses, the greater degree of SSD should be correlated with greater degree of condition dependence, especially in organisms with male-biased SSD. However, in *D. prolongata*, the only species with very exaggerated male-biased SSD, particularly in the frontal legs, we saw the greatest degree of condition dependence in more intermediate traits, such as the wing, while the legs exhibited moderate condition dependence. The males of these species did have a greater degree of condition dependence, which suggests that, generally, the larger sex tends to be more sensitive to condition manipulation. Since the most exaggerated traits, tibia length and width, were not the most condition dependent (predicted by condition dependence theory) (Bonduriansky 2007c; Bonduriansky 2007b; Stillwell et al. 2010; Cotton et al. 2004b; Rowe and Houle 1996), our results suggest that for this species the adaptive canalization hypothesis may provide a better explanation (Fairbairn 2005). However, this hypothesis needs to be tested more explicitly.

D. prolongata is considered to be an "evolutionary singularity" because it is so drastically different compared to its close relatives. However, species that are more closely related to *D. prolongata*, *D. fuyamai*, *D. elegans* and *D. ficusphila*, all have male-biased SSD in tibia length, while the other species are nearly monomorphic. Male-biased SSD

in *D. prolongata* may not be an evolutionary singularity after all, and this species may have used existing genetic and phenotypic variation in order to evolve to exaggerated proportions.

In most species, leg segment sizes tend to be monomorphic even in species with overall female-biased SSD. Sexual selection may operate differently among the different traits within each species and its interaction with other selective forces can cause different allometric relationships for the different traits, particularly in the legs. This highlights the importance of measuring multiple traits when studying sexual dimorphism, and cautions us against generalizing patterns of sexual dimorphism from single trait measurements among species or from combining measurements of different traits in meta-analyses.

The inter-specific patterns of evolution for SSD and condition dependence were more complex. For most traits we saw relatively strong phylogenetic signal for SSD at both HC and LC, with the exception of tibia width. Condition dependence, on the other hand, had much lower phylogenetic signal and seem to have more random evolutionary patterns. We did not observe correlated evolution between SSD and condition dependence in any traits. This suggests that inter-specifically we do not see correlated evolution of SSD and condition dependence in the *melanogaster* species subgroup.

Although this study is among the first to test the evolution of condition dependence and sexual dimorphism in a comparative context it does have some caveats. The way that the condition manipulation was performed is not optimal. We originally planned to expose the different species to a series of diluted food treatments during development as a way to alter condition and to make sure the treatment affects each species appropriately. However, even lowest dilution food treatments had too weak an effect to allow us to effectively study condition dependence. We decided to perform the staggered starvation experiment instead. However, starvation can affect stress pathways and can

have unwanted effects on phenotype although it provides a consistent way to affect condition. With the starvation treatments we did see strong enough effect that allowed us to test the condition dependence hypotheses. For most of the species we had only 1-2 strains which limits our ability to make more general conclusions about both intra- and inter-specific patterns. For a comparative study, although we had over 3000 specimens, we had a total of 27 species which may be considered a relatively low number and this may in fact influence the estimates we got from the models.

We also aimed to examine wing shape and SShD in a similar context, but due to unforeseen circumstances were not able to complete the data acquisition. I would be very interested to see whether wing shape and SShD have similar patterns as wing size and SSD for wing size, and whether SShD and condition dependence have more correlated evolution than SSD and condition dependence. In the future, we can also use the measurements of the intermediate cohorts in the analysis in order to examine a more continuous decline in condition among each species. We will use alternative tools that allow us to perform PGLS by considering both intra and inter-specific variation.

In conclusion, although we observe intra-specific correlation of condition dependence and SSD in species within the *melanogaster* species group that have primarily female-biased SSD, we observe no inter-specific co-evolution of SSD and condition dependence. In the future it would be interesting to examine the proximate causes of these effects by exploring what are the developmental mechanisms that drive the intra-specific patterns. Examining the evolutionary patterns in more taxonomic groups would also be very helpful as it will provide further tests of the condition dependence hypotheses of sexual dimorphism.

Chapter 5

Conclusion

The main theme of this thesis is the influence of environmental variation on phenotypic variation. I addressed this theme in three different contexts: (1) canalization, (2) micro-evolutionary patterns of sexual dimorphism and (3) macro-evolutionary patterns of sexual dimorphism. In the first study (Chapter 2), I examined the effect of environmental variation in the context of canalization. I studied two populations of *D. melanogaster*, one of which has undergone genetic decanalizaiton as a result of strong directional selection for greater size. The main goal of this study was to examine whether the genetically decanalized population has increased phenotypic variability as a result of variable environment. I examined environmental canalization at three different levels: (1) within-individual variation represented by fluctuating asymmetry, (2) within-line variation represented by variation among individuals within each strain, and (3) differences in reaction norms for temperature. The first two levels are used to quantify micro-environmental canalization while the third is used to quantify macro-environmental canalization. Despite the increased expression of genetic mutations in the genetically decanalized population, we found no differences in environmental canalization at any level in the two populations. We concluded that genetic and environmental canalization may be uncorrelated and that different mechanisms facilitate these two processes. This study is one of the few studies that directly tests the congruence hypothesis

of environmental and genetic canalization, and it is the only one that tests it in natural populations with naturally occurring mutations where we have direct measurement of genetic decanalization. The literature offers mixed support for the congruence hypothesis; this study does not support it. This study examines a single case of naturally occurring genetic decanalization; to further establish the generality of these results, similar studies in more populations and/or taxa are required. Examining the genomic architecture of these populations can give us some insights into the mechanisms that facilitated the breakdown of genetic canalization in the high-altitude population.

In the second study (Chapter 3), the main goal was to examine how strong adaptive evolution affects the interplay between sexual dimorphism and condition dependence. We used the same two altitudinally diverged populations from Chapter 2 and examined phenotypic plasticity due to diet and temperature manipulation of wing size and shape within each sex, and the response of SSD and SShD within and between the two populations to each treatment. We also examined the allometric vectors for wing shape and how they differ as a result of sex, adaptive differences and plasticity. Finally, we attempted to partition the allometric and non-allometric portions of SShD within each population and treatment. Although both SSD and SShD decreased as a result of poor quality diet within each population, there were no differences in condition dependence of sexual dimorphism between the two populations. Sex, adaptive divergence and diet had small to moderate effects in allometric vectors of wing shape, while temperature had relatively larger effects. Partitioning SShD into its allometric and non-allometric components was challenging because our data violated the assumption of common allometry between the sexes. We concluded that both allometric and non-allometric components a considerable portion to total SShD. This study highlights the importance of examining SSD and SShD and wing shape allometry in the context of plasticity and adaptive divergence because (1) the results suggest that despite the drastic phenotypic changes of wing size and shape due to adaptation to high-altitude, SSD, SShD and their condition

dependence may be conserved; (2) although the adaptive changes in wing size in the high-altitude population are substantial, size-shape allometry for the wing is more influenced by the plastic response to environmental factors such as temperature; and finally (3) although it may be important to understand the contribution of allometric and non-allometric portions of SShD to total SShD, more robust methods are necessary to do this effectively. Extending this experiment to measure multiple traits would provide us with more information about the overall static allometry rather than just for the shape-size allometry of the wing. Since we saw drastic differences in condition dependence of sexual dimorphism in larval weight, examining the developmental mechanisms that facilitate the phenotypic variation in the adults due to the adaptive and plastic response would be informative. We also performed metabolic assays to measure the macro-nutrient content of larva raised at the different diet treatments in order to have a more robust measure of condition. However, the metabolic assays that we used were not sensitive enough to detect the subtle differences in our very small larval samples. If this experiment is repeated in the future, my recommendation is to collect much larger larval samples in order to allow for greater sensitivity of the assays.

In the third study (Chapter 4), the main goal was to examine the evolutionary patterns of sexual dimorphism and condition dependence and to test whether there is correlated evolution of these two main sources of phenotypic variation among species from the *melanogaster* species subgroup. We manipulated condition in 27 different species from this group by limiting nutrition at different duration during development and then measured the thorax, frontal leg segments and wing area. We examined the intra-specific patterns of sexual dimorphism and condition dependence by examining their correlation among the different traits within each species. We examined the inter-specific evolutionary patterns of sexual dimorphism and condition dependence by modeling their evolution under different evolutionary models, estimating their evolutionary rates and phylogenetic

signal. The results suggest that at the intra-specific level sexual dimorphism and condition dependence are correlated, with some notable exceptions. However, at the inter-specific level sexual dimorphism and condition dependence seem to evolve more or less independently. Surprisingly, we did not observe the predicted correlation of sexual dimorphism and condition dependence in *D. prolongata*, the only species with male-biased SSD and exaggerated leg size, which exhibited the greatest condition dependence in the wing (relatively moderate SSD) rather than the leg (relatively high male-biased SSD). The results from this study demonstrate that: (1) we cannot assume all traits exhibit the same degree and direction of sexual dimorphism and degree of condition dependence within and among species and we need to be cautious when presenting data from multiple traits; (2) the leg within males has the highest evolutionary rate; (3) species more closely related to *D. prolongata* tend to have traits that are either monomorphic or male-biased, suggesting that the evolution of male-biased SSD in *D. prolongata* may not be an evolutionary singularity and may have been facilitated by preexisting phentotypic and genotypic variation in that direction; (4) unlike many comparative studies, especially in vertebrates, where data is often collected from limited amount of specimens within a species, this study demonstrates that both intra- and inter-specific variation are important, especially when it comes to condition dependence and plasticity; (5) contrary to theoretical predictions, sexual dimorphism and condition dependence do not seem to co-evolve at the inter-specific level, at least in the *melanogaster* species group. Of the studies presented here, this study is furthest from completion. We originally planned to include wing shape measurements and measurements of cell density across the wing. However, due to both unforeseen complications in experimental design as well as restrictions due to the global pandemic, this data was not collected in time for the defence of this thesis. We plan to have this data collected for the publication of this study. In the future, examining species in other taxonomic groups, especially ones in which there are more variable magnitudes and directions of SSD would be allow us to

fully test the predictions of the condition dependence hypotheses and to examine the relationship between sexual dimorphism and and condition dependence.

Appendix A

Chapter 2 Supplement

Supplemental Figures

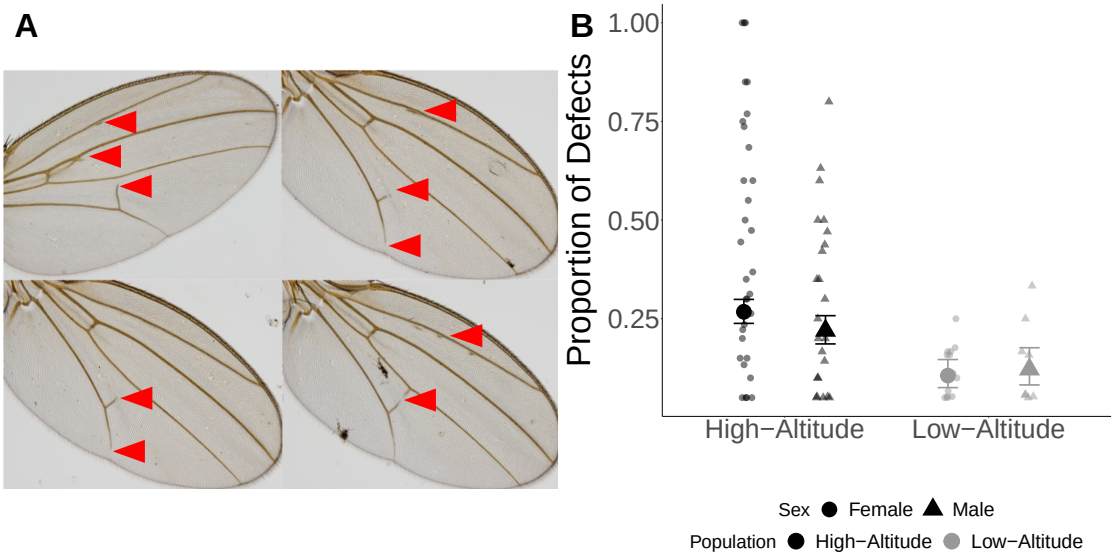

FIGURE A-S1 : High-altitude population has a higher frequency of wing defects compared to the low-altitude population. (A) Examples of wing defects seen in the high-altitude population (red arrowheads pointing to venation defects). Venation defects represented include additional longitudinal veins (top left) or small pieces of vein material (top left and top and bottom right), incomplete posterior cross vein (all 4 wings) and incomplete L5 vein (bottom left) (B) Proportion of wing venation defects within lines is much greater in the high-altitude population than the low-altitude population. Error bars represent 95% CIs.

Figure A-S2 : Within-line measures of variability for wing shape are not correlated with proportion of wing defects (TV - Total variance: r = 0.16 95% CI −0.26 - 0.53;Ecc - Eccentricity: r = −0.24 95% CI −0.59 - 0.18) or the low-altitude population (Total variance: r = 0.34 95% CI −0.24 - 0.74; eccentricity: r = −0.33 95% CI −0.73 - 0.24)

Figure A-S3 : Within-line wing size variation measured as Levene's Deviates. (A) Consistent with the results using the coefficient of variation, Levene's deviates are similar for the high- and low-altitude populations. Error bars represent 95% CIs. (B) The two measures of within-line variation, Levene's Deviates and CV, are highly correlated (high-altitude r = 0.89 95% CIs 0.80 - 0.94; low-altitude r = 0.98 95% CIs 0.94 - 0.99)

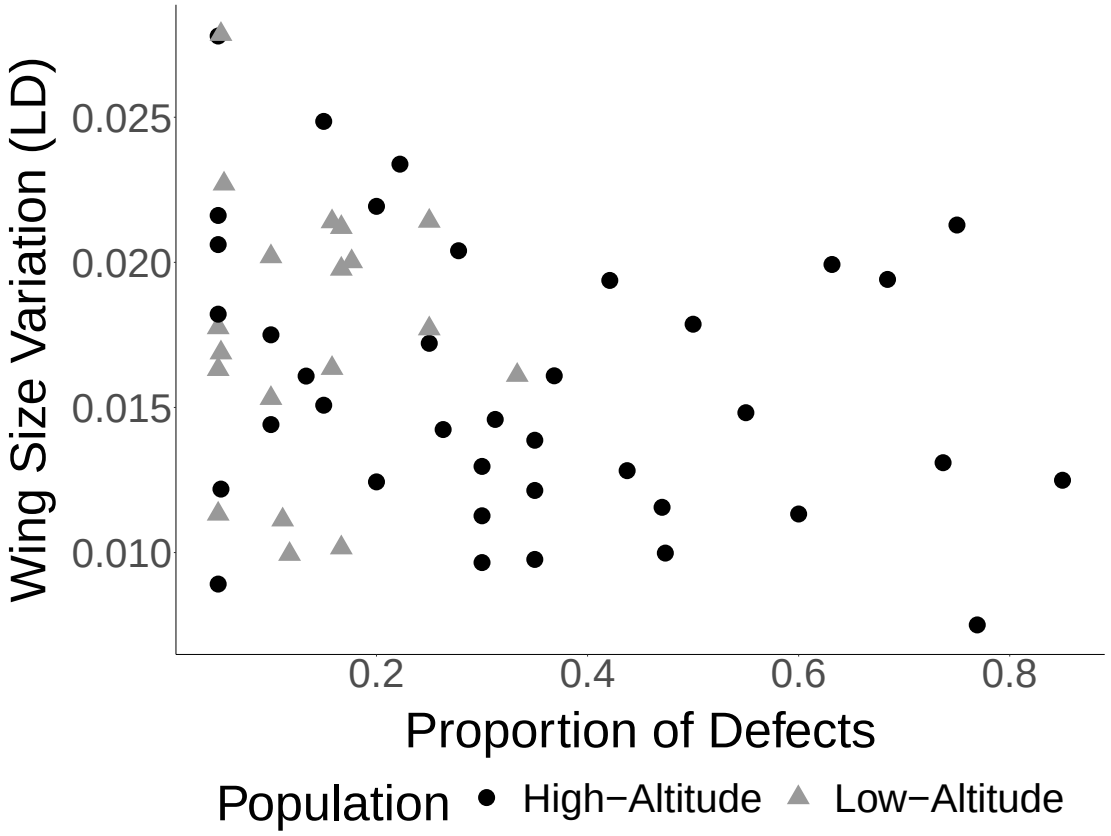

Figure A-S4 : Within-line variation for wing size measured as Levene's deviates are not correlated with within-line proportion of defects in either the high-altitude population(r = −0.27 95% CIs −0.54 - 0.055) and the low-altitude population (r = −0.0032 95% CIs −0.46 - 0.45).

Figure A-S5 : Within-line measures of variability for wing shape measured as wing shape integration using the relative standard deviation of the eigenvalues (rSDE - multiplied by 10000 and rSDE2) are not correlated with proportion of wing defects (rSDE: r = 7.6×10^{-3} 95% CI −0.40 - 0.41; rSDE2: r = −0.29 95% CI −0.62 - 0.12) or the low-altitude population (rSDE: r = 0.12 95% CI −0.44 - 0.61; rSDE2: r = −0.17 95% CI −0.65 - 0.39)

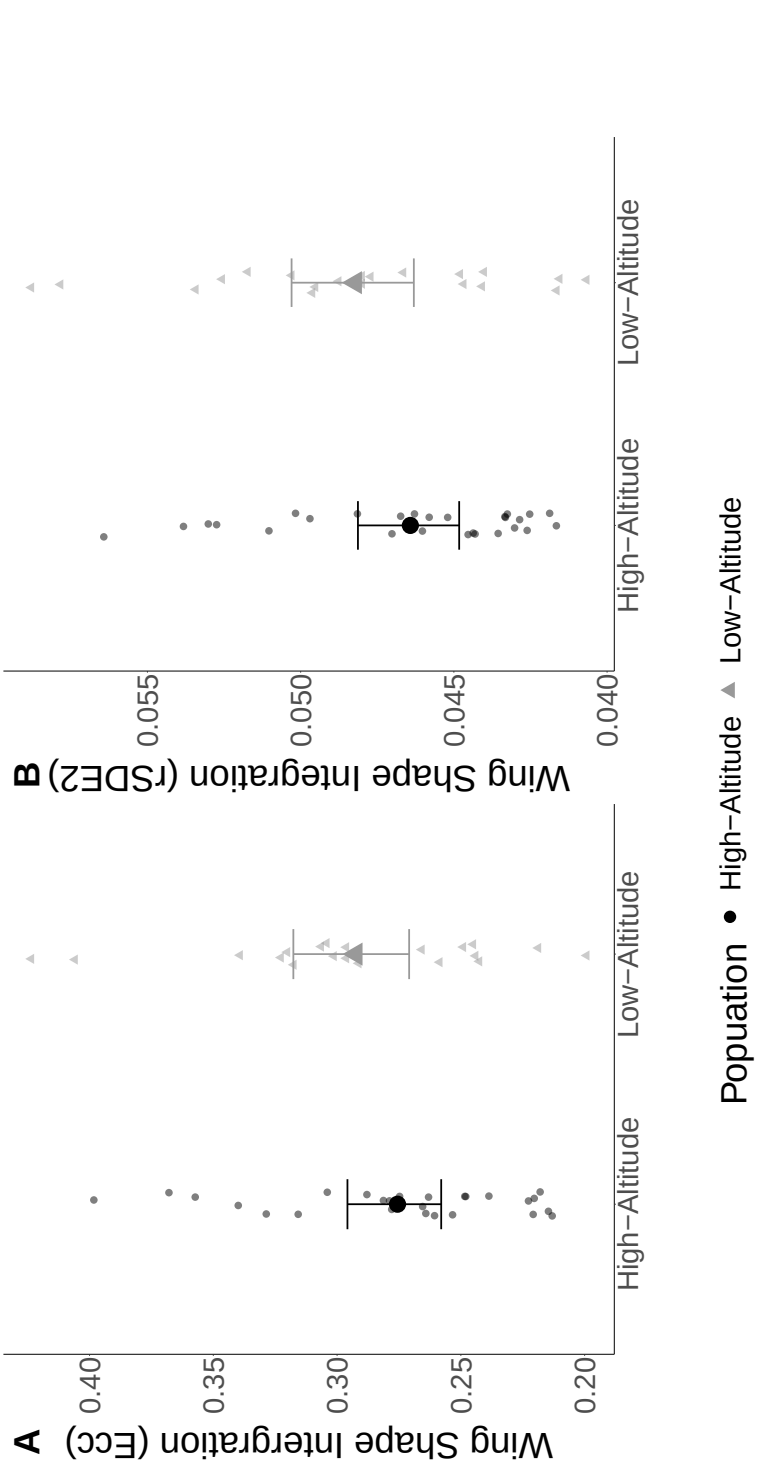

Figure A-S6 : Within-line variation of wing shape measured as wing shape integration using matrix eccentricity and the relative standard deviation (rSDE2) of the eigenvalues of VCV matrix are similar in the high- and low-altitude populations. Error bars represent 95% CIs.

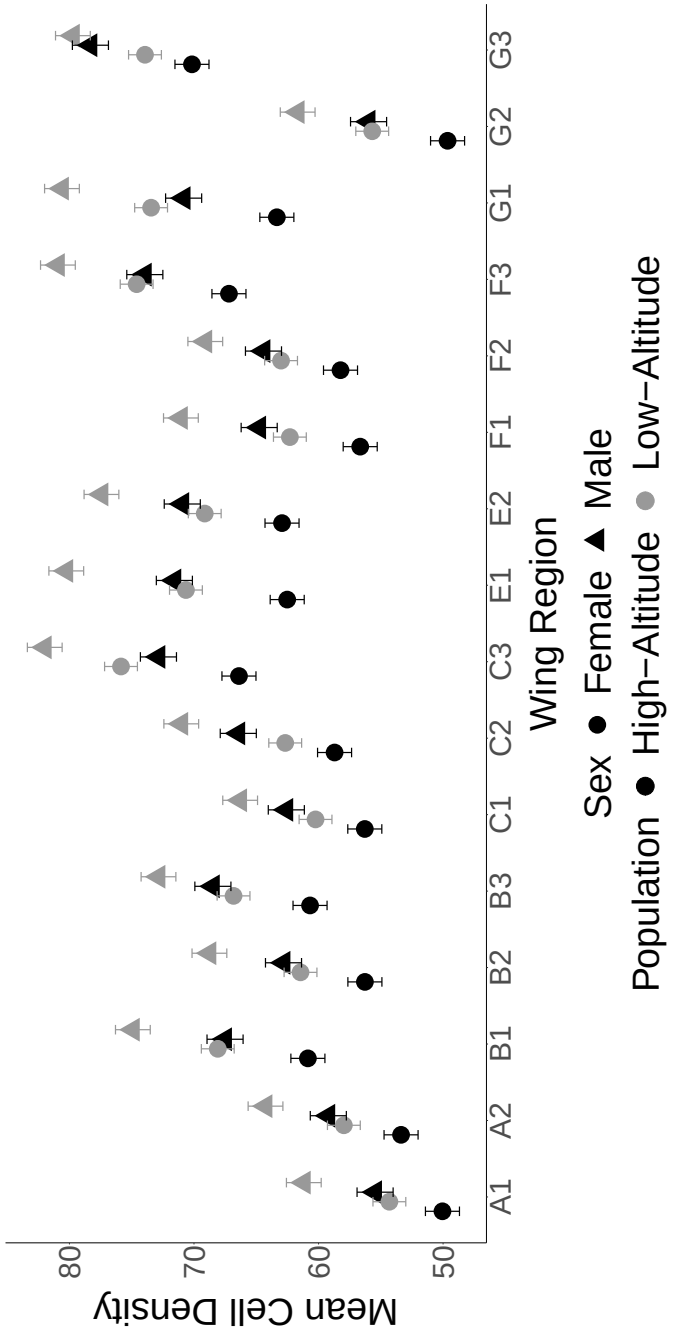

Figure A-S7 : Mean cell density across the 16 measurement wing regions. Wing density varies by wing region, sex and population. Error bars are 95% CI.

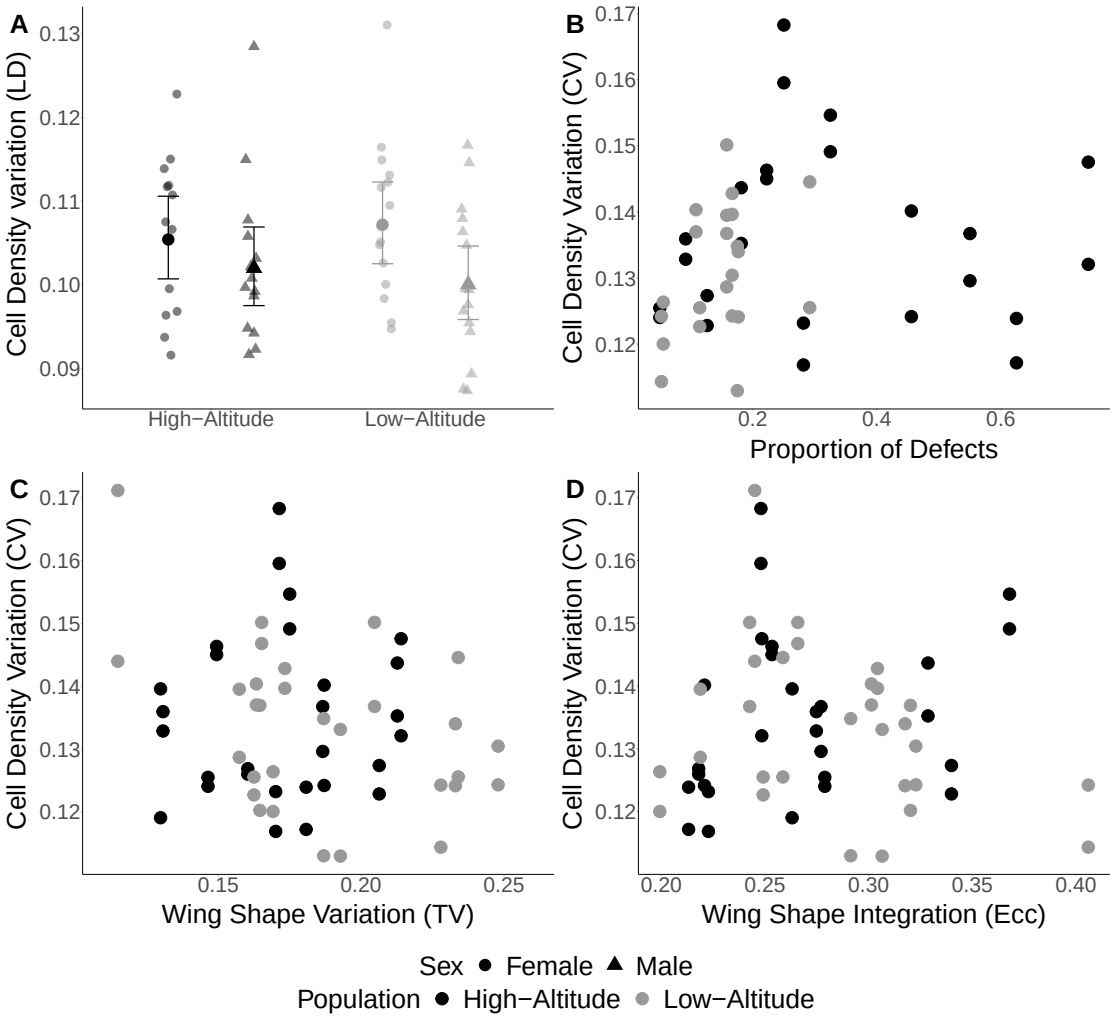

Figure A-S8 : Within-line variation for cell density measured as Levene's Deviates and association between within-line variation for cell density with within-line wing defects, and within-line variation for wing shape. (A) As seen using CV, within-line variation for cell density using Levene's deviates is similar between high- and low-altitude populations. Error bars represent 95% CIs. (B) There is little association between cell density CV and proportion of defects in either the high-altitude (r = −0.043 95% −0.44 - 0.37) or low-altitude populations (r = 0.36 95% −0.076 - 0.68). Within-line variation (total variance) (C) and integration (eccentricity) (D) for wing shape are not correlated with within-line variation for cell density (TV - Total variance $r = -0.22$ 95% CIs −0.45 - 0.04) , Ecc - Eccentricity $r = -0.04$ 95% CIs −0.29 - 0.22)

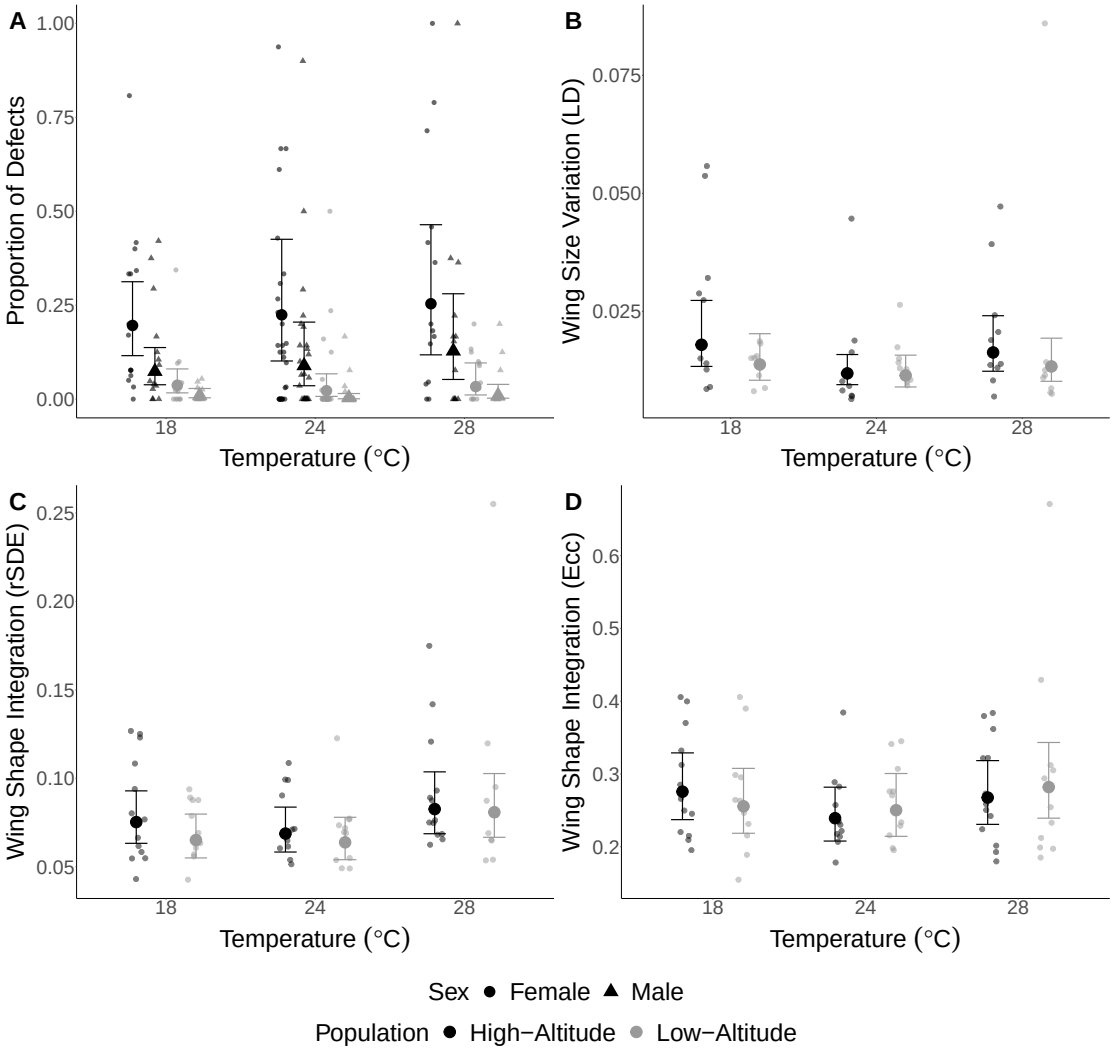

Figure A-S9 : Proportion of wing defects and alternative measures of within-line variation for wing size and shape for the high- and low-altitude population at different developmental temperatures. (A) Proportion of defects are similar across the different developmental temperatures with the high-altitude population having consistently greater proportion of defects than the low-altitude population. (B) Within-line variation for wing size measured as Levene's Deviates and within-line variation for wing shape measured as (C) rSDE (multiplied by a factor of 10000) and (D) Eccentricity are similar in the high- and low-altitude populations across developmental temperatures. Error bars represent 95% CIs.

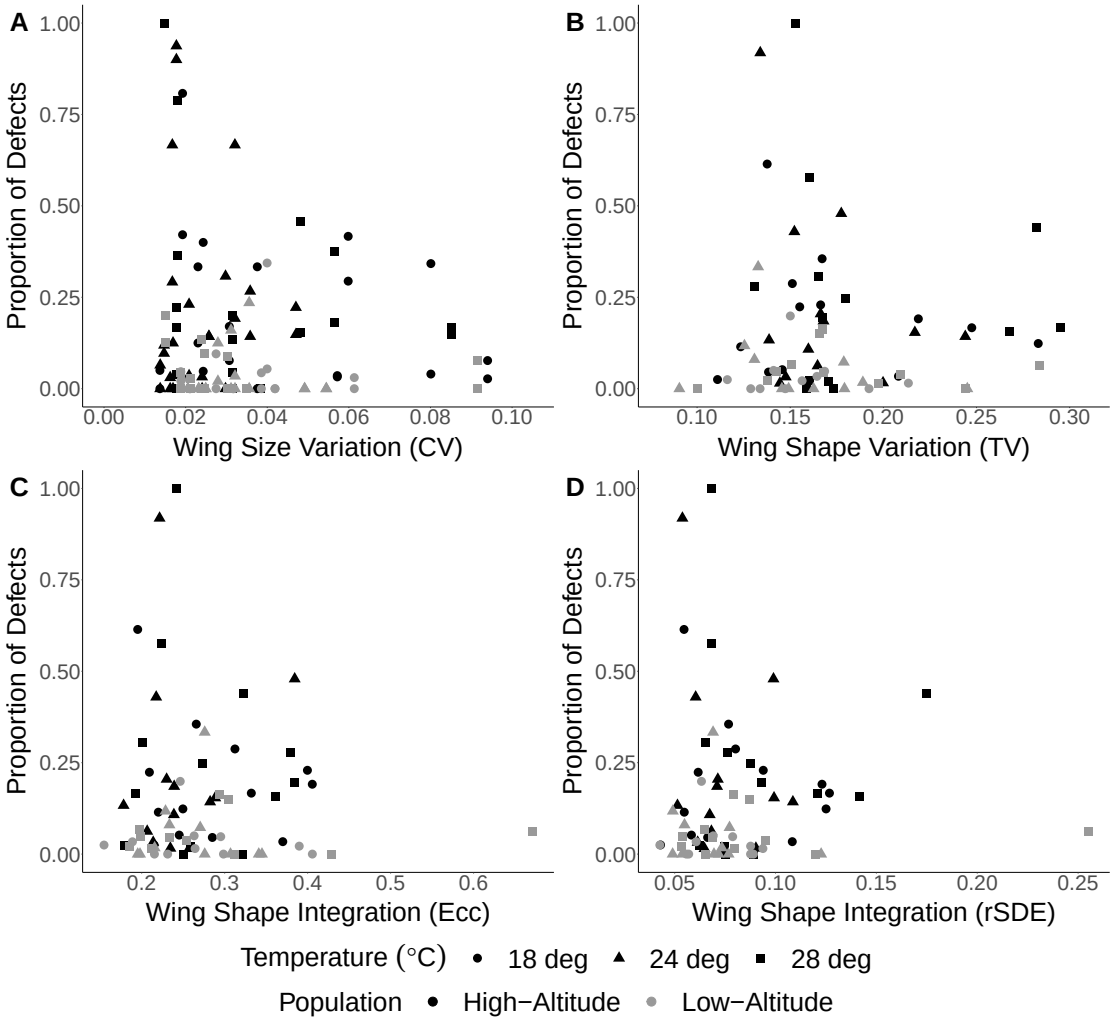

Figure A-S10 : Little evidence for correlation between measures of within-line variation for (A) wing size (CV) and (B-D) wing shape (total variance, eccentricity and rSDE) with proportion of defects for the high- and low-altitude populations at different developmental temperatures

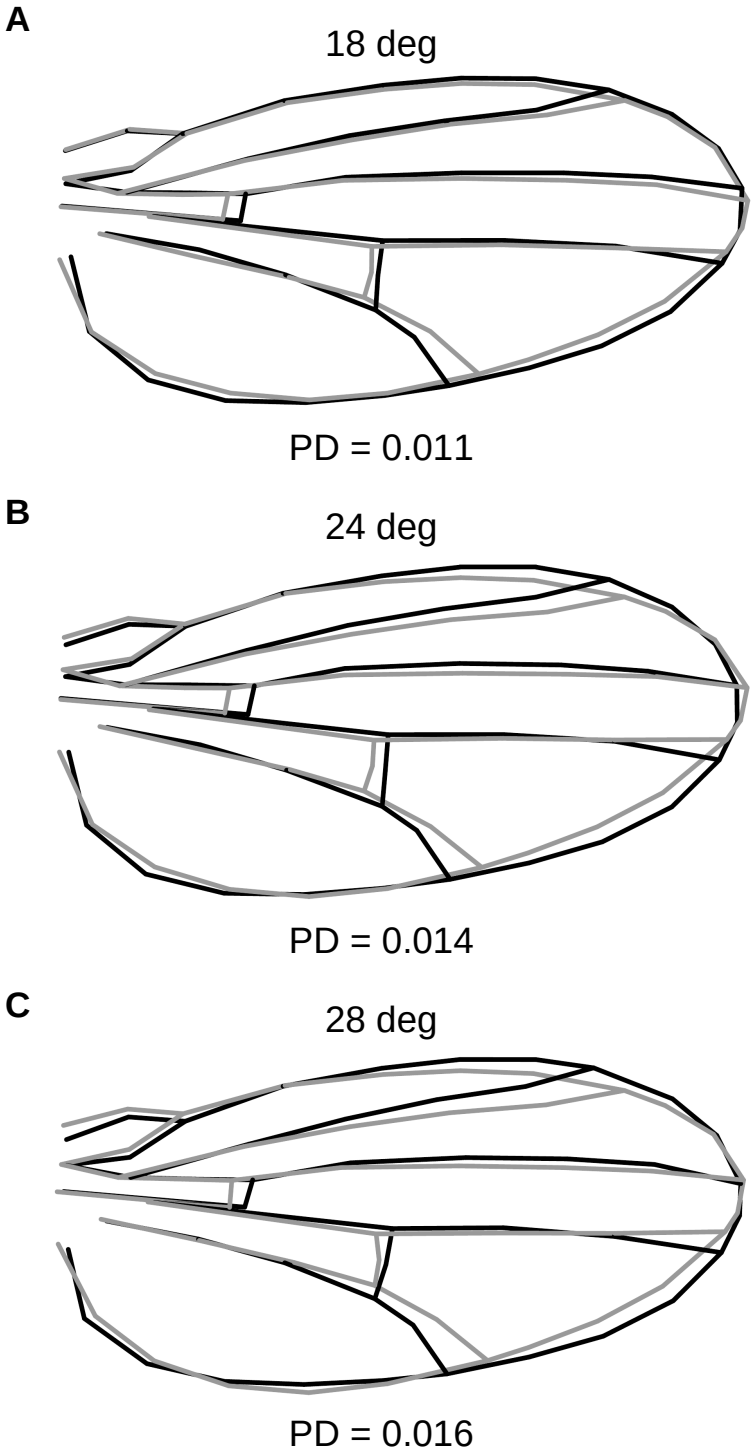

Figure A-S11 : Mean wing shape differences between the high- and low-altitude populations at different temperatures (females). The difference in shape measured as PD (Procrustes distance) is increasing with temperature

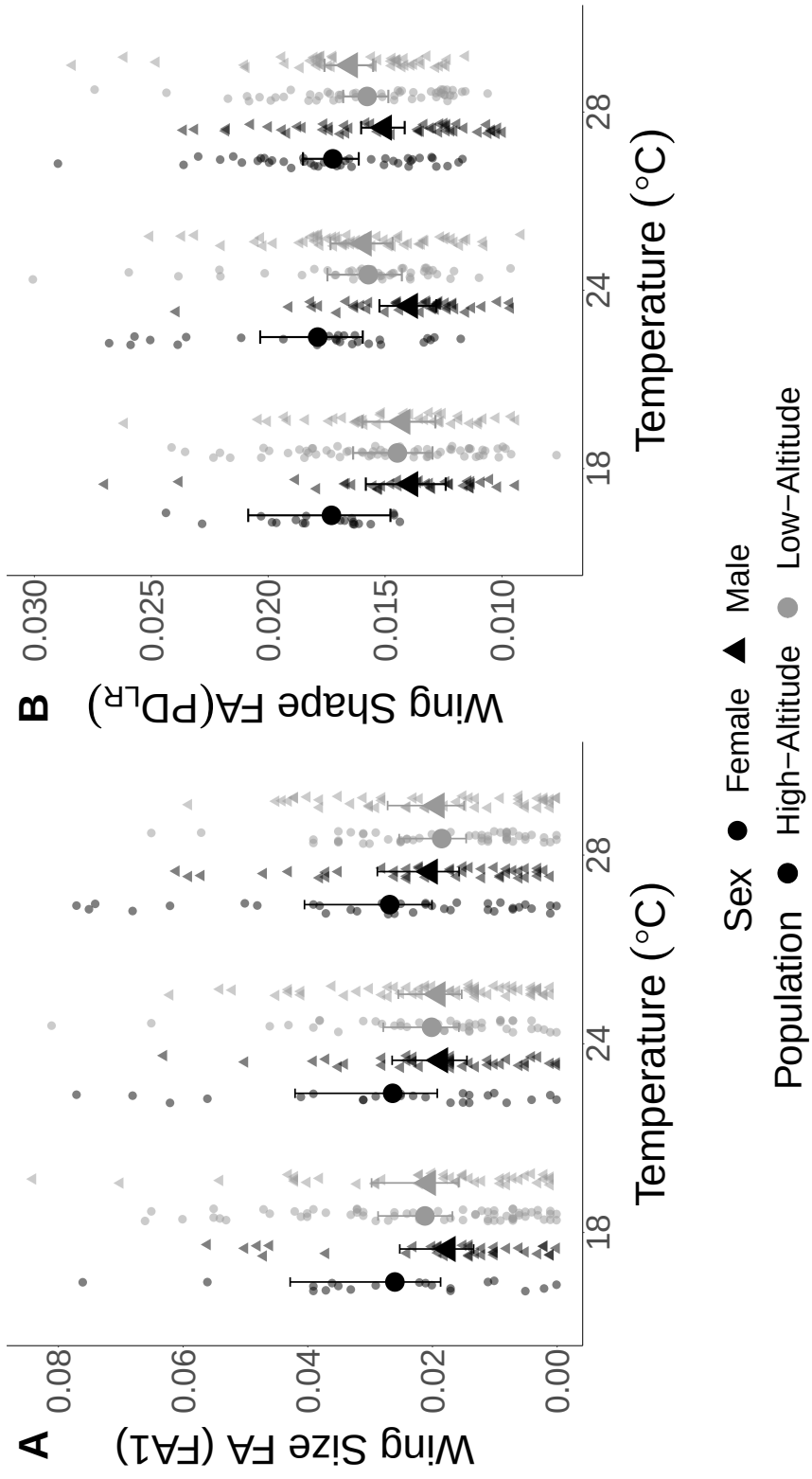

Figure A-S12 : Fluctuating Asymmetry for wing size and shape represented by (A) FA1 and (B) Procrustes distance between the left and right wing (PD_{LR}). High-altitude females have consistently greater wing size FA and and wing shape FA across the three developmental temperature treatments. Large symbols represent population means, small symbols represent individuals, error bars represent 95% CIs

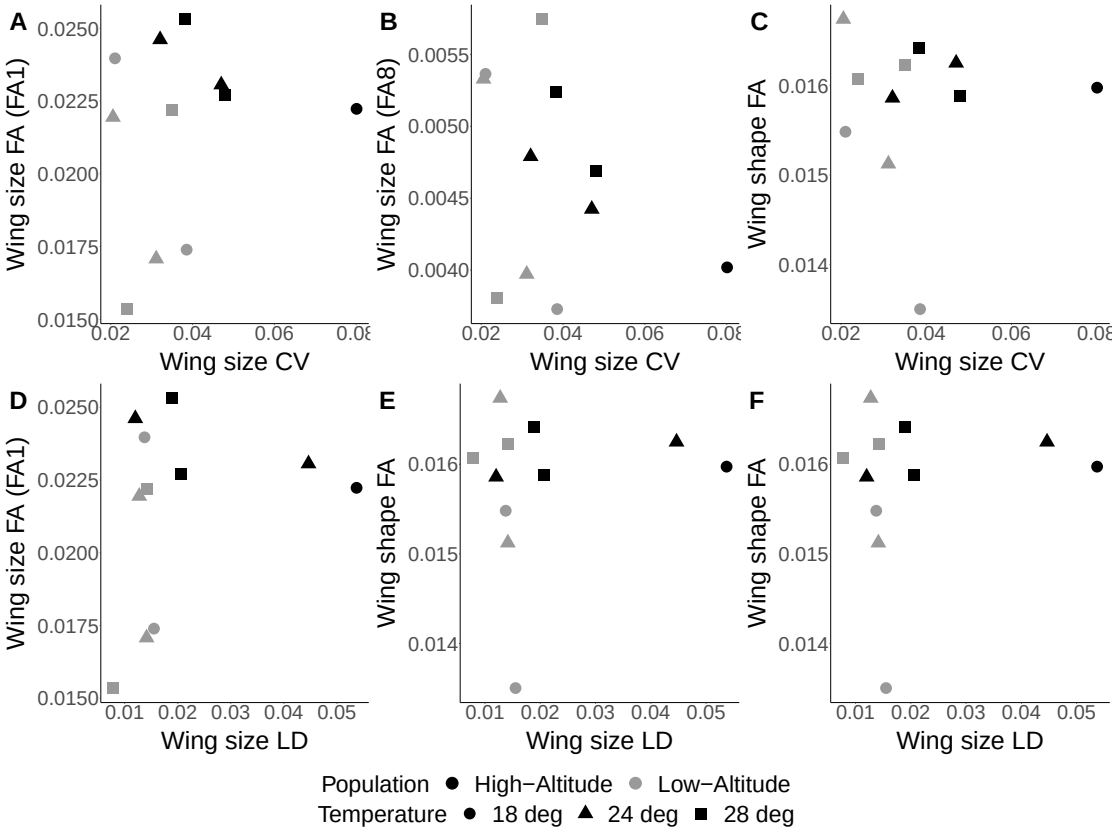

Figure A-S13 : Fluctuating asymmetry for wing size and shape vs. measures of variability for wing size. Correlations were not calculated as FA was measured for only two strains per population (A) Plot of FA1 with wing size CV (B) FA8 with wing size CV (C) Procrustes distance between the left and right wing (PD_{LR}) with wing size CV (D) FA1 with wing size LD (E) FA8 with wing size LD (F)(PD_{LR}) with wing size LD.

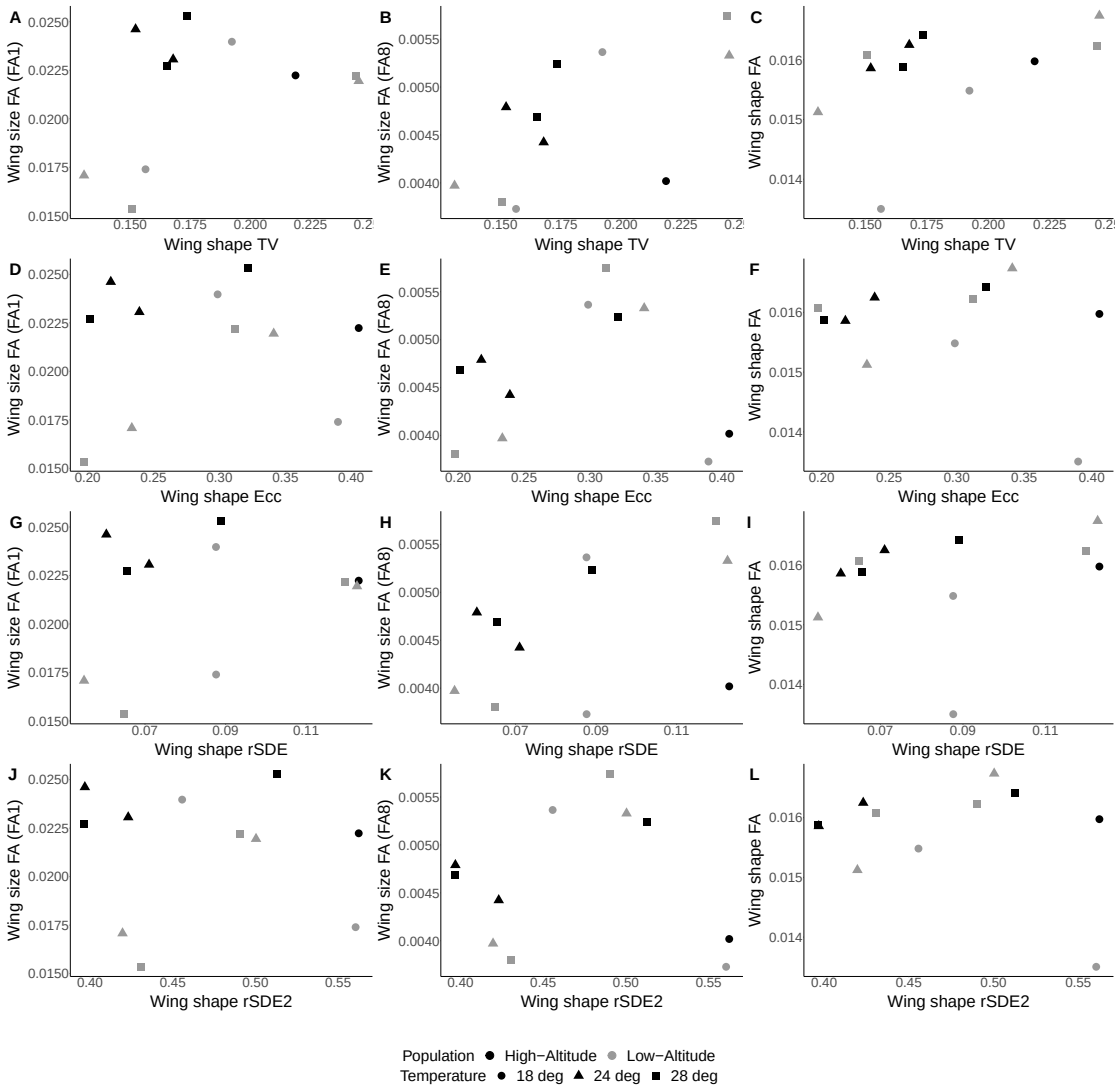

Figure A-S14 : Fluctuating asymmetry for wing size and shape vs. measures of variability for wing shape.(A) FA1 and wing shape total variance (B) FA8 with wing shape total variance (C) Procrustes distance between the left and right wing (PD_{LR}) with wing shape total variance (D) FA1 with wing shape eccentricity (E) FA8 with wing shape eccentricity (F)(PD_{LR}) with wing shape eccentricity (G) FA1 correlated with wing shape rSDE (H) FA8 with wing shape rSDE (I)(PD_{LR}) with wing shape rSDE (J) FA1 with wing shape rSDE2 (K) FA8 with wing shape rSDE2 (L) PD_{LR}) with wing shape rSDE2.

Supplemental Tables

Table A-S1: Sample sizes of fly strains for micro-environmental canalization

Line	Population	N_F	N_M	Line	Population	N_F	N_M
ef101	High-Altitude	19	20	zi124	Low-Altitude	20	18
ef112	High-Altitude	20	20	zi159	Low-Altitude	18	15
ef115	High-Altitude	19	20	zi160	Low-Altitude	17	12
ef117	High-Altitude	20	19	zi186	Low-Altitude	17	17
ef119	High-Altitude	18	14	zi197	Low-Altitude	19	19
ef122	High-Altitude	18	20	zi216	Low-Altitude	16	17
ef131	High-Altitude	20	20	zi217	Low-Altitude	19	20
ef135	High-Altitude	19	20	zi251	Low-Altitude	19	14
ef136	High-Altitude	16	20	zi254	Low-Altitude	12	17
ef15	High-Altitude	15	20	zi311	Low-Altitude	17	19
ef16	High-Altitude	20	19	zi322	Low-Altitude	23	14
ef19	High-Altitude	20	19	zi337	Low-Altitude	19	20
ef1	High-Altitude	20	17	zi357	Low-Altitude	20	20
ef25	High-Altitude	20	20	zi360	Low-Altitude	18	14
ef31	High-Altitude	20	19	zi366	Low-Altitude	18	20
ef39	High-Altitude	20	20	zi383	Low-Altitude	19	19
ef3	High-Altitude	18	14	zi403	Low-Altitude	17	16
ef54	High-Altitude	20	19	zi455	Low-Altitude	20	19
ef59	High-Altitude	19	13	zi461	Low-Altitude	14	18
ef65	High-Altitude	19	19	zi507	Low-Altitude	20	18
ef67	High-Altitude	11	17				
ef73	High-Altitude	18	16				
ef75	High-Altitude	8	18				
ef7	High-Altitude	14	19				
ef86	High-Altitude	19	20				
ef98	High-Altitude	14	20				
ef9	High-Altitude	17	20				

Table A-S2: Sample sizes of fly strains for Temperature Plasticity and Macro-environmental canalization

Line	Population	N_{18F}	N_{24F}	N_{28F}	N_{18M}	N_{24M}	N_{28M}
ef112	High-Altitude	28	10	22	18	23	9
ef117	High-Altitude	7	22	8	9	5	8
ef119	High-Altitude	20	24	11	16	9	8
ef122	High-Altitude	8	14	6	11	19	18
ef131	High-Altitude	26	27	41	35	39	22
ef136	High-Altitude	12	20	16	14	20	10
ef15	High-Altitude	13	19	21	19	24	14
ef19	High-Altitude	7	12	9	6	13	10
ef39	High-Altitude	7	13	17	12	21	23
ef65	High-Altitude	13	20	4	8	14	6
ef73	High-Altitude	36	21	12	23	26	8
ef16	High-Altitude	42	27	43	36	26	30
ef98	High-Altitude	25	16	13	19	20	8
zi124	Low-Altitude	47	20	31	39	21	25
zi159	Low-Altitude	41	72	42	39	60	31
zi186	Low-Altitude	26	8	21	37	8	23
zi216	Low-Altitude	26	47	10	29	56	4
zi251	Low-Altitude	20	32	17	23	47	28
zi254	Low-Altitude	40	40	40	38	49	36
zi311	Low-Altitude	22	22	15	13	16	12
zi360	Low-Altitude	9	17	5	20	9	4
zi366	Low-Altitude	4	12	8	14	6	2
zi367	Low-Altitude	8	35	23	8	23	10
zi455	Low-Altitude	32	32	15	35	37	16

Table A-S3: Recipe for 1.5:1 Protein:Sugar Food used in Temperature Plasticity/Macro-environmental canalization and FA experiments

Ingredient	Amount
Water	4250 ml
Black strap molasses	20 g
Fancy table molasses	20 g
Cornmeal	20 g
Carageenan	27 g
Yeast	280 g
Sucrose	75 g
Propionic acid	12 ml
Methylparaben	2.5 g
Ethanol	25 ml

Table A-S4: Linear mixed estimates for the contributions of sex, population and their interaction on wing size

Effect	Chisq	Df	Pr(>Chisq)
Sex	4.6×10^3	1	$< 2.2 \times 10^{-16}$
Pop	9.5×10^2	1	$< 2.2 \times 10^{-16}$
Sex:Pop	1.0×10^2	1	$< 2.2 \times 10^{-16}$

Table A-S5: Results from Multivariate Procrustes ANOVA testing the effects of wing size, population, sex and all interactions on wing shape

Effect	Df	SS	MS	Rsq	F	Z	p (>F)
Size	1	5.7×10^{-2}	5.7×10^{-2}	5.8×10^{-2}	2.7×10^2	11	5.0×10^{-4}
Pop	1	1.1×10^{-1}	1.1×10^{-1}	1.1×10^{-1}	11	4.7	5.0×10^{-4}
Sex	1	1.0×10^{-2}	1.0×10^{-2}	1.0×10^{-2}	47	9	5.0×10^{-4}
Size:Pop	1	3.3×10^{-3}	3.3×10^{-3}	3.3×10^{-3}	16	6.4	5.0×10^{-4}
Size:Sex	1	1.3×10^{-3}	1.3×10^{-3}	1.3×10^{-3}	6.0	4.5	5.0×10^{-4}
Pop:Sex	1	5.5×10^{-4}	5.5×10^{-4}	5.6×10^{-4}	2.6	2.7	5.0×10^{-4}
Pop:Line	45	4.5×10^{-1}	1.0×10^{-2}	4.6×10^{-1}	47	35	5.0×10^{-4}
Size:Pop:Sex	1	4.0×10^{-4}	3.9×10^{-4}	4.0×10^{-4}	1.8	4.0	5.0×10^{-4}
Residuals	1.6×10^3	3.5×10^{-1}	2.1×10^{-4}	3.5×10^{-1}			
Total	1.7×10^3	9.8×10^{-1}					

Table A-S6: Results from generalized mixed effects model testing the effects of population and sex and their interaction on within-line among-individual wing size CV

Effect	Chisq	Df	Pr(>Chisq)
Pop	0.13	1	0.72
Sex	1.0×10^{-3}	1	0.98
Pop:Sex	0.41	1	0.52

Table A-S7: Results from generalized mixed effects model testing the effects of sex and population and their interaction on within-line among-individual wing size Levene's deviates

Effect	Chisq	Df	Pr(>Chisq)
Sex	0.84	1	0.36
Pop	1.5	1	0.22
Sex:Pop	0.20	1	0.66

Table A-S8: Results from generalized mixed effects model testing the effects of population and sex and their interaction on within-line proportion of wing defects

Effect	LR Chisq	Df	Pr(>Chisq)
Pop	44	1	3.6×10^{-11}
Sex	2.5	1	0.11
Pop:Sex	1.7	1	0.20

Table A-S9: Results from generalized mixed effects model testing the effects of population on within-line total variance for wing shape

Effect	LR Chisq	Df	Pr(>Chisq)
Pop	5.6×10^{3}	1	0.94

Table A-S10: Results from generalized mixed effects model testing the effects of population on within-line rSDE for wing shape

Effect	Chisq	Df	Pr(>Chisq)
Pop	0.29	1	0.59

Table A-S11: Results from generalized mixed effects model testing the effects of population on within-line eccentricity for wing shape

Effect	LR Chisq	Df	Pr(>Chisq)
Pop	1.3	1	0.25

Table A-S12: Results from generalized mixed effects model testing the effects of population on within-line rSDE2 for wing shape

Effect	Chisq	Df	Pr(>Chisq)
Pop	2.0	1	0.16

Table A-S13: Results from linear mixed effects model testing the effects of wing region, sex, population and their interactions on cell density across 16 different regions of the wing

Effect	Chisq	Df	Pr(>Chisq)
WingReg	1.9×10^4	15	$< 2.2 \times 10^{-16}$
Sex	5.6×10^2	1	$< 2.2 \times 10^{-16}$
Pop	31	1	2.0×10^{-8}
WingReg:Sex	1.1×10^2	15	$< 2.2 \times 10^{-16}$
WingReg:Pop	4.0×10^2	15	$< 2.2 \times 10^{-16}$
Sex:Pop	0.31	1	0.58
WingReg:Sex:Pop	20	15	0.16

Table A-S14: Results from generalized mixed effects model testing the effects of population and sex and their interaction on within-line among-individual cell density CV

Effect	Chisq	Df	Pr(>Chisq)
Pop	4.2×10^{-2}	1	0.84
Sex	10	1	1.0×10^{-3}
Pop:Sex	0.27	1	0.60

Table A-S15: Results from generalized mixed effects model testing the effects of population and sex and their interaction on within-line among-individual cell density Levene's deviates

Effect	Chisq	Df	Pr(>Chisq)
Sex	14	1	2.0×10^{-4}
Pop	3.0×10^{-3}	1	0.95
Sex:Pop	0.63	1	0.43

Table A-S16: Results from linear mixed effects model testing the effects of temperature, sex, population and their interactions on wing size

Effect	Chisq	Df	Pr(>Chisq)
$Temp^2$	6.5×10^2	2	$< 2.2 \times 10^{-16}$
Sex	1.8×10^3	1	$< 2.2 \times 10^{-16}$
Pop	1.1×10^2	1	$< 2.2 \times 10^{-16}$
Sex:$Temp^2$	16	2	3.0×10^{-4}
Pop:$Temp^2$	20	2	1.0×10^{-4}
Sex:Pop	22	1	4.0×10^{-6}
Sex:Pop:$Temp^2$:	8.7	2	1.3×10^{-2}

Table A-S17: Results from Multivariate Procrustes ANOVA testing the effects of wing size, sex, temperature, population and all interactions on wing shape

Effect	Df	SS	MS	Rsq	F	Z	p (>F)
Size	1	7.6×10^{-2}	7.6×10^{-2}	3.6×10^{-2}	3.4×10^{2}	12	5.0×10^{-4}
Sex	1	0.14	0.14	6.7×10^{-2}	6.3×10^{2}	13	5.0×10^{-4}
Temp	1	0.13	0.13	6.1×10^{-2}	5.8×10^{2}	13	5.0×10^{-4}
^{2}	1	2.0×10^{-2}	2.0×10^{-2}	9.4×10^{-3}	89	9.6	5.0×10^{-4}
Pop	1	5.90×10^{-2}	5.9×10^{-2}	2.8×10^{-2}	1.7	1.1	0.14
Size:Sex	1	2.6×10^{-3}	2.6×10^{-3}	1.5×10^{-3}	12	5.8	5.0×10^{-4}
Size:Temp	1	3.8×10^{-3}	3.8×10^{-3}	1.8×10^{-3}	17	6.4	5.0×10^{-4}
Size:^{2}	1	1.4×10^{-3}	1.4×10^{-3}	6.6×10^{-4}	6.2	4.4	5.0×10^{-4}
Size:Pop	1	2.0×10^{-2}	2.0×10^{-2}	9.4×10^{-3}	89	10	5.0×10^{-4}
Sex:Temp	1	3.9×10^{-3}	3.9×10^{-3}	1.9×10^{-3}	18	6.5	5.0×10^{-4}
Sex:^{2}	1	1.5×10^{-3}	1.5×10^{-3}	7.3×10^{-4}	6.9	4.7	5.0×10^{-4}
Sex:Pop	1	6.9×10^{-3}	6.9×10^{-3}	3.3×10^{-3}	31	7.5	5.0×10^{-4}
Pop:Temp	1	1.3×10^{-2}	1.3×10^{-2}	6.2×10^{-3}	59	9.0	5.0×10^{-4}
Pop:^{2}	1	4.4×10^{-3}	4.4×10^{-3}	2.1×10^{-3}	20	6.8	5.0×10^{-4}
Pop:Line	25	0.89	3.5×10^{-2}	0.42	1.6×10^{2}	42	5.0×10^{-4}
Size:Sex:Temp	1	5.0×10^{-4}	5.0×10^{-4}	2.4×10^{-4}	2.0	4.4	5.0×10^{-4}
Size:Sex:^{2}	1	4.5×10^{-4}	4.5×10^{-4}	2.1×10^{-4}	2.2	4.7	5.0×10^{-4}
Size:Sex:Pop	1	1.4×10^{-3}	1.4×10^{-3}	6.8×10^{-4}	6.4	7.2	5.0×10^{-4}
Size:Pop:Temp	1	6.0×10^{-4}	6.0×10^{-4}	2.9×10^{-4}	2.7	5.2	5.0×10^{-4}
Size:Pop:^{2}	1	1.7×10^{-3}	1.7×10^{-3}	8.1×10^{-4}	7.6	7.6	5.0×10^{-4}
Sex:Pop:Temp	1	9.8×10^{-4}	9.8×10^{-4}	4.7×10^{-4}	4.4	6.4	5.0×10^{-4}
Sex:Pop:^{2}	1	3.2×10^{-4}	3.2×10^{-4}	1.5×10^{-4}	1.4	3.7	5.0×10^{-4}
Residuals	3.3×10^{3}	0.73	2.2×10^{-4}	0.35			
Total	3.3×10^{3}	2.1					

Table A-S18: Results from generalized mixed effects model testing the effects population, temperature and their interaction on within-line among-individual CV for wing size

	Chisq	Df	Pr(>Chisq)
Pop	5.0×10^{-4}	1	0.99
Temp	8.1	2	1.8×10^{-2}
Pop:Temp	5.2	2	7.5×10^{-2}

Table A-S19: Results from generalized mixed effects model testing the effects of population, temperature and thier interaction on within-line among-individual Levene's deviates for wing size

	Chisq	Df	Pr(>Chisq)
Pop	0.67	1	0.41
Temp	14	2	1.0×10^{-3}
Pop:Temp	4.4	2	0.11

Table A-S20: Results from generalized mixed effects model testing the effects of population, temperature and their interaction on within-line among-individual total variance for wing shape

Effect	Chisq	Df	Pr(>Chisq)
Pop	0.44	1	0.51
Temp	10	2	6.0×10^{-3}
Pop:Temp	0.85	2	0.65

Table A-S21: Results from generalized mixed effects model testing the effects of population, temperature and their interaction on within-line among-individual eccentricity for wing shape

Effect	Chisq	Df	Pr(>Chisq)
Pop	4.0×10^{-3}	1	0.95
Temp	3.7	2	0.15
Pop:Temp	0.91	2	0.63

Table A-S22: Results from generalized mixed effects model testing the effects of population, temperature and their interaction on within-line among-individual rSDE for wing shape

Effect	Chisq	Df	Pr(>Chisq)
Pop	0.88	1	0.35
Temp	4.5	2	0.11
Pop:Temp	1.2	2	0.56

Table A-S23: Results from generalized mixed effects model testing the effects of population, temperature and their interaction on within-line among-individual rSDE2 for wing shape

Effect	Chisq	Df	Pr(>Chisq)
Pop	1.5	1	0.22
Temp	4.2	2	0.12
Pop:Temp	1.3	2	0.52

Table A-S24: Results from generalized mixed effects model testing the effects of temperature, population, sex and all interactions on within-line proportion of wing defects

Effect	Chisq	Df	Pr(>Chisq)
Temp	1.2	2	0.55
Sex	70	1	$< 2.2 \times 10^{-16}$
Pop	15	1	1.0×10^{-4}
Temp:Sex	0.94	2	0.62
Temp:Pop	2.4	2	0.30
Sex:Pop	1.8	1	0.19
Temp:Sex:Pop	0.52	2	0.77

Table A-S25: Results from generalized mixed effects model testing the effects of sex, population, temperature and all interactions on FA1

Effect	Chisq	Df	Pr(>Chisq)
Sex	3.2	1	7.2×10^{-2}
Pop	1.1	1	0.28
Temp	0.11	2	0.94
Sex:Pop	3.9	1	4.7×10^{-2}
Sex:Temp	0.34	2	0.84
Pop:Temp	1.0	2	0.60
Sex:Pop:Temp	9.4×10^{-2}	2	0.95

Table A-S26: Results from generalized mixed effects model testing the effects of sex, population, temperature and all interactions on FA8

Effect	Chisq	Df	Pr(>Chisq)
Sex	9.0×10^{-3}	1	0.92
Pop	1.0×10^{-2}	1	0.92
Temp	1.0	2	0.60
Sex:Pop	10	1	1.0×10^{-3}
Sex:Temp	0.80	2	0.67
Pop:Temp	2.4	2	0.29
Sex:Pop:Temp	0.84	2	0.66

Table A-S27: Results from generalized mixed effects model testing the effects of sex, population, temperature and all interactions on PD_{LR}

Effect	Chisq	Df	Pr(>Chisq)
Sex	12	1	4.0×10^{-4}
Pop	0.77	1	0.38
Temp	5.2	2	7.5×10^{-2}
Sex:Pop	28	1	1.2×10^{-7}
Sex:Temp	3.2	2	0.20
Pop:Temp	2.2	2	0.34
Sex:Pop:Temp	0.91	2	0.64

Table A-S28: Results from generalized model testing the effects of wing size, sex, population, temperature and all interactions on wing shape FA after correcting for DA

	LR Chisq	Df	Pr(>Chisq)
Pop	1.4	1	0.24
Sex	4.1	1	0.043
Temp	0.90	2	0.64
Size	3.2	1	0.075
Pop:Sex	1.3	1	0.25
Pop:Temp	2.0	2	0.37
Pop:Size	1.3	1	0.26
Sex:Temp	1.6	2	0.44
Sex:Size	0.070	1	0.79
Temp:Size	1.0	2	0.60
Pop:Sex:Temp	0.15	2	0.93
Pop:Sex:Size	2.7	1	0.10
Pop:Temp:Size	8.8	2	0.012
Sex:Temp:Size	2.1	2	0.36

Table A-S29: Results from wing size ANOVA on repeated measurements using side, individual and their interaction as effects in order to estimate measurement error

Effect	Sum Sq	Df	F value	Pr(>F)
Side	4.0×10^{-4}	1	14	3.0×10^{-4}
Inds	1.0×10^{2}	76	4.2×10^{4}	$< 2.2 \times 10^{-16}$
Side:Inds	0.31	76	1.3×10^{2}	$< 2.2 \times 10^{-16}$
Residuals	5.0×10^{-3}	154		

Table A-S30: Results from wing shape multivariate procrustes ANOVA on repeated measurements using side, individual and their interaction as effects in order to estimate Wing shape Measurement Error ANOVA

Effect	Df	SS	MS	Rsq	F	Z	Pr(>F)
Side	1	8.7×10^{-4}	8.7×10^{-4}	5.0×10^{-3}	15	4.8	1.0×10^{-3}
Inds	76	0.15	1.9×10^{-3}	0.86	33	25	1.0×10^{-3}
Side:Inds	76	1.4×10^{-2}	1.9×10^{-4}	8.4×10^{-2}	3.2	28	1.0×10^{-3}
Residuals	154	9.1×10^{-3}	5.9×10^{-5}	5.3×10^{-2}			
Total	307	0.17					

Appendix B

Chapter 3 Supplement

Supplemental Figures

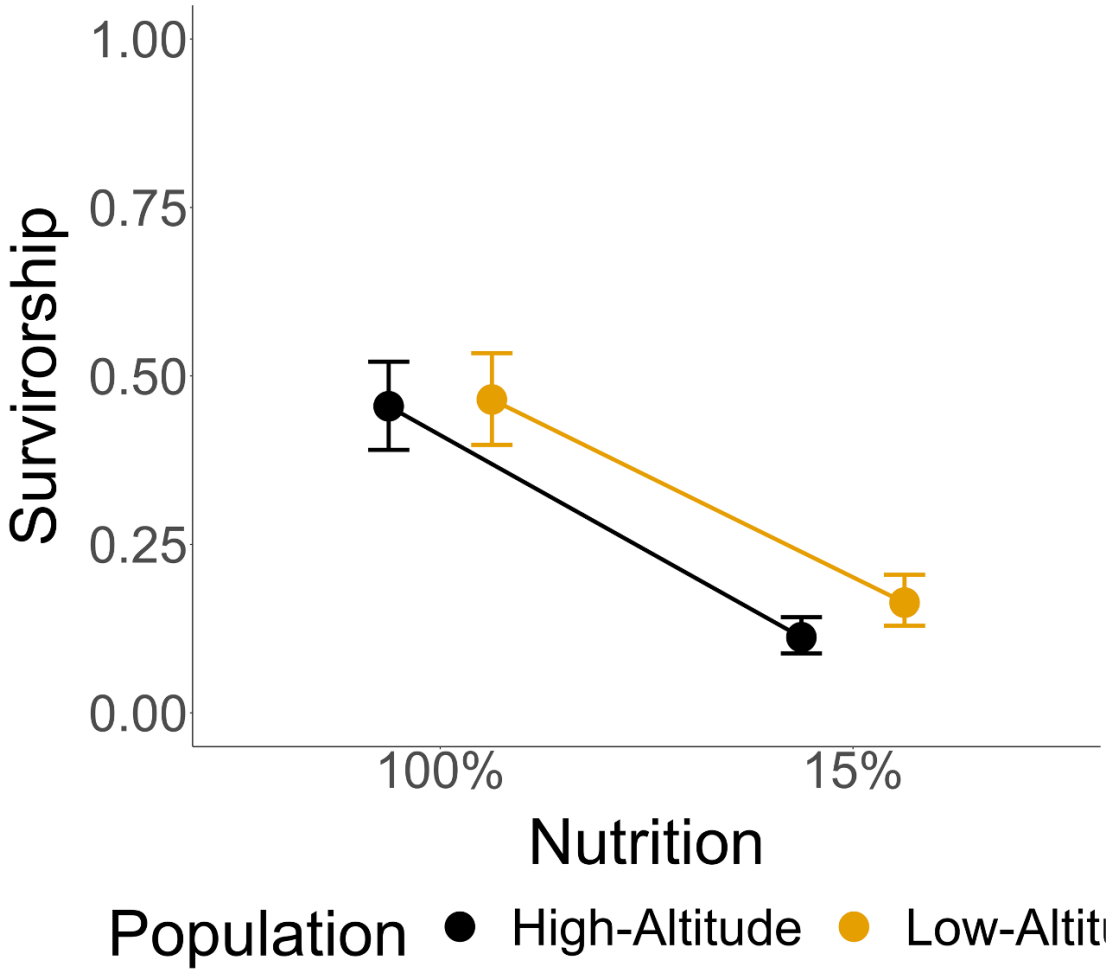

Figure B-S1: Survival Nutrition

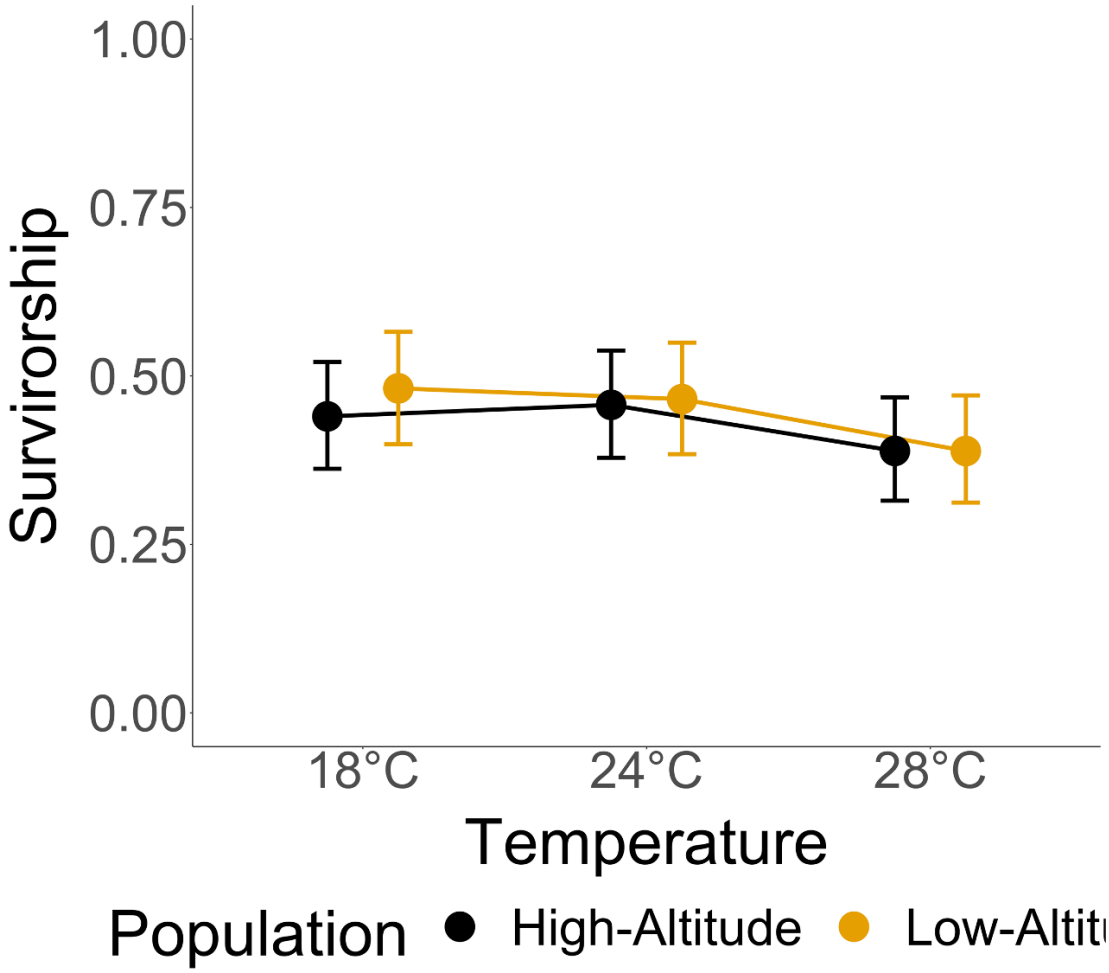

Figure B-S2: Survival Temperature

Table B-S1: Sample sizes of fly strains for Nutritional manipulation

Line	Population	$N_{100\%F}$	$N_{15\%F}$	$N_{100\%F}$	$N_{15\%M}$
ef112	High-Altitude	10	13	23	9
ef117	High-Altitude	37	5	27	5
ef119	High-Altitude	24	13	9	19
ef131	High-Altitude	27	22	39	19
ef136	High-Altitude	42	10	40	2
ef15	High-Altitude	41	2	47	6
ef19	High-Altitude	12	6	13	6
ef39	High-Altitude	48	59	58	56
ef59	High-Altitude	7	6	8	12
ef65	High-Altitude	28	5	25	4
ef73	High-Altitude	43	18	63	23
ef16	High-Altitude	27	25	26	24
ef98	High-Altitude	16	18	20	12
Total	**High-Altitude**	**362**	**202**	**398**	**197**
zi124	Low-Altitude	56	23	43	24
zi159	Low-Altitude	72	30	60	24
zi160	Low-Altitude	22	29	19	10
zi186	Low-Altitude	8	37	8	28
zi216	Low-Altitude	47	17	56	10
zi251	Low-Altitude	44	29	71	34
zi254	Low-Altitude	71	48	81	46
zi311	Low-Altitude	45	55	43	41
zi357	Low-Altitude	31	14	33	9
zi360	Low-Altitude	50	34	39	24
zi366	Low-Altitude	33	39	28	22
zi367	Low-Altitude	35	19	23	17
zi455	Low-Altitude	32	16	37	22
zi455	Low-Altitude	55	31	38	24
Total	**Low-Altitude**	**601**	**421**	**579**	**335**

Table B-S2: Sample sizes of fly strains for Temperature manipulation

Line	Population	N_{18F}	N_{18M}	N_{28F}	N_{28M}
ef112	High-Altitude	28	18	22	9
ef117	High-Altitude	25	41	23	27
ef119	High-Altitude	20	16	11	9
ef131	High-Altitude	26	35	41	27
ef136	High-Altitude	31	45	31	32
ef15	High-Altitude	33	32	49	40
ef19	High-Altitude	7	6	9	10
ef39	High-Altitude	34	48	44	52
ef59	High-Altitude	15	10	5	3
ef65	High-Altitude	21	17	6	8
ef73	High-Altitude	65	61	35	32
ef16	High-Altitude	42	36	43	30
ef98	High-Altitude	25	19	13	8
Total	**High-Altitude**	**372**	**374**	**632**	**287**
zi124	Low-Altitude	73	70	65	60
zi159	Low-Altitude	41	39	42	31
zi160	Low-Altitude	31	21	0	0
zi186	Low-Altitude	26	37	21	23
zi216	Low-Altitude	26	29	10	4
zi251	Low-Altitude	37	38	38	40
zi254	Low-Altitude	90	71	67	70
zi311	Low-Altitude	47	29	44	32
zi357	Low-Altitude	25	18	17	19
zi360	Low-Altitude	36	49	40	35
zi366	Low-Altitude	23	33	16	15
zi367	Low-Altitude	8	8	23	10
zi455	Low-Altitude	32	35	15	16
zi461	Low-Altitude	0	0	37	18
Total	**Low-Altitude**	**495**	**477**	**435**	**373**

Table B-S3: ANOVA for wing size fitting sex, population and nutrition as fixed effects and line as random effect

	Chisq	Df	Pr(>Chisq)
Sex	2.1×10^{3}	1	$< 2.0 \times 10^{-16}$
Pop	1.2×10^{2}	1	$< 2.0 \times 10^{-16}$
Nut	1.0×10^{2}	1	$< 2.0 \times 10^{-16}$
Sex:Pop	1.6×10^{-2}	1	9.0×10^{-1}
Sex:Nut	76	1	$< 2.0 \times 10^{-16}$
Pop:Nut	1.9	1	1.6×10^{-1}
Sex:Pop:Nut	5.8×10^{-1}	1	0.44

Table B-S4: ANOVA for wing size fitting sex, population and temperature as fixed effects and line as random effect

	Chisq	Df	Pr(>Chisq)
Sex	3.0×10^{3}	1	$< 2.0 \times 10^{-16}$
Pop	1.1×10^{2}	1	$< 2.0 \times 10^{-16}$
Temp	9.5×10^{2}	2	$< 2.0 \times 10^{-16}$
Sex:Pop	7.0×10^{-1}	1	4.0×10^{-1}
Sex:Temp	8.5×10^{1}	2	$< 2.0 \times 10^{-16}$
Pop:Temp	4.1	2	1.3×10^{-1}
Sex:Pop:Temp	2.5	2	2.9×10^{-1}

Table B-S5: MANOVA table for wing shape fitting size, sex, population and nutrition as fixed effects

Effect	Df	SS	MS	Rsq	F	Z	p (>F)
Size	1	1.5×10^{-1}	1.5×10^{-1}	8.1×10^{-2}	4.9	2.9	3.0×10^{-3}
Pop	1	2.1×10^{-1}	2.1×10^{-1}	1.1×10^{-1}	6.7	3.5	$< 1.0 \times 10^{-3}$
Sex	1	4.4×10^{-2}	4.4×10^{-2}	2.3×10^{-2}	39	6.3	$< 1.0 \times 10^{-3}$
Nut	1	2.2×10^{-2}	2.2×10^{-2}	1.2×10^{-2}	33	6.3	$< 1.0 \times 10^{-3}$
Pop:Line	25	7.8×10^{-1}	3.1×10^{-2}	4.1×10^{-1}	1.6×10^{2}	31	$< 1.0 \times 10^{-3}$
Size:Pop	1	3.1×10^{-3}	3.1×10^{-3}	1.6×10^{-3}	1.0×10^{-1}	-3.5	1.0
Size:Sex	1	1.8×10^{-3}	1.8×10^{-3}	9.5×10^{-4}	1.6	1.2	1.1×10^{-1}
Pop:Sex	1	8.3×10^{-4}	8.3×10^{-4}	4.4×10^{-4}	7.4×10^{-1}	-6.4×10^{-1}	7.2×10^{-1}
Size:Nut	1	7.0×10^{-4}	7.0×10^{-4}	3.7×10^{-4}	1.0	-6.7×10^{-2}	5.3×10^{-1}
Pop:Nut	1	2.1×10^{-3}	2.1×10^{-3}	1.1×10^{-3}	3.1	2.6	8.0×10^{-3}
Sex:Nut	1	2.7×10^{-3}	2.7×10^{-3}	1.4×10^{-3}	6.9	4.7	$< 1.0 \times 10^{-3}$
Size:Pop:Line	25	2.6×10^{-2}	1.0×10^{-3}	1.4×10^{-2}	5.2	1.4×10^{1}	$< 1.0 \times 10^{-3}$
Pop:Sex:Line	25	2.8×10^{-2}	1.1×10^{-3}	1.5×10^{-2}	5.7	1.6×10^{1}	$< 1.0 \times 10^{-3}$
Size:Pop:Sex	1	1.2×10^{-3}	1.2×10^{-3}	6.6×10^{-4}	1.1	5.6×10^{-1}	2.9×10^{-1}
Pop:Nut:Line	25	1.7×10^{-2}	6.8×10^{-4}	8.9×10^{-3}	3.4	1.5×10^{1}	$< 1.0 \times 10^{-3}$
Size:Pop:Nut	1	4.4×10^{-4}	4.4×10^{-4}	2.3×10^{-4}	1.1	3.7×10^{-1}	3.5×10^{-1}
Pop:Sex:Nut	1	7.2×10^{-4}	7.2×10^{-4}	3.8×10^{-4}	1.8	1.5	6.9×10^{-2}
Size:Pop:Sex:Line	25	7.3×10^{-3}	2.9×10^{-4}	3.8×10^{-2}	1.5	4.4	$< 1.0 \times 10^{-3}$
Size:Pop:Nut:Line	25	7.8×10^{-3}	3.1×10^{-4}	4.1×10^{-3}	1.6	5.3	$< 1.0 \times 10^{-3}$
Pop:Sex:Nut:Line	25	1.0×10^{-3}	4.0×10^{-4}	5.2×10^{-3}	2.0	7.7	$< 1.0 \times 10^{-3}$
Size:Pop:Sex:Nut	1	5.0×10^{-4}	5.0×10^{-4}	2.6×10^{-4}	1.3	8.2×10^{-1}	2.0×10^{-1}
Size:Pop:Sex:Nut:Line	25	6.7×10^{-3}	2.7×10^{-4}	3.5×10^{-3}	1.3	3.2	2.0×10^{-3}
Residuals	2.9×10^{3}	5.7×10^{-2}	2.0×10^{-4}	3.0×10^{-1}			
Total	3.1×10^{3}	1.9					

Table B-S6: MANOVA table for wing shape fitting size, sex, population and temperature as fixed effects

Effect	Df	SS	MS	Rsq	F	Z	p (>F)
Size	1	1.2×10^{-1}	1.2×10^{-1}	3.8×10^{-2}	2.4	1.5	7.9×10^{-2}
Pop	1	3.0×10^{-1}	3.0×10^{-1}	9.6×10^{-2}	5.9	3.2	2.0×10^{-3}
Sex	1	1.2×10^{-1}	1.2×10^{-1}	3.8×10^{-2}	68	7.9	1.0×10^{-3}
Temp	2	1.2×10^{-1}	6.0×10^{-2}	3.8×10^{-2}	38	7.2	1.0×10^{-3}
Pop:Line	25	1.3	5.1×10^{-2}	4.0×10^{-1}	2.5×10^{2}	29	1.0×10^{-3}
Size:Pop	1	1.2×10^{-2}	1.2×10^{-2}	3.9×10^{-3}	2.4×10^{-1}	-1.5	9.2×10^{-1}
Size:Sex	1	6.2×10^{-3}	6.2×10^{-3}	2.0×10^{-3}	3.5	2.9	2.0×10^{-3}
Pop:Sex	1	1.2×10^{-3}	1.2×10^{-3}	3.6×10^{-4}	6.4×10^{-1}	-9.3×10^{-1}	8.3×10^{-1}
Size:Temp	2	5.2×10^{-3}	2.6×10^{-3}	1.6×10^{-3}	1.7	1.5	6.7×10^{-2}
Pop:Temp	2	1.0×10^{-2}	5.0×10^{-3}	3.1×10^{-3}	3.2	3.7	1.0×10^{-3}
Sex:Temp	2	4.4×10^{-3}	2.2×10^{-3}	1.4×10^{-3}	4.6	5.0	1.0×10^{-3}
Size:Pop:Line	25	5.5×10^{-2}	2.2×10^{-3}	1.7×10^{-2}	11	28	1.0×10^{-3}
Pop:Sex:Line	25	4.5×10^{-2}	1.8×10^{-3}	1.4×10^{-2}	9.0	21	1.0×10^{-3}
Size:Pop:Sex	1	1.1×10^{-3}	1.1×10^{-3}	3.5×10^{-4}	6.2×10^{-1}	-8.2×10^{-1}	8.0×10^{-1}
Pop:Temp:Line	48	7.4×10^{-2}	1.5×10^{-3}	2.3×10^{-2}	7.8	21	1.0×10^{-3}
Size:Pop:Temp	2	1.7×10^{-3}	8.5×10^{-4}	5.4×10^{-4}	5.5×10^{-1}	-1.6	9.5×10^{-1}
Pop:Sex:Temp	2	1.4×10^{-3}	6.9×10^{-4}	4.3×10^{-4}	1.4	3.3	2.0×10^{-3}
Size:Pop:Sex:Line	25	1.1×10^{-2}	4.5×10^{-4}	3.6×10^{-3}	2.3	8.5	1.0×10^{-3}
Size:Pop:Temp:Line	48	2.0×10^{-2}	4.3×10^{-4}	6.5×10^{-3}	2.1	14	1.0×10^{-3}
Pop:Sex:Temp:Line	48	2.3×10^{-3}	4.8×10^{-4}	7.3×10^{-3}	2.4	15	1.0×10^{-3}
Size:Pop:Sex:Temp	2	1.1×10^{-3}	5.3×10^{-4}	3.3×10^{-4}	1.1	1.1	3.3×10^{-1}
Size:Pop:Sex:Temp:Line	48	1.1×10^{-2}	2.4×10^{-4}	3.6×10^{-3}	1.2	3.1	1.0×10^{-3}
Residuals	4.8×10^{3}	9.4×10^{-1}	2.0×10^{-4}	3.0×10^{-1}			
Total	5.1×10^{3}	3.2					

Table B-S7: ANOVA for larval weight fitting block, sex, population and nutrition as fixed effects and line as random effect

	Chisq	Df	Pr(>Chisq)
Block	4.8	1	2.8×10^{-2}
Sex	1.5×10^{2}	1	$< 2.2 \times 10^{-16}$
Pop	24	1	1.2×10^{-6}
Nut	2.9×10^{2}	1	$< 2.2 \times 10^{-16}$
Sex:Pop	8.9×10^{-1}	1	3.5×10^{-1}
Sex:Nut	17	1	3.7×10^{-5}
Pop:Nut	25	1	5.0×10^{-7}
Sex:Pop:Nut	2.8	1	9.3×10^{-2}

Table B-S8: Generalized linear mixed model for survivorship fitting population and nutrition as fixed effects and line as a random effect

	Chisq	Df	Pr(>Chisq)
Pop	1.2	1	2.6×10^{-1}
Nut	2.2×10^{3}	1	$< 2.2 \times 10^{-16}$
Pop:Nut	31	1	3.015×10^{-8}

Table B-S9: Generalized linear mixed model for survivorship fitting population and temperature as fixed effects and line as a random effect

	Chisq	Df	Pr(>Chisq)
Pop	7.7×10^{-2}	1	7.8×10^{-1}
Temp	85	2	$< 2 \times 10^{-16}$
Pop:Temp	5.7	2	5.7×10^{-2}

Table B-S10: Calculating Partitioned SShD in two ways

Pop	Treatment	Tot SShD	Allom SShD	Non-Allom SShD	Allom SShD (subt)	Non-Allom SShD (subt)
HA	100% food, 24	0.0145	0.0110	0.0151	−0.0005	0.0036
LA	100% food, 24	0.0152	0.0113	0.0092	0.0059	0.0039
HA	15% food, 24	0.0120	0.0085	0.0111	0.0008	0.0039
LA	15% food, 24	0.0122	0.0096	0.0085	0.0036	0.0026
HA	100% food, 18	0.0143	0.0074	0.0176	−0.0033	0.0069
LA	100% food, 18	0.0137	0.0102	0.0131	0.0006	0.0035
HA	100% food, 28	0.0125	0.0084	0.0131	−0.0006	0.0040
LA	100% food, 28	0.0153	0.0129	0.0144	0.0008	0.00246

Appendix C

Chapter 4 Supplement

Supplemental Figures

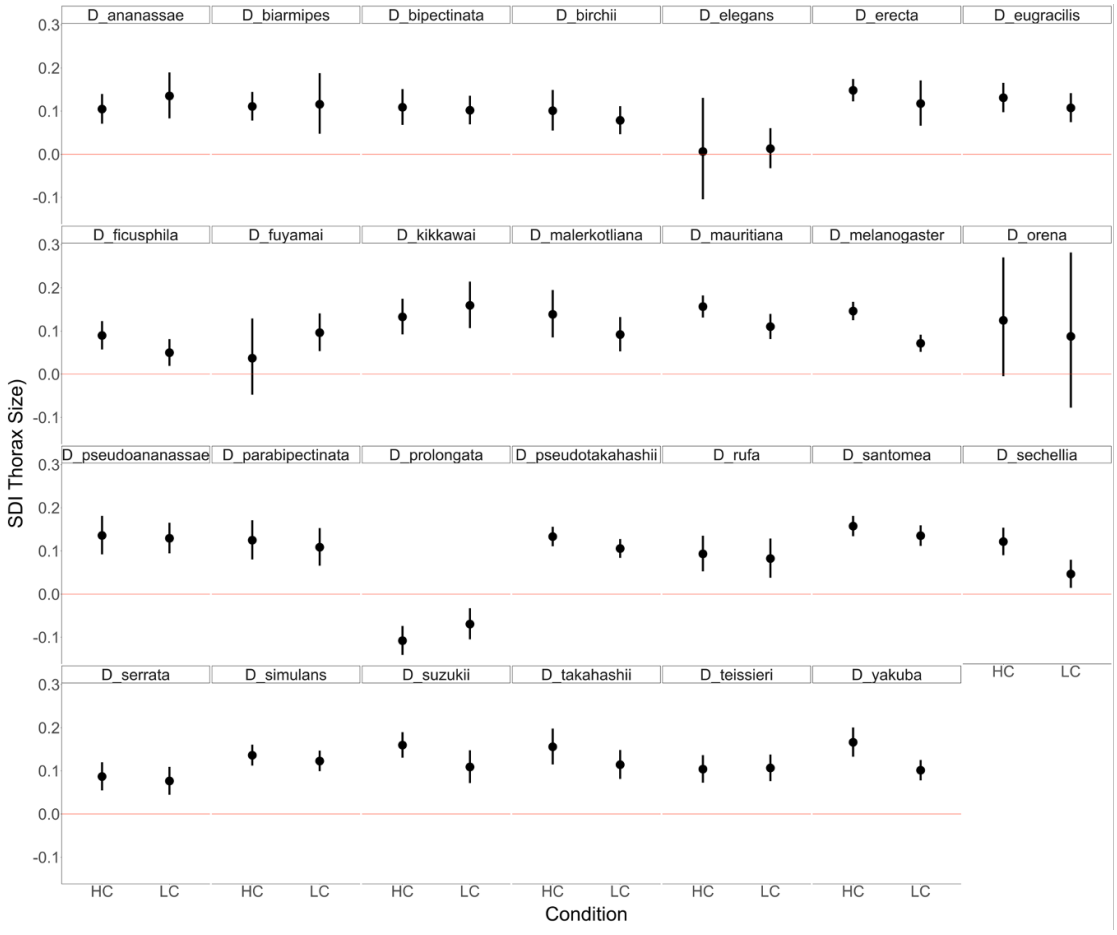

Figure C-S1: Thorax length SDI

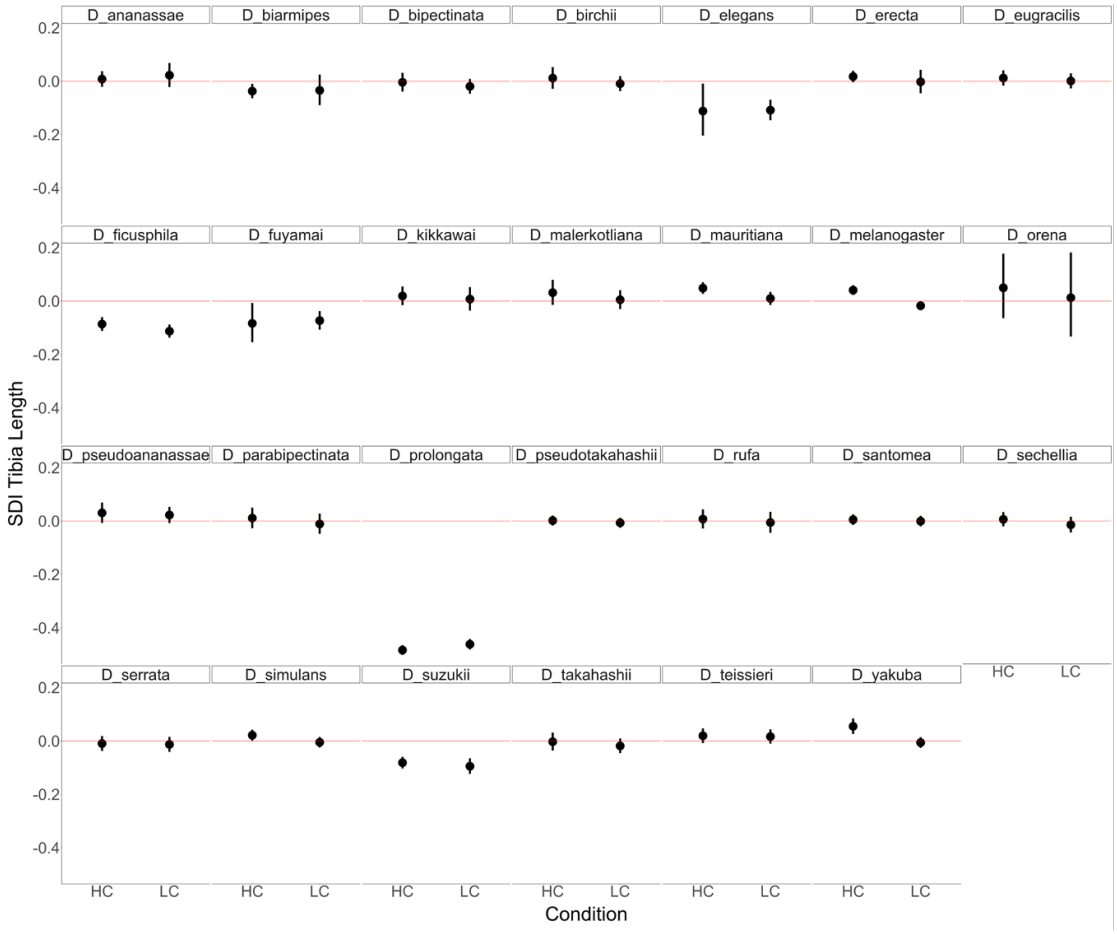

Figure C-S2: Tibia Length SDI

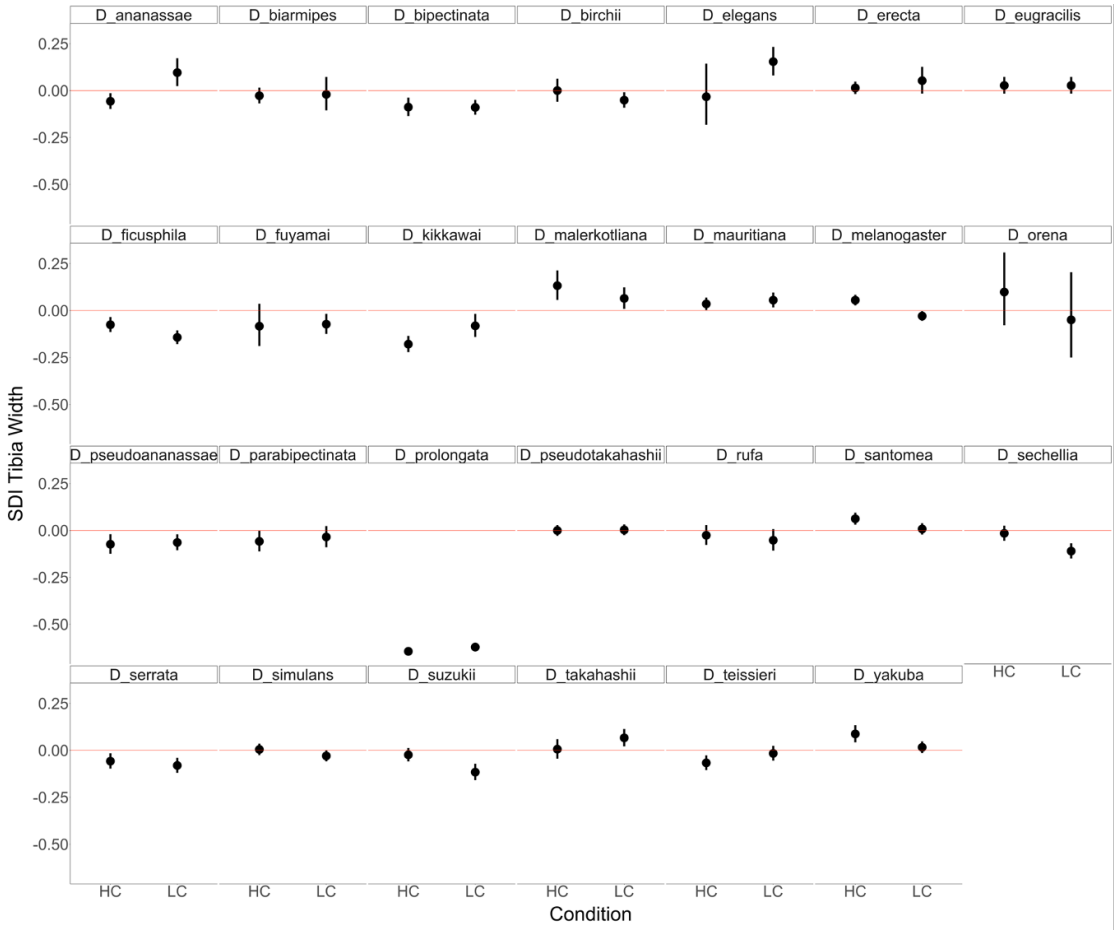

Figure C-S3: Tibia Width SDI

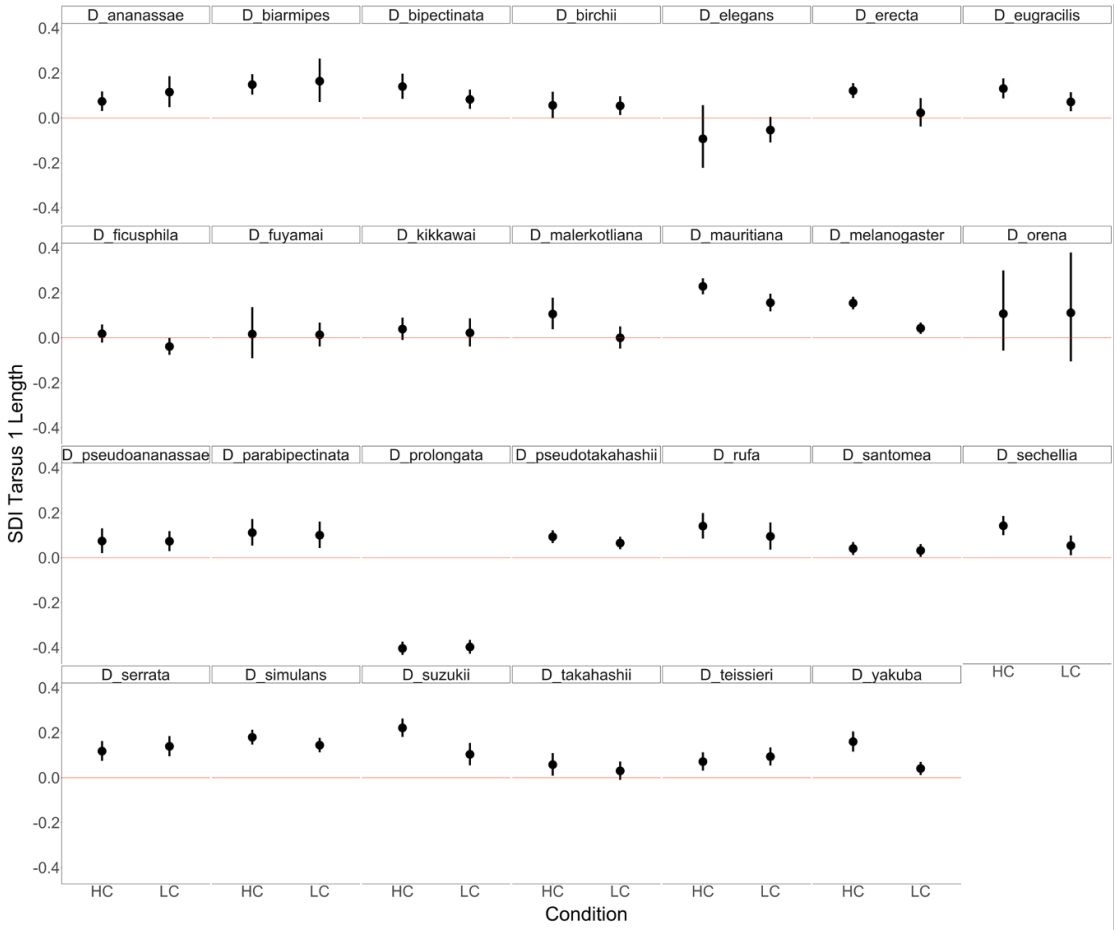

Figure C-S4: Tarsus Length SDI

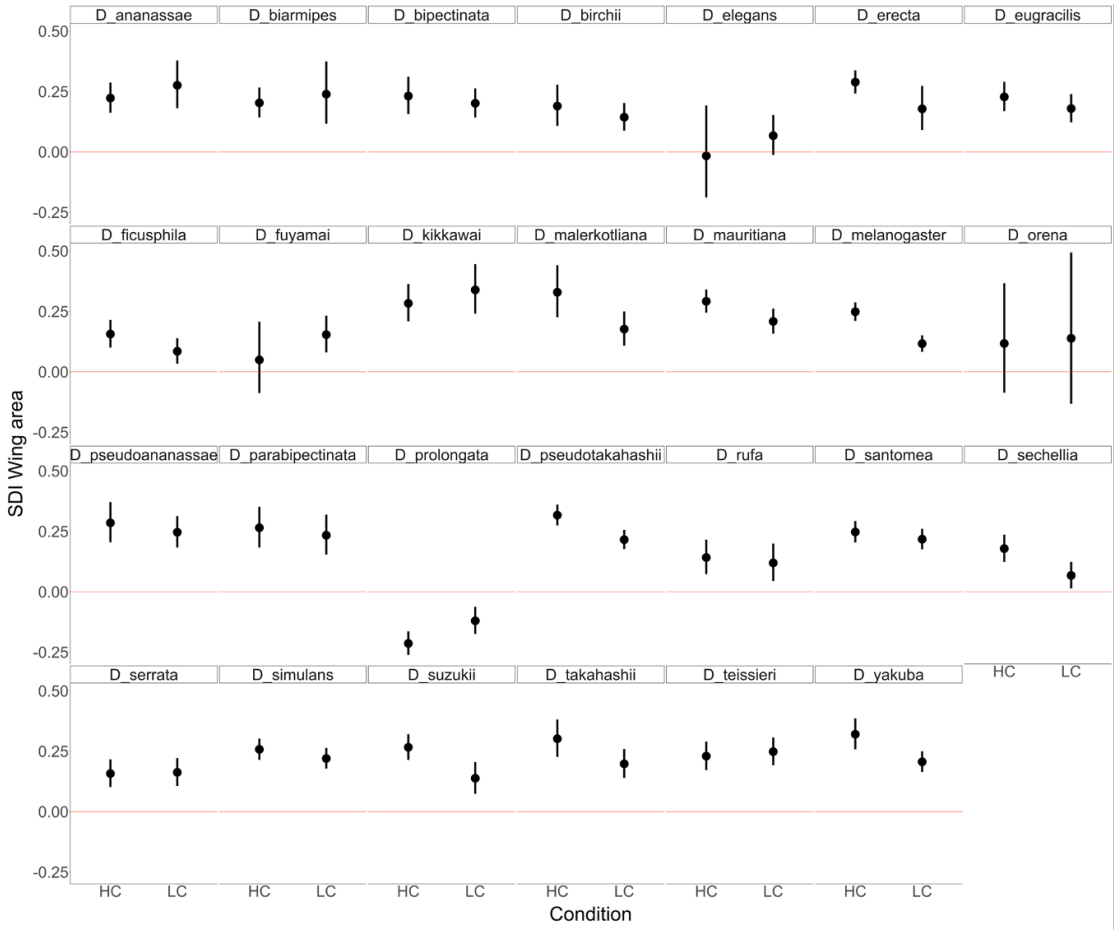

Figure C-S5: Wing Area SDI

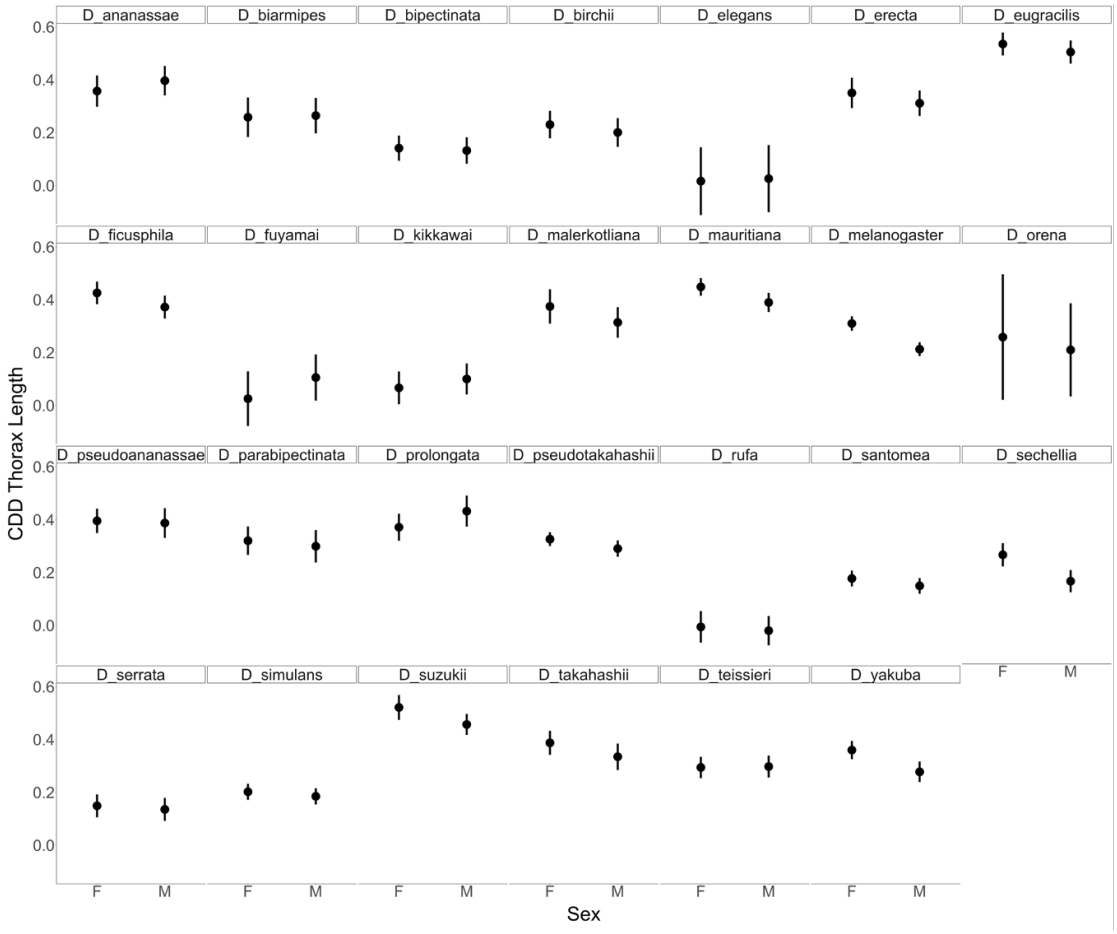

Figure C-S6: Thorax length CDD

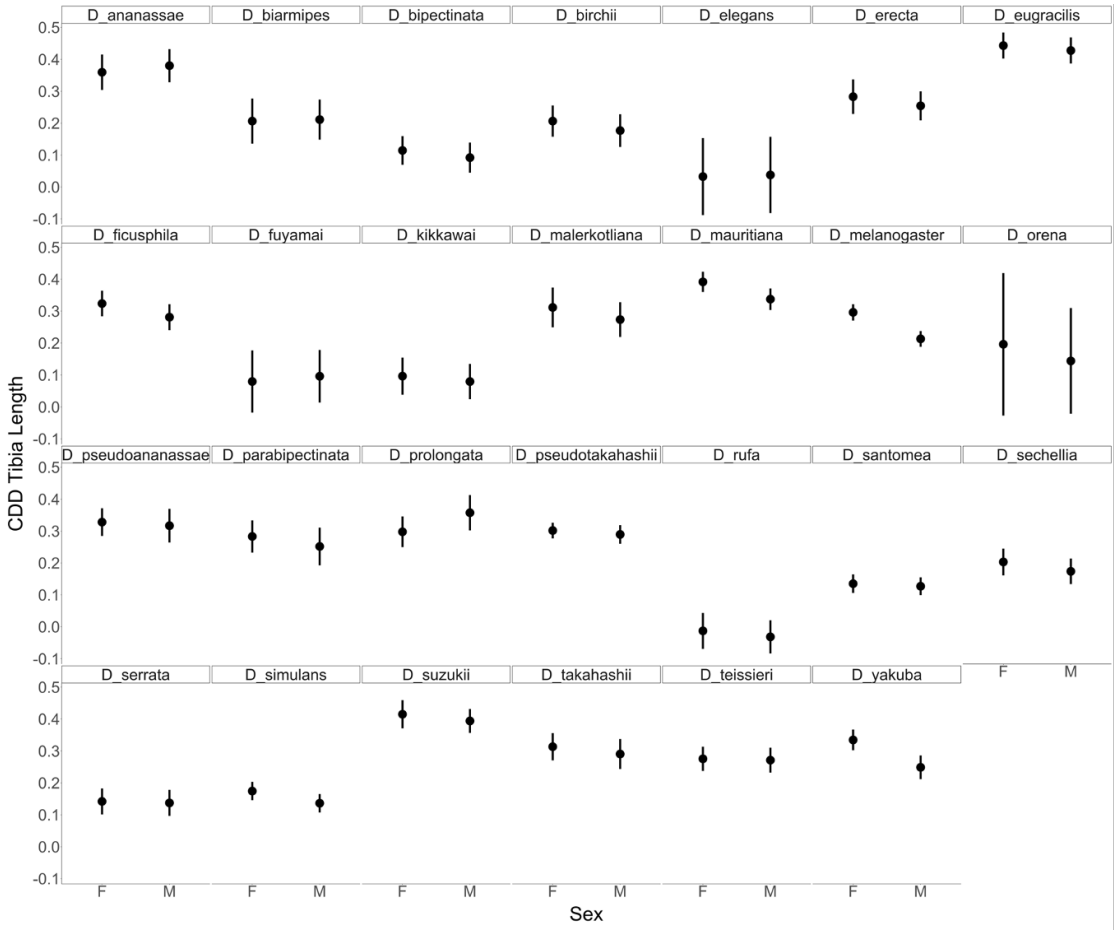

Figure C-S7: Tibia Length CDD

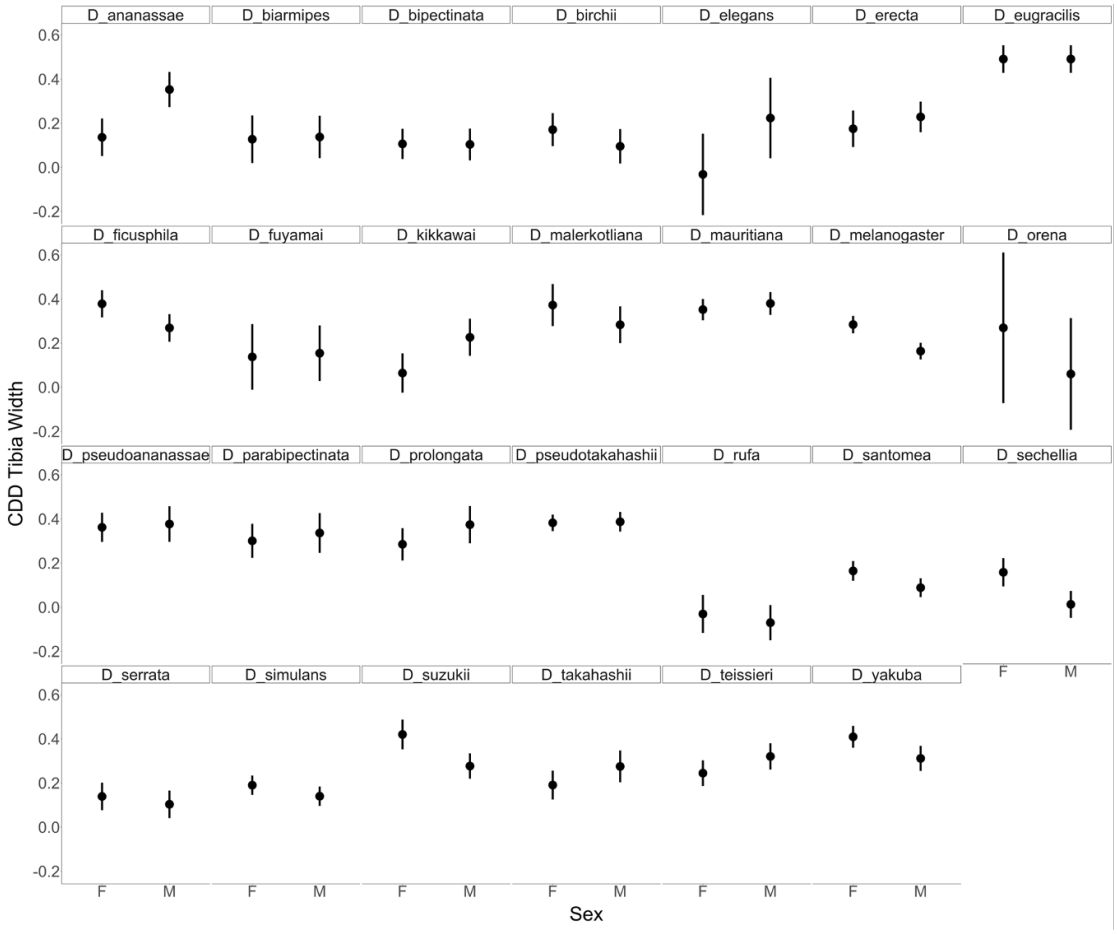

Figure C-S8: Tibia Width CDD

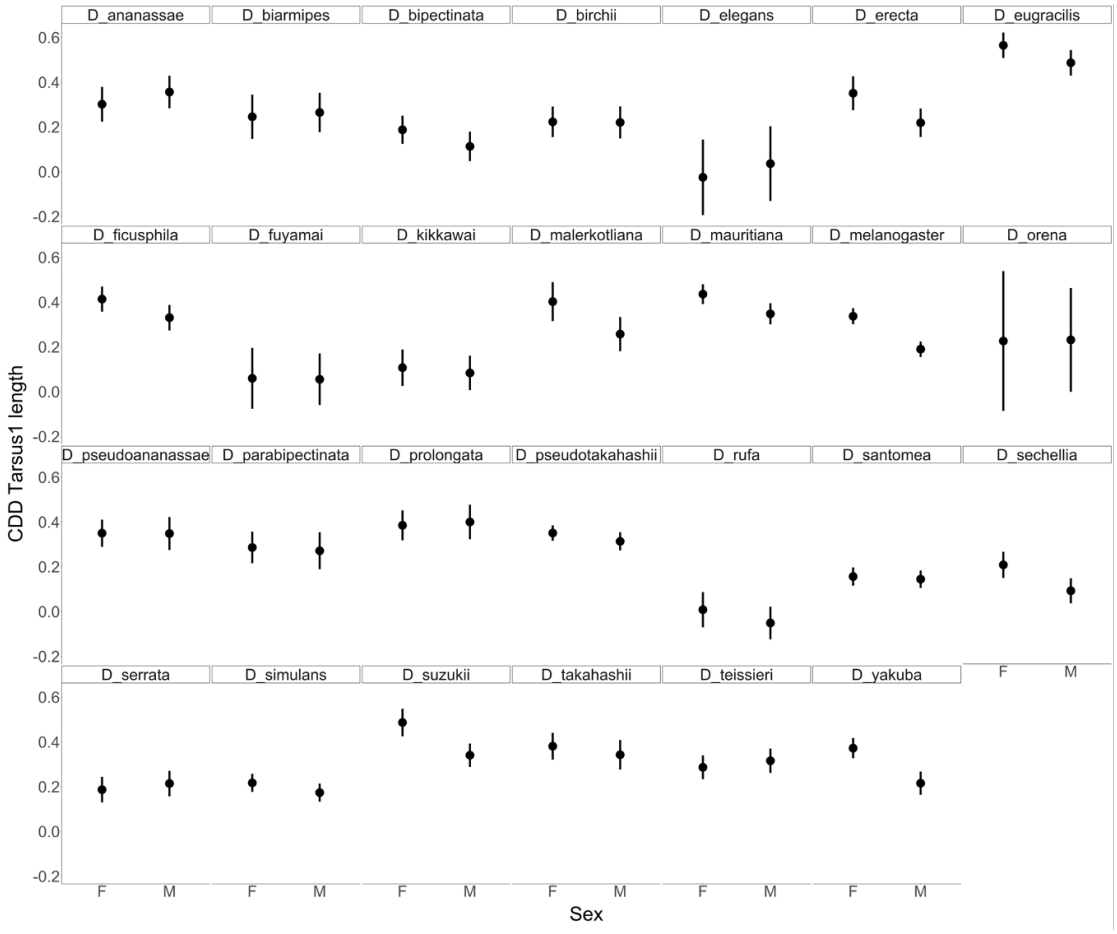

Figure C-S9: Tarsus Length CDD

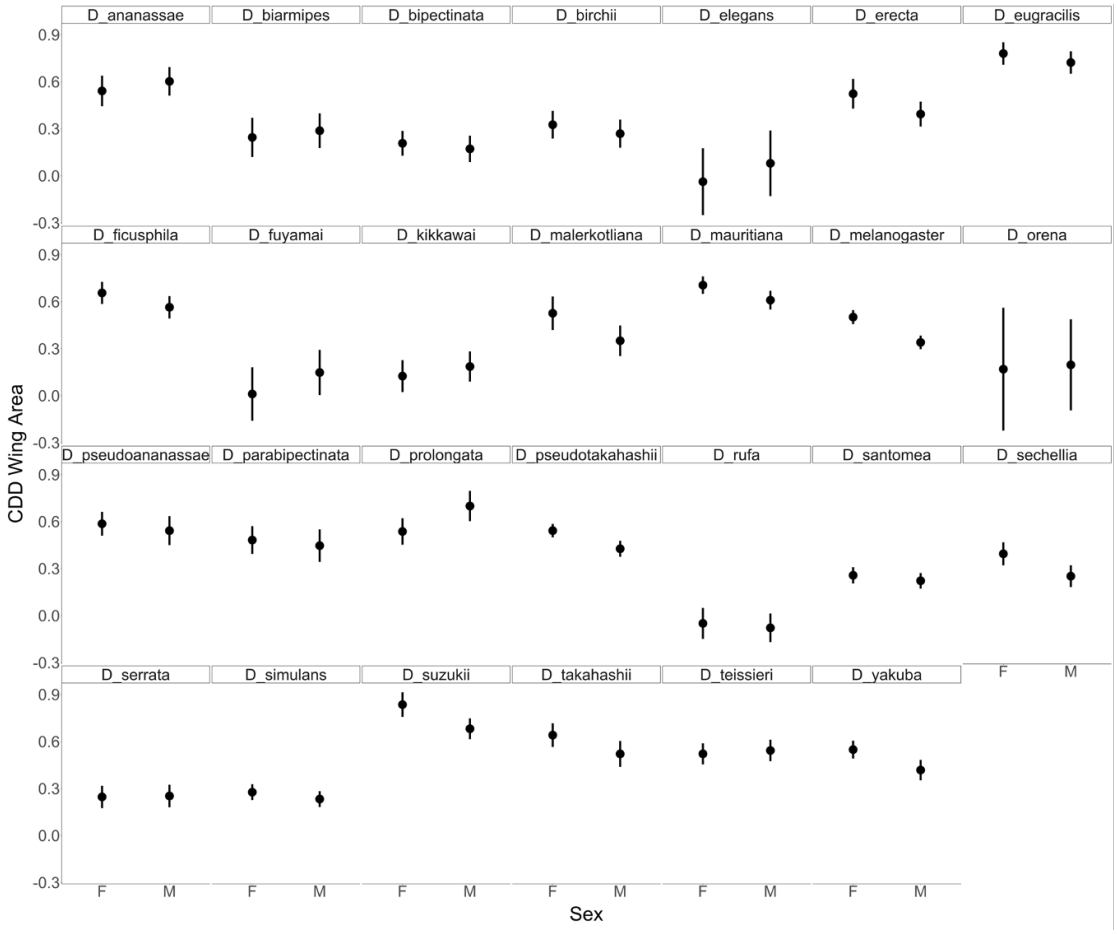

Figure C-S10: Wing Area CDD

Supplemental Tables

Table C-S1: List of species and their sources. Abbreviations for Source include: DSSC - *Drosophila* species stock centre; HAA - High altitude African; LAA - Low altitude African; LC - Local collection; KL - Kopp Lab; LL - Levine Lab; ML - Matute Lab; RL - Rebeiz Lab; HL - Hoffmann Lab, JL - Jaenike Lab

Species	Strains	Source	N_{HC_F}	N_{HC_M}	N_{LC_F}	N_{LC_M}
D. ananassae	1	KL	30	27	11	14
D. biarmipes	1	KL	31	30	6	8
D. bipectinata	1	KL	21	18	30	30
D. birchii	1	DSSC	16	14	30	30
D. elegans	1	KL	2	2	12	14
D. erecta	2	DSSC and LL	58	49	10	16
D. eugracilis	1	KL	30	30	30	30
D. ficusphila	1	KL	30	30	32	30
D. fuyamai	1	KL	3	5	22	14
D. kikkawai	1	KL	28	16	10	17
D. malerkotliana	1	KL	10	14	20	21
D. mauritiana	2	DSSC and ML	60	52	43	38
D. melanogaster	3	HAA, LAA and LC	75	82	77	83
D. orena	2	RL	1	10	1	1
D. pseudoananassae	1	KL	24	14	30	25
D. parabipectinata	1	KL	23	13	17	18
D. prolongata	1	KL	17	22	30	13
D. pseudotakahashii	1	HL	77	62	90	57
D. rufa	1	KL	18	20	14	17
D. santomea	2	KL and ML	68	64	58	65
D. sechellia	2	DSSC and LL	33	34	26	30
D. serrata	1	KL	30	30	30	30
D. simulans	1	ML and LC	60	60	61	60
D. suzukii	2	JL and LC	40	44	19	30
D. takahashii	1	KL	25	18	30	30
D. teissieri	2	ML	34	31	35	35
D. yakuba	2	KL and DSSC	40	27	58	60

Table C-S2: Recipe for 1:2 Protein:Carbohydrate ratio Food for 1L

Ingredient	Amount
Water	$850ml$
Cornmeal	$5g$
Carageenan	$5.4g$
Yeast	$100g$
Sucrose	$100g$
Propionic acid	$2.4ml$
Methylparaben	$2.5g$
Ethanol	$5ml$

Bibliography

Abbott, J. K., Bedhomme, S., and Chippindale, A. K. (Sept. 2010). Sexual conflict in wing size and shape in Drosophila melanogaster. *Journal of Evolutionary Biology* 23(9), 1989–1997. ISSN: 1010061X.

Abouheif, E. and Fairbairn, D. J. (Oct. 1997). A comparative analysis of allometry for sexual size dimorphism: Assessing Rensch's rule. *American Naturalist* 149(3), 540–562. ISSN: 00030147.

Adams, D., Collyer, M., and Kaliontzopoulou, A. (2018). *Geomorph: Software for geometric morphometric analyses. R package version 3.0.6.*

Adams, D. C., Glynne, E., and Kaliontzopoulou, A. (Sept. 2020). Interspecific allometry for sexual shape dimorphism: Macroevolution of multivariate sexual phenotypes with application to Rensch's rule. *Evolution* 74 (9), 1908–1922. ISSN: 0014-3820.

Adams, D. C., Rohlf, F. J., and Slice, D. E. (2013). A field comes of age: Geometric morphometrics in the 21st century. *Hystrix* 24 (1). ISSN: 03941914.

Adler, M. I., Cassidy, E. J., Fricke, C., and Bonduriansky, R. (2013). The lifespan-reproduction trade-off under dietary restriction is sex-specific and context-dependent. *Experimental gerontology* 48(6), 539–548.

Agrawal, A. F. and Stinchcombe, J. R. (Mar. 2009). How much do genetic covariances alter the rate of adaptation? *Proceedings of The Royal Society B: Biological sciences* 276(1659), 1183–1191.

Alicchio, R. and Palenzona, D. L. (1971). Changes of sexual dimorphism values in Drosophila melanogaster. *Italian Journal of Zoology* 38(1), 75–84.

Alpatov, W. W. (1930). Phenotypical variation in body and cell size of *Drosophila Melanogaster*. *The Biological Bulletin* 58(1), 85–103.

Ancel, L. W. and Fontana, W. (2000). Plasticity, evolvability, and modularity in RNA. *Journal of Experimental Zoology* 288(3), 242–283.

Andersson, M. (June 1982). Sexual selection, natural selection and quality advertisement. *Biological Journal of the Linnean Society* 17 (4), 375–393. ISSN: 10958312.

Andersson, M. (July 1986). Evolution Of Condition-Dependent Sex Ornaments And Mating Preferences - Sexual Selection Based On Viability Differences. *Evolution* 40 (4), 804–816. ISSN: 0014-3820.

Andersson, M. (1994). *Sexual Selection*.

Arnold, S. J. (1994). Bateman's principles and the measurement of sexual selection in plants and animals. *American Naturalist* 144 (Suppl.). ISSN: 00030147.

Arnold, S. (Jan. 1983). Morphology, Performance and Fitness. *American Zoologist* 23(2), 347–361.

Arnold, S. (Jan. 2003). Performance surfaces and adaptive landscapes. *Integrative And Comparative Biology* 43(3), 367–375.

Atkinson, D. (1994). *Temperature and Organism Size—A Biological Law for Ectotherms?*

Atkinson, D. (2010). *Ectotherm life-history responses to developmental temperature*.

Azevedo, R. B. R., James, A. C., McCabe, J., and Partridge, L. (Oct. 1998). Latitudinal variation of wing:thorax size ratio and wing-aspect ratio in *Drosophila melanogaster*. *Evolution* 52(5), 1353–1362. ISSN: 00143820.

Badyaev, A. V. (2002). Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. *Trends in Ecology & Evolution* 17(8), 369–378. ISSN: 01695347.

Bagheri-Chaichian, H., Blanchard, J. L., Chao, L., Cheverud, J. M., Elena, S. F., Fontana, W., Gibson, G., Hansen, T. F., Krakauer, D., Lewontin, R. C., et al. (2003). Evolution and detection of genetic robustness. *Evolution* 57(9), 1959–1972.

Baroiller, J. F., DCotta, H., Bezault, E., Wessels, S., and Hoerstgen-Schwark, G. (2009). *Tilapia sex determination: Where temperature and genetics meet.*

Bastide, H., Lange, J. D., Lack, J. B., Yassin, A., and Pool, J. E. (Nov. 2016). A Variable Genetic Architecture of Melanic Evolution in *Drosophila melanogaster*. *Genetics* 204(3), 1307–1319.

Bateman, A. J. (1948a). Intra-sexual selection in Drosophila. *Heredity* 2 (3). ISSN: 13652540.

Bateman, A. J. (1948b). Intra-sexual selection in Drosophila. *Heredity* 2(3), 349–368.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting Linear Mixed-Effects Models Using *lme4*. *Journal of Statistical Software* 67(1), 1–48.

Baur, J., Roy, J., Schäfer, M. A., Puniamoorthy, N., Blanckenhorn, W. U., and Rohner, P. T. (2020). Intraspecific mating system evolution and its effect on complex male secondary sexual traits: Does male–male competition increase selection on size or shape? *Journal of Evolutionary Biology* 33 (3). ISSN: 14209101.

Berger, D., Grieshop, K., Lind, M. I., Goenaga, J., Maklakov, A. A., and Arnqvist, G. (2014). Intralocus sexual conflict and environmental stress. *Evolution* 68(8), 2184–2196.

Berns, M. C. (Mar. 2013). The Evolution of Sexual Dimorphism: Understanding Mechanisms of Sexual Shape Differences. In: *Sexual Dimorphism*. InTech.

Berry, J. F. and Shine, R. (Jan. 1980). Sexual size dimorphism and sexual selection in turtles (order testudines). *Oecologia* 44(2), 185–191. ISSN: 00298549.

Biewener, A. A. (1990). Biomechanics of mammalian terrestrial locomotion. *Science* 250(4984), 1097–1103.

Bird, M. A. and Schaffer, H. (1972). A study of the genetic basis of the sexual dimorphism for wing length in Drosophila melanogaster. *Genetics* 72(3), 475–487.

Bitner–Mathe, B. C. and Klaczko, L. B. (1999). Plasticity of Drosophila melanogaster wing morphology: effects of sex, temperature and density. *Genetica* 105(2), 203–210. ISSN: 00166707.

Blanckenhorn, W. U. (2000). The evolution of body size: what keeps organisms small? *The quarterly review of biology* 75(4), 385–407.

Blanckenhorn, W. U., Meier, R., and Teder, T. (2007a). Rensch's rule in insects: patterns among and within species. *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*.

Blanckenhorn, W. U., Stillwell, R. C., Young, K. A., Fox, C. W., and Ashton, K. G. (2006). When Rensch meets Bergmann: does sexual size dimorphism change systematically with latitude? *Evolution* 60(10), 2004–2011.

Blanckenhorn, W. U., Dixon, A. F. G., Fairbairn, D. J., Foellmer, M. W., Gibert, P., Linde, K. van der, Meier, R., Nylin, S., Pitnick, S., Schoff, C., Signorelli, M., Teder, T., and Wiklund, C. (Feb. 2007b). Proximate Causes of Rensch's Rule: Does Sexual Size Dimorphism in Arthropods Result from Sex Differences in Development Time? *The American Naturalist* 169(2), 245–257. ISSN: 0003-0147.

Blanckenhorn, W. U., Preziosi, R. F., and Fairbairn, D. J. (July 1995). Time and energy constraints and the evolution of sexual size dimorphism ? to eat or to mate? *Evolutionary Ecology* 9(4), 369–381. ISSN: 0269-7653.

Block, M. D. and Stoks, R. (2003). Adaptive sex-specific life history plasticity to temperature and photoperiod in a damselfly. *Journal of Evolutionary Biology* 16 (5). ISSN: 1010061X.

Blows, M. W. (2007). A tale of two matrices: Multivariate approaches in evolutionary biology. *Journal of Evolutionary Biology* 20 (1). ISSN: 1010061X.

Blows, M. W. and Mcguigan, K. (Nov. 2014). The distribution of genetic variance across phenotypic space and the response to selection. *Molecular Ecology* 24(9), 2056–2072.

Bolstad, G. H., Cassara, J. A., Marquez, E., Hansen, T. F., Van Der Linde, K., Houle, D., and Pelabon, C. (Oct. 2015). Complex constraints on allometry revealed by artificial selection on the wing of Drosophila melanogaster. *Proceedings of the National*

Academy of Sciences of the United States of America 112(43), 13284–13289. ISSN: 10916490.

Bonduriansky, R. (Apr. 2009). Condition dependence of developmental stability in the sexually dimorphic fly Telostylinus angusticollis (Diptera: Neriidae). *Journal of Evolutionary Biology* 22(4), 861–872. ISSN: 1010061X.

Bonduriansky, R. (May 2006). Convergent evolution of sexual shape dimorphism in Diptera. *Journal of Morphology* 267(5), 602–611. ISSN: 0362-2525.

Bonduriansky, R. (Apr. 2007a). Sexual selection and allometry: A critical reappraisal of the evidence and ideas. *Evolution* 61 (4), 838–849. ISSN: 00143820.

Bonduriansky, R. (Jan. 2007b). The Evolution of Condition-Dependent Sexual Dimorphism. *The American Naturalist* 169 (1), 9–19. ISSN: 0003-0147.

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*. Ed. by D. J. Fairbairn, W. U. Blanckenhorn, and T. Szekely. New York, NY: Oxford University Press. Chap. 17, 176–184.

Bonduriansky, R. and Chenoweth, S. F. (May 2009). Intralocus sexual conflict. *Trends in Ecology & Evolution* 24 (5), 280–288. ISSN: 0169-5347.

Bonduriansky, R. and Rowe, L. (2005a). Intralocus sexual conflict and the genetic architecture of sexually dimorphic traits in Prochyliza xanthostoma (Diptera: Piophilidae). *Evolution* 59(9), 1965–1975.

Bonduriansky, R. and Rowe, L. (Jan. 2005b). Sexual selection, genetic architecture, and the condition dependence of body shape in the sexually dimorphic fly prochyliza xanthostoma (Piophilidae). *Evolution* 59 (1), 138–151. ISSN: 0014-3820.

Bonneaud, C., Marnocha, E., Herrel, A., Vanhooydonck, B., Irschick, D. J., and Smith, T. B. (2016). Developmental plasticity affects sexual size dimorphism in an anole lizard. *Functional Ecology* 30(2), 235–243.

Bookstein, F. L. (1991). *Morphometric tools for landmark data: geometry and biology.* Cambridge University Press.

Borenstein, E. and Ruppin, E. (2006). Direct evolution of genetic robustness in microRNA. *Proceedings of the National Academy of Sciences* 103(17), 6593–6598.

Breuker, C. J., Patterson, J. S., and Klingenberg, C. P. (2006). A single basis for developmental buffering of *Drosophila* wing shape. *PLoS ONE* 1, e7.

Brooks, M. E., Kristensen, K., van Benthem, K. J., Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M., and Bolker, B. M. (2017). glmmTMB Balances Speed and Flexibility Among Packages for Zero-inflated Generalized Linear Mixed Modeling. *The R Journal* 9(2), 378–400.

Bubliy, O. A. and Loeschcke, V. (June 2002). Effect of low stressful temperature on genetic variation of five quantitative traits in drosophila melanogaster. *Heredity* 89(1), 70–75. ISSN: 0018067X.

Bubliy, O. A., Loeschcke, V., and Imasheva, A. G. (Mar. 2001). Genetic variation of morphological traits in Drosophila melanogaster under poor nutrition: Isofemale lines and offspring-parent regression. *Heredity* 86(3), 363–369. ISSN: 0018067X.

Bull, J. J. and Vogt, R. C. (1979). Temperature-dependent sex determination in turtles. *Science* 206 (4423). ISSN: 00368075.

Butler, M. A. and Losos, J. B. (2002). Multivariate sexual dimorphism, sexual selection, and adaptation in greater antillean anolis lizards. *Ecological Monographs* 72 (4). ISSN: 00129615.

Camara, M. D. and Pigliucci, M. (1999). Mutational contributions to genetic variance-covariance matrices: an experimental approach using induced mutations in *Arabidopsis thaliana*. *Evolution* 53(6), 1692–1703.

Carreira, V. P., Mensch, J., and Fanara, J. J. (Nov. 2008). Body size in Drosophila: genetic architecture, allometries and sexual dimorphism. *Heredity* 102(3), 246–256.

Carreira, V. P., Soto, I. M., Mensch, J., and Fanara, J. J. (2011). Genetic basis of wing morphogenesis in Drosophila: sexual dimorphism and non-allometric effects of shape variation. *BMC developmental biology* 11, 32.

Cassidy, E. J., Bath, E., Chenoweth, S. F., and Bonduriansky, R. (2014). Sex-specific patterns of morphological diversification: Evolution of reaction norms and static allometries in neriid flies. *Evolution* 68(2), 368–383.

Ceballos, C. P. and Valenzuela, N. (2011). The Role of Sex-specific Plasticity in Shaping Sexual Dimorphism in a Long-lived Vertebrate, the Snapping Turtle Chelydra serpentina. *Evolutionary Biology* 38 (2). ISSN: 00713260.

Chandler, C. H., Ofria, C., and Dworkin, I. (2013). Runaway sexual selection leads to good genes. *Evolution: International Journal of Organic Evolution* 67(1), 110–119.

Chandler, C. H., Chari, S., Kowalski, A., Choi, L., Tack, D., DeNieu, M., Pitchers, W., Sonnenschein, A., Marvin, L., Hummel, K., Marier, C., Victory, A., Porter, C., Mammel, A., Holms, J., Sivaratnam, G., and Dworkin, I. (Nov. 2017). How well do you know your mutation? Complex effects of genetic background on expressivity, complementation, and ordering of allelic effects. *PLoS Genetics* 13(11), e1007075.

Chari, S. and Dworkin, I. (2013). The Conditional Nature of Genetic Interactions: The Consequences of Wild-Type Backgrounds on Mutational Interactions in a Genome-Wide Modifier Screen. *PLoS Genetics* 9(8). Ed. by J. Zhang, e1003661. ISSN: 1553-7404.

Chazot, N., Panara, S., Zilbermann, N., Blandin, P., Le Poul, Y., Cornette, R., Elias, M., and Debat, V. (2016). Morpho morphometrics: shared ancestry and selection drive the evolution of wing size and shape in Morpho butterflies. *Evolution* 70(1), 181–194.

Cheng, C. and Houle, D. (2020). Predicting multivariate responses of sexual dimorphism to direct and indirect selection. *The American Naturalist* 196(4), 391–405.

Cheng, C. and Kirkpatrick, M. (2017). Environmental plasticity in the intersexual correlation and sex bias of gene expression. *Journal of Heredity* 108(7), 754–758.

Cheverud, J. M., Rutledge, J. J., and Atchley, W. R. (1983). Quantitative genetics of development: Genetic correlations among age-specific trait values and the evolution of ontogeny. *Evolution* 37(5), 895–905. ISSN: 00143820.

Clarke, G. M., Yen, J. L., and McKenzie, J. A. (2000). Wings and bristles: character specificity of the asymmetry phenotype in insecticide-resistant strains of Lucilia cuprina. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 267(1455), 1815–1818.

Clarke, G. and McKenzie, J. (1987). Developmental stability of insecticide resistant phenotypes in blowfly; a result of canalizing natural selection. *Nature* 325(6102), 345–346.

Collet, J. M., Fuentes, S., Hesketh, J., Hill, M. S., Innocenti, P., Morrow, E. H., Fowler, K., and Reuter, M. (2016). Rapid evolution of the intersexual genetic correlation for fitness in Drosophila melanogaster. *Evolution* 70(4), 781–795.

Collyer, M. and Adams, D.-R. (2020). Linear model evaluation with randomized residuals in a permutation procedure. *R package version 0.5* 2.

Connallon, T. (2010). Genie capture, sex linkage, and the heritability of fitness. *American Naturalist*. ISSN: 00030147.

Connallon, T. (2015). The geography of sex-specific selection, local adaptation, and sexual dimorphism. *Evolution*. ISSN: 15585646.

Connallon, T. and Clark, A. G. (Dec. 2010). Sex linkage, sex-specific selection, and the role of recombination in the evolution of sexually dimorphic gene expression. *Evolution* 64(12), 3417–3442. ISSN: 00143820.

Connallon, T., Debarre, F., and Li, X. Y. (2018). Linking local adaptation with the evolution of sex differences. *Philosophical Transactions of the Royal Society B: Biological Sciences* 373(1757). ISSN: 14712970.

Connallon, T. and Hall, M. D. (2016). Genetic correlations and sex-specific adaptation in changing environments. *Evolution*. ISSN: 15585646.

Cotton, S., Fowler, K., and Pomiankowski, A. (May 2004a). Condition dependance of sexual ornament size and variation in the stalk-eyed fly Cyrtodiopsis dalmanni (Diptera:Diopsidae). *Evolution* 58(5), 1038–1046.

Cotton, S., Fowler, K., and Pomiankowski, A. (Apr. 2004b). Do sexual ornaments demonstrate heightened condition-dependent expression as predicted by the handicap hypothesis? *Proceedings of the Royal Society B: Biological Sciences* 271 (1541), 771–783. ISSN: 0962-8452.

Cox, R. M., Butler, M. A., and John-Alder, H. B. (July 2007). The evolution of sexual size dimorphism in reptiles. In: *Sex, Size and Gender Roles*. Oxford University Press, 38–49.

Cox, R. M. and Calsbeek, R. (2009). Sexually antagonistic selection, sexual dimorphism, and the resolution of intralocus sexual conflict. *American Naturalist*. ISSN: 00030147.

Cox, R. M., Skelly, S. L., and John-Alder, H. B. (2003). A comparative test of adaptive hypotheses for sexual size dimorphism in lizards. *Evolution*. ISSN: 00143820.

Crespo, E., Vicente, L., and SaSousa, P. (2002). Morphological variability of Podarcis hispanica (Sauria: lacertidae) in Portugal. *Amphibia-Reptilia* 23(1), 55–69.

Darwin, C. (1874). *The Descent of Man, and Selection in Relation to Sex*. 2nd ed. Vol. 2. New York: D Appleton and Company, 1–475.

David, J. R. and Bocquet, C. (Feb. 1975). Evolution in a cosmopolitan species: Genetic latitudinal clines in Drosophila melanogaster wild populations. *Experientia* 31(2), 164–166. ISSN: 00144754.

David, J. R., Gibert, P., Mignon-Grasteau, S., Legout, H., Petavy, G., Beaumont, C., and Moreteau, B. (2003). Genetic variability of sexual size dimorphism in a natural population ofDrosophila melanogaster: An isofemale-line approach. *Journal of genetics* 82(3), 79–88.

David, J., Moreteau, B., Gauthier, J., Petavy, G., Stockel, A., and Imasheva, A. (1994). Reaction norms of size characters in relation to growth temperature in *Drosophila*

melanogaster: an isofemale lines analysis. *Genetics Selection Evolution* 26(3), 229–251. ISSN: 1297-9686.

David, P., Hingle, A., Greig, D., Rutherford, A., Pomiankowski, A., and Fowler, K. (1998). Male sexual ornament size but not asymmetry reflects condition in stalk-eyed flies. *Proceedings of the Royal Society B: Biological Sciences* 265 (1411), 2211–2216. ISSN: 0962-8452.

Davies, A. G., Game, A. Y., Chen, Z., Williams, T. J., Goodall, S., Yen, J. L., McKenzie, J. A., and Batterham, P. (1996). Scalloped wings is the *Lucilia cuprina* Notch homologue and a candidate for the Modifier of fitness and asymmetry of diazinon resistance. *Genetics* 143(3), 1321–1337.

Day, T. and Bonduriansky, R. (Aug. 2004). Intralocus Sexual Conflict Can Drive the Evolution of Genomic Imprinting. *Genetics* 167(4), 1537–1546. ISSN: 0016-6731.

Debat, V., Begin, M., Legout, H., and David, J. R. (Dec. 2003). Allometric and nonallometric components of Drosophila wing shape respond differently to developmental temperature. *Evolution; international journal of organic evolution* 57(12), 2773–84. ISSN: 0014-3820.

Debat, V., Bloyer, S., Faradji, F., Gidaszewski, N., Navarro, N., Orozco-terWengel, P., Ribeiro, V., Schlotterer, C., Deutsch, J. S., and Peronnet, F. (Oct. 2011). Developmental Stability: A Major Role for Cyclin G in *Drosophila melanogaster*. *PLoS Genetics* 7(10), e1002314.

Debat, V., Debelle, A., and Dworkin, I. (2009). Plasticity, canalization, and developmental stability of the *Drosophila* wing: Joint effects of mutations and developmental temperature. *Evolution* 63(11), 2864–2876. ISSN: 00143820.

Debat, V., Milton, C. C., Rutherford, S., Klingenberg, C. P., and Hoffmann, A. A. (Jan. 2006). Hsp90 and the quantitative variation of wing shape in *Drosophila melanogaster*. *Evolution* 60(12), 2529–2538.

Delph, L. F., Steven, J. C., Anderson, I. A., Herlihy, C. R., and Brodie III, E. D. (2011). Elimination of a genetic correlation between the sexes via artificial correlational selection. *Evolution: International Journal of Organic Evolution* 65(10), 2872–2880.

Dillon, M. E., Frazier, M. R., and Dudley, R. (2006a). Into thin air: physiology and evolution of alpine insects. *Integrative and Comparative Biology* 46(1), 49–61.

Dillon, M. E., Frazier, M. R., and Dudley, R. (Feb. 2006b). Into thin air: Physiology and evolution of alpine insects. *Integrative and Comparative Biology* 46(1), 49–61. ISSN: 1540-7063.

Dittmar, E. L., Oakley, C. G., Conner, J. K., Gould, B. A., and Schemske, D. W. (Apr. 2016). Factors influencing the effect size distribution of adaptive substitutions. *Proceedings Of The Royal Society B: Biological Sciences* 283(1828), 20153065.

Dmitriew, C. and Blanckenhorn, W. U. (Nov. 2014). Condition dependence and the maintenance of genetic variance in a sexually dimorphic black scavenger fly. *Journal of Evolutionary Biology* 27 (11), 2408–2419. ISSN: 1010061X.

Dobens, A. C. and Dobens, L. L. (Aug. 2013). FijiWings: An Open Source Toolkit for Semiautomated Morphometric Analysis of Insect Wings. *G3* 3(8), 1443–1449.

Dobzhansky, T. (Apr. 1929). The influence of the quantity and quality of chromosomal material on the size of the cells in *Drosophila melanogaster*. *Wilhelm Roux Archiv fur Entwicklungsmechanik der Organismen* 115(3), 363–379.

Dryden, I. and Mardia, K. (1998). *Statistical analysis of shape*. Wiley.

Dun, R. and Fraser, A. (1959). Selection for an Invariant · Character, Vibrissa Number, in the House Mouse. *Australian Journal of Biological Sciences* 12(4), 506–523.

Dworkin, I. (2005a). A study of canalization and developmental stability in the sternopleural bristle system of *Drosophila melanogaster*. *Evolution* 59(7), 1500–1509. ISSN: 00143820.

Dworkin, I. (Jan. 2005b). Canalization, cryptic variation, and developmental buffering: A critical examination and analytical perspective. In: *Variation*. Academic Press, 131–158.

Dworkin, I. (2005c). Evidence for canalization of Distal-less function in the leg of *Drosophila melanogaster*. *Evolution and Development* 7(2), 89–100. ISSN: 1520-541X.

Eisen, E. and Hanrahan, J. (1972). Selection for sexual dimorphism in body weight of mice. *Australian journal of biological sciences* 25(5), 1015–1024.

Emlen, D. J. (1994). Environmental Control of Horn Length Dimorphism in the Beetle Onthophagus acuminatus (Coleoptera: Scarabaeidae). *Proceedings of the Royal Society B: Biological Sciences* 256 (1346), 131–136. ISSN: 0962-8452.

Emlen, D. J. (1997). Diet alters male horn allometry in the beetle Onthophagus acuminatus (Coleoptera: Scarabaeidae). *Proceedings of the Royal Society of London. Series B: Biological Sciences* 264(1381), 567–574.

Emlen, D. J. and Nijhout, H. F. (1999). Hormonal control of male horn length dimorphism in the dung beetle Onthophagus taurus (Coleoptera: Scarabaeidae). *Journal of Insect Physiology* 45(1), 45–53.

Emlen, D. J., Warren, I. A., Johns, A., Dworkin, I., and Lavine, L. C. (2012). A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. *Science* 337(6096), 860–864.

Endler, J. A. (1980). Natural Selection on Color Patterns in Poecilia reticulata. *Evolution* 34 (1). ISSN: 00143820.

Eshel, I. and Matessi, C. (Aug. 1998). Canalization, Genetic Assimilation and Preadaptation: A Quantitative Genetic Model. *Genetics* 149(4), 2119–2133.

Evans, K. M., Bernt, M. J., Kolmann, M. A., Ford, K. L., and Albert, J. S. (2019). Why the long face? Static allometry in the sexually dimorphic phenotypes of Neotropical electric fishes. *Zoological Journal of the Linnean Society* 186(3), 633–649.

Ewing, A. W. (1983). Functional aspects of Drosophila courtship. *Biological Reviews* 58(2), 275–292.

Ewing, A. W. and Bennet-Clark, H. (1968). The courtship songs of Drosophila. *Behaviour* 31(3-4), 288–301.

Ewing, A. W. (1964). The influence of wing area on the courtship behaviour of Drosophila melanogaster. *Animal Behaviour* 12(2), 316–320. ISSN: 00033472.

Fabian, D. K., Lack, J. B., Mathur, V., Schlotterer, C., Schmidt, P. S., Pool, J. E., and Flatt, T. (2015). Spatially varying selection shapes life history clines among populations of *Drosophila melanogaster* from sub-Saharan Africa. *Journal of Evolutionary Biology* 28(4), 826–840. ISSN: 1010061X.

Fairbairn, D. J. (Nov. 1997). Allometry for sexual size dimorphism: Pattern and process in the coevolution of body size in males and females. *Annual Review of Ecology and Systematics* 28(1), 659–687. ISSN: 00664162.

Fairbairn, D. J. and Preziosi, R. F. (Aug. 1994). Sexual selection and the evolution of allometry for sexual size dimorphism in the water strider, Aquarius remigis. *American Naturalist* 144(1), 101–118. ISSN: 00030147.

Fairbairn, D. J. (1990). Factors influencing sexual size dimorphism in temperate waterstriders. *The American Naturalist* 136(1), 61–86.

Fairbairn, D. J. (2005). Allometry for sexual size dimorphism: Testing two hypotheses for Rensch's rule in the water strider Aquarius remigis. In: vol. 166.

Fairbairn, D. J. (2007a). Introduction: The enigma of sexual size dimorphism. *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*. ISSN: 10752846.

Fairbairn, D. J. (2007b). Sexual dimorphism in the water strider, Aquarius remigis: A case study of adaptation in response to sexually antagonistic selection. *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*, 97–105.

Felix, M.-A. and Barkoulas, M. (Aug. 2015). Pervasive robustness in biological systems. *Nature Reviews Genetics* 16(8), 483–496.

Fernandez-Montraveta, C. and Moya-Larano, J. (2007). Sex-specific plasticity of growth and maturation size in a spider: implications for sexual size dimorphism. *Journal of evolutionary biology* 20(5), 1689–1699.

Fernandez-Montraveta, C. and Marugan-Lobon, J. (2017). Geometric morphometrics reveals sex-differential shape allometry in a spider. *PeerJ* 5, e3617.

Fernández-Montraveta, C. and Marugán-Lobón, J. (2017). Geometric morphometrics reveals sex-differential shape allometry in a spider. *PeerJ* 2017 (7). ISSN: 21678359.

Fischer, K. and Fiedler, K. (2000). Sex-related differences in reaction norms in the butterfly Lycaena tityrus (Lepidoptera: Lycaenidae). *Oikos* 90(2), 372–380.

Fischer, K. and Fiedler, K. (2002). Reaction norms for age and size at maturity in response to temperature: a test of the compound interest hypothesis. *Evolutionary Ecology* 16(4), 333–349.

Fisher, R. (1958). The genetical theory of natural selection. *Dover Publication* 22, 127–130. ISSN: 0016-6731.

Flatt, T. (Sept. 2005). The evolutionary genetics of canalization. *The Quarterly review of biology* 80(3), 287–316.

Foellmer, M. W. and Moya-Larano, J. (2007). Sexual size dimorphism in spiders: patterns and processes. *Sex, size and gender roles: Evolutionary studies of sexual size dimorphism*, 71–81.

Fox, C. W., Bush, M. L., Roff, D. A., and Wallin, W. G. (2004). Evolutionary genetics of lifespan and mortality rates in two populations of the seed beetle, Callosobruchus maculatus. *Heredity* 92 (3). ISSN: 0018067X.

Fraimout, A., Jacquemart, P., Villarroel, B., Aponte, D. J., Decamps, T., Herrel, A., Cornette, R., and Debat, V. (2018). Phenotypic plasticity of Drosophila suzukii wing to developmental temperature: implications for flight. *Journal of Experimental Biology* 221(13).

Franklin, D., Oxnard, C. E., O'Higgins, P., and Dadour, I. (2007). Sexual dimorphism in the subadult mandible: quantification using geometric morphometrics. *Journal of forensic sciences* 52(1), 6–10.

Gallach, M. and Betran, E. (May 2011). Intralocus sexual conflict resolved through gene duplication. *Elsevier Current Trends* 26(5), 222–228. ISSN: 01695347.

Gebhardt, M. D. and Stearns, S. C. (1988). Reaction norms for developmental time and weight at eclosion in Drosophila mercatorum. *Journal of Evolutionary Biology* 1(4), 335–354.

Geiler-Samerotte, K., Sartori, F. M., and Siegal, M. L. (2019). Decanalizing thinking on genetic canalization. *Seminars in cell & developmental biology* 88, 54–66.

Gibert, P., Capy, P., Imasheva, A., Moreteau, B., Morin, J. P., Petavy, G., and David, J. R. (Mar. 2004). Comparative analysis of morphological traits among Drosophila melanogaster and D. simulans: Genetic variability, clines and phenotypic plasticity. In: *Genetica*. Vol. 120. 1-3. Springer, 165–179.

Gibert, P., Moreteau, B., and David, J. R. (1999). Phenotypic plasticity of abdominal pigmentation in Drosophila kikkawai: multiple interactions between a major gene, sex, abdomen segment and growth temperature. *Genetica* 105(2), 165–176.

Gibson, G. and Helden, S. van (Nov. 1997). Is function of the *Drosophila* homeotic gene Ultrabithorax canalized? *Genetics* 147(3), 1155–1168.

Gibson, G. and Wagner, G. (Apr. 2000). Canalization in evolutionary genetics: a stabilizing theory? *BioEssays* 22(4), 372–380.

Gidaszewski, N. A., Baylac, M., and Klingenberg, C. P. (May 2009). Evolution of sexual dimorphism of wing shape in the Drosophila melanogaster subgroup. *BMC Evolutionary Biology* 9(1), 110. ISSN: 1471-2148.

Gilchrist, A. S., Azevedo, R. B., Partridge, L., and OHiggins, P. (Feb. 2000). Adaptation and constraint in the evolution of Drosophila melanogaster wing shape. *Evolution and Development* 2(2), 114–124. ISSN: 1520541X.

Gonzalez-Gaitan, M., Capdevila, M. P., and Garcia-Bellido, A. (June 1994). Cell proliferation patterns in the wing imaginal disc of *Drosophila*. *Mechanisms of Development* 46(3), 183–200.

Goodall, C. (1991). Procrustes methods in the statistical analysis of shape. *Journal of the Royal Statistical Society: Series B (Methodological)* 53(2), 285–321.

Gosden, T. P., Shastri, K.-L., Innocenti, P., and Chenoweth, S. F. (2012). The B-matrix harbors significant and sex-specific constraints on the evolution of multicharacter sexual dimorphism. *Evolution: International Journal of Organic Evolution* 66(7), 2106–2116.

Gotoh, H., Miyakawa, H., Ishikawa, A., Ishikawa, Y., Sugime, Y., Emlen, D. J., Lavine, L. C., and Miura, T. (2014). Developmental link between sex and nutrition; doublesex regulates sex-specific mandible growth via juvenile hormone signaling in stag beetles. *PLoS Genet* 10(1), e1004098.

Green, R. M., Fish, J. L., Young, N. M., Smith, F. J., Roberts, B., Dolan, K., Choi, I., Leach, C. L., Gordon, P., Cheverud, J. M., Roseman, C. C., Williams, T. J., Marcucio, R. S., and Hallgrimsson, B. (Dec. 2017). Developmental nonlinearity drives phenotypic robustness. *Nat Commun* 8(1), 1970.

Groth, B. R., Huang, Y., Monette, M. J., and Pool, J. E. (Aug. 2018). Directional selection reduces developmental canalization against genetic and environmental perturbations in *Drosophila* wings. *Evolution* 72(8), 1708–1715. ISSN: 00143820.

Haber, A. (Dec. 2011). A Comparative Analysis of Integration Indices. *Evolutionary Biology* 38(4), 476–488. ISSN: 00713260.

Haber, A. and Dworkin, I. (2017). Disintegrating the fly: A mutational perspective on phenotypic integration and covariation. *Evolution* 71(1), 66–80. ISSN: 00143820.

Hallgrimsson, B., Brown, J. J. Y., Ford-Hutchinson, A. F., Sheets, H. D., Zelditch, M. L., and Jirik, F. R. (Jan. 2006). The brachymorph mouse and the developmental-genetic basis for canalization and morphological integration. *Evolution & Development* 8(1), 61–73.

Hallgrimsson, B., Jamniczky, H., Young, N. M., Rolian, C., Parsons, T. E., Boughner, J. C., and Marcucio, R. S. (Nov. 2009). Deciphering the Palimpsest: Studying the Relationship Between Morphological Integration and Phenotypic Covariation. *Evolutionary Biology* 36(4), 355–376.

Hallsson, L. R. and Bjorklund, M. (2012). Selection in a fluctuating environment and the evolution of sexual dimorphism in the seed beetle Callosobruchus maculatus. *Journal of Evolutionary Biology* 25(8), 1564–1575.

Hansen, T. F. and Houle, D. (Sept. 2008). Measuring and comparing evolvability and constraint in multivariate characters. *Journal Of Evolutionary Biology* 21(5), 1201–1219.

Have, T. M. V. D. and Jong, G. D. (1996). Adult size in ectotherms: Temperature effects on growth and differentiation. *Journal of Theoretical Biology* 183 (3). ISSN: 00225193.

Hayden, E., Weikert, C., and Wagner, A. (2012). Directional Selection Causes Decanalization in a Group I Ribozyme. *PLoS ONE* 7(9), e45351.

Head, G. (1995). Selection on fecundity and variation in the degree of sexual size dimorphism among spider species (Class Araneae). *Evolution*, 776–781.

Hedrick, A. V. and Temeles, E. J. (1989). The evolution of sexual dimorphism in animals: Hypotheses and tests. *Trends in Ecology and Evolution* 4(5), 136–138. ISSN: 01695347.

Hermisson, J., Hansen, T. F., and Wagner, G. P. (2003). Epistasis in polygenic traits and the evolution of genetic architecture under stabilizing selection. *The American Naturalist* 161(5), 708–734.

Hermisson, J. and Wagner, G. P. (2004). The population genetic theory of hidden variation and genetic robustness. *Genetics* 168(4), 2271–2284.

Herrel, A., Moore, J. A., Bredeweg, E. M., and Nelson, N. J. (2010). Sexual dimorphism, body size, bite force and male mating success in tuatara. *Biological Journal of the Linnean Society* 100 (2). ISSN: 00244066.

Herrel, A., Schaerlaeken, V., Moravec, J., and Ross, C. F. (2009). Sexual shape dimorphism in tuatara. *Copeia* (4). ISSN: 00458511.

Hill, G. E. (2011). Condition-dependent traits as signals of the functionality of vital cellular processes. *Ecology Letters* 14 (7), 625–634. ISSN: 1461023X.

Hirst, A. G., Horne, C. R., and Atkinson, D. (2015). Equal temperature–size responses of the sexes are widespread within arthropod species. *Proceedings of the Royal Society B: Biological Sciences* 282 (1820). ISSN: 14712954.

Hodkinson, I. D. (Aug. 2005). Terrestrial insects along elevation gradients: species and community responses to altitude. *Biological Reviews* 80(03), 489. ISSN: 1464-7931.

Hoffmann, A. A. and Weeks, A. R. (Feb. 2007). Climatic selection on genes and traits after a 100 year-old invasion: A critical look at the temperate-tropical clines in Drosophila melanogaster from eastern Australia. *Genetica* 129(2), 133–147. ISSN: 00166707.

Houle, D., Bolstad, G. H., Linde, K. van der, and Hansen, T. F. (Aug. 2017a). Mutation predicts 40 million years of fly wing evolution. *Nature* 548(7668), 447–450.

Houle, D., Bolstad, G. H., Van Der Linde, K., and Hansen, T. F. (2017b). Mutation predicts 40 million years of fly wing evolution. *Nature* 548(7668). ISSN: 14764687.

Houle, D. and Fierst, J. (2012). Properties of spontaneous mutational variance and covariance for wing size and shape in Drosophila melanogaster. *Evolution*, no–no.

Houle, D. and Kondrashov, A. S. (2002). Coevolution of costly mate choice and condition-dependent display of good genes. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 269(1486), 97–104.

Houle, D., Mezey, J., Galpern, P., and Carter, A. (Dec. 2003). Automated measurement of *Drosophila* wings. *BMC Evolutionary Biology* 3, 25.

Hu, Y., Xie, Y., Zhu, F., Wang, C., and Lei, C. (2010). Variation in sexual size dimorphism among populations: Testing the differential-plasticity hypothesis. *Entomologia Experimentalis et Applicata* 137 (2), 204–209. ISSN: 00138703.

Huey, R. B., Moreteau, B., Moreteau, J. C., Gibert, P., Gilchrist, G. W., Ives, A. R., Garland, T., and David, J. R. (2006). Sexual size dimorphism in a Drosophila clade, the D. obscura group. *Zoology* 109(4), 318–330. ISSN: 09442006.

Imasheva, A. G., Bosenko, D. V., and Bubli, O. A. (Feb. 1999). Variation in morphological traits of Drosophila melanogaster (fruit fly) under nutritional stress. *Heredity* 82(2), 187–192. ISSN: 0018067X.

Ingleby, F., Innocenti, P., Rundle, H., and Morrow, E. (2014). Between-sex genetic covariance constrains the evolution of sexual dimorphism in D rosophila melanogaster. *Journal of evolutionary biology* 27(8), 1721–1732.

Iwasa, Y. and Pomiankowski, A. (June 1994). The evolution of mate preferences for multiple sexual ornaments. *Evolution* 48 (3), 853–867. ISSN: 00143820.

Iwasa, Y. and Pomiankowski, A. (1995). Continual change in mate preferences. *Nature* 377 (6548). ISSN: 00280836.

Iwasa, Y. and Pomiankowski, A. (Sept. 1999). Good parent and good genes models of handicap evolution. *Journal of Theoretical Biology* 200 (1), 97–109. ISSN: 00225193.

Iwasa, Y., Pomiankowski, A., and Nee, S. (Sept. 1991). The evolution of costly mate preferences II. The "Handicap" principle. *Evolution* 45(6), 1431–1442. ISSN: 00143820.

Jakob, E. M., Marshall, S. D., and Uetz, G. W. (1996). Estimating fitness: A comparison of body condition indices. *Oikos* 77 (1), 61–67. ISSN: 00301299.

James, A. C., Azevedo, R. B., and Partridge, L. (1997). Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. *Genetics* 146(3), 881–890. ISSN: 00166731.

Jensen, H., Sæther, B.-E., Ringsby, T. H., Tufto, J., Griffith, S., and Ellegren, H. (2003). Sexual variation in heritability and genetic correlations of morphological traits in house sparrow (Passer domesticus). *Journal of evolutionary biology* 16(6), 1296–1307.

Johnstone, R. (Feb. 1995). Sexual selection, honest advertisement and the handicap principle: Reviewing the evidence. *Biological Reviews* 70 (1), 1–65. ISSN: 1469185X.

Jones, A. G., Arnold, S. J., and Bürger, R. (Aug. 2003). Stability of the G-matrix in a population experiencing pleiotropic mutation, stabilizing selection, and genetic drift. *Evolution* 57(8), 1747–1760. ISSN: 00143820.

Kaliontzopoulou, A., Carretero, M. A., and Adams, D. C. (2015). Ecomorphological variation in male and female wall lizards and the macroevolution of sexual dimorphism in relation to habitat use. *Journal of Evolutionary Biology* 28 (1). ISSN: 14209101.

Kaliontzopoulou, A., Adams, D. C., Meijden, A. van der, Perera, A., and Carretero, M. A. (2012). Relationships between head morphology, bite performance and ecology in two species of Podarcis wall lizards. *Evolutionary Ecology* 26(4), 825–845.

Kaliontzopoulou, A., Carretero, M. A., and LLorente, G. A. (Dec. 2008a). Head shape allometry and proximate causes of head sexual dimorphism in Podarcis lizards: Joining linear and geometric morphometrics. *Biological Journal of the Linnean Society* 93(1), 111–124. ISSN: 00244066.

Kaliontzopoulou, A., Carretero, M. A., and Llorente, G. A. (2008b). Head shape allometry and proximate causes of head sexual dimorphism in Podarcis lizards: joining linear and geometric morphometrics. *Biological Journal of the Linnean Society* 93(1), 111–124.

Karan, D., Morin, J.-P., Gibert, P., Moreteau, B., Scheiner, S. M., and David, J. R. (2000). The genetics of phenotypic plasticity. IX. Genetic architecture, temperature, and sex differences in Drosophila melanogaster. *BioOne* 54(3), 1035–1040. ISSN: 0014-3820.

Karl, I. and Fischer, K. (2008). Why get big in the cold? Towards a solution to a life-history puzzle. *Oecologia* 155(2), 215–225.

Katayama, N., Abbott, J. K., Kjærandsen, J., Takahashi, Y., and Svensson, E. I. (Oct. 2014). Sexual selection on wing interference patterns in Drosophila melanogaster. *Proceedings of the National Academy of Sciences* 111(42), 15144–15148. ISSN: 0027-8424.

Kawano, K. (Mar. 2006). Sexual dimorphism and the making of oversized male characters in beetles (Coleoptera). *Annals of the Entomological Society of America* 99 (2), 327–341. ISSN: 00138746.

Kawecki, T. J. (2000). The evolution of genetic canalization under fluctuating selection. *Evolution* 54(1), 1–12.

Kemp, D. J., Reznick, D. N., Grether, G. F., and Endler, J. A. (2009). Predicting the direction of ornament evolution in Trinidadian guppies (Poecilia reticulata). *Proceedings of the Royal Society B: Biological Sciences* 276 (1677). ISSN: 14712970.

Kendall, D. G. (1977). The diffusion of shape. *Advances in applied probability* 9(3), 428–430.

Kirkpatrick, M. (1986). The handicap mechanism of sexual selection does not work. *The American Naturalist* 127(2), 222–240.

Kirkpatrick, M. (June 2009). Patterns of quantitative genetic variation in multiple dimensions. *Genetica* 136(2), 271–284.

Kleisner, K., Tureček, P., Roberts, S. C., Havlíček, J., Valentova, J. V., Akoko, R. M., Leongómez, J. D., Apostol, S., Varella, M. A., and Saribay, S. A. (Dec. 2021). How and why patterns of sexual dimorphism in human faces vary across the world. *Scientific Reports* 11 (1), 1–14. ISSN: 20452322.

Klepsatel, P., Galikova, M., De Maio, N., Huber, C. D., Schlotterer, C., and Flatt, T. (Dec. 2013). Variation in thermal performance and reaction norms among populations of *Drosophila melangoaster*. *Evolution* 67(12), 3573–3587. ISSN: 00143820.

Klepsatel, P., Gáliková, M., Huber, C. D., and Flatt, T. (2014). Similarities and differences in altitudinal versus latitudinal variation for morphological traits in *Drosophila melangoaster*. *Evolution* 68(5), 1385–1398.

Klingenberg, C. P. (June 2016). Size, shape, and form: concepts of allometry in geometric morphometrics. *Development Genes and Evolution* 226 (3), 113–137. ISSN: 1432041X.

Klingenberg, C. P. (Mar. 2019). Phenotypic Plasticity, Developmental Instability, and Robustness: The Concepts and How They Are Connected. *Frontiers in Ecology and Evolution* 7, 56.

Klingenberg, C. P. and McIntyre, G. S. (1998). Geometric Morphometrics of Developmental Instability: Analyzing Patterns of Fluctuating Asymmetry with Procrustes Methods. *Evolution* 52(5), 1363. ISSN: 00143820.

Klingenberg, C. P. and Zaklan, S. D. (2000). Morphological integration between developmental compartments in the *Drosophila* wing. *Evolution* 54(4), 1273–1285.

Kokko, H., Brooks, R., McNamara, J. M., and Houston, A. I. (2002). The sexual selection continuum. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 269(1498), 1331–1340.

Kopp, A., Duncan, I., and Carroll, S. B. (2000). Genetic control and evolution of sexually dimorphic characters in Drosophila. *Nature* 408(6812), 553–559.

Kopp, A. and True, J. R. (2002). Evolution of male sexual characters in the oriental Drosophila melanogaster species group. *Evolution & development* 4(4), 278–291.

Koyama, T., Mendes, C., and Mirth, C. K. (2013). Mechanisms regulating nutrition-dependent developmental plasticity through organ-specific effects in insects. *Frontiers in physiology* 4, 263.

Kuo, C. Y., Lin, Y. T., and Lin, Y. S. (2009). Sexual size and shape dimorphism in an agamid lizard, Japalura swinhonis (Squamata: Lacertilia: Agamidae). *Zoological Studies* 48 (3). ISSN: 10215506.

Kupfer, A. (Sept. 2007). Sexual size dimorphism in amphibians: An overview. In: *Sex, Size and Gender Roles: Evolutionary Studies of Sexual Size Dimorphism*. Oxford University Press.

Lack, J. B., Monette, M. J., Johanning, E. J., Sprengelmeyer, Q. D., and Pool, J. E. (2016a). Decanalization of wing development accompanied the evolution of large wings in high-altitude *Drosophila*. *Proceedings of the National Academy of Sciences* 113(4), 1014–1019. ISSN: 0027-8424.

Lack, J. B., Yassin, A., Sprengelmeyer, Q. D., Johanning, E. J., David, J. R., and Pool, J. E. (2016b). Life history evolution and cellular mechanisms associated with increased size in high-altitude *Drosophila*. *Ecology and Evolution* 6(16), 5893–5906. ISSN: 20457758.

Lande, R. (1979). Quantitative genetic analysis of multivariate evolution applied to brain:body size allometry. *Evolution* 33 (1Part2). ISSN: 1558-5646.

Lande, R. (1980). Sexual Dimorphism, Sexual Selection, and Adaptation in Polygenic Characters. *Evolution* 34(2), 292. ISSN: 00143820.

Lande, R. (2009). Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of evolutionary biology* 22(7), 1435–1446.

Lande, R. and Arnold, S. (Nov. 1983a). The Measurement of Selection on Correlated Characters. *Evolution* 37(6), 1210–1226.

Lande, R. and Arnold, S. J. (1983b). The Measurement of Selection on Correlated Characters. *Evolution* 37 (6). ISSN: 00143820.

Lang, J. W. and Andrews, H. V. (1994). Temperature-dependent sex determination in crocodilians. *Journal of Experimental Zoology* 270 (1). ISSN: 1097010X.

Laporte, M., Berrebi, P., Claude, J., Vinyoles, D., Pou-Rovira, Q., Raymond, J.-C., and Magnan, P. (2018). The ecology of sexual dimorphism in size and shape of the freshwater blenny Salaria fluviatilis. *Current zoology* 64(2), 183–191.

Lasne, C., Hangartner, S. B., Connallon, T., and Sgro, C. M. (2018). Cross-sex genetic correlations and the evolution of sex-specific local adaptation: Insights from classical trait clines in Drosophila melanogaster. *Evolution*. ISSN: 15585646.

Lavine, L., Gotoh, H., Brent, C. S., Dworkin, I., and Emlen, D. J. (2015). Exaggerated trait growth in insects. *Annual review of entomology* 60, 453–472.

Layzer, D. (1980). Genetic variation and progressive evolution. *The American Naturalist* 115(6), 809–826.

Ledon-Rettig, C. C., Pfennig, D. W., Chunco, A. J., and Dworkin, I. (2014). *Cryptic genetic variation in natural populations: a predictive framework*.

Lefranc, A. and Bundgaard, J. (2000). The influence of male and female body size on copulation duration and fecundity in Drosophila melanogaster. *Hereditas* 132(3), 243–247.

Lehner, B. (2010). Genes confer similar robustness to environmental, stochastic, and genetic perturbations in yeast. *PloS one* 5(2), e9035.

Leips, J. and Mackay, T. F. (2000). Quantitative trait loci for life span in Drosophila melanogaster: Interactions with genetic background and larval density. *Genetics* 155 (4). ISSN: 00166731.

Levy, S. F. and Siegal, M. L. (2008). Network Hubs Buffer Environmental Variation in *Saccharomyces cerevisiae*. *PLoS Biology* 6(11). Ed. by A. Levchenko, e264. ISSN: 1545-7885.

Lewis, Z., Wedell, N., and Hunt, J. (2011). Evidence for strong intralocus sexual conflict in the Indian meal moth, Plodia interpunctella. *Evolution: International Journal of Organic Evolution* 65(7), 2085–2097.

Linde, K. van der and Houle, D. (2008). A supertree analysis and literature review of the genus Drosophila and closely related genera (Diptera, Drosophilidae). *Insect Systematics & Evolution* 39(3), 241–267.

Lovich, J. E. and Gibbons, J. W. (Jan. 1992). *Review of techniques for quantifying sexual size dimorphism.*

Lyons, E., Miller, D., and Meagher, T. (1994). Evolutionary dynamics of sex ratio and gender dimorphism in Silene latifolia: I. Environmental effects. *Journal of Heredity* 85(3), 196–203.

Magnusson, A., Skaug, H., Nielsen, A., Berg, C., Kristensen, K., Maechler, M., and Brooks, M. (2017). Generalized linear mixed models using template model builder. *GlmmTMB. R Packag, version 0.1* 3.

Mansourian, S., Enjin, A., Jirle, E. V., Ramesh, V., Rehermann, G., Becher, P. G., Pool, J. E., and Stensmyr, M. C. (Dec. 2018). Wild African Drosophila melanogaster Are Seasonal Specialists on Marula Fruit. *Current Biology* 28(24), 3960–3968.e3. ISSN: 09609822.

Mardia, K. and Dryden, I. (1989). The statistical analysis of shape data. *Biometrika* 76(2), 271–281.

Markow, T. A., Bustoz, D., and Pitnick, S. (1996). Sexual selection and a secondary sexual character in twoDrosophilaspecies. *Animal Behaviour* 52(4), 759–766.

Martin-Serra, A., Figueirido, B., and Palmqvist, P. (2014). A three-dimensional analysis of morphological evolution and locomotor performance of the carnivoran forelimb. *PLoS One* 9(1), e85574.

Masaki, S. (1967). Geographic variation and climatic adaptation in a field cricket (Orthoptera: Gryllidae). *Evolution*, 725–741.

Mathews, K. W., Cavegn, M., and Zwicky, M. (2017). Sexual dimorphism of body size is controlled by dosage of the X-chromosomal gene Myc and by the sex-determining gene tra in Drosophila. *Genetics* 205(3), 1215–1228.

Matthews, G., Hangartner, S., Chapple, D. G., and Connallon, T. (2019a). Quantifying maladaptation during the evolution of sexual dimorphism. *Proceedings of the Royal Society B* 286(1908), 20191372.

Matthews, G., Hangartner, S., Chapple, D. G., and Connallon, T. (2019b). Quantifying maladaptation during the evolution of sexual dimorphism. *Proceedings of the Royal Society B: Biological Sciences*. ISSN: 14712954.

Mayer, C. and Hansen, T. F. (Oct. 2017). Evolvability and robustness: A paradox restored. *Journal of Theoretical Biology* 430, 78–85.

McCann, T. S., Fedak, M. A., and Harwood, J. (1989). Parental investment in southern elephant seals, Mirounga leonina. *Behavioral Ecology and Sociobiology* 25 (2). ISSN: 14320762.

Mcguigan, K. and Blows, M. W. (Apr. 2007). The phenotypic and genetic covariance structure of *Drosphilid* wings. *Evolution* 61(4), 902–911.

McKenzie, J. A. and Clarke, G. M. (1988). Diazinon resistance, fluctuating asymmetry and fitness in the Australian sheep blowfly, *Lucilia cuprina*. *Genetics* 120(1), 213–220.

Menezes, B. F., Vigoder, F. M., Peixoto, A. A., Varaldi, J., and Bitner-Mathe, B. C. (2013). The influence of male wing shape on mating success in Drosophila melanogaster. *Animal Behaviour*. ISSN: 00033472.

Mezey, J. G. and Houle, D. (2005a). The dimensionality of genetic variation for wing shape in Drosophila melanogaster. *Evolution* 59(5), 1027–1038.

Mezey, J. G. and Houle, D. (May 2005b). The dimensionality of genetic variation for wing shape in Drosophila melanogaster. *Evolution* 59(5), 1027–1038.

Mezey, J. G., Houle, D., and Nuzhdin, S. V. (Apr. 2005). Naturally segregating quantitative trait loci affecting wing shape of Drosophila melanogaster. *Genetics* 169(4), 2101–2113.

Miller, C. W., McDonald, G. C., and Moore, A. J. (Nov. 2016). The tale of the shrinking weapon: seasonal changes in nutrition affect weapon size and sexual dimorphism, but not contemporary evolution. *Journal of Evolutionary Biology* 29 (11), 2266–2275. ISSN: 1010061X.

Millington, J. W., Brownrigg, G. P., Chao, C., Sun, Z., Basner-Collins, P. J., Wat, L. W., Hudry, B., Miguel-Aliaga, I., and Rideout, E. J. (2021). Female-biased upregulation of insulin pathway activity mediates the sex difference in Drosophila body size plasticity. *Elife* 10, e58341.

Moczek, A. P. and Emlen, D. J. (1999). Proximate determination of male horn dimorphism in the beetle Ontophagus taurus (Coleoptera: Scarabaeidae). *Journal of Evolutionary Biology* 12(1), 27–37.

Monnet, J. M. and Cherry, M. I. (Nov. 2002). Sexual size dimorphism in anurans. *Proceedings of the Royal Society B: Biological Sciences* 269(1507), 2301–2307. ISSN: 14712970.

Morimoto, J., Pizzari, T., and Wigby, S. (2016). Developmental environment effects on sexual selection in male and female Drosophila melanogaster. *PLoS One* 11(5), e0154468.

Morin, J., Moreteau, B., Petavy, G., Imasheva, A., and David, J. (1996). Body size and developmental temperature in Drosophila simulans: comparison of reaction norms with sympatric Drosophila melanogaster. *Genetics Selection Evolution* 28(5), 415–436.

Morris, M. R. J., Kaufman, R., and Rogers, S. M. (2019). Heterozygosity and asymmetry: Ectodysplasin as a form of genetic stress in marine threespine stickleback. *Evolution* 73(2), 378–389.

Morrissey, M. B. (2016). Meta-analysis of magnitudes, differences and variation in evolutionary parameters. *Journal of Evolutionary Biology* 29(10), 1882–1904.

Moya-Laraño, J., Macías-Ordóñez, R., Blanckenhorn, W. U., and Fernández-Montraveta, C. (2008). Analysing body condition: Mass, volume or density? *Journal of Animal Ecology* 77 (6), 1099–1108. ISSN: 00218790.

Navarro, J., Kaliontzopoulou, A., and González-Solís, J. (2009). Sexual dimorphism in bill morphology and feeding ecology in Cory's shearwater (Calonectris diomedea). *Zoology* 112 (2). ISSN: 09442006.

Norman, M. D., Paul, D., Finn, J., and Tregenza, T. (2002). First encounter with a live male blanket octopus: The world's most sexually size-dimorphic large animal. *New Zealand Journal of Marine and Freshwater Research* 36 (4). ISSN: 11758805.

O'Grady, P. M. and DeSalle, R. (2018). Phylogeny of the genus Drosophila. *Genetics* 209(1), 1–25.

OHiggins, P. and Dryden, I. L. (1993). Sexual dimorphism in hominoids: further studies of craniofacial shape differences in Pan, Gorilla and Pongo. *Journal of Human Evolution* 24(3), 183–205.

Oliver, J. C. and Monteiro, A. (July 2011). On the origins of sexual dimorphism in butterflies. *Proceedings of the Royal Society B: Biological Sciences* 278(1714), 1981–1988. ISSN: 09628452.

Olsson, M., Shine, R., Wapstra, E., Ujvari, B., and Madsen, T. (2002). Sexual dimorphism in lizard body shape: The roles of sexual selection and fecundity selection. *Evolution* 56 (7). ISSN: 00143820.

Orgogozo, V., Morizot, B., and Martin, A. (2015). The differential view of genotype-phenotype relationships. *Frontiers in Genetics* 6, 179.

Oudin, M. J., Bonduriansky, R., and Rundle, H. D. (2015). Experimental evidence of condition-dependent sexual dimorphism in the weakly dimorphic antler fly Protopiophila litigata (Diptera: Piophilidae). *Biological Journal of the Linnean Society* 116 (1), 211–220. ISSN: 10958312.

Paaby, A. B. and Rockman, M. V. (Apr. 2014). Cryptic genetic variation: evolution's hidden substrate. *Nature Reviews Genetics* 15(4), 247–258.

Paaby, A. B., White, A. G., Riccardi, D. D., Gunsalus, K. C., Piano, F., and Rockman, M. V. (2015). Wild worm embryogenesis harbors ubiquitous polygenic modifier variation. *eLife* 4, e09178.

Palmer, A. R. and Strobeck, C. (1986). Fluctuating Asymmetry: Measurement, Analysis, Patterns. *Annual Review of Ecology and Systematics* 17(1), 391–421. ISSN: 0066-4162.

Palmer, A. R. and Strobeck, C. (2003). Fluctuating Asymmetry Analyses Revisited. In: *Developmental Instability: Causes and Consequences*, 279–319.

Palmer, A. R. (1994). Fluctuating asymmetry analyses: a primer. In: *Contemporary Issues in Genetics and Evolution*, 335–364.

Partridge, L., Barrie, B., Fowler, K., and French, V. (Aug. 1994). Evolution and Development of Body Size and Cell Size in *Drosophila melanogaster* in Response to Temperature. *Evolution* 48(4), 1269–1276. ISSN: 00143820.

Partridge, L., Ewing, A., and Chandler, A. (1987). Male size and mating success in Drosophila melanogaster: the roles of male and female behaviour. *Animal Behaviour* 35(2), 555–562.

Partridge, L. and Farquhar, M. (1983). Lifetime mating success of male fruitflies (Drosophila melanogaster) is related to their size. *Animal Behaviour* 31(3), 871–877.

Pavlicev, M., Cheverud, J. M., and Wagner, G. P. (2009). Measuring Morphological Integration Using Eigenvalue Variance. *Evolutionary Biology* 36(1), 157–170. ISSN: 0071-3260.

Peck, L. S. and Maddrell, S. H. (Nov. 2005). Limitation of size by hypoxia in the fruit fly *Drosophila melanogaster*. *Journal of Experimental Zoology Part A: Comparative Experimental Biology* 303A(11), 968–975. ISSN: 1548-8969.

Pelabon, C., Hansen, T., Carter, A., and Houle, D. (2006). Response of fluctuating and directional asymmetry to selection on wing shape in *Drosophila melanogaster*. *Journal of Evolutionary Biology* 19(3), 764–776.

Pelabon, C., Hansen, T. F., Carter, A. J. R., and Houle, D. (July 2010). Evolution of variation and variability under fluctuating, stabilizing, and disruptive selection. *Evolution* 64(7), 1912–1925.

Penn, D. J. and Szamado, S. (2020). The Handicap Principle: how an erroneous hypothesis became a scientific principle. *Biological Reviews* 95(1), 267–290.

Pesevski, M. and Dworkin, I. (2020). Genetic and environmental canalization are not associated among altitudinally varying populations of Drosophila melanogaster. *Evolution*. ISSN: 15585646.

Petrie, M., Tim, H., and Carolyn, S. (1991). Peahens prefer peacocks with elaborate trains. *Animal Behaviour* 41 (2). ISSN: 00033472.

Pincheira-Donoso, D. and Hunt, J. (2017). Fecundity selection theory: concepts and evidence. *Biological Reviews* 92(1). ISSN: 1469185X.

Pitchers, W., Wolf, J. B., Tregenza, T., Hunt, J., and Dworkin, I. (July 2014). Evolutionary rates for multivariate traits: the role of selection and genetic variation. *Phil. Trans. R. Soc. B* 369(1649), 20130252.

Pitchers, W., Nye, J., Marquez, E. J., Kowalski, A., Dworkin, I., and Houle, D. (Feb. 2019a). A Multivariate Genome-Wide Association Study of Wing Shape in *Drosophila melanogaster*. *Genetics* 211(4), 1429–1447.

Pitchers, W., Nye, J., Marquez, E. J., Kowalski, A., Dworkin, I., and Houle, D. (Apr. 2019b). A Multivariate Genome-Wide Association Study of Wing Shape in *Drosophila melanogaster*. *Genetics* 211(4), 1429–1447. ISSN: 0016-6731.

Pitchers, W., Pool, J. E., and Dworkin, I. (2013). Altitudinal clinal variation in wing size and shape in African *Drosophila melanogaster*: one cline or many? *Evolution* 67(2), 438–452.

Poissant, J., Morrissey, M. B., Gosler, A. G., Slate, J., and Sheldon, B. C. (2016). Multivariate selection and intersexual genetic constraints in a wild bird population. *Journal of evolutionary biology* 29(10), 2022–2035.

Poissant, J., Wilson, A. J., and Coltman, D. W. (2010). Sex-specific genetic variance and the evolution of sexual dimorphism: A systematic review of cross-sex genetic correlations. *Evolution* 64(1), 97–107. ISSN: 00143820.

Pomiankowski, A. and Iwasa, Y. (Aug. 1993). Evolution of multiple sexual preferences by Fisher's runaway process of sexual selection. *Proceedings of the Royal Society B: Biological Sciences* 253 (1337), 173–181. ISSN: 14712970.

Pomiankowski, A. and Moller, A. P. (1995). A Resolution of the Lek Paradox. *Proceedings of the Royal Society B: Biological Sciences* 260(1357), 21–29. ISSN: 0962-8452.

Pomiankowski, A. and Iwasa, Y. (Apr. 1998). Runaway ornament diversity caused by Fisherian sexual selection. *Proceedings of the National Academy of Sciences of the United States of America* 95 (9), 5106–5111. ISSN: 00278424.

Pool, J. E., Corbett-Detig, R. B., Sugino, R. P., Stevens, K. A., Cardeno, C. M., Crepeau, M. W., Duchen, P., Emerson, J. J., Saelao, P., Begun, D. J., and Langley, C. H. (2012a). Population Genomics of sub-Saharan *Drosophila melanogaster*: African diversity and non-African admixture. *PLoS Genetics* 8(12), e1003080.

Pool, J. E., Braun, D. T., and Lack, J. B. (Oct. 2016). Parallel Evolution of Cold Tolerance within Drosophila melanogaster. *Molecular Biology and Evolution* 34(2), 349–360. ISSN: 0737-4038.

Pool, J. E., Corbett-Detig, R. B., Sugino, R. P., Stevens, K. A., Cardeno, C. M., Crepeau, M. W., Duchen, P., Emerson, J. J., Saelao, P., Begun, D. J., and Langley, C. H. (Dec. 2012b). Population Genomics of Sub-Saharan Drosophila melanogaster: African Diversity and Non-African Admixture. *PLoS Genetics* 8(12). Ed. by H. S. Malik, e1003080. ISSN: 1553-7404.

Preziosi, R. F., Fairbairn, D. J., Roff, D. A., and Brennan, J. M. (1996). Body size and fecundity in the waterstrider Aquarius remigis: a test of Darwin's fecundity advantage hypothesis. *Oecologia* 108(3), 424–431.

Preziosi, R. F. and Fairbairn, D. J. (1997). Sexual size dimorphism and selection in the wild in the waterstrider Aquarius remigis: Lifetime fecundity selection on female total length and its components. *Evolution* 51 (2). ISSN: 00143820.

Proulx, S. and Phillips, P. (Jan. 2005). The Opportunity for Canalization and the Evolution of Genetic Networks. *The American Naturalist* 165(2), 147–162.

Punzalan, D., Cooray, M., Helen Rodd, F., and Rowe, L. (2008). Condition dependence of sexually dimorphic colouration and longevity in the ambush bug Phymata americana. *Journal of evolutionary biology* 21(5), 1297–1306.

Punzalan, D., Delcourt, M., and Rundle, H. (2014). Comparing the intersex genetic correlation for fitness across novel environments in the fruit fly, Drosophila serrata. *Heredity* 112(2), 143–148.

R Core Team (2018). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria.

Ray, R. P., Nakata, T., Henningsson, P., and Bomphrey, R. J. (Mar. 2016). Enhanced flight performance by genetic manipulation of wing shape in Drosophila. *Nature Communications* 7(1), 1–8. ISSN: 20411723.

Reeve, J. P. and Fairbairn, D. J. (1996). Sexual size dimorphism as a correlated response to selection on body size: an empirical test of the quantitative genetic model. *Evolution* 50(5), 1927–1938.

Reeve, J. P. and Fairbairn, D. J. (1999). Change in sexual size dimorphism as a correlated response to selection on fecundity. *Heredity* 83 (6). ISSN: 0018067X.

Reiss, M. J. (1989). *The allometry of growth and reproduction*. Cambridge University Press.

Rendel, J. (1963). The Nature of Concealed Genotypic Variation. In: *Proceedings-National Poultry Breeders*, 4–16.

Rensch, B. (1960). *Evolution Above the Species Level*. Columbia biological series. Columbia University Press.

Rhen, T. (Feb. 2000). Sex-limited mutations and the evolution of sexual dimorphism. *Evolution* 54(1), 37–43. ISSN: 00143820.

Rice, W. R. (July 1984). Sex Chromosomes and the Evolution of Sexual Dimorphism. *Evolution* 38(4), 735. ISSN: 00143820.

Rice, W. R. (1992). Sexually antagonistic genes: Experimental evidence. *Science* 256 (5062). ISSN: 00368075.

Rice, W. and Chippindale, A. (2001). Intersexual ontogenetic conflict. *Journal of Evolutionary Biology* 14(5), 685–693.

Rideout, E. J., Narsaiya, M. S., and Grewal, S. S. (2015). The sex determination gene transformer regulates male-female differences in Drosophila body size. *PLoS genetics* 11(12), e1005683.

Rockman, M. V. (Dec. 2008). Reverse engineering the genotype-phenotype map with natural genetic variation. *Nature* 456(7223), 738–744.

Rohde, K., Dreher, E., and Hochkirch, A. (2015). Sex-specific phenotypic plasticity in response to the trade-off between developmental time and body size supports the dimorphic niche hypothesis. *Biological Journal of the Linnean Society* 115(1), 48–57.

Rohner, P. T., Blanckenhorn, W. U., and Schäfer, M. A. (2017). Critical weight mediates sex-specific body size plasticity and sexual dimorphism in the yellow dung fly Scathophaga stercoraria (Diptera: Scathophagidae). *Evolution & development* 19(3), 147–156.

Rohner, P. T., Teder, T., Esperk, T., Lüpold, S., and Blanckenhorn, W. U. (2018a). The evolution of male-biased sexual size dimorphism is associated with increased body size plasticity in males. *Functional ecology* 32(2), 581–591.

Rohner, P. T. and Blanckenhorn, W. U. (2018). A Comparative Study of the Role of Sex-Specific Condition Dependence in the Evolution of Sexually Dimorphic Traits. *The American Naturalist*. ISSN: 0003-0147.

Rohner, P. T., Pitnick, S., Blanckenhorn, W. U., Snook, R. R., Bachli, G., and Lupold, S. (2018b). Interrelations of global macroecological patterns in wing and thorax size, sexual size dimorphism, and range size of the Drosophilidae. *Ecography*. ISSN: 16000587.

Rohner, P. T., Teder, T., Esperk, T., Lüpold, S., and Blanckenhorn, W. U. (Feb. 2018c). The evolution of male-biased sexual size dimorphism is associated with increased body size plasticity in males. *Functional Ecology* 32 (2). Ed. by G. Davidowitz, 581–591. ISSN: 13652435.

Roitberg, E. S., Eplanova, G. V., Kotenko, T. I., Amat, F., Carretero, M. A., Kuranova, V. N., Bulakhova, N. A., Zinenko, O. I., and Yakovlev, V. (2015). Geographic variation of life-history traits in the sand lizard, Lacerta agilis: testing Darwin's fecundity-advantage hypothesis. *Journal of Evolutionary Biology* 28(3), 613–629.

Rolff, J., Armitage, S. A., and Coltman, D. W. (2005). Genetic constraints and sexual dimorphism in immune defense. *Evolution* 59(8), 1844–1850.

Rowe, L. and Houle, D. (1996). The Lek Paradox and the Capture of Genetic Variance by Condition Dependent Traits. *Proceedings of the Royal Society B: Biological Sciences* 263(1375), 1415–1421. ISSN: 0962-8452.

Russell, L., Henrik, S., Jonathon, L., Paul, B., and Maxime, H. (2018). Estimated marginal means, aka least-squares means. *The American Statistician* 34, 216–221.

Sanger, T. J., Sherratt, E., McGlothlin, J. W., Brodie III, E. D., Losos, J. B., and Abzhanov, A. (2013). Convergent evolution of sexual dimorphism in skull shape using distinct developmental strategies. *Evolution* 67(8), 2180–2193.

Sasaki, M., Hedberg, S., Richardson, K., and Dam, H. G. (2019). Complex interactions between local adaptation, phenotypic plasticity and sex affect vulnerability to warming in a widespread marine copepod. *Royal Society open science* 6(3), 182115.

Schaefer, K., Mitteroecker, P., Gunz, P., Bernhard, M., and Bookstein, F. L. (Dec. 2004). Craniofacial sexual dimorphism patterns and allometry among extant hominids. *Annals of Anatomy* 186(5-6), 471–478. ISSN: 09409602.

Scharloo, W. (1991). Canalization: Genetic and Developmental Aspects. *Annual Review Of Ecology And Systematics* 22, 65–93.

Schluter, D. (Oct. 1996). Adaptive Radiation Along Genetic Lines of Least Resistance. *Evolution* 50(5), 1766–1774.

Schwarzkopf, L. (2005a). Sexual dimorphism in body shape without sexual dimorphism in body size in water skinks (Eulamprus quoyii). *Herpetologica* 61(2), 116–123.

Schwarzkopf, L. (2005b). Sexual dimorphism in body shape without sexual dimorphism in body size in water skinks (Eulamprus quoyii). *Herpetologica* 61 (2). ISSN: 00180831.

Selander, R. K. (1966). Sexual dimorphism and differential niche utilization in birds. *The Condor* 68(2), 113–151.

Sessegolo, C., Burlet, N., and Haudry, A. (2016). Strong phylogenetic inertia on genome size and transposable element content among 26 species of flies. *Biology letters* 12(8), 20160407.

Shine, R. (1988). The evolution of large body size in females: a critique of Darwin's "fecundity advantage' model. *American Naturalist*. ISSN: 00030147.

Shine, R. (1991). Intersexual dietary divergence and the evolution of sexual dimorphism in snakes. *American Naturalist* 138 (1). ISSN: 00030147.

Shine, R. (1994). Sexual size dimorphism in snakes revisited. *Copeia* (2), 326–346. ISSN: 00458511.

Shine, R. (1989). Ecological Causes for the Evolution of Sexual Dimorphism: A Review of the Evidence. *The Quarterly Review of Biology* 64(4), 419–461. ISSN: 0033-5770.

Shingleton, A. W., Masandika, J. R., Thorsen, L. S., Zhu, Y., and Mirth, C. K. (2017). The sex-specific effects of diet quality versus quantity on morphology in Drosophila melanogaster. *Royal Society open science* 4(9), 170375.

Shingleton, A. W., Estep, C. M., Driscoll, M. V., and Dworkin, I. (2009). Many ways to be small: different environmental regulators of size generate distinct scaling relationships in Drosophila melanogaster. *Proceedings of the Royal Society of London B: Biological Sciences*.

Shu, W., Bo, X., Ni, M., Zheng, Z., and Wang, S. (2007). *In silico* genetic robustness analysis of microRNA secondary structures: potential evidence of congruent evolution in microRNA. *BMC Evolutionary Biology* 7(1), 223.

Siegal, M. L. and Bergman, A. (2002). Waddington's canalization revisited: developmental stability and evolution. *Proceedings of the National Academy of Sciences* 99(16), 10528–10532.

Siegal, M. L. and Leu, J.-Y. (Nov. 2014). On the Nature and Evolutionary Impact of Phenotypic Robustness Mechanisms. *Annual Review of Ecology, Evolution, and Systematics* 45, 496–517.

Simons, A. M. and Roff, D. A. (1996). The effect of a variable environment on the genetic correlation structure in a field cricket. *Evolution* 50(1), 267–275.

Singh, A. and Punzalan, D. (2018). The strength of sex-specific selection in the wild. *Evolution* 72(12), 2818–2824.

Siomava, N., Wimmer, E. A., and Posnien, N. (2016). Size relationships of different body parts in the three dipteran species Drosophila melanogaster, Ceratitis capitata and Musca domestica. *Development genes and evolution* 226(3), 245–256.

Slatkin, M. (1984). Ecological Causes of Sexual Dimorphism. *Source: Evolution* 3867216(3), 622–630. ISSN: 00143820.

Smith, J. M. (1976). Sexual selection and the handicap principle. *Journal of Theoretical Biology* 57(1), 239–242.

Smith, R. J. (1999). Statistics of sexual size dimorphism. *Journal of Human Evolution* 36(4), 423–458.

Soto, I., Carreira, V., Soto, E., and Hasson, E. (2008). Wing morphology and fluctuating asymmetry depend on the host plant in cactophilic *Drosophila*. *Journal of Evolutionary Biology* 21(2), 598–609.

Sprengelmeyer, Q. D., Mansourian, S., Lange, J. D., Matute, D. R., Cooper, B. S., Jirle, E. V., Stensmyr, M. C., and Pool, J. E. (Mar. 2020). Recurrent Collection of Drosophila melanogaster from Wild African Environments and Genomic Insights into Species History. *Molecular Biology and Evolution* 37(3). Ed. by A. Larracuente, 627–638. ISSN: 0737-4038.

Starostova, Z., Kubicka, L., and Kratochvil, L. (2010). Macroevolutionary pattern of sexual size dimorphism in geckos corresponds to intraspecific temperature-induced variation. *Journal of evolutionary biology* 23(4), 670–677.

Stearns, S., Kaiser, M., and Kawecki, T. (1995). The differential canalization of fitness components against environmental perturbations in *Drosophila melanogaster*. *J. Evol. Biol* 8, 539–557.

Stearns, S. C. and Kawecki, T. J. (1994). Fitness sensitivity and the canalization of life-history traits. *Evolution* 48(5), 1438–1450.

Stewart, A. D. and Rice, W. R. (2018). Arrest of sex-specific adaptation during the evolution of sexual dimorphism in Drosophila. *Nature ecology & evolution* 2(9), 1507–1513.

Stillwell, R. C. and Davidowitz, G. (2010). Sex differences in phenotypic plasticity of a mechanism that controls body size: implications for sexual size dimorphism. *Proceedings. Biological sciences / The Royal Society* 277 (1701), 3819–26. ISSN: 1471-2954.

Stillwell, R. C., Blanckenhorn, W. U., Teder, T., Davidowitz, G., and Fox, C. W. (Jan. 2010). Sex Differences in Phenotypic Plasticity Affect Variation in Sexual Size Dimorphism in Insects: From Physiology to Evolution. *Annual Review of Entomology* 55 (1), 227–245. ISSN: 0066-4170.

Stillwell, R. C. and Fox, C. W. (2005). Complex patterns of phenotypic plasticity: Interactive effects of temperature during rearing and oviposition. *Ecology* 86 (4). ISSN: 00129658.

Stillwell, R. C. and Fox, C. W. (2007). Environmental effects on sexual size dimorphism of a seed-feeding beetle. *Oecologia* 153 (2), 273–280. ISSN: 00298549.

Stillwell, R. C., Wallin, W. G., Hitchcock, L. J., and Fox, C. W. (2007). Phenotypic plasticity in a complex world: Interactive effects of food and temperature on fitness components of a seed beetle. *Oecologia* 153 (2). ISSN: 00298549.

Suvorov, A., Kim, B. Y., Wang, J., Armstrong, E. E., Peede, D., DAgostino, E. R., Price, D. K., Wadell, P., Lang, M., Courtier-Orgogozo, V., et al. (2021). Widespread introgression across a phylogeny of 155 Drosophila genomes. *BioRxiv*, 2020–12.

Svensson, E. I., Goedert, D., Gomez-Llano, M. A., Spagopoulou, F., Nava-Bolanos, A., and Booksmythe, I. (2018). Sex differences in local adaptation: what can we learn from reciprocal transplant experiments? *Philosophical Transactions of the Royal Society B: Biological Sciences* 373(1757), 20170420.

Szollhosi, G. J. and Derenyi, I. (2009). Congruent evolution of genetic and environmental robustness in micro-RNA. *Molecular biology and evolution* 26(4), 867–874.

Sztepanacz, J. L. and Houle, D. (2019). Cross-sex genetic covariances limit the evolvability of wing-shape within and among species of Drosophila. *Evolution* 73(8), 1617–1633.

Sztepanacz, J. L. and Houle, D. (2021). Allometry constrains the evolution of sexual dimorphism in Drosophila across 33 million years of divergence. *Evolution*.

Takahashi, K. H. (Feb. 2017). Little effect of HSP90 inhibition on the quantitative wing traits variation in *Drosophila melanogaster*. *Genetica* 145(1), 9–18.

Takahashi, K. H., Okada, Y., and Teramura, K. (July 2011). Genome-wide deficiency mapping of the regions responsible for temporal canalization of the developmental processes of *Drosophila melanogaster*. *The Journal of Heredity* 102(4), 448–457.

Takahashi, K. H., Rako, L., Takano-Shimizu, T., Hoffmann, A. A., and Lee, S. F. (2010). Effects of small Hsp genes on developmental stability and microenvironmental canalization. *BMC Evolutionary Biology* 10, 284.

Teder, T. and Tammaru, T. (2005). Sexual size dimorphism within species increases with body size in insects. *Oikos* 108 (2). ISSN: 00301299.

Temeles, E. J., Miller, J. S., and Rifkin, J. L. (2010). Evolution of sexual dimorphism in bill size and shape of hermit hummingbirds (Phaethornithinae): A role for ecological causation. *Philosophical Transactions of the Royal Society B: Biological Sciences* 365 (1543). ISSN: 14712970.

Temeles, E. J., Pan, I. L., Brennan, J. L., and Horwitt, J. N. (2000). Evidence for ecological causation of sexual dimorphism in a hummingbird. *Science* 289 (5478). ISSN: 00368075.

Testa, N. D. and Dworkin, I. (2016). The sex-limited effects of mutations in the EGFR and TGF-β signaling pathways on shape and size sexual dimorphism and allometry in the Drosophila wing. *Development Genes and Evolution* 226 (3). ISSN: 1432041X.

Testa, N. D., Ghosh, S. M., and Shingleton, A. W. (Mar. 2013). Sex-Specific Weight Loss Mediates Sexual Size Dimorphism in Drosophila melanogaster. *PLoS ONE* 8(3). Ed. by F. Pichaud, e58936. ISSN: 1932-6203.

Tigreros, N. and Lewis, S. (2011). Direct and correlated responses to artificial selection on sexual size dimorphism in the flour beetle, Tribolium castaneum. *Journal of evolutionary biology* 24(4), 835–842.

Tracy, C. B., Nguyen, J., Abraham, R., and Shirangi, T. R. (Jan. 2020). Evolution of sexual size dimorphism in the wing musculature of Drosophila. *PeerJ* 2020(1), e8360. ISSN: 21678359.

Trivers, R. L. (1996). *Parental investment and sexual selection.*

Valenzuela, N., Adams, D. C., Bowden, R. M., and Gauger, A. C. (2004). Geometric morphometric sex estimation for hatchling turtles: a powerful alternative for detecting subtle sexual shape dimorphism. *Copeia* 2004(4), 735–742.

Van Valen, L. (May 1974). Multivariate structural statistics in natural history. *Journal of Theoretical Biology* 45(1), 235–247. ISSN: 10958541.

Van Valen, L. (2005). The statistics of variation. In: *Variation*. Ed. by B. Hallgrimsson and B. K. Hall. Elsevier. Chap. 3, 29–47.

Vieira, C., Pasyukova, E. G., Zeng, Z. B., Hackett, J. B., Lyman, R. F., and Mackay, T. F. (2000). Genotype-environment interaction for quantitative trait loci affecting life span in Drosophila melanogaster. *Genetics* 154 (1). ISSN: 00166731.

Vincent, S. E., Herrel, A., and Irschick, D. J. (2004). Sexual dimorphism in head shape and diet in the cottonmouth snake (Agkistrodon piscivorus). *Journal of Zoology* 264 (1). ISSN: 09528369.

Visser, J., Hermisson, J., Wagner, G., Meyers, L., Bagheri-Chaichian, H., Blanchard, J., Chao, L., Cheverud, J., Elena, S., Fontana, W., Gibson, G., Hansen, T., Krakauer, D., Lewontin, R., Ofria, C., Rice, S., Dassow, G., Wagner, A., and Whitlock, M. (Sept. 2003). Perspective: Evolution and Detection of Genetic Robustness. *Evolution* 57(9), 1959–1972.

Waddington, C. H. (1957). The strategy of the genes. A discussion of some aspects of theoretical biology. With an appendix by H. Kacser. *The strategy of the genes A discussion of some ...*

Waddington, C. H. (1942). Canalization of development and the inheritance of acquired characters. *Nature* 150(3811), 563–565.

Wagner, G. P., Booth, G., and Bagheri-Chaichian, H. (1997). A Population Genetic Theory of Canalization. *Evolution* 51(2), 329–347. ISSN: 00143820.

Walsh, B. and Blows, M. W. (Jan. 2009). Abundant Genetic Variation plus Strong Selection - Multivariate Genetic Constraints: A Geometric View of Adaptation. *Annual Review of Ecology, Evolution, and Systematics* 40, 41–59.

Walzer, A. and Schausberger, P. (2014). Canalization of body size matters for lifetime reproductive success of male predatory mites (Acari: Phytoseiidae). *Biological Journal of the Linnean Society* 111(4), 889–899.

Webb, T. J. and Freckleton, R. P. (Sept. 2007). Only Half Right: Species with Female-Biased Sexual Size Dimorphism Consistently Break Rensch's Rule. *PLoS ONE* 2(9). Ed. by P. Bennett, e897. ISSN: 1932-6203.

Weber, K., Eisman, R., Morey, L., Patty, A., Sparks, J., Tausek, M., and Zeng, Z. B. (Oct. 1999). An analysis of polygenes affecting wing shape on chromosome 3 in Drosophila melanogaster. *Genetics* 153(2), 773–786.

Weber, K. E. (July 1990a). Increased selection response in larger populations. I. Selection for wing-tip height in Drosophila melanogaster at three population sizes. *Genetics* 125(3), 579–584.

Weber, K. E. (Dec. 1990b). Selection on wing allometry in Drosophila melanogaster. *Genetics* 126(4), 975–989.

Weber, K., Johnson, N., Champlin, D., and Patty, A. (Mar. 2005). Many P-element insertions affect wing shape in Drosophila melanogaster. *Genetics* 169(3), 1461–1475.

Weston, E. M., Friday, A. E., and Liò, P. (2007). Biometric evidence that sexual selection has shaped the hominin face. *PloS one* 2(8), e710.

Wilder, S. M., Raubenheimer, D., and Simpson, S. J. (2016). Moving beyond body condition indices as an estimate of fitness in ecological and evolutionary studies. *Functional Ecology* 30 (1), 108–115. ISSN: 13652435.

Wilkinson, G. S. (1987). Equilibrium analysis of sexual selection in Drosophila melanogaster. *Evolution* 41(1), 11–21.

Williams, T. M. and Carroll, S. B. (2009). Genetic and molecular insights into the development and evolution of sexual dimorphism. *Nature reviews. Genetics* 10(11), 797–804. ISSN: 1471-0056.

Wyman, M. J., Stinchcombe, J. R., and Rowe, L. (2013). A multivariate view of the evolution of sexual dimorphism. *Journal of Evolutionary Biology* 26(10), 2070–2080. ISSN: 1010061X.

Wyman, M. J., Agrawal, A. F., and Rowe, L. (June 2010). Condition-dependence of the sexually dimorphic transcriptome in drosophila melanogaster. *Evolution* 64(6), 1836–1848. ISSN: 00143820.

Zahavi, A. (1975). Mate selection—a selection for a handicap. *Journal of theoretical Biology* 53(1), 205–214.

Zahavi, A. (1977). The cost of honesty (further remarks on the handicap principle). *Journal of theoretical Biology* 67(3), 603–605.

Zelditch, M. L., Swiderski, D. L., and Sheets, H. D. (2012). *Geometric morphometrics for biologists: a primer*. academic press.

Zinna, R., Emlen, D., Lavine, L. C., Johns, A., Gotoh, H., Niimi, T., and Dworkin, I. (2018). Sexual dimorphism and heightened conditional expression in a sexually selected weapon in the Asian rhinoceros beetle. *Molecular ecology* 27(24), 5049–5072.