A study of various components of egg investment in laboratory populations of Drosophila melanogaster adapted to larval crowding

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Certificate of Examination

This is to certify that the dissertation titled "A study of various components of egg investment in laboratory populations of Drosophila melanogaster adapted to larval crowding" submitted by **Mr. Sohit Chobhiyal** (Reg. No. MS15155) 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: June 30, 2020

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.

Sohit Chobhiyal

Dated: June 30, 2020

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. N. G. Prasad

(Supervisor)

Acknowledgements

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be selfless like my parents and hard-working like my sister. I am genuinely thankful to their unparalleled an unselfish love.

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Notation

MB	**M**elanogaster **B**aseline
MCU (CU)	**M**elanogaster **C**rowded as larvae **U**ncrowded as adults
LD	**L**ow larval **D**ensity
HD	**H**igh larval **D**ensity
ANOVA	**A**nalysis **O**f **V**ariance

Contents

Abstract

Parental investment is a significant contributing factor to the offspring's survival and fitness and has direct implications on the fitness of the parents' as well. Parental investment is, in turn, under the influence of various genetic and non-genetic factors. Females and males employ different reproduction tactics, due to the inherent asymmetry of parental contribution toward producing offspring, to maximize their respective fitness. Therefore, it is essential to study these factors in order to understand how parental investment evolves and how fluctuating environmental conditions would shape parental investment. With inspiration from a few previous studies, I tried to study various components of egg investment in populations of *Drosophila melanogaster* adapted to larval-crowding. Apart from other interesting observations, I have found out that populations selected for larval crowding laid significantly larger eggs in comparison to their baseline populations (while laying few). I also observed that males of the selected lines significantly reduce their mates' fecundity.

Chapter 1

Introduction

Life-history theories attempt to explain how forces of natural selection would shape the way in which organisms pass on their resources into their progeny (David Reznick, 2010). Life-history of an organism constitutes the sum total of events occurring from birth through death: survival, growth, reproductive development, progeny production and lifespan. To ensure maximal survival and reproduction, and thus fitness, all life-history traits must evolve continuously (Houle, 2001) and independent from one another. Nevertheless, the so-called *Darwinian demons* (Law, 1979) do not exist in nature. Firstly, because nature does not select for all traits independently at optimal levels due to the existence of certain broader genetic and physiological networks connecting those traits. Finally, since resources, abundance and acquisition thereof, are limited in nature which leads to the existence of trade-offs in evolution among various life-history traits (Prasad & Joshi, 2003). The constraint of a finite resource pool, to draw energy from, causes trade-offs to impose a limit on an organism's potential for fitness. Hence, in such a scenario, increased investment in one trait could lead to a reduction in another. For example, a trade-off between maximizing lifespan and maximizing body size (Shenoi & Prasad, 2015). An ideal resource allocation strategy is one that enables an organism to achieve an optimal life-history strategy to achieve maximal fitness level. A simple Y model of resource allocation (Noordwijk & de Jong, 1986) attempts to explain trade-offs between such negatively correlated traits.

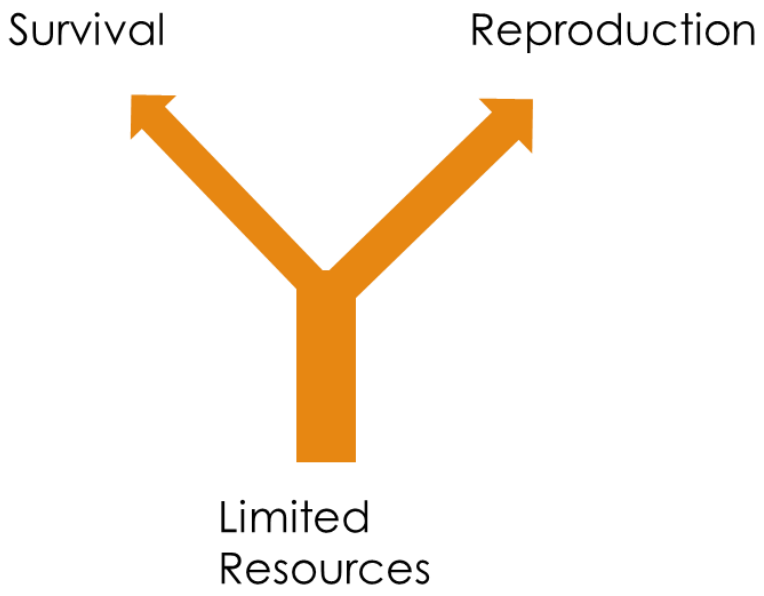

Figure 1.1: Y-model of resource allocation depicting a trade-off between two traits

In the face of constraints posed by the environment, the various reproductive investment strategies also become subject to trade-offs. For example, between maximizing progeny size and maximizing progeny number (Stearns, 1976). Parental investment in the progeny is a life-history trait subject to various environmental/non-genetic and intrinsic/genetic factors that shape the dynamics of intergenerational changes in phenotype and fitness. The genetic mode of inheritance involves the transmission of DNA sequence variation. In contrast, non-genetic inheritance involves transmission of non-DNA-based (soft inheritance, Lamarckian inheritance, transgenerational epigenetic effects, non-Mendelian inheritance, parental effects, fetal programming, carry-over effects, and cell memory) factors from parents to progeny which alter the offspring phenotype. Non-genetic factors mostly constitute information regarding the ancestral environment, which would enable the past generation to prime their progeny to such conditions for a better chance at survival. It has been shown that acquired environmental condition is transferrable across generations via parental effects (Bonduriansky & Head, 2007). Since non-genetic inheritance can decouple phenotypic change from genotype (Bonduriansky & Day, 2009), and also owing to the fact that these modes of inheritance operate in parallel, it becomes imperative to understand such mechanisms in order to understand the evolutionary history of a life-history trait and its response to current natural selection.

A study conducted by Hallsson, Chenoweth & Bonduriansky (2012) on seed beetle, *Callososbuchus maculatus*, elucidates very elegantly how the relative importance of genetic and non-genetic mechanisms of inheritance is related to the degree of trait plasticity. They predicted and found out that a more plastic trait would respond strongly to non-genetic maternal and paternal effects, whereas, less plastic traits would remain less affected by such effects. A study conducted by Mousseau et al. (1997) demonstrated how female *Stator limbatus*, a seed beetle, alter their egg size depending upon the host seed. The females laid larger and fewer eggs on a host plant with more robust seed coats and vice versa. Switching the host plant caused the females to readjust their investment strategy accordingly. This study provided evidence for egg size being an adaptively plastic trait in the seed beetle and the existence of a trade-off between offspring quality and quantity. Thus, females can increase their fitness by optimizing the balance between investing more per offspring and producing more offspring. A trade-off between the two strategies seems to be an obvious outcome in a limiting environment.

Females can not only alter their progeny's phenotype through genes, but also by shaping the developmental environment early in life (Mousseau & Fox, 1998). Such maternal effects can be transmitted as: biomolecules like nutrients and hormones, environmental influences like temperature or prenatal/natal environment and, behaviorally through maternal care. Females' interaction with various male genotypes can influence their investment too (Pischedda et al., 2010). Female assessment of male quality during courtship and copulation could be critical for a female to invest accordingly (Galeotti P, 2005).

Males of many insect species have shown to manipulate female investment into progeny to maximize their own fitness. Paternal effects are important as they represent a source of variation in phenotype and fitness of the progeny (Crean & Bonduriansky, 2014; Wolf, J.B. et al., 1997). These effects are, however, largely mediated through maternal responses. This is because eggs contribute a larger quantity of cytoplasm to the zygote and thus control the early development. Nevertheless, various modes of transmission of paternal effects can occur in species exhibiting a substantial paternal investment component. For example, male ejaculates may contain anti-microbial peptides (drosonin and drosomycin in *Drosophila*) (reviewed by T. Chapman, 2001), a complex blend of

accessory gland proteins and lipids which can alter female receptivity, ovulation and oogenesis (Wolfner, 1997).

Nutritional environment of parents along with the parental effects arising from it, can alter the reproductive investment in offspring. Vijendravarma et al. (2009) observed that parental larval diet can influence reproductive investment in *Drosophila*, as parents raised on poor larval diet laid 3-6% heavier eggs in contrast to the ones raised on standard diet. A similar study conducted by Prasad et al. (2003) established congruent results illuminating how egg investment patterns can exhibit plasticity when parental environmental conditions are subject to stress.

In order to understand how egg investment evolves in a population subject to developmental stress, it becomes of vital importance to examine the various aforementioned components that are involved. We tried to investigate the same in laboratory populations of *Drosophila melanogaster* adapted to larval crowding that have evolved longer adult lifespan (Shenoi, SZ Ali & Prasad, 2015), increased courtship (Shenoi et al., 2016), larger testes and a number of adult traits. To this end, we used crosses of different parental combinations (which were in turn subject to different larval density treatments) to answer the following questions (using egg volume to quantify said investment):

a. Does the environment play a role in shaping egg investment?
b. Is the variation in egg investment inherited maternally or paternally?
c. Is there any trade-off between fecundity and egg size?

Chapter 2

General Experimental Methods

2.1 Fly Populations Used

The study was carried out on two sets of large outbred populations of Drosophila melanogaster. One selected for adaptation to larval crowding, **M**elanogaster **C**rowded as larvae and **U**ncrowded as adults or MCU, and the corresponding baseline population **M**elanogaster **B**aseline or MB. Four independent replicate populations, referred to as *Blocks*, and are maintained for both sets of populations on a 21- day discrete generation cycle. Standard lab conditions were observed which included temperature being maintained at 25°C with a relative humidity of 95%, a light cycle of 24-hours and a diet of standard cornmeal-charcoal food. Per replicate, the adult census for MBs is ~2500 individuals and ~1900 individuals MCUs. These populations were derived in the lab of Prof. Amitabh Joshi at the Evolutionary and Organismal Unit, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), where they underwent 75 generations of selection. Now they continue to be maintained in the EBL (Evolutionary Biology Lab), IISER Mohali.

MCU and MB have originally been derived for JB populations (Sheeba et al., 1998) which were maintained under standard laboratory conditions on a discrete 21-day generation cycle for up to 280 generations. In 2006, the JB populations (JB 1-4) were combined and re-sampled, thus producing a single large population called Melanogaster Baseline (MGB). After 15 generations, MBs were split and grouped into four replicate populations called MBs (1-4). From each MB replicate, the selection lines of MCUs (1-4) were created and henceforth have been maintained independently under the previously mentioned standard laboratory conditions.

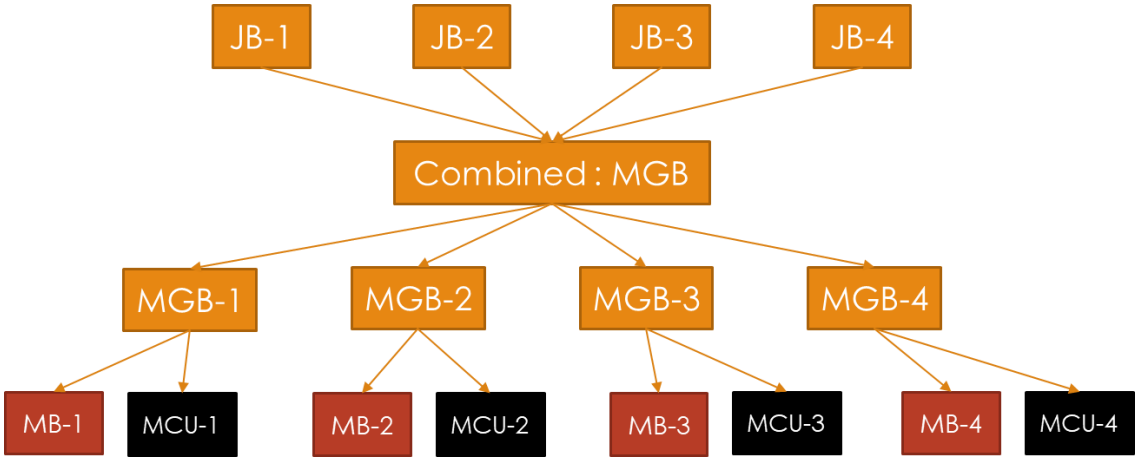

Figure 2.1: Lineage of MB & MCU populations

2.2 Population Maintenance

2.2.1 MB

Maintaining a 21-day discrete generation cycle, eggs collection is done from 21-days old females (post eclosion) of the previous generation. Subsequently, the eggs are then placed into glass vials (25mm diameter & 90mm height) which are prepped with cornmeal charcoal food (6-8 mL) with the density maintained between 60-80 eggs per vial and not exceeding. For each replicate, forty such vials are collected. By the 12th day almost all the adult flies enclose, and upon reaching this stage they are transferred into a Plexiglas cage measuring at 24cm × 19cm × 14cm. A petri dish is pre-placed within, containing cornmeal-charcoal food, and a wet cotton piece to main ample relative humidity, both of which are changed and provided afresh every alternate day. The cages are maintained until both the stock and the backup egg collection is complete. On 18th day, post eclosion, fresh plates are provided with the addition of live yeast paste and two days after i.e. on 20th day, cut plates are provided to the cages and flies are given 18-hours window to oviposit. Eggs are then collected from this plate for the next generation.

2.2.2 MCU

The derived MCU population is also maintained under the same standard laboratory conditions as the MB's, the only difference being in the amount of food provided to each vial and the density of eggs collected per vial. On 21st day post egg collection, eggs are again collected for the next generation in vials that contain 1.5 ml of cornmeal-charcoal

food at a density of 800 eggs collected per vial. For each block, 25 vials are collected. The flies these populations, however, have a broad eclosion pattern and start eclosing 8th day post egg collection. This is done daily till 18th day post egg collection to prevent adult crowding in vials.

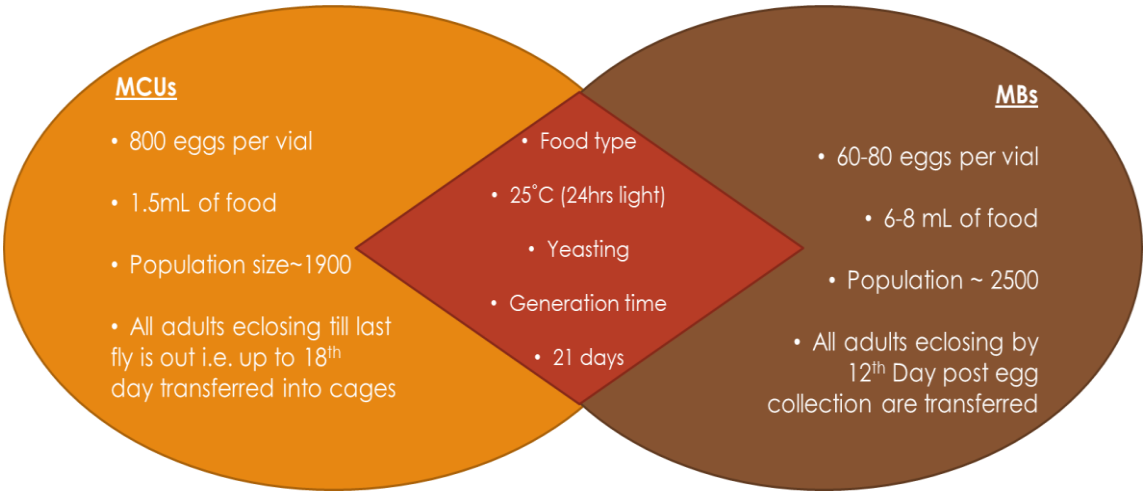

Figure 2.2: Stock maintenance of MBs and MCUs

2.3 Standardization of Flies

Standardization of flies is done by relaxing selection pressure for one generation in MCUs and maintaining them in same conditions as the baseline population. This is done to account for potential non-genetic parental effects. To this effect, backup populations are maintained in the same Plexiglas cage setup. On 22nd day post egg collection, cut plate is given to the stock cages for a time period of 18 hours. Egg collection from these cut plate is done with a density of 350-400 eggs per bottle with *ad libitum* amount of food. On 12th day, these eclosed standardized flies are dumped into cages. Eggs collected from the standardized flies were used for the experiments.

Chapter 3

Experimental Methods & Procedures

3.1 Experimental Setup

Four crosses of different male-female combinations were used in this experiment:

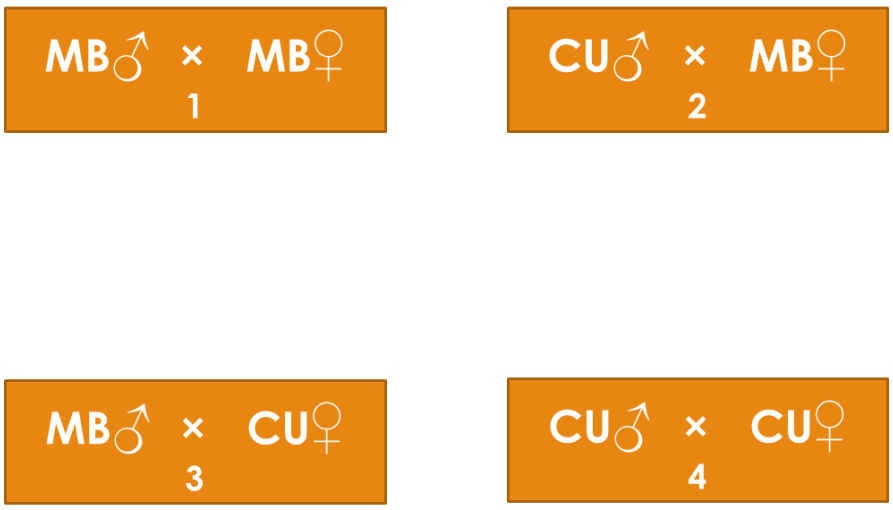

Figure3.1: Crosses performed in the experiment

Cross 1 was performed to study the baseline distribution of egg size of the original population, and Cross 2 to produce a distribution for the adapted population. Crosses 2 and 3 represent the reciprocal crosses to check for parental contributions. All the crosses were performed for two density treatments to check for environmental effects (larval crowding):

a. LD – low density treatment with 60 eggs/vial
b. HD – high density treatment with 600 eggs/vial

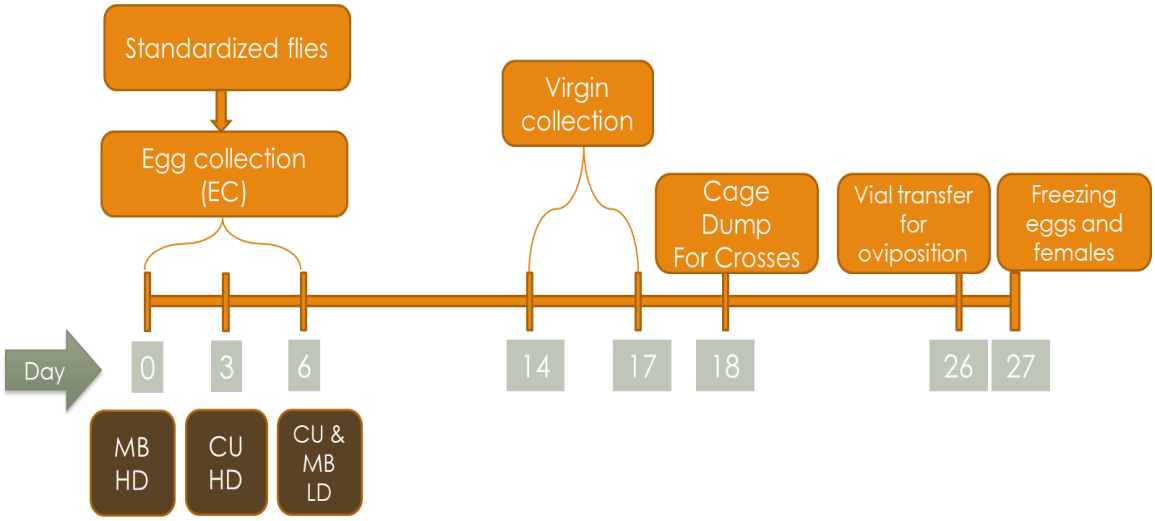

Figure 3.2: Schematic timeline of the experimental setup

Egg collection was done form the standardized flies over three different days for different populations and treatments in standard-cornmeal charcoal food. Flies of these populations exhibit different eclosion patterns depending upon the population and the density treatment. Therefore, to synchronize the peak of eclosion eggs were collected in this manner (which has been standardized by Shenoi et al.). This method also ensured that age of the flies were matched to eliminate any age-related effects. Virgins were collected after a first discard dump of initially eclosed flies, and maintained in separate male and female vial bunches. Virgin collection was done from 8th to 11th day post egg collection. Flies were flipped into fresh food vials on every alternate day.

For each cross, 100 virgin females and males were dumped into a Plexiglas cage (amounting to a total of 8 cages per block) on 12th day post egg collection. These 100 pairs of in each cross were allowed to interact and mate for 7 days during which the cages were maintained following the standard protocol with food plates being changed every alternate day. On the 7th day of interaction, the cages were provided with *ad libitum* yeasted food plates. Two days later, 30 female flies (chosen at random) from each cage were transferred into food vials and allowed to lay eggs over a period of 18 hours. At the end of the egg-laying period, females from each vial were secured in micro-centrifuge tubes and then stored in -80 along with the eggs for the subsequent measurements. Each such vial consisted of a food filled falcon cap, ensuring an easy dismount for storing and imaging eggs.

3.2 Imaging & Measurement Protocols

3.2.1 Imaging eggs for Volume measurement

Eggs from individual females of different treatment were transferred from the food surface to an agar petri-plate. The eggs were then arranged together, with their flattened sides on the agar surface, and imaged under an imaging microscope (Leica Digital Microscope) with built in reference scale for measurement.

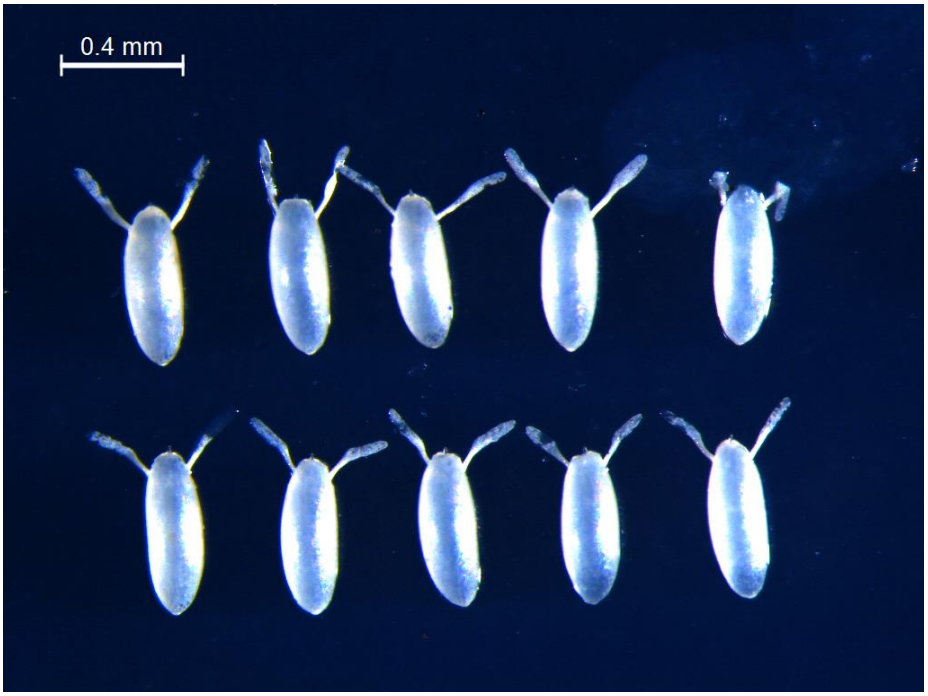

Figure 3.3: Image of eggs arranged on agar plate, taken form a single female

From every female of different treatments, a maximum of 10 eggs were imaged. So, 240 such images were taken for each block. The magnification was kept constant across all images.

Measurements of *polar axis* and the *equatorial diameter* were obtained from these images. The shape of a *Drosophila* egg was considered to be a prolate spheroid (Pischedda et al., 2010) in order to quantize the volume. The following formula could then be used to approximate the volume of an egg:

$$V = \frac{1}{6}\pi ab2$$

Where, a = polar axis

b = equatorial axis

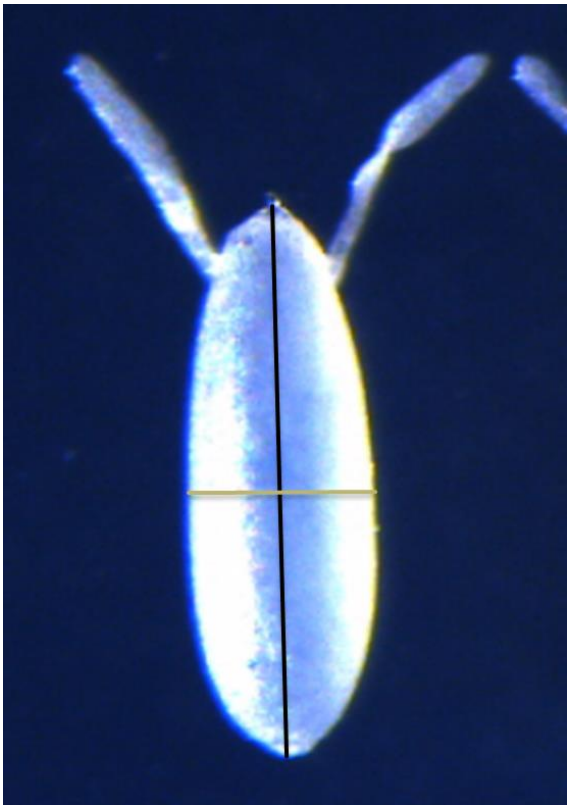

Figure 3.4: The vertical line through the egg represents the polar axis while the horizontal line across the center represents the equatorial diameter.

The measurements were taken using ImageJ version 1.8.0 using the constant reference scale produced by the Leica Digital Microscope.

3.2.2 Body-size Measurement: Thorax length

Body-size measurements of females were taken in order to normalize their corresponding egg volume data for comparisons. Thorax length was used as a proxy for body-size. In order to align the females for imaging thorax length, the wings and limbs had to be dissected out. The females were immediately dissected and imaged after being removed from the freezer to preserve their size. Wing length was not used as a proxy here as it does not exhibit as much plasticity as thorax, upon being subject to nutritional stress.

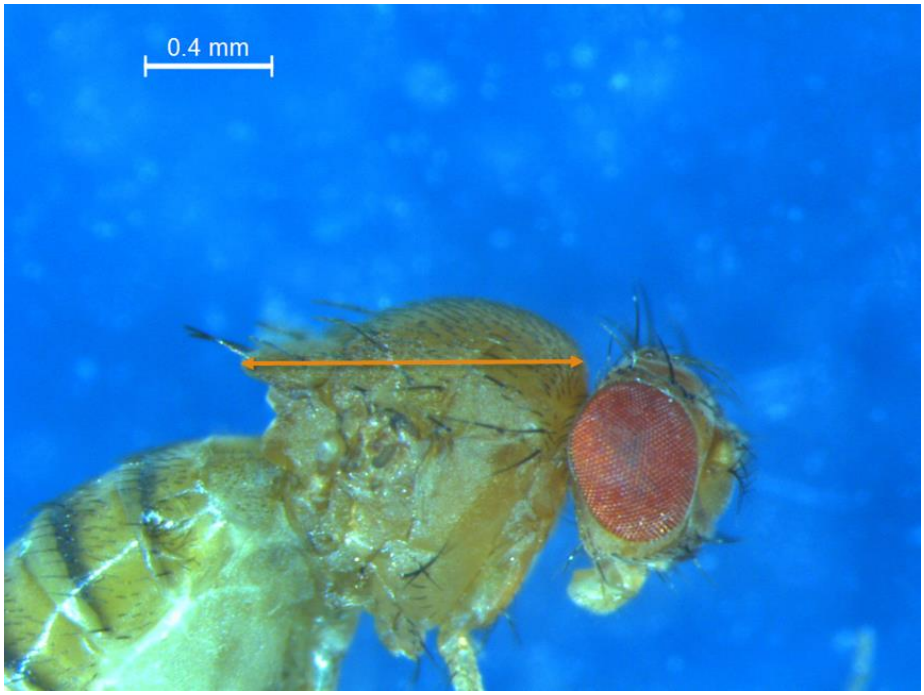

Figure3.5: Thorax length measurement done as shown in the image.

240 such images were taken and measured for each block. All measurements were again done using ImageJ version 1.8.0.

Statistical analysis of the data was done employing linear mixed models using the R package lme4. Selection regime, Sex and Density treatment were modelled as fixed factors while Blocks were modelled as random intercepts.

3.3 Fecundity Data Collection

An egg count was also maintained per female for all treatments. This would make it possible to investigate whether there exists a trade-off between the quantity and quality of offspring and also to check whether adaptation to larval crowding has an effect on fecundity. Maintaining an egg count data was made simpler due to the method in which the females were made to lay eggs.

Chapter 4

Results & Analyses

Statistical analysis of the data was done employing linear mixed models using the R package lme4. Selection regime, Sex, and Density treatment were modelled as fixed factors while Blocks were modelled as random intercepts. The result of analyses on various datasets have been briefly discussed below.

4.1 Absolute Egg Volume

A multi-variable ANOVA (Satterthwaite's method) was performed with female ID, male ID and density as fixed factors with block as a random factor, and following results were observed:

Absolute Egg Volume						
	Sum Sq	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**p value**
Female	3.07E-05	3.07E-05	1	427	420.7605	**< 2E-16**
Male	8.76E-08	8.76E-08	1	2	1.2017	0.3873
Density	2.20E-06	2.20E-06	1	427.01	30.1777	**6.79E-08**
Female:Male	1.49E-07	1.49E-07	1	427	2.0406	0.1539
Female:Density	1.43E-06	1.43E-06	1	427.03	19.58	**1.23E-05**
Male:Density	7.94E-08	7.94E-08	1	427.01	1.0897	0.2971
Female:Male:Density	4.50E-09	4.50E-09	1	427.03	0.0623	0.8031

Table 4.1 Multi-variate ANOVA table for Absolute Egg Volume

Female ID has a significant effect ($p<0.000001$) on absolute egg volume with MCU females laying larger eggs when compared to MB females in both density treatments and irrespective of the kind of male they were mated to. Male ID, on the other hand, did not have any significant effect on absolute egg volume.

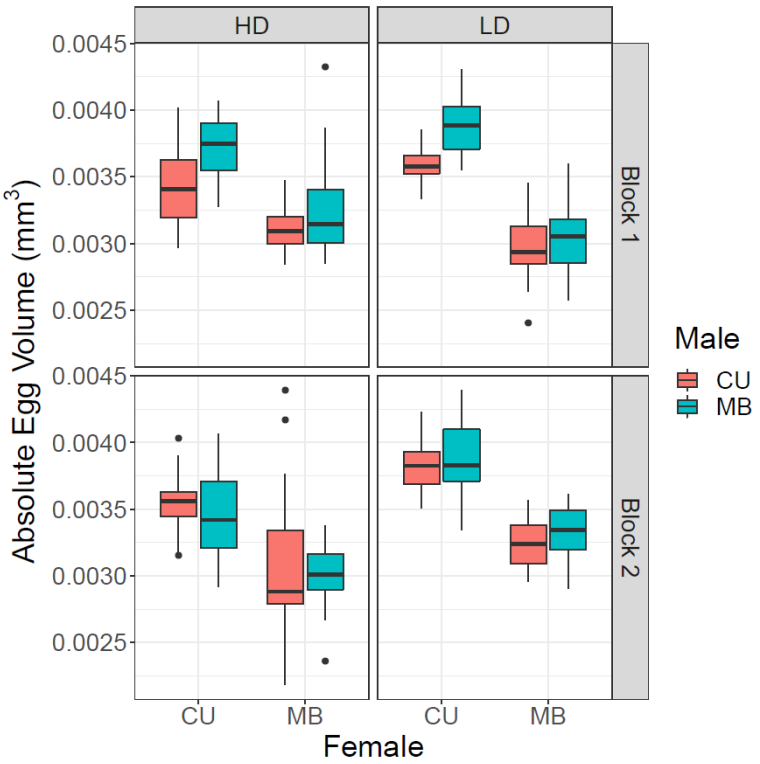

Figure 4.1: Graphical distribution of Absolute Egg Volume

Density also shows a significant effect ($p<0.000001$) on absolute egg volume with a general trend of LD treatment females laying eggs of larger volume compared to HD treatment females. The interaction between female ID and density is also significant ($p<0.00001$). As can be seen from Figure 4.1, MCU females of LD treatment consistently laid larger eggs compared to that of their HD counterparts. But the trend seems to be opposite for MB females of Block 1.

4.2 Body-size Comparison: Thorax Length

A mixed model ANOVA was performed with female ID and density as fixed factors, and block as a random factor.

Thorax Length						
	Sum Sq	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**p value**
Female	0.0002	0.0002	1	432	0.1468	0.70183
Density	11.3477	11.3477	1	432	6969.7668	**<2E-16**
Female:Density	0.0054	0.0054	1	432.2	3.3092	0.06958

Table 4.2 ANOVA table for female Body-size comparison

Female ID did not show any significant effect on thorax length when observed across the same density treatment in both the blocks. Density, however, exhibited a very strong effect ($p<0.000001$) on the body size.

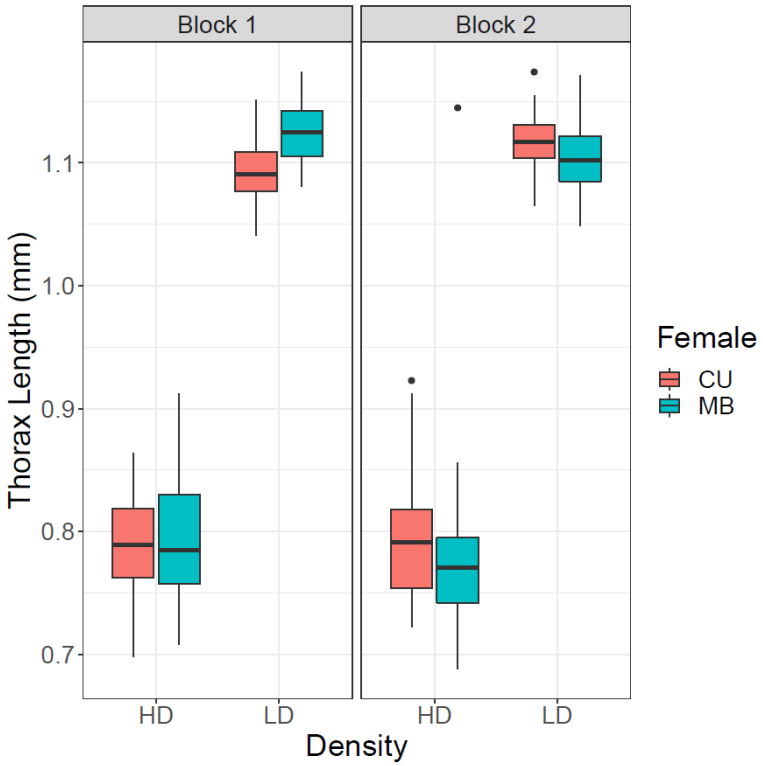

Figure 4.2: Graphical distribution of female Body-size

The lopsided difference in the thorax length between HD treatment and LD treatment females can clearly be seen in Figure 4.2.

4.3 Standardized Egg Volume

A multi-variable ANOVA was performed separately for the two density treatments due to as we were getting singular fits. Also because the denominator used for standardization of

LD treatment eggs, i.e. thorax length, was much larger in comparison to HD counterparts, the variances were much smaller in LD (Figure 4.3).

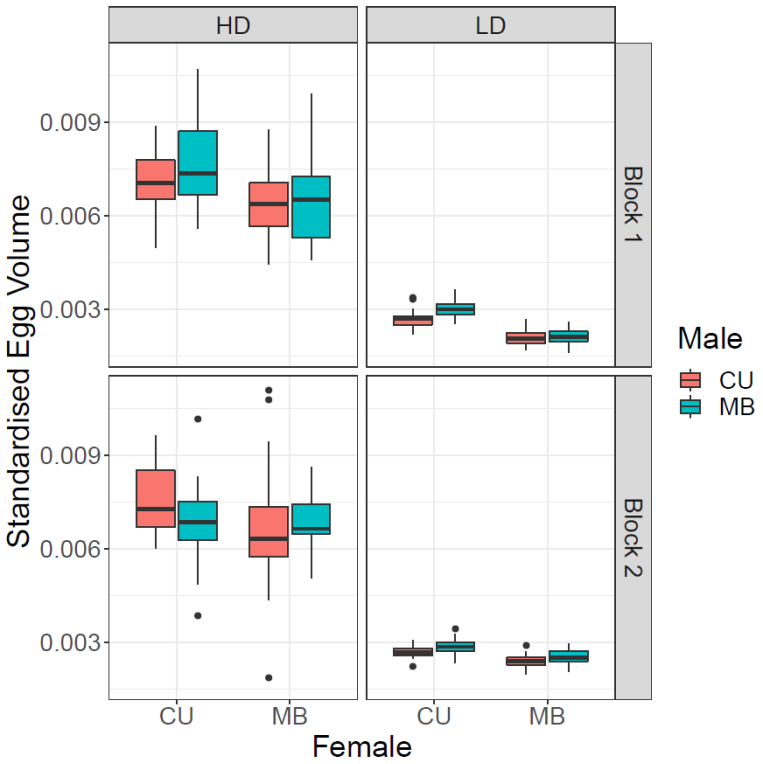

Figure 4.3: Graphical distribution of Standardized Egg Volume

Relative Egg Volume - Low Density					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Female	1	1.53E-05	1.53E-05	213.6444	**< 2.2E-16**
Male	1	1.51E-06	1.51E-06	21.1476	**7.19E-06**
Female:Male	1	3.81E-07	3.81E-07	5.3233	**0.02198**
Residuals	219	1.57E-05	7.15E-08		

Table 4.3 ANOVA table for relative egg volume in LD treatment

Female ID exhibits a strong significant effect ($p<0.000001$) on standardized egg volume with MCU females laying larger egg volume, when normalized with body size. Male ID also has a significant effect and it is evident from Figure 4.3 that MB males seem

to induce females to lay larger eggs. Consequentially, the interaction term between male and female ID is also significant.

Relative Egg Volume - High Density					
	Df	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Female	1	2.1E-05	2.15E-05	12.4927	**0.000503**
Male	1	0	2.80E-09	0.0016	0.967697
Female:Male	1	6E-07	6.04E-07	0.3518	0.553706
Residuals	210	3.61E-04	1.72E-06		

Table 4.4 ANOVA table for relative egg volume in HD treatment

The variances are much larger in HD treatment dataset due to variation in thorax length and also owing to the lengths being smaller in comparison to LD treatment flies. Female ID has a significant effect ($p<0.001$) in HD treatment as well. Here too, MCUs had showed a similar trend with MCU females laying larger (standardized) eggs. Male ID has no significant effect on (standardized) egg size in HD treatment flies.

4.4 Fecundity

Analysis was done using generalized mixed models and the errors were assumed to follow a Poisson distribution. The program assumes one variable from each treatment (in an alphabetic manner) and sets its default value to a zero and then compares the other variable against it. Here, MCU and HD treatments were given the default value.

Fecundity				
Fixed effect	**Estimate**	**Std. Error**	**z value**	**p value**
(Intercept)	3.2567	0.0262	124.17	**<2E-16**
FemaleMB Females	0.1131	0.0368	3.0770	**0.0021**
MaleMB Males	0.1259	0.0360	3.5010	**0.0005**
DensityLD	0.8573	0.0310	27.6530	**<2E-16**
FemaleMB Females:MaleMB Males	-0.0175	0.0507	-0.3450	0.7302
FemaleMB Females:DensityLD	0.0823	0.0435	1.8940	0.0583
MaleMB Males:DensityLD	0.0033	0.0432	0.0760	0.9391
FemaleMB Females:MaleMB Males:DensityLD	-0.0690	0.0600	-1.1490	0.2504

Table 4.5 Statistical table for analysis of Fecundity (Block as random factor)

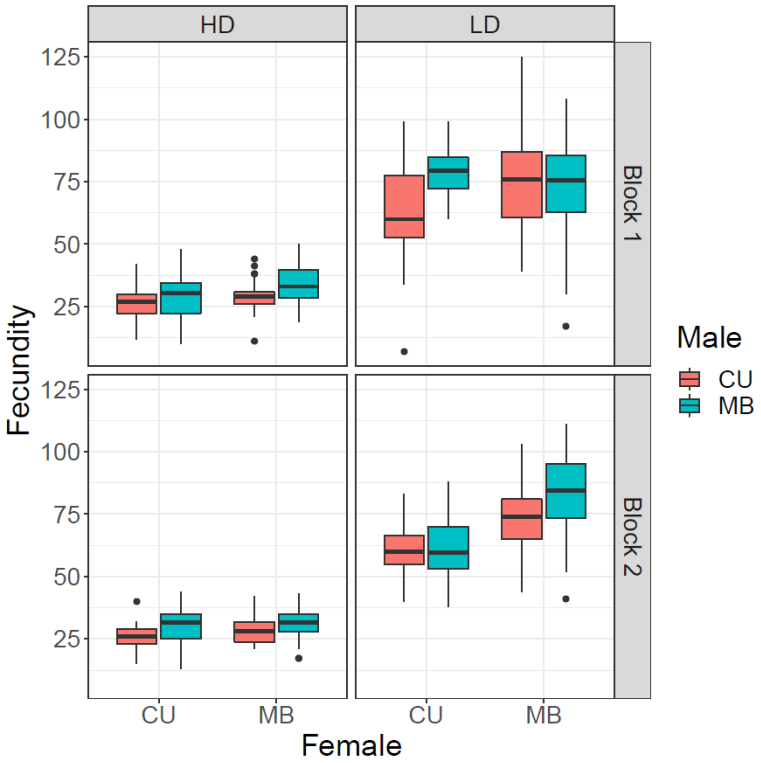

Figure 4.4 Graphical distribution of data on Fecundity across treatments

MB females have significantly higher fecundity compared to MCU females ($p<0.01$). MB males too have an effect on female fecundity ($p<0.001$) as the females to them show higher fecundity in both density treatments. Density strongly affects fecundity ($p<0.000001$) and as expected, LD treatment flies lay more number of eggs in comparison to their HD counterparts.

4.5 Fecundity vs. Egg Volume Correlation

Correlation tests were conducted individually for each density treatment in each block as the entire data did not fit into the linear model. We fit linear models and tested if the slopes were significantly different from zero by calculation a t-statistic. It was observed that only the cross of MB flies with itself, in high density treatment, exhibited a positive correlation between fecundity and egg size which, contrary to the expectation of a negative correlation.

Correlation between Fecundity and Egg Size						
Block 1						
			Estimate	**Std. Error**	**t value**	**p value**
Low Density	CU*CU	(Intercept)	3.74E-03	8.34E-05	44.874	**< 2E-16**
		EC	-2.31E-06	1.29E-06	-1.788	0.0855
	CU*MB	(Intercept)	4.30E-03	3.06E-04	14.05	**4.50E-13**
		EC	-5.50E-06	3.88E-06	-1.42	0.168
	MB*CU	(Intercept)	2.90E-03	1.79E-04	16.167	**9.54E-15**
		EC	1.09E-06	2.32E-06	0.471	0.642
	MB*MB	(Intercept)	3.05E-03	1.57E-04	19.412	**< 2E-16**
		EC	-5.93E-08	2.11E-06	-0.028	0.978
High Density	CU*CU	(Intercept)	3.24E-03	2.24E-04	14.471	**3.06E-14**
		EC	7.07E-06	8.28E-06	0.854	0.401
	CU*MB	(Intercept)	3.55E-03	1.52E-04	23.334	**< 2E-16**
		EC	5.64E-06	5.04E-06	1.118	0.274
	MB*CU	(Intercept)	3.29E-03	1.42E-04	23.12	**< 2E-16**
		EC	-6.05E-06	4.75E-06	-1.274	0.215
	MB*MB	(Intercept)	2.55E-03	2.81E-04	9.075	**3.16E-09**
		EC	2.10E-05	8.03E-06	2.61	**0.0153**
Block 2						
			Estimate	**Std. Error**	**t value**	**p value**
Low Density	CU*CU	(Intercept)	3.99E-03	2.38E-04	16.778	**4.07E-15**
		EC	-2.51E-06	3.88E-06	-0.647	0.524
	CU*MB	(Intercept)	3.74E-03	2.56E-04	14.63	**1.86E-13**
		EC	2.26E-06	4.11E-06	0.55	0.587
	MB*CU	(Intercept)	3.43E-03	1.73E-04	19.836	**< 2E-16**
		EC	-2.45E-06	2.30E-06	-1.066	0.296
	MB*MB	(Intercept)	3.13E-03	1.91E-04	16.381	**7.08E-16**
		EC	2.27E-06	2.26E-06	1.003	0.324
High Density	CU*CU	(Intercept)	3.21E-03	1.80E-04	17.865	**< 2E-16**
		EC	1.36E-05	6.85E-06	1.977	0.0583
	CU*MB	(Intercept)	3.45E-03	2.92E-04	11.831	**5.74E-12**
		EC	3.20E-07	9.52E-06	0.034	0.973
	MB*CU	(Intercept)	2.86E-03	4.99E-04	5.723	**9.34E-06**
		EC	8.20E-06	1.68E-05	0.487	0.631
	MB*MB	(Intercept)	3.18E-03	2.19E-04	14.517	**2.21E-13**
		EC	-5.32E-06	6.97E-06	-0.763	0.453

Table 4.6 Dataset of p-values corresponding to the respective correlation tests of various crosses

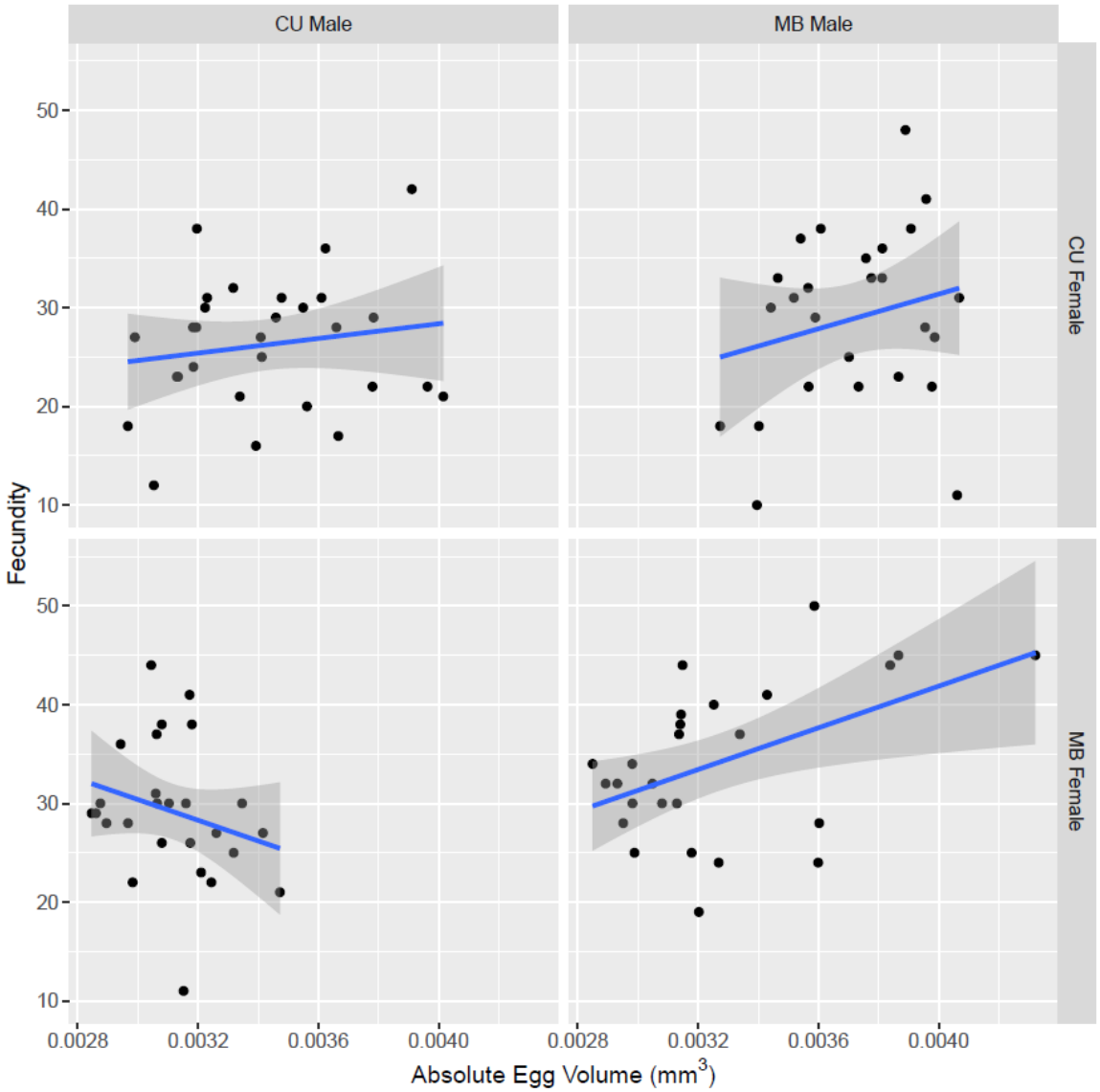

Figure 4.5 Correlation graphs of HD treatment crosses in Block 1

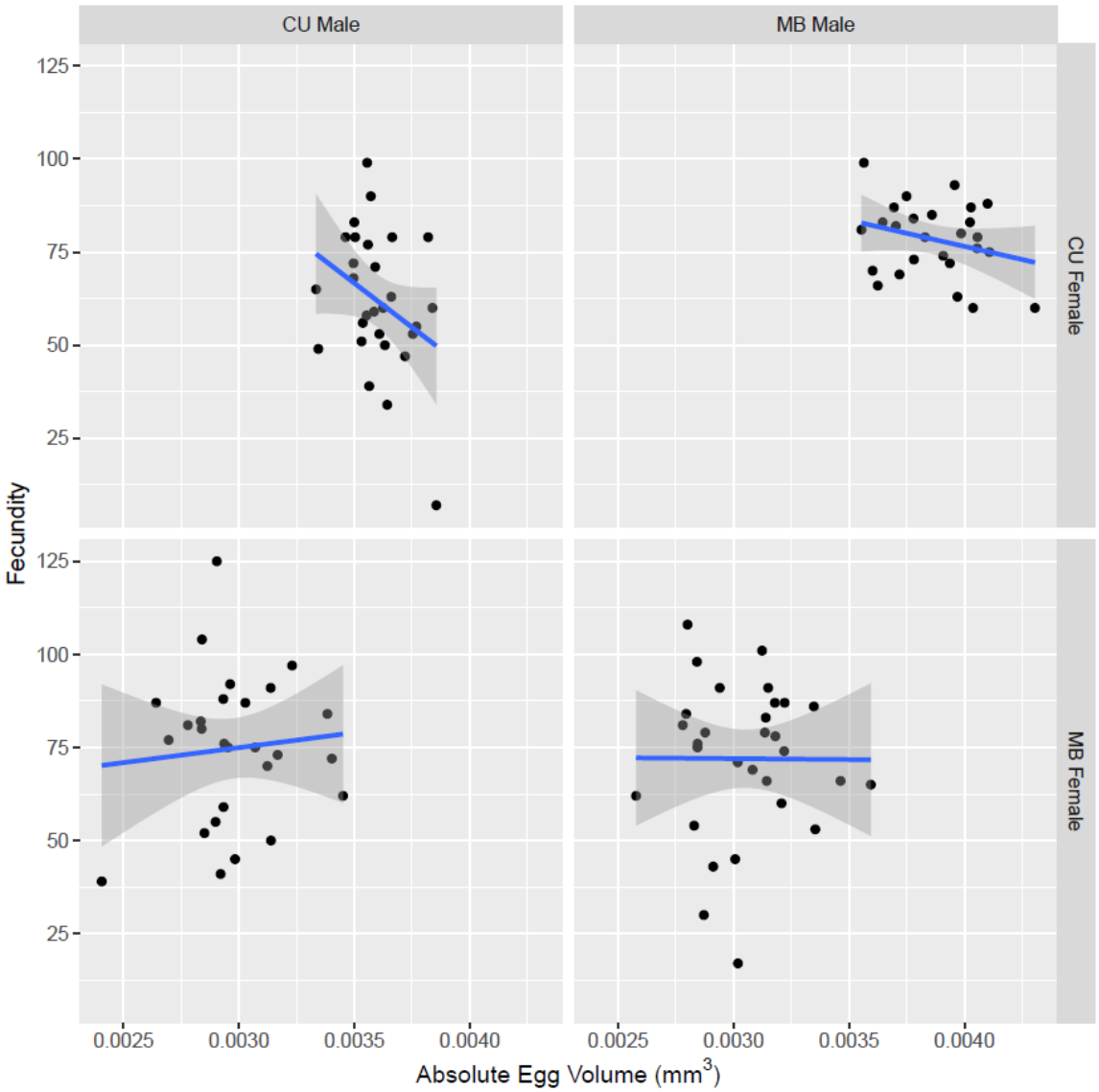

Figure 4.6 Correlation graphs of LD treatment crosses in Block 1

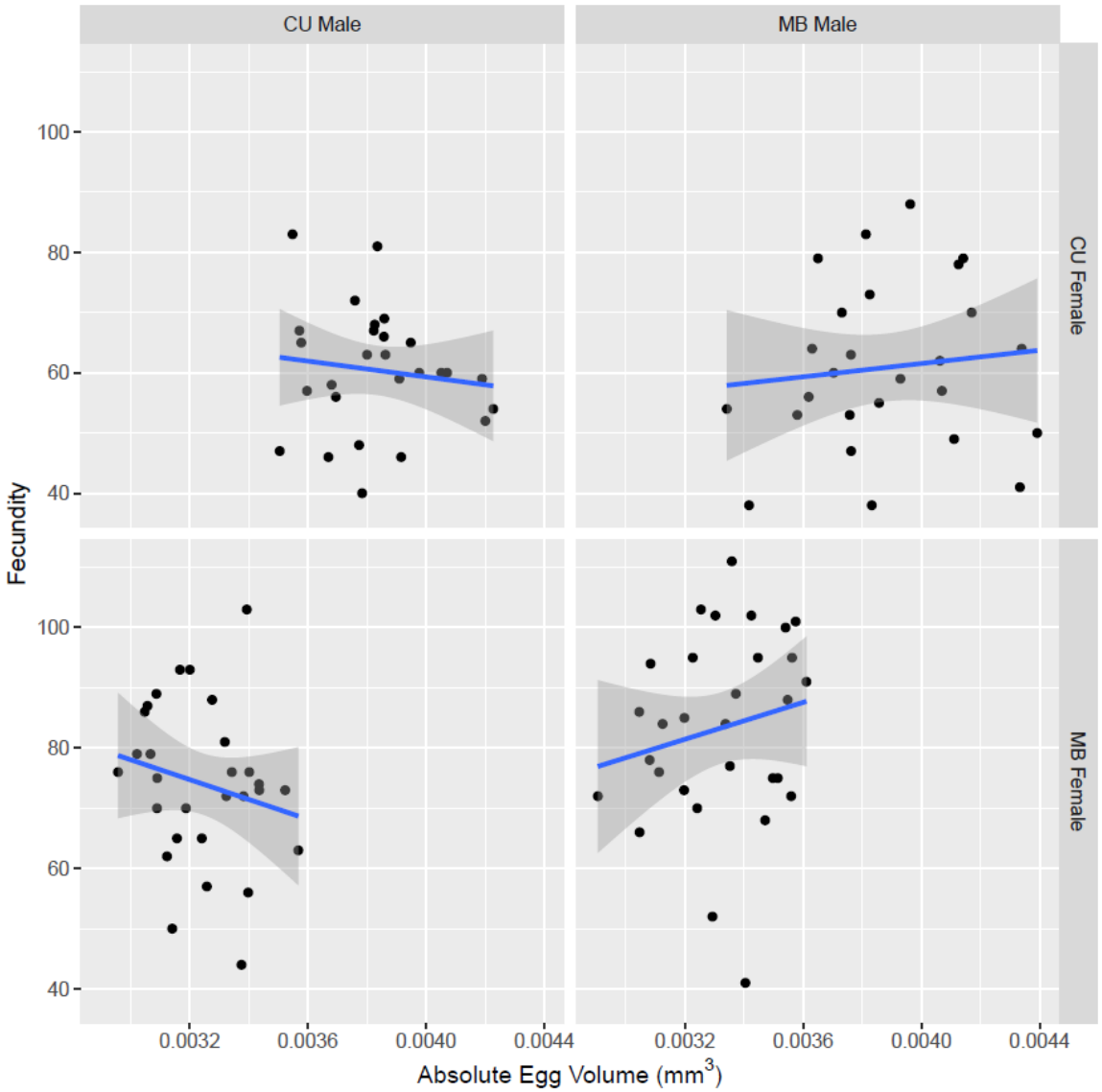

Figure 4.7 Correlation graphs of HD treatment crosses in Block 2

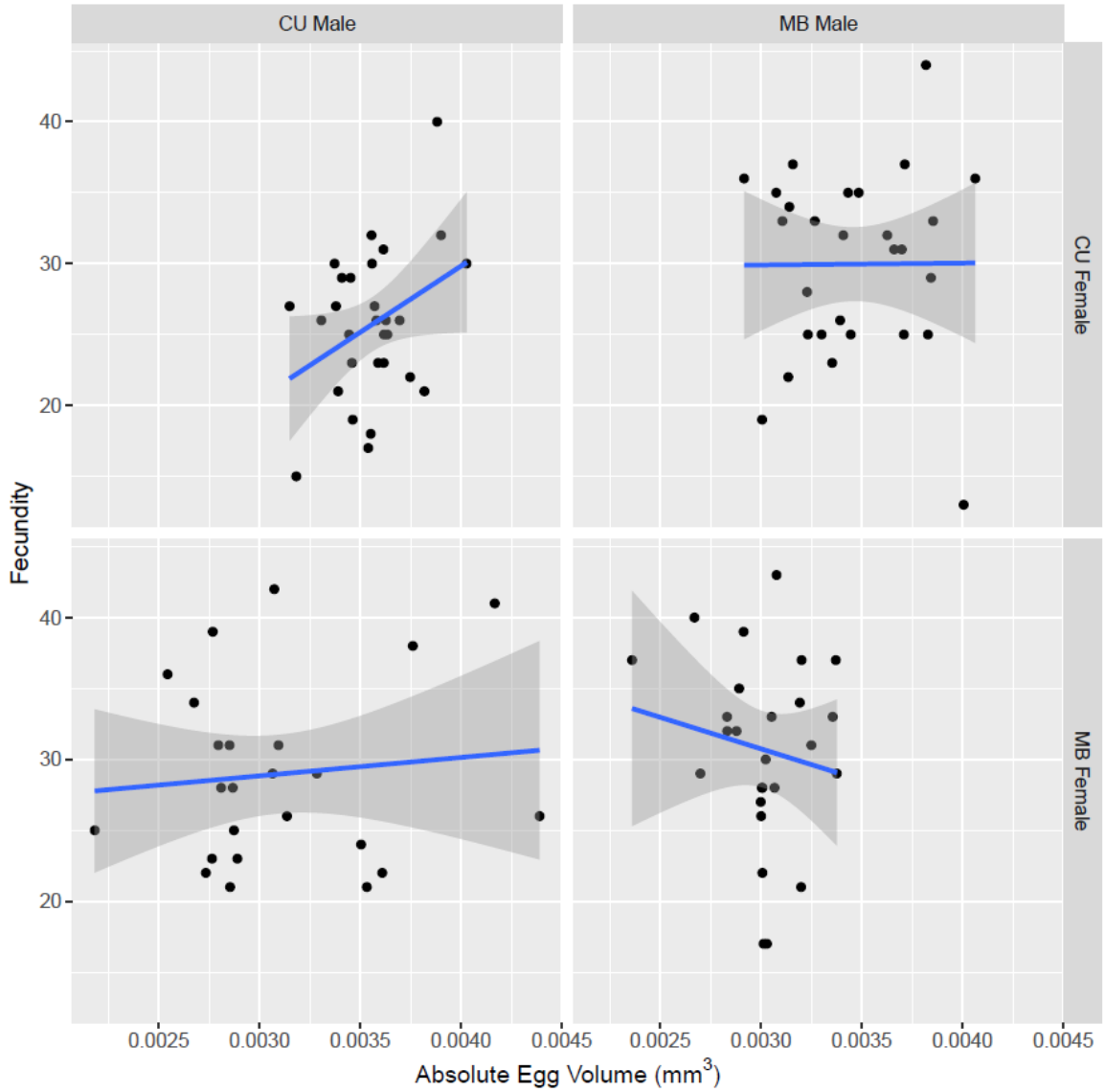

Figure 4.8 Correlation graphs of LD treatment crosses in Block 2

There is not much evidence to say that a correlation between fecundity and egg size for these individual crosses.

Chapter 5

Discussion

It has already been established that larval crowding, a form of density-dependent selection, is a potent source of selection, leading to alternative life histories due to trade-offs in performance across densities. Males and females exhibit different strategies trying to balance between maximizing their respective fitness at a personal expense of parental investment. In *Drosophila*, a highly promiscuous species, males would benefit from maximizing the number of mating and somehow inducing the females (physically or physiologically) to invest more in their progeny. On the other hand, females, having to spend much more resources towards their progeny, would benefit from assessing the males for quality before mating and trying to avoid too much mating.

We found out that MCUs, a population adapted to larval crowding, laid eggs with greater volume (both absolute and standardized), in both HD and LD conditions compared to its baseline population, MBs. However, MCU females were less fecund than their baseline population. Hence, based on such a negative correlation, there seems to exist a trade-off between egg investment and egg number in the population selected for larval crowding.

Males did not show any significant contribution to absolute egg volume. However, MB males did induce females (irrespective of their identity) to produce eggs with significantly greater standardized egg volume in LD treatment. This could possibly be attributed to the fact that standardized egg volume, as a unit of measurement, is greatly influenced by the thorax length, which was considerably large in LD treatment. Hence, causing a sharp reduction in variance in what otherwise seems to be an overlapping dataset. Male identity also had a significant effect on fecundity. MB males induced females to be more fecund compared to MCU males (or in other words, females mated with MCU females tend to be

less fecund). MCU males have evolved increased courtship frequency (Shenoi et al. 2016) and also increased the size of accessory glands (unpublished data from EBL, IISER Mohali). Components of male ejaculate in *Drosophila* can induce females to lay more eggs in the short-run (Chapman et al., 1995). However, continual exposure to courtships is costly to females and can be detrimental in the long run (Fowler & Partridge, 1989). This could possibly explain why, in this study, females mated to MCU males, over a period of ~10 days are less fecund than those mated to MB males.

Combined data of the two density treatments revealed a negative effect of nutritional restriction on the absolute egg size. Fecundity, likewise, was found to be lower for HD treatment. This observation is in line with other studies that suggest how poor larval nutritional environment can reduce the number of ovarioles, the egg-producing units (Hodin & Riddiford, 2003). Another peculiar result of density treatment was on body-size. Previously conducted studies in our lab had consistently reported that selection had a significant effect on body-size and that MCUs were smaller than MBs. On the other hand, this study did not find any significant effect of selection on body size with a p-value of 0.069. There were some outliers in the body-size distribution, and since only two blocks were analyzed, any concrete statement on change in the body-size cannot be made.

Correlations of egg volume vs. fecundity did not produce any significant trend. These correlations reflect a within generation trade-off, unlike an evolutionary trade-off which was observed in the MCU population, as discussed earlier.

In this study, it was found that females adapted to larval crowding lay larger eggs while laying fewer. A study can be done to determine how this larger egg volume confer benefits to the survival of the larvae in food-limiting toxic environments, for example by protecting its contents or by providing more space to pack reserve nutrients to provide an initial edge to the larvae. It was also observed that males adapted to larval crowding cause a reduction in their mates' fecundity. To this effect, it would be interesting to find out how these males affect the fecundity of their mates over different mating windows.

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