Evolution of Condition-dependent Sexual dimorpism in crowding-adapted populations of *Drosophila melanogaster*

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Certificate of Examination

This is to certify that the dissertation titled "**Evolution of Condition-dependent Sexual dimorpism in crowding-adapted populations of *Drosophila melanogaster***" submitted by Mr. Mayank Kashyap (Reg. No. MS16098) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

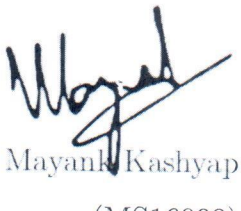
Dr. Manjari Jain	Dr. Rhitoban Ray Choudhury	Dr. N. G. Prasad (Supervisor)

Dated: April 24, 2021

Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. N. G. Prasad at the Indian Institute of Science Education and Research Mohali.

This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.



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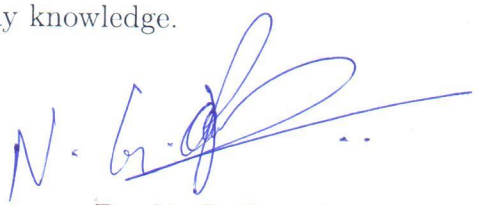

Dr. N. G. Prasad

(Supervisor)

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Abstract

Sexual dimorphism is a product of some form of differential selection between males and females. Such traits should also have a history of directional selection for exaggeration in any one of the sexes. Because of this persistent directional selection, these traits are also expected to evolve a form of heightened condition-dependence. Moreover, theory also predicts a coevolution between sexual dimorphism and condition-dependence itself such that the two eventually evolve a positive covariation. Phenotypic evidence of this comes from a wide-ranging set of taxa which includes species with highly exaggerated display traits and even species which show more typical levels of sexual dimorphism. Empirical studies of condition-dependent sexual dimorphism have predominantly resorted to single generation manipulations and fail to address evolutionary consequences of resource limitation. Sexual dimorphism also results for optimal resource allocation in the two sexes and therefore including evolution in a resource limiting environment could provide us with key insights. In this study, I manipulated larval rearing density (thereby, manipulating condition) in baseline populations of *Drosophila melanogaster* and also in populations subjected to more than 250 generations of adaptation in crowded developmental environment. While dimorphism in body size did increase as the rearing density decreased (i.e., increasing condition) in both control and selected populations, control populations nearly lost all their dimorphism in high density whereas selected populations were better able to maintain their sexual dimorphism even in high density. Among traits, both control and selected populations showed positive covariation between condition-dependence and sexual dimorphism. My results suggest a shared developmental and genetic basis for condition-dependence and sexual dimorphism in both control and selected populations. Moreover, selected populations maintaining sexual dimorphism in high density suggest evolution of some optimal resource allocation mechanisms that help in maintaining this costly dimorphism.

Chapter 1

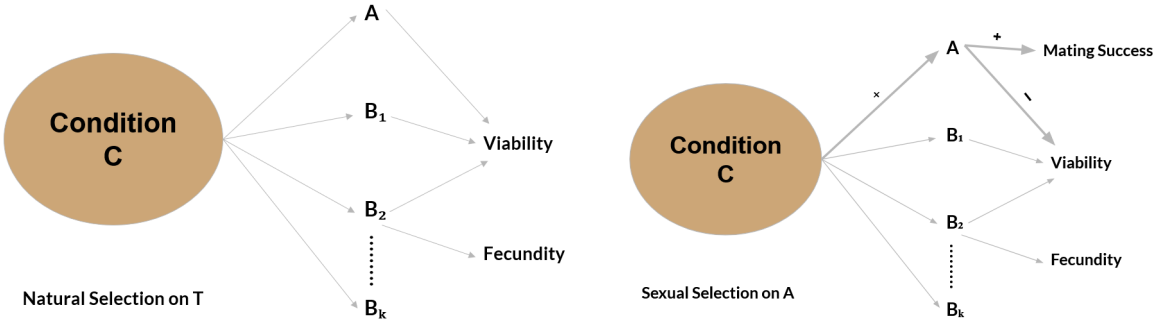
Introduction

Condition of an organism can be defined as a function of amount of metabolic resources an individual acquires over its life-history and the efficiency by which it the acquired resource pool translates into the fitness of an organism (Rowe et al., 1996). Thus, condition effectively represents a combination of genetically and environmentally determined variation that can have a substantial impact on fitness of an organism. Condition-dependence is a form of developmental plasticity that acts as a link between the degree of trait expression and condition of an individual (Bonduriansky, 2007).

Sexual dimorphism, differences between the two sexes of a species, very often manifests in terms of morphological differences in body size, shape and exaggeration of secondary sexual traits (Andersson, 1982). Although, sexual dimorphism can result from a history of natural selection, it is usually thought of as a product of sexual selection. The persistent nature of sexual selection coupled with the divergent reproductive interests of the two sexes results in the sex specific nature of selection (Fairbairn et al., 1994; Price, 1984). Regardless of the evolutionary history that resulted in the sexual dimorphism one can conclusively say that sexual dimorphism ultimately arises because of some form of differential selection between sexes. The two opposing forces, sexually antagonistic selection and the constraints posed by a largely shared genome between sexes, interact and results into the extent of sexual dimorphism that you observe (Lande, 1980).

For sexually dimorphic traits at least one of the sexes would have been subjected to directional selection for exaggeration. (Andersson, 1982; Nur et al., 1984) argued

that for all such traits a component of fitness is a function of investment in them such that higher is the investment higher is the trait expression which subsequently results in increase in some component of fitness. Expression of such traits is hence heavily dependent on cost-benefit balance, costs which it bears because of less amount of resources left for other fitness components or in case of a sexually selected trait viability costs offset the benefits associated with increase in fitness and halt the evolutionary exaggeration of such traits. (Ge, 2011; Rowe et al., 1996) predicted that such traits would evolve condition-dependence because of this cost-benefit balance. It is easy to understand this prediction from the life history pictured in (figure 1.1), initially the focal fitness component A is under natural selection only but as we impose sexual selection to A trade-offs associated with increased allocation to A start to show up. Condition-dependence is expected to arise in these cases because only higher condition individuals can pay the marginal costs associated with exaggeration in A and further increase their fitness (Rowe et al., 1996). Although the explanation given here is for sexually selected traits it can be extended for any sexually dimorphic trait if you consider them as directionally selected trait for exaggeration in at least one of the sexes.



(a) Negligible correlation between focal trait **A** and fitness components

(b) Divergent selection on **A** (mating success and viability) leads to a correlation with fitness components

Figure 1.1: Relationship between different traits and fitness components

It also follows from the model described in (figure 1.1) that the strength of condition-dependence that evolves is some function of strength of selection and the costs associated with the exaggerated display of the trait (Rowe et al., 1996). Therefore, both

condition-dependence and extent of sexual dimorphism are dependent on selection which subsequently results in positive association between the two (Bonduriansky, 2007; Bonduriansky et al., 2005). Because of this coevolution between condition-dependence and sexual dimorphism we can expect that 1) within a trait the extent of sexual dimorphism should be greater as compared to low condition individuals 2) among traits there should be a positive correlation between condition-dependence and sexual dimorphism such that highly dimorphic traits have heightened condition-dependence as compared to weakly dimorphic traits. Both condition-dependence and sexual dimorphism represent variation across different axis, for the latter it is phenotypic variation across sexes and for the former this axis is condition or to simplify it further availability of metabolic resources. Therefore, it is not a very wise decision to study them in a single individual at just one point rather they should be thought of as reaction norms.

Condition-dependence of sexually selected displays/ ornaments have received a great amount of empirical support (Cotton et al., 2004). Numerous studies have supported the idea for sexually dimorphic traits also when tested against some environmental factor suspected to alter the condition (Bonduriansky, 2007; Bonduriansky et al., 2005; David et al., 1994; Karan et al., 2000; Post et al., 1999; Punzalan et al., 2008). Condition-dependent sexual dimorphism (CDSD) has been shown in sex biased transcriptome also in *Drosophila melanogaster* (Wyman et al., 2010). Even after such great empirical support for this idea only a few studies have gone a step further to study the association between condition-dependence and sexual dimorphism (Bonduriansky, 2007; Bonduriansky et al., 2005; Oudin et al., 2015). To the best of my knowledge all the studies mentioned above are single generation manipulation studies and empirical support for evolutionary consequences on the relationship between condition-dependence and sexual dimorphism is lacking. My study will provide novel insights to the field by investigating the phenomenon of CDSD in a population evolving in a resource limited developmental environment for more than 150 generations.

Chapter 2

Study System

2.1 Experimental System

For this project, I use fruit fly *Drosophila melanogaster* (Phylum: Arthropoda, Class: Insecta, Order: Diptera, Family: Drosophilidae) as my model system.
Drosophila melanogaster is a holometabolous insect meaning its life cycle has four distinct stages: egg, larva, pupa and the adult fly (2.1). In its natural habitat adult fruit flies feed on overripe or rotten fruit. Females oviposit on the fruits as well and larvae eat the food they were laid on.
Drosophila melanogaster is a commonly used model organism in evolutionary genetic studies. It is widely used for its low maintenance costs, short generation time, small size, and relative ease of use. Majority of the metabolic resource acquisition happens in larval stages. Amount of resources acquired during larval stages can have a substantial impact on the adults. This is particularly important in the context of this theses because here I use larval diet manipulation to generate low and high condition individuals.

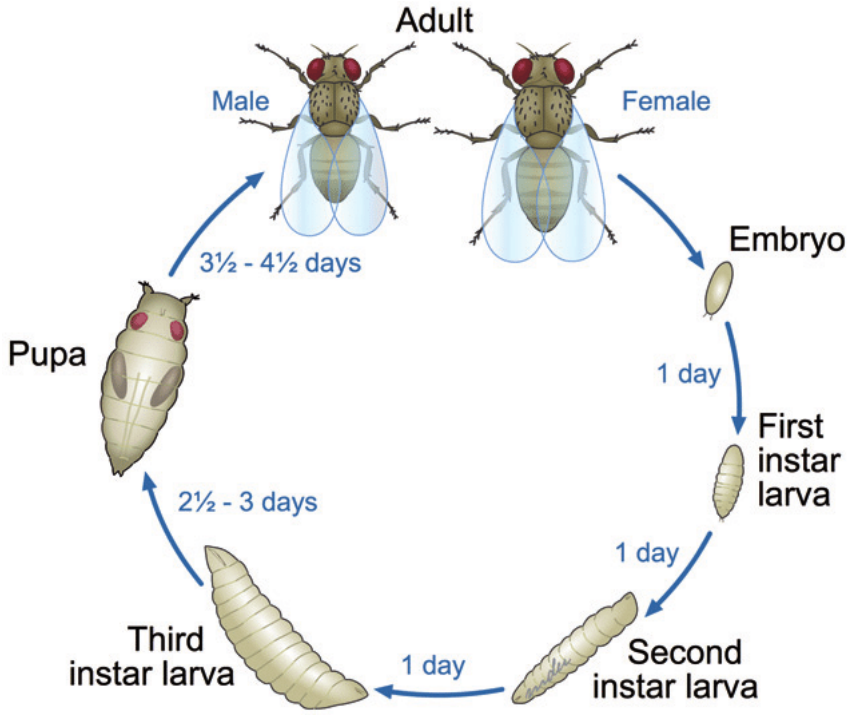


Figure 2.1: *Drosophila melanogaster* life cycle (modified from Ong etal., 2014)

2.2 Flystocks

All the stocks used are maintained on charcoal-cornmeal food (see A for food recipe). A total of eight sets of populations were used in this study, four of these populations are called Melanogaster Baseline (henceforth MB) and the other half are called Crowded as larvae, Uncrowded as adults (henceforth CU).

MB's were derived from JB stock populations (Shenoi, Ali, et al., 2016), which were historically derived from Mueller's UU populations (Sheeba et al., 1998). They are maintained at 25 °C, 80-90 % RH, 24-hour light photoperiod and in a 21-day discrete generation cycle. A total of 40 vials per replicate population are collected each generation at the density of 60-70 eggs per vial (25mm diameter * 90mm height). Each vial contains 8-10 ml of standard charcoal cornmeal food. On 12th day when adults have eclosed out of pupa, they are dumped into a plexiglass cage (24 * 19 * 14 cm). Adults are supplied with a petri plate containing charcoal cornmeal food and a wet cotton ball to maintain humidity levels. Population size per MB replicate population is approximately 2400-2800 adults. Starting from the day of eclosion fresh food plates are provided on alternate days i.e., on day 12, 14 and 16. On 18th day, adults are provided with a fresh food plate supplemented with ad-libitum yeast and

subsequently on 20th day a fresh food plate is provided for 18 hours. These eggs are then collected at the above-mentioned density for next generation.

CU's were derived from similar subscript MB replicate by selecting for larval crowding every generation. Selection is imposed at larval stage by crowding them at 800 eggs per vial containing 1.5 ml of standard charcoal-cornmeal food. As a response to larval crowding mean development time increases and eclosion range also broadens (Santos et al., 1997). As a result, CU adults start eclosing out of the pupa from day 8 and henceforth are dumped every day into a plexiglass cage (24 * 19 * 14 cm) till day 18. They are also provided with fresh food plates every alternate day to not apply any form of conscious selection on them as adults. From day 18 they receive a similar treatment to MB's i.e., they are provided with a fresh food plate supplemented with ad-libitum yeast. On day 20 they are also provided with oviposition plate for 18 hours, from which eggs for next generation are collected. 24 vials per replicate CU population is collected every generation. At their respective rearing density approximately 90-100 flies survive till adulthood. Therefore, effective population size per replicate population is approximately 2000-2400 adults. All of 8 CU and MB populations are handled similarly and on same day and are treated as statistical blocks in our analyses.

2.3 Standardisation and Generation of Experimental Flies

To eliminate all non-genetic parental effects flies are subjected to the process of standardisation. This involves one generation of common garden rearing and eliminates non-genetic parental effects which may arise due to difference in environmental conditions between CU and MB populations (Rose, 1984). From stock populations eggs are collected at the density of 300 eggs per standard fly culture bottle containing approximately 50 ml of food. 4 such bottles are collected to make up an adult population size of 1200 per population. Rest of the maintenance is similar to MB maintenance regime.

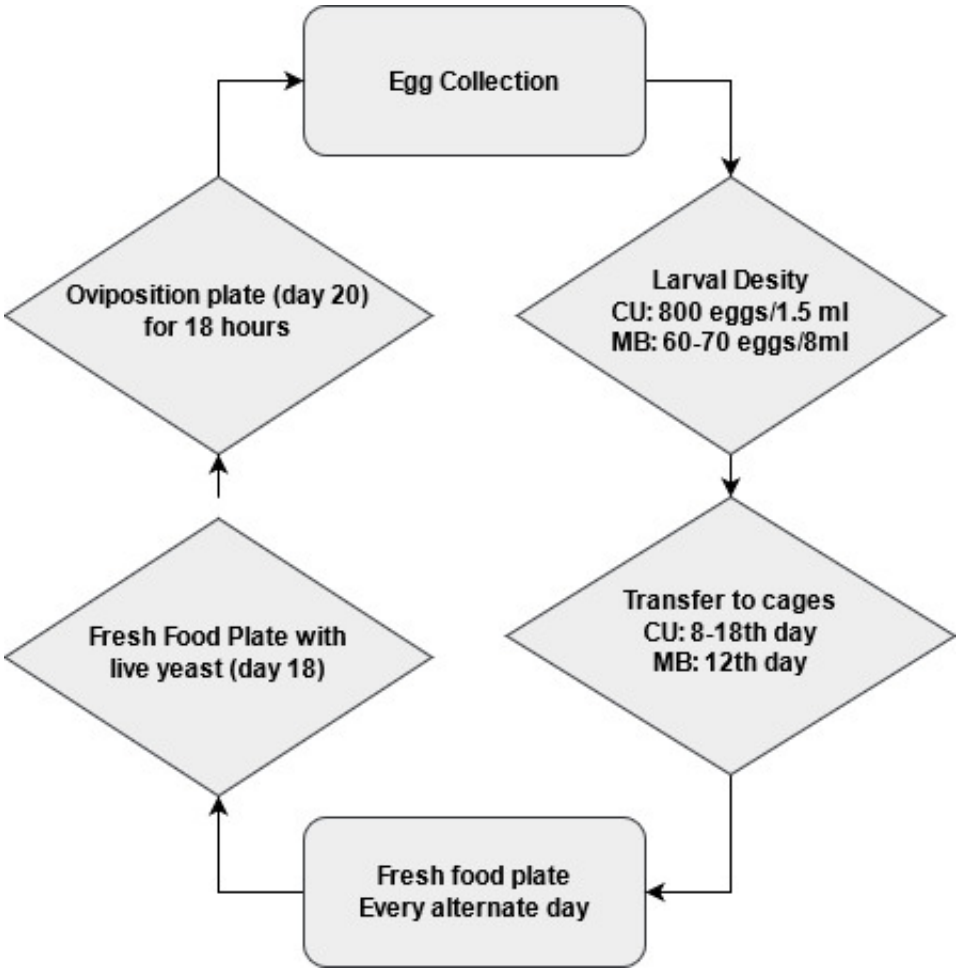


Figure 2.2: Population maintenance of CU's and MB's

Chapter 3

Methods

3.1 Experimental Setup

To manipulate condition in this study larval rearing density was varied in both CU and MB. Three density treatments were chosen, a high-density treatment (600 eggs per vial containing 2 ml of charcoal cornmeal food), an intermediate-density treatment (300 eggs per vial containing 2 ml of food) and a low-density treatment (60 eggs per 6 ml of food). A high-density treatment of 800 eggs per vial containing 2 ml of food (native CU density) could have been more appropriate here but (Shenoi, Ali, et al., 2016) previously found that MB populations show less than 5% survivorship at this density. Thus, a density of 600 eggs per 2ml of food was chosen. Larval crowding as well as adaptation to larval crowding has been known to affect development time by decreasing the mean of the distribution and increasing the variance of the distribution (Moya et al., 1985). Therefore, egg collections for different selection regime and different density treatments were done on different days to age match all the flies. Maintenance of these flies were similar to the stock populations, high-density and intermediate-density flies from both MB and CU populations were transferred into Plexiglas cages (12*11*11 cm) daily (similar to the maintenance of CU populations) once they started eclosing in the culture vials and low-density treatments from both MB and CU populations were transferred to the Plexiglas cages (12*11*11 cm) on 12th day post egg collection (similar to the maintenance of MB populations). The procedure of transferring crowded flies i.e., high/intermediate density flies daily and low-density flies on 12th day post egg collection is a standard practise in studies of

larval crowding and this is done to ensure no resource limitation in the adult stages (Nagarajan et al., 2016; SARANGI et al., 2016; Shenoi, Banerjee, et al., 2016).

3.2 Morphometric Data

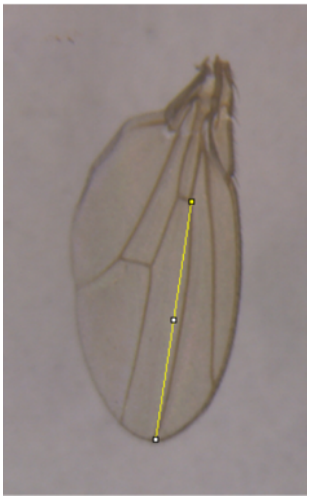
After dumping low-density flies on 12th day post egg collection, all the density treatments from both populations were given a 24-hour period to allow their cuticle to sclerotize. Following this 24-hour period flies were frozen at -20°C and reserved for morphological measurements. (Chechi et al., 2017) showed that freezing the flies at -20°C does not affect their morphological measurements. From these frozen flies 15 flies for each sex per selection regime*density treatment were randomly chosen for morphological measurements. For each fly right foreleg and both wings were dissected under a compound microscope using fine dissection forceps. Subsequently, following parts of the dissected fly were imaged (using a digital camera attached with microscope) for linear measurements: femur, tibia, right wing (for L3 vein length), left wing (for L3 vein length) and thorax.

3.3 Image analyses

Image J version 1.50b (National Institute of Health) was used to perform all linear measurements mentioned above. An image of standard stage micrometre (1mm) glass slide served as a reference for each organ (for absolute length/pixels). For wing vein, a straight-line length from anterior cross vein to the end of the second longitudinal vein was used (Markow et al., 1994). Thorax length was measured from where the neck meets the pronotum to the posterior tip of the scutellum. Femur and tibia length was measured from severed right forelegs using a method similar to (Shingleton et al., 2009) (see 3.1).

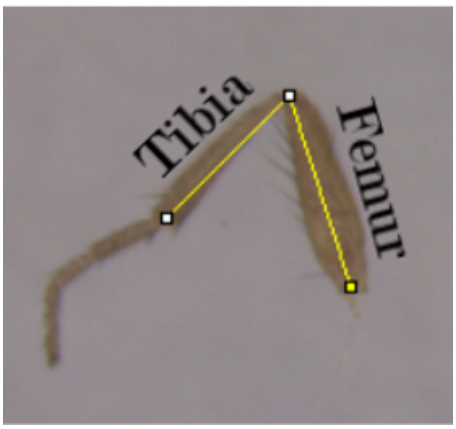
3.4 Statistical Analyses

All the traits covaried positively as one would expect from multiple measures of the same trait i.e., body size. Therefore, principal component 1 (PC1) of the correlation matrix of all traits across three density treatments, both sexes and both selection



(a) Wing Vein Length

(b) Thorax Length



(c) Femur and Tibia Length

Figure 3.1: Linear Measurements of Body Shape Components

regimes was used as a measure of body size. Use of PC1 instead of commonly used measures like wing vein length or thorax length ensures that maximal variation is encapsulated for further analyses (Oudin et al., 2015). Further, allometric scaling of all the traits with PC1 showed no heterogeneity of slopes whereas allometric scaling of wing vein length and thorax length with rest of the traits varied significantly across diet treatments. This makes an even stronger argument for using PC1 as a proxy for body size.

To analyse effects of density treatment, selection regime, sex and their interaction on body size following linear mixed effects model was setup using "lme4" and "lmerTest" on R (R Core Team, 2018).

$$PC1 = Sx + T + Se + Sx:T + Se:T + Sx:Se + Sx:Se:T$$

Sx = Sex, Se = Selection and T = Treatment

Block was added as a random factor and the rest were fixed factors.

Relationship between condition-dependence and sexual dimorphism can only be investigated if you have a set of multiple different traits, not multiple measures of the same traits. Therefore, all the traits were corrected for body-size before the analyses. Body-size correction for each trait was done by taking residuals from linear regression of the trait and PC1 (excluding focal trait). To investigate correlation between sexual dimorphism and condition-dependence, a sexual dimorphism index was calculated for each trait (mean trait value for females – mean trait value for males) and a condition-dependence index (mean trait value in low density - mean trait value in high density).

Chapter 4

Results

4.1 Multivariate body size

PC1 of all traits across density treatment*Selection*Sex was highly negatively correlated with each individual body size related trait. Therefore, for the sake of simplicity and to view correct trends modulus of PC1 is plotted in 4.1 and 4.2.

I found a significant sexual dimorphism in body size. As expected, females were significantly larger as compared to the males. Body size also showed condition-dependence as the three density treatments differed significantly from each other with size gradually increasing from high density to low density. Additionally, I also found a significant effect of selection. MB flies on an average were significantly larger than CU flies.

To see condition-dependence in sexual dimorphism, one should get a significant interaction between the density treatment and sex which was the case with our data. Sexual dimorphism in body size gradually increased on improving the condition (which in our case was done by decreasing rearing density) 4.1.

A closer investigation at this condition-dependent sexual dimorphism revealed that the significant treatment*sex interaction was majorly due to loss of sexual dimorphism in MB populations 4.2. Multiple comparison also verified this as the difference between males and females from MB populations in high density treatment was non-significant whereas selected populations still maintained a significant sexual dimorphism (see B).

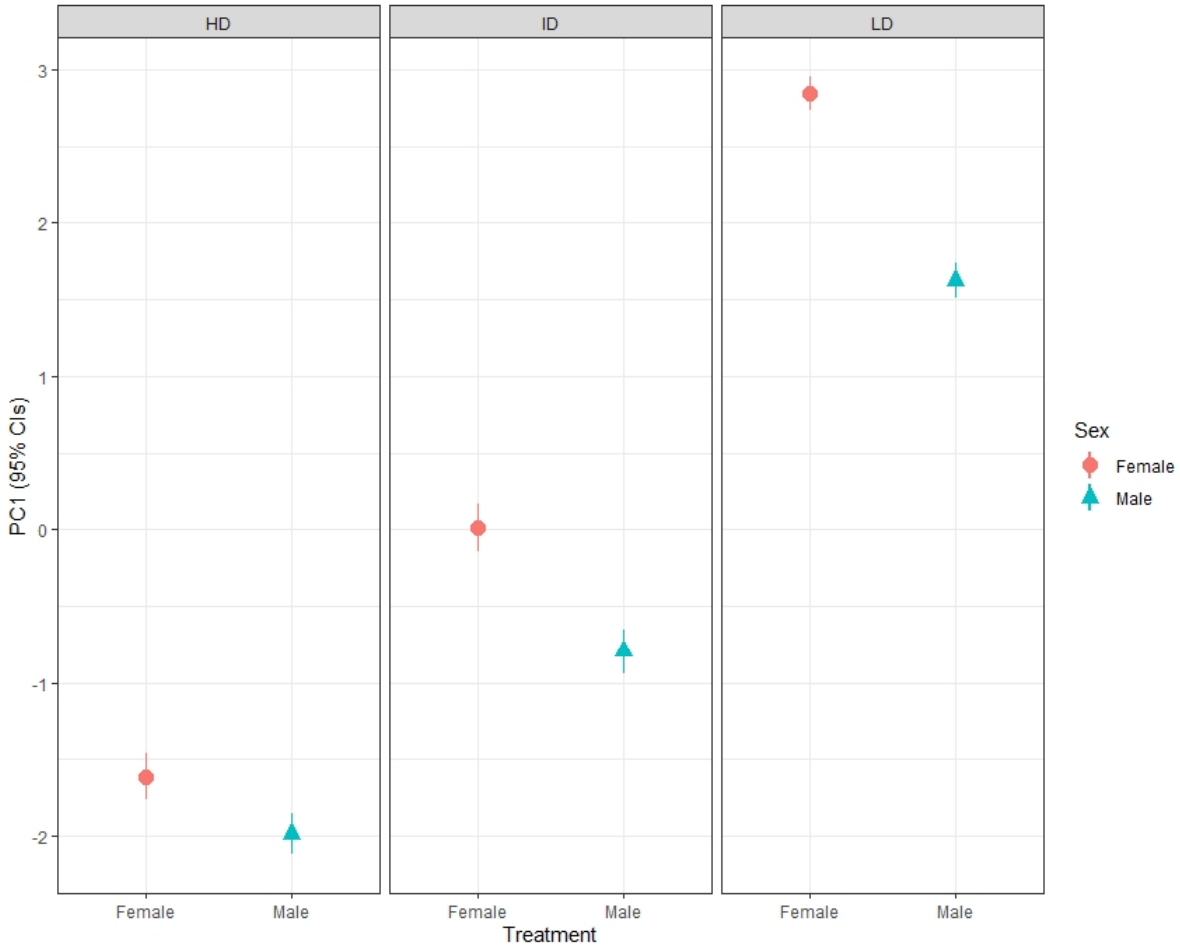


Figure 4.1: Sexual dimorphism across different density treatments. Density increases from left to right

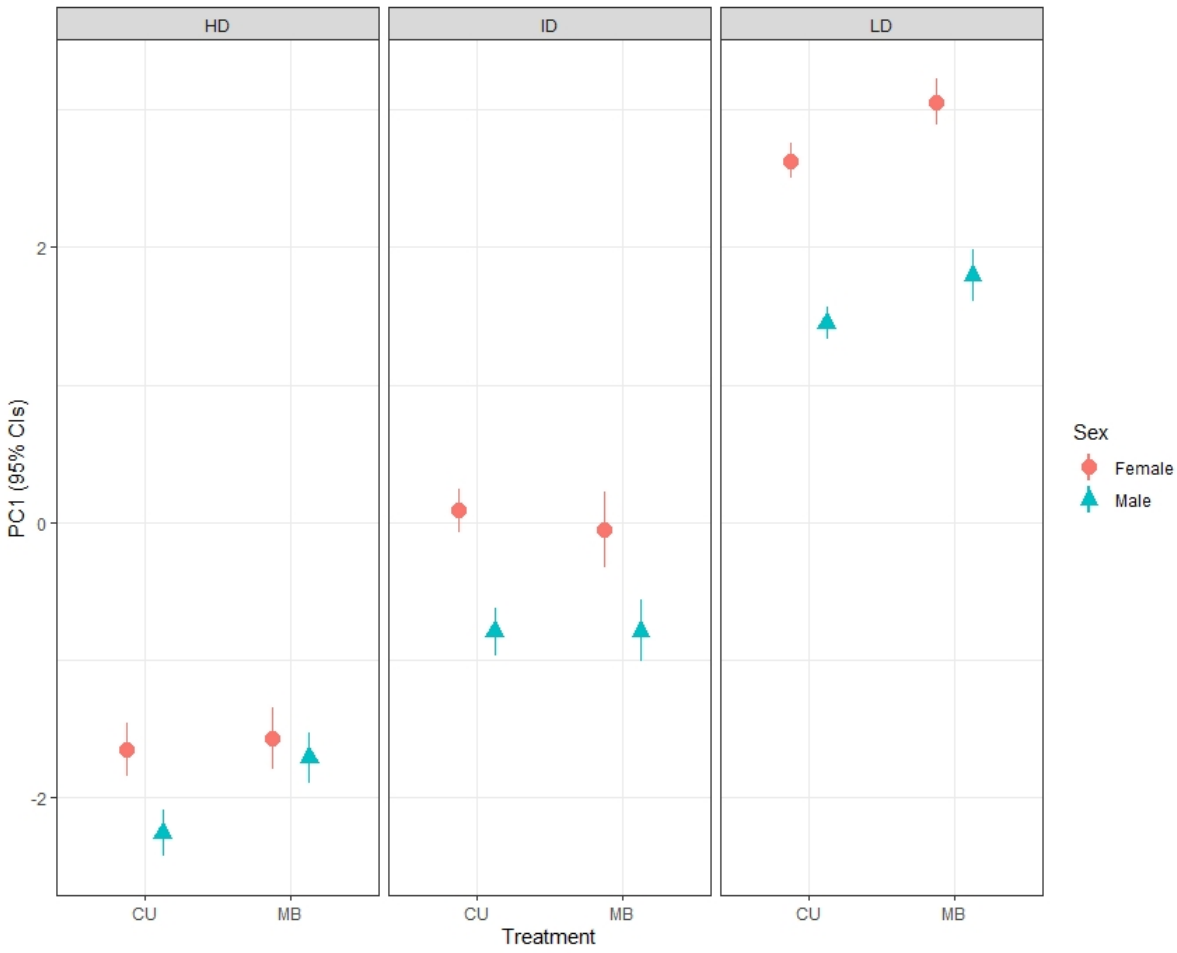


Figure 4.2: Sexual dimorphism in MB's and CU's across different density treatments

	Chisq	Df	$\Pr(>\chi^2)$
(Intercept)	103.39	1	< 2.2e-16
Treatement	1161.93	2	< 2.2e-16
Sex	23.40	1	< 1.318e-06
Selection	0.42	1	0.5186
Treatement:Sex	10.18	2	0.0062
Treatement:Selection	10.92	2	0.0043
Sex:Selection	6.85	1	0.0089
Treatement:Sex:Selection	4.90	2	0.0862

Table 4.1: Multivariate body size

4.2 Relationship between condition-dependence and sexual dimorphism

Among different body shape components there was a significant positive correlation between extent of sexual dimorphism and the strength of condition-dependence in both MB and CU populations. Although, I found a non-significant difference between regression slopes of MB's and CU's. There was a trend of higher slope in control populations as compared to the crowding adapted populations.

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	0.02	1	254.42	0.0039
sdi	0.04	1	453.84	0.0022
Residuals	0.00	2		

Table 4.2: Regression model results for CU's

	Sum Sq	Df	F value	Pr(>F)
(Intercept)	0.11	1	83.76	0.0117
sdi	0.05	1	35.92	0.0267
Residuals	0.00	2		

Table 4.3: Regression model for MB's

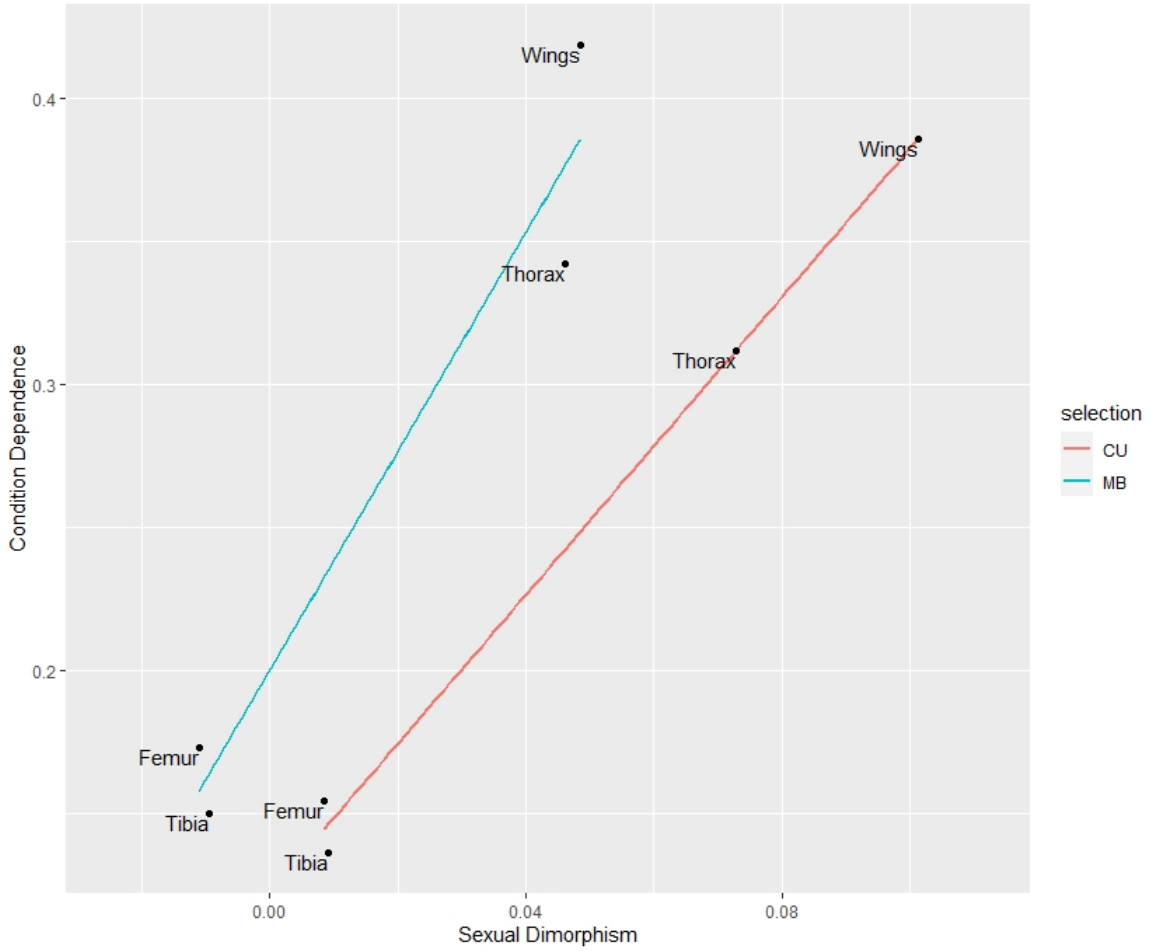


Figure 4.3: Among trait variation in CU's and MB's

Chapter 5

Discussion

In this thesis, I tried to ascertain if *Drosophila melanogaster* shows variability in extent of sexual dimorphism across a gradient of nutritional environment (i.e., different conditions). I also looked at how multiple generations of adaptation to larval crowding affects this variability of sexual dimorphism across condition gradient.

5.1 Coevolution of condition-dependence and sexual dimorphism

Directional selection for exaggeration in one of the sexes (specifically, higher body size in females for our case) results in displacement of that sex from viability selected phenotype and subsequently leads to a more pronounced sexual dimorphism. This displacement from viability selected phenotype also represents the costs incurred by the directionally selected sex and therefore the degree of condition-dependence favoured by the selection (Rowe et al., 1996). A joint dependency of condition-dependence and sexual dimorphism on selection was thus predicted by the theory. This join dependency should lead to the coevolution between condition-dependence and sexual dimorphism (Oudin et al., 2015). My results provide a strong support for this prediction. In an analysis of multiple traits (i.e., different body shape components) which vary in the extent of sexual dimorphism, I found that extent of sexual dimorphism was postively correlated with condition-dependence. Highly dimorphic traits (wings and thorax) showed more condition-dependence as compared to weakly dimorphic traits (femur and tibia). This pattern was consistent in both control and selected

populations and multiple generations of adaptation to larval crowding did not affect the relationship between condition-dependence and sexual dimorphism. These results are in line with the theoretical predictions and previous empirical findings and suggest that condition-dependence and sexual dimorphism share a common genetic and developmental basis (Bonduriansky, 2007; Oudin et al., 2015; Wyman et al., 2010).

5.2 Within trait variation in MB's

Within a trait (multivariate body size in our case), the pattern of sexual dimorphism in control populations was similar to what (Bonduriansky, 2007) described as incomplete sexual dimorphism. The flies started off with a near body size monomorphism in high density treatments (low condition environment) and transitioned to a very pronounced sexual dimorphism in low density (high condition). High density males and females were almost indistinguishable from each other, commonly used identification marks which are used sex the flies apart (for example dark black coloration at the tip of abdomen in males) were almost completely lost. Other insect species which are known to show an incomplete sexual dimorphism in low condition are *Prochyliza xanthostoma* (Bonduriansky, 2006), *Onthophagus* dung beetles where in low condition males lose their horns and become more female like (Emlen, 1994; Nijhout et al., 1998). In contrast there are several insect species which show complete sexual dimorphism even in the lowest possible condition for example, diopsid (stalk-eyed) fly *Cyrtodiopsis dalmanni* (Cotton et al., 2004).

Several empirical studies have found support for phenotypic plasticity in sexual dimorphism in response to nutrition (David et al., 1994; Karan et al., 2000; Post et al., 1999). Our results show that extent of sexual dimorphism is variable in response to environmental factors that can influence condition of an individual. Moreover, strong positive covariation between strength of condition-dependence and extent of sexual dimorphism suggests that these both are biologically inseparable, sexual dimorphism itself is a product of condition-dependence. This more than creates a need to study these two concepts in consortium. This not only will give us better understanding of sexual dimorphism but also will help us understand the common genetic architecture of condition-dependence and sexual dimorphism. Although, there have been numer-

ous theoretical and empirical studies which have laid light on genetic architecture of sexual dimorphism (Rhen, 2000; Rice, 1984; Rice et al., 2002; T et al., 2004) but all these models fail to address common genetic basis for condition-dependence and sexual dimorphism.

5.3 Within trait variation in CU's

Sexual dimorphism can be thought of as a product of optimal resource allocation controlled by pleiotropic effect from sex-linked allocation genes (Bonduriansky, 2007a). If an organism is exposed to a low resource environment (which would eventually lead to lower condition) this optimal resource allocation would be compromised. Effects of this would be visible in the sex directionally selected for exaggeration subsequently leading to lesser extent of sexual dimorphism. This is what happens in MB populations, in low resource environment they show a monomorphism for multivariate body size. This effect is most likely due to reduction of body size in females (Multiple comparisons revealed a non-significant difference between CU female – MB female and CU female – MB male). Crowding adapted populations also show a similar trend of decreasing sexual dimorphism as the condition worsens. There's one very interesting thing different from MB's here though, they do not show a complete loss of sexual dimorphism in high density. This is probably because they have adapted an optimal resource allocation in high density and are not exposed to the novel stress of crowding as the MB's experience.

In summary, this study demonstrates a strong condition-dependence in sexually dimorphic multivariate body size. This condition-dependence is also affected by multiple generations of adaptation in resource limiting developmental environment. Our results also show the positive covariation between condition-dependence and sexual dimorphism. Such a covariation is most likely a result of joint dependency of the two on the selection which would ultimately lead to coevolution.

Appendix A

Food Recipes

Charcoal-Cornmeal Food To make 1L of charcoal-cornmeal food, the following ingredients are used:

- 40g of dry yeast
- 40g of refined sugar
- 2g of agar
- 100g of cornmeal
- 100g of cornmeal

All ingredients are mixed in a bowl with 1100 ml water and cooked in a pressure cooker until the first whistle. The cooker is then taken off the gas and further cooking is allowed till the pressure releases. Food is cooled down to 60 $\circ$C followed by addition of preservatives in the form of methyl paraben (1 g), ethanol (10 ml) and propionic acid (10 ml).

Appendix B

Supplementary Material

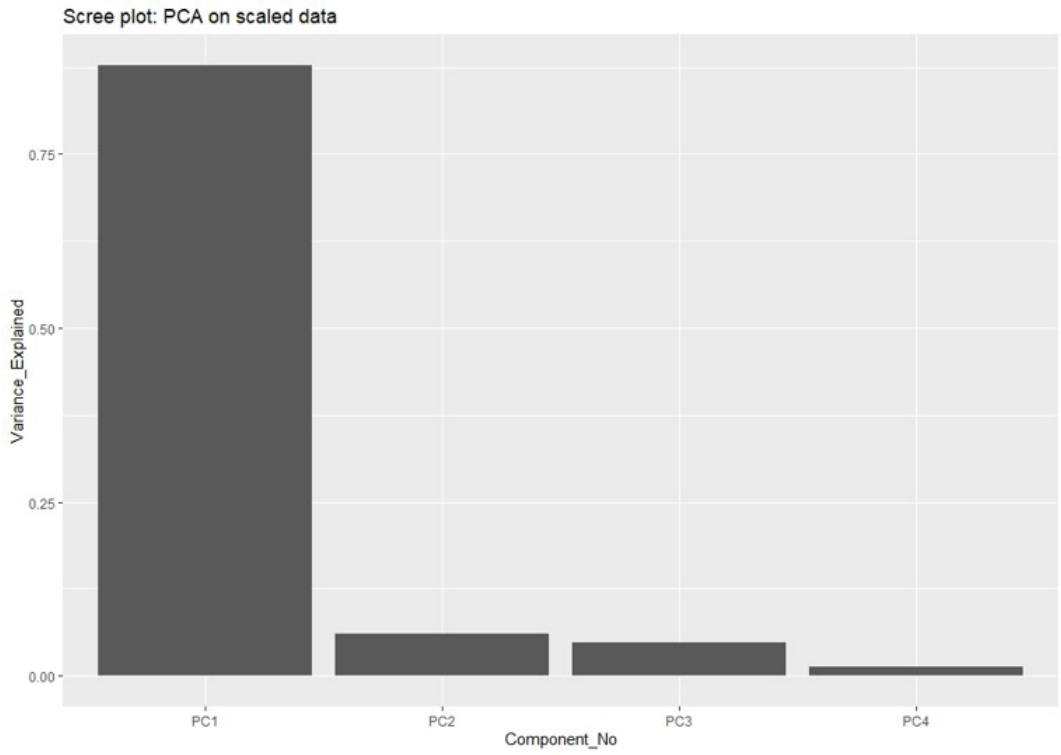
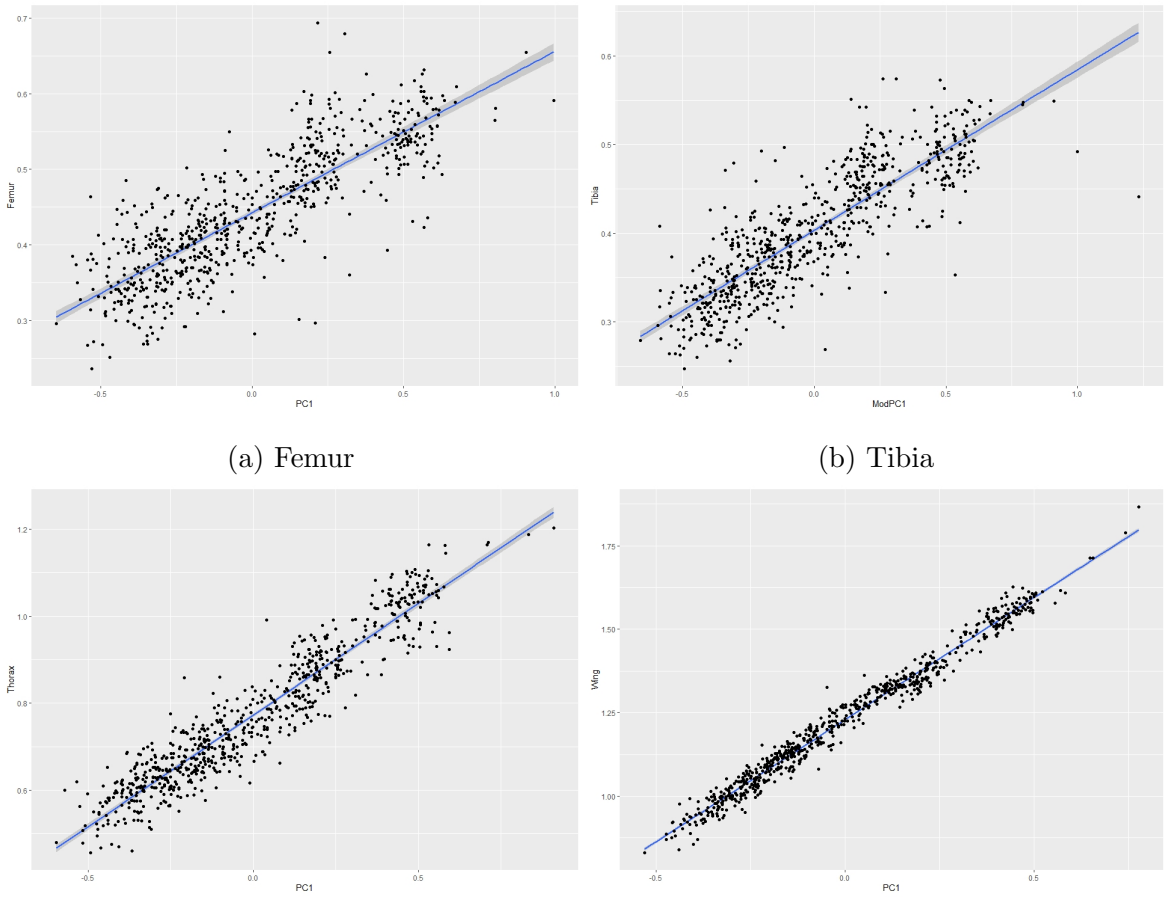


Figure B.1: Variation explained by each Principal component



(a) Femur

(b) Tibia

(c) Thorax

(d) Wings

Figure B.2: Linear regression of body shape components against PC1

contrast	estimate	SE	df	t.ratio	p.value
Treatement = HD					
CU Female - MB Female	0.0811	0.1233	695.00	0.658	0.9128
CU Female - CU Male	-0.6077	0.1233	695.00	-4.929	<.0001
CU Female - MB Male	-0.0615	0.1233	695.00	-0.499	0.9593
MB Female - CU Male	-0.6888	0.1233	695.00	-5.586	<.0001
MB Female - MB Male	-0.1426	0.1233	695.00	-1.157	0.6543
CU Male - MB Male	0.5462	0.1233	695.00	4.430	0.0001
Treatement = ID					
CU Female - MB Female	-0.1423	0.1233	695.00	-1.154	0.6558
CU Female - CU Male	-0.8834	0.1233	695.00	-7.165	<.0001
CU Female - MB Male	-0.8769	0.1233	695.00	-7.112	<.0001
MB Female - CU Male	-0.7411	0.1233	695.00	-6.010	<.0001
MB Female - MB Male	-0.7346	0.1233	695.00	-5.958	<.0001
CU Male - MB Male	0.0065	0.1233	695.00	0.053	0.9999
Treatement = LD					
CU Female - MB Female	0.4422	0.1249	695.13	3.540	0.0024
CU Female - CU Male	-1.1781	0.1238	695.02	-9.514	<.0001
CU Female - MB Male	-0.8257	0.1244	695.06	-6.640	<.0001
MB Female - CU Male	-1.6202	0.1244	695.09	-13.026	<.0001
MB Female - MB Male	-1.2679	0.1249	695.02	-10.152	<.0001
CU Male - MB Male	0.3524	0.1238	695.02	2.846	0.0236

Degrees-of-freedom method: kenward-roger

P value adjustment: tukey method for comparing a family of 4 estimates

Table B.1: Multiple comparisons for Multivariate body size

contrast	estimate	SE	df	t.ratio	p.value
CU - MB	-1.2260	0.5744	4	-2.134	0.0997

Table B.2: Linear Model Results: MB-CU slope comparison

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