A study of female reproductive investment in populations of *Drosophila melanogaster* adapted to larval crowding

Lokesh Kumar

A dissertation submitted for the partial fulfillment of BS-MS dual degree in Science

Indian Institute of Science Education and Research Mohali
April 25, 2014

Certificate of Examination

This is to certify that the dissertation titled "A study of female reproductive investment in populations of *Drosophila melanogaster* adapted to larval crowding" submitted by Ms. Lokesh Kumar (Reg. No. MS09078) for the partial fulfillment 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. Rachna Chaba Dr. N. G. Prasad
(Supervisor)
Dated: April 25, 2014

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.

Lokesh Kumar
Dated: April 25, 2012

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)

Acknowledgement

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I would love to thanks my parents for all their care and faith in me, which makes this whole thing possible. Again, a special thanks to Vinesh bhaiya for their extraordinary care and support.

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Notation

1. MCU – **M**elanogaster **C**rowded as larvae **U**ncrowded as adults
2. MB – **M**elanogaster **B**aseline
3. L-H – **L**ow density **H**igh food
4. H-H – **H**igh density **H**igh food
5. H-L – **H**igh density **L**ow food
6. ANOVA – **An**alysis **O**f **Va**riance

Contents

Abstract

Maternal nutritional status at larval stage or as adults affects their offspring's fitness. It was known that the male genotype affects his mate's reproductive investment. Two hypotheses are there for maternal investment with respect to their mates. (A) Females assess male's ability at the time of courtship and copulation and invest accordingly in their offspring. (B) Males manipulate females to invest more in offspring just after mating. In the light of these previous studies, I tried to focus on the maternal effects in populations of *Drosophila melanogaster* adapted to larval crowding. I have found that selected populations, MCUs despite of their smaller body-size laid significantly larger eggs when compared to their ancestral control line, the MBs. I also found that there exists phenotypic plasticity in terms of body-size with respect to selection as well as larval density.

CHAPTER-1

Introduction

Organisms differ remarkably in how they develop, the time they take to grow and become mature, the number and size of offspring they produce, and how long they live. Together, these factors between birth and death of an organism make up its life history. Theories of life-history evolution try to explain how evolutionary forces and constraints shape the life-history traits of organisms to optimize their survival and reproduction in the face of ecological challenges posed by the environment (Stearns 1992, Roff 1992, Stearns 2000).

The fitness of an organism would be maximal if survival and reproduction would be maximal at all ages or stages of an organism. In principle then, the basic problem of life history evolution is: all life history traits should always evolve so as to maximize survival and reproduction and thus fitness (Houle 2001). This would very rapidly lead to the evolution of *Darwinian demons* (Law 1979) that would take over the world, i.e. organisms start to reproduce as soon as they are born, produce an infinite number of offspring, and live forever. However such organisms do not exist in the real world. The reason for this is that life history traits cannot evolve independent of each other (so that natural selection can independently select for all traits to their optimal level) because they are at some level connected to a broader physiological and/or genetic network and have to draw resources from a common resource pool. Resources (abundance and/or acquisition), however, are limited, which leads to trade off in the evolution of these traits (Prasad and Joshi, 2003).

A simple model of such trade-off is the Y model (Noordwijk et al 1986). Consider two traits competing for resource from a common finite pool (Figure 1.1). It is then obvious that if allocation in one is increased, it has to be decreased in the other. Now if such a negative correlation is present between traits (phenotypes), it is plausible that the correlation has some genetic basis. Therefore if increase (therefore higher allocation) in one trait is selected for, the other trait will suffer. A classic example of this is the trade-off between early and late life fecundity seen in *Drosophila melanogaster* (Rose 1984). Flies selected for postponed

senescence evolved higher late life fecundity but suffered lower early life fecundity, showing evolutionary trade-off between these two traits.

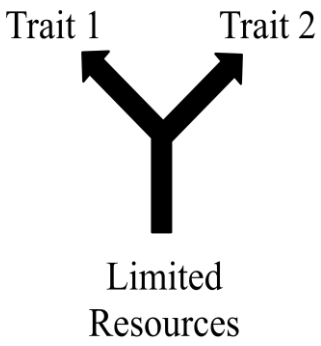

Figure 1.1 Y-model for trade-off.

Female investment in progeny is an interesting area to study because since it is an important contributing factor in the offspring of its progeny and therefore has implications on the female's own fitness. A female's fitness can increase by investing more in each of their offspring or by producing more number of offspring. Since both these traits (progeny production and investment per progeny) depend on the same resource pool, they can potentially show trade-off. Besides, the amount of resources a female can invest in its offspring can vary depending on a number of factors, such as female condition (e.g. age or nutritional status), life history, environmental variation etc. For example, it has been shown that in *Drosophila* maternal interaction with mates can influence their investment in offspring.

In a study done by Pischedda et al (2010), it has been shown that Male genotype influences the amount of resources their mates invest in reproduction. Two hypotheses for this observation are that female assess male quality during courtship and copulation and alter investment in offspring accordingly, or that males manipulate female to invest heavily in offspring produced soon after mating Maternal nutritional status can also alter the reproductive investment in offspring. Parental larval diet can influence their investment in egg size and offspring traits on *Drosophila* (Vijendravarma et al 2009). According to this study, if mother's nutritional status predicts the nutritional environment of offspring, it would be adaptive for mothers experiencing nutritional stress to prime their offspring fitness for a

better tolerance to poor environment. They report that parents raised on poor larval food (that contains no protein supplement in form of dietary Yeast) laid 3-6% heavier eggs than parents raised on standard food.

In another study, Prasad et al (2003), have shown that mean weight per egg of eggs laid by mothers raised on poor food was about 28% greater than that of eggs laid by mother raised on rich food (Contain double the amount of protein supplement in form of dietary Yeast than standard food). They have also shown that there is an interaction between maternal and larval nutritional level on larval, and therefore, egg to adult survivorship. Larva whose mothers were reared on poor food and who themselves were reared on rich food had higher survivorship than cases where both the focal larva and mothers were reared on either poor food or rich food or where the larva was reared on poor food but mothers were reared in rich food. These studies demonstrate that if mothers face stress during their development, they invest more in each egg showing plasticity in maternal investment. However, how maternal investment evolves in a population subjected to developmental stress and how it is related to other related traits like fecundity remains poorly understood.

The present study tries to address this issue. Larval density during development is a potent force of developmental stress (Mueller et al 1988) We subjected laboratory populations of *Drosophila melanogaster* to high larval density in low amount of food (600 eggs/1.5 ml food compared to baseline, 60-80eggs/6ml food) and selected for adaptation to larval crowding. Adaptation to larval crowding has resulted in the evolution of multiple adult traits (longevity, mating behavior, etc) in these populations (Shenoi et al Unpublished data). Previous studies (Prasad et al 2003, Vijendravarma et al. 2009) have shown that larval stress in a single generation can affect maternal investment in eggs. Therefore we asked:

a. Can such investment evolve when flies are selected for adaptation to larval stress?
b. How does the plasticity in investment change if the selected flies are reared in across different rearing environment (larval density and amount food) or adult environment (presence/absence of yeast as egg-laying stimulus)?

CHAPTER-2

General Experimental Methods

2.1 Fly populations used:

We used two sets of populations of *Drosophila melanogaster* for this study one selected for adaptation to larval crowding, MCU (**M**elanogaster **C**rowded as larvae **U**ncrowded as adults) and its baseline MB (**M**elanogaster **B**aseline). Both populations are maintained as four independent replicate populations (Blocks) in a 21-day discrete generation cycle at standard laboratory conditions i.e. 25℃, 95% relative humidity, standard corn-meal charcoal food; in 24 hour light. For each of the replicate in MB's, the adult census size is ~ 2500 individuals and in the MCU's it is ~1900 individuals. The four MB populations were originally derived from long-term laboratory population of *D. melanogaster* called JB populations (Sheeba et al 1998). In the year 2006, the four JB population were mixed together to form a single population called MB (Melanogaster Baseline). After ten generations the single MB population was split into four replicate populations called MB 1-4 and since then are independently maintained under standard laboratory conditions.

The maintenance of MB population is done in the following way: Eggs collected from 21 days old (post egg collection in the previous generation) females are transferred into glass vials (25mm diameter × 90mm height) containing corn meal-charcoal food(6-8 ml) at a density of 60-80 eggs per vial. Forty such vials are collected for each independent replicate and are incubated under standard laboratory conditions (as mentioned above). Day-10 from egg collection is the peak eclosion day and by day-12 almost all the adult flies have eclosed. On the same day these flies are transferred into a Plexiglas cage (24 x 19 x 14 cm) containing a Petri-plate of corn meal-charcoal food and wet cotton to maintain high relative humid environment. After flies are transferred into cages fresh food plate is provided every alternate day. On day-18 post egg-collection fresh food plates are given to the flies with live yeast paste. Two days later, on day-20 fresh food cut-plates are provided to the flies and are allowed to oviposit for next 18 hours. On the next egg collection is done and the next generation is started after maintaining the MBs for 15 generations, MCUs were derived from

them by selecting for larval crowding. The MCU populations are also maintained under the same standard laboratory conditions in which MB populations are maintained but for MCU, egg collection is done in vials containing only 1.5ml of corn meal-charcoal food at a density of 800 eggs per vial. Twenty five such vials are collected per population. From 2011 these populations are maintained in our lab and these sets of experiments are performed when these populations have undergone selection for more than 130 generations.

MCU-1 was derived from MB-1 and so on other replicates and are independently maintained as separate populations. MCUs connected to MBs by the same replicate numbers are the direct descendants and hence they were treated as statistical blocks in analyses.

2.2 Standardization of Experimental flies:

We relax the selection pressure for one generation in MCU and maintain them in the baseline condition. We call this process standardization and the flies thus generated are standardized flies. For standardization, eggs are collected at a density of 60-80 eggs /vial in 6-8 ml of corn meal-charcoal food for both the populations and are maintained under the same standard laboratory conditions. This is done to get rid of non-genetic maternal effect.

2.3 Egg Collection for Experiment:

Egg collection for the experimental flies is done from standardized flies. Agar plates are prepared by dissolving 1.2mg of bacteriological agar in 100ml of water in a beaker. This solution when solidified in petri-plates is used for egg collection.

Since MCUs are selected for adaptation to larval crowding, it is observed that their eclosion pattern (period) is more spread out (3-4 days) as compared to that of MB's. In MCUs it was observed that flies start enclosing from day-8 post egg collection and eclosion ends around day-18 post egg collection. Therefore, in these experiments, in order to synchronize the peak eclosion for different density treatments, egg collection is spread over 3-4 days according to experiments.

2.4 Imaging of egg for volume Measurement:

Eggs from different treatments are taken on agar Petri-plate and arranged in particular under microscope (ZEISS, 10X magnification) as shown in the figure.

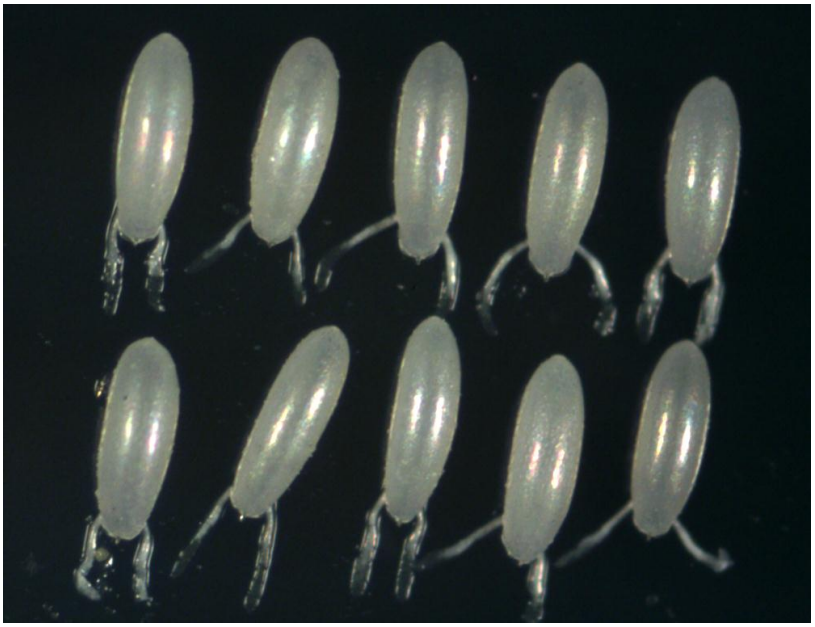

Fig.2.1. image of eggs arranged in particular fashion for volume measurement

10 eggs arranged together on their "backs" (the flattened side of the egg) under microscope which is further connected to a camera (ZEISS, AxioCam ICc 1). All the imaging is done at 40X (eye piece 10X, objective 1X) optical zoom.

2.5 Egg Volume Measurement:

To quantify maternal reproductive investment, egg volume measurement is done for both the experiments.

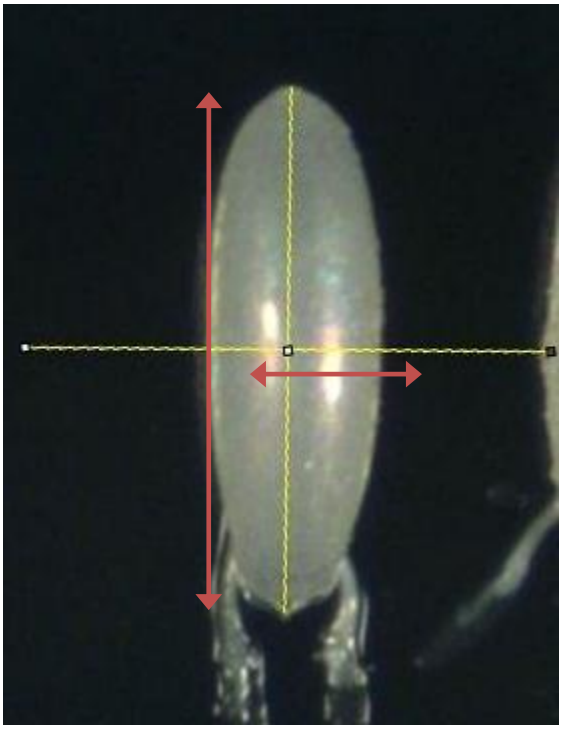

Figure 2.2 Showing Polar axis and equatorial diameter for egg volume measurement.

Measurement of egg volume is done by considering the shape of *Drosophila egg* as prolate spheroid (Pischedda et al 2010). Formula used for the measurement of volume of the egg is

$$V = \frac{4}{3}\pi a b^2$$

Where, a = semi-major axis

b = semi-minor axis

but measuring semi-major axis and semi-minor axis from these images of eggs is not that much accurate, so for the ease of experiment and for more accuracy, measurement is done for *Polar axis* and *Equatorial Diameter*. New formula for the volume measurement of egg using these new factors, which is used in the experiment is

$$V = \frac{1}{6}\pi AB^2$$

Where, A = polar axis

B = Equatorial axis

There are total 816 such images from both the experiment, containing 8160 eggs which are measured thrice at different time points. All measurements were done using Image J version 1.47v.

2.5 Body-size Measurement:

Egg volume measurement was done to quantify maternal investment. In order to normalize the data for egg volume, body-size measurement was done. Here in this experiment, length of thorax was measured as this can be used as a proxy for body-size. We sort 40 female *drosophila* from each treatment and freeze them at -20℃. For thorax length measurement, their wings and limbs were removed to arrange them properly for imaging.

Figure 2.3 Thorax length measurement was done as a proxy for body-size.

2.6 Data Analysis:

Data for both the experiments for egg volume and body size are analyzed by using Multi-variable mixed model analysis of variance (ANOVA) with different treatments as fixed variable and block as random factor. Multiple comparisons were implemented using Tukey's Honesty Significance Difference (HSD) wherever required.

CHAPTER-3

Experimental Set-up and Procedures

3.1 Experiment 1: Effect *of crowding at larval stage on maternal reproductive investment*.

3.1. a. Experimental set-up:

There are three density treatments for both the population in same amount of food (5 mL corn-meal charcoal food). These density treatments are:

(1) 60 eggs/vial, which is native condition for MB

(2) 600 eggs/vial, which native condition for MCU density wise

(3) 300 eggs/vial, which is an intermediate density

All these density treatments are reared the in same amount of standard corn-meal charcoal food (~5 mL)

Imaging of *Drosophila* eggs for volume measurement is done at two different ages. We include two ages for analysis to check is there any effect of aging on maternal investment. Age-II is day-13 post-eclosion, on which egg collection for the next generation is done under its standard maintenance protocol regime. Age-I is day-4 post-eclosion, So we thought it would be interesting to check maternal investment at this time point since they are young adults.

To get rid of non-genetic parental effect, eggs collection for the experiment was done from the set of standard flies. Egg collection is done in a period of 3 days to synchronize the pick of eclosion. On day-1 egg collection is done for MB's for 600 density in each vial with a survivorship rate of ~20-25 % and 8 such vial are collected. On day-2 egg collection is done for MCU's for 600 density in each vial with a survival rate of ~30 % and 6 such vials are collected. On day-3, egg collection is done for the remaining two densities for both the

populations. For 300 density with survivorship ~35-40% each vial and 8 such vials are collected and for 60 density, 15 vials are collected for each population.

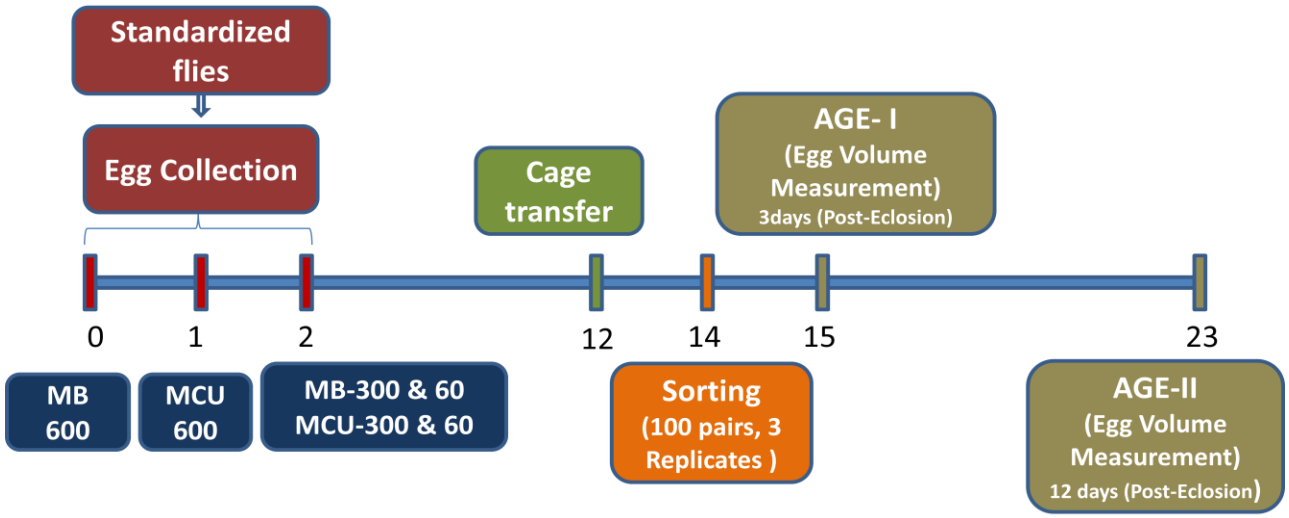

Figure 3.1. Schematic representation of the experimental set-up for experiment-1

From day-10, flies start eclosing. They are dumped into cages at a regular interval of 6 hrs for next 3 more days. Food plates were also provided to them on each alternate day. On day-14, 100 mating pairs were sorted from each treatment for experiment which are maintained in smaller cages and three such replicates were there for each treatment. On day-15, which is Age-I of the experiment. So fresh food plate were provided to collect egg for 6 hr window for volume measurement. For next 8 days, fresh food plates were provided on every alternate day. On day-23, which Age-II of the experiment. So again fresh food were provided to collect egg for volume measurement.

After the imaging part for both the ages, 40 female flies were sorted from each treatment and freezed at -20℃ for body size measurement. There for four blocks (replicates) of both the populations.

3.1. b. Egg Volume measurement:

Egg volume measurement is done in order to quantify maternal reproductive investment. After imaging eggs for both the ages, measurement is done using Image J version 1.47v. Polar axis and equatorial diameter were measured thrice independently to average out the volume for each egg . There are total 108 such images (10 eggs/image) per block including both the ages. So there were total 4320 (1080×4) such eggs which are measured thrice. Data was analyzed by doing multi-variable mixed model analysis of variance (ANOVA) with age, selection regime and density as fixed-factor and block as random factor.

3.1. c. Body-size measurement:

For body-size measurement, we use thorax length as a proxy for body size. It was proved that thorax length can be used as a proxy for body-size. We sort 40 female *drosophila* from each treatment and freeze them at -20℃. For thorax length measurement, their wings and limbs were removed to arrange them properly for imaging. 30 flies were imaged from all the three density treatment and for both the populations per block. So total 720 (30×3×2×4) females were imaged for thorax length measurement. Thorax length were obtained by averaging three independent measurements. Data was analyzed by doing Two-factor mixed model analysis of variance (ANOVA) with thorax length and selection regime as fixed factor and block as random factor.

3.2 Experiment 2*: Effect of larval density and food on maternal reproductive investment and also of yeast as adult food on maternal investment.*

3.2. a. Experimental set-up:

In this experiment we exclude the intermediate density treatment i.e. 300 eggs/vial as it is observed from the previous experiment that there is no significant effect of density treatment on maternal investment. Here three more treatment are introduced which are combinations of larval density and larval food quantity. Three treatments are:

(1). **L-H** : treatment includes low density of 60 eggs/vial in 5 mL of standard food. MB's are maintained in this condition in laboratory under their standard maintenance protocol.

(2) **H-H** : treatment includes high density of 600 eggs/vial in 5 mL of standard food.

(3) **H-L** : treatment includes high density of 600 eggs/vial in only 1.5 mL of food. MCU's are maintained in this condition in the laboratory under their standard maintenance protocol.

One more factor introduced here was the yeast. Yeast is like a protein supplement for the *Drosophila.* Fresh food plates were provided with *ad libitum* live yeast paste for 48 hr window before the egg collection for egg volume measurement. It was studied that there is an exponential increase in the number of eggs laid by female provided yeast paste with their food.

Egg collection was done from set of standard flies in a period of 4 days, to synchronize the pick of eclosion. On day-0, eggs collection was done from MB's for the H-L treatment. Egg collection for the H-H treatment from MB's was done on day-1. Egg collection for both the treatment H-L and H-H from MCU's was done on day-2. Day-3 was the day for egg collection from both the populations MB's and MCU's for L-H treatment. Eclosion of adult flies start from day-10. Adult flies were transferred into cages regularly in an interval of 6hr till day-14 when eclosion ends. Sorting was done on day-15 for 100 mating pairs from all the treatments, two such replicates were sorted for experimental purpose. One replicate was provided yeast with fresh food-plate and another replicate without yeast for 48hr window. Egg collection for imaging purpose for egg volume measurement was done on day-17(Age-

I). For next 8 days fresh food plates were provided on every alternate day. Again on day-22 one replicate of all the treatments were provided yeast with food-plate and another without yeast. On Day-24 (Age-II), fresh food-plate were provided (6hr window) for egg collection for imaging for egg volume measurement.

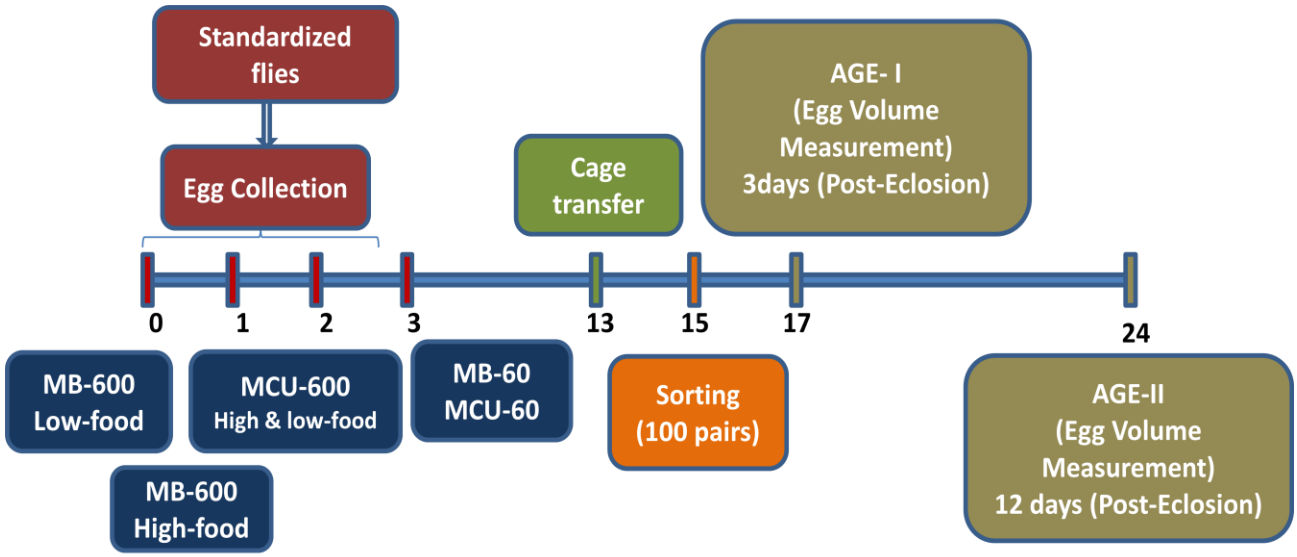

Figure 3.2. Schematic representation of experimental set-up for experiment-2

3.2. b. Egg volume measurement:

Using Image J version 1.47v, images were analyzed for polar-axis and equatorial-diameter measurement. Including all the treatment and both the ages there were 96 images (10 eggs/ image) from a single block. There were total 960 eggs from each block and there were four such blocks. So there were total 3840 (960×4) such eggs which were measured, thrice, at different times so as to counter for experimenter bias.

CHAPTER-4

Results

4.1 Experiment 1: Effect *of crowding at larval stage on maternal reproductive investment*.

4.1. A. Effect of larval density treatments on egg volume

Values were obtained after performing multi-variable mixed model analysis of variance (ANOVA) using selection, age and density as fixed variables and block as a random factor.

Table 4.1 Data-table obtained by multi-variable mixed model analysis of variance (ANOVA)

Source	SS	MS Num	DF Num	F ratio	prob > F
Selection Regime	0.0009565	0.0009565	1	37.8235778	**0.00864881***
Block	3.67E-05	1.22E-05	3	0.16053278	0.91636176
Density	5.70E-06	2.85E-06	2	0.5550895	0.60091307
Age	2.73E-08	2.73E-08	1	0.0003879	0.98552315
Selection Regime × Block	0.00007587	0.00002529	3	1.80650092	0.30369863
Selection Regime × Density	1.50E-05	7.49E-06	2	0.6050974	0.57625209
Selection Regime × Age	6.33E-06	6.33E-06	1	0.74564093	0.45137122
Block × Density	3.08E-05	5.13E-06	6	0.31907726	0.90149637
Block × Age	0.00021116	7.04E-05	3	5.76930407	0.09421277
Density × Age	4.12E-06	2.06E-06	2	0.19492377	0.82790829
Selection Regime × Block × Density	0.00007427	1.24E-05	6	1.80288237	0.2457826
Selection Regime × Block ×Age	2.55E-05	8.49E-06	3	1.23618026	0.37598267
Selection Regime ×Density × Age	1.12E-05	5.61E-06	2	0.81728841	0.48539814
Block × Density × Age	6.35E-05	1.06E-05	6	1.54080534	0.30639991
Selection Regime × Block × Density ×Age	4.12E-05	6.87E-06	6	7.84627371	2.02E-08

Three density treatments at a) 60, b) 300 and c) 600 eggs/vial kept in standard corn-meal charcoal food (5 ml) were used for analysis. Results revealed that there is no significant effect of crowding at larval stage on egg volume.

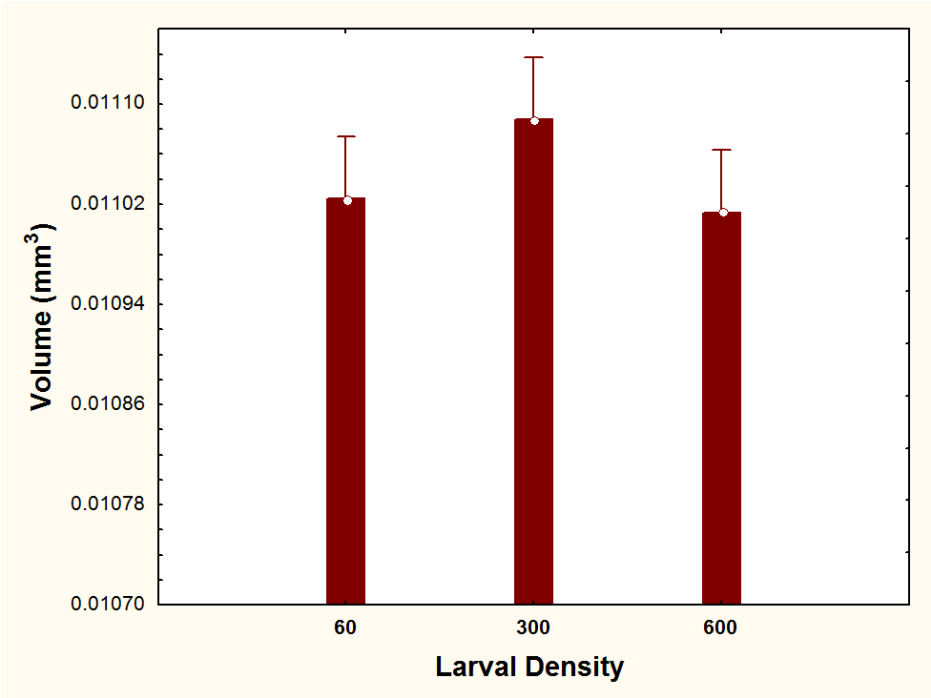

Figure 4.1 graph between larval density and egg volume (mm^3)

When all the three treatments were included in the analysis, MCUs were found to be laying eggs of larger volume compared to their ancestral control population, MBs. This result seemed consistent with the idea that MCU being a population exposed to larval crowding for many generations, were laying eggs with higher volume, thereby packing higher amount of resources in each egg ensuring that the offspring gets adequate resources when exposed to an environment where there is intense competition for resources .

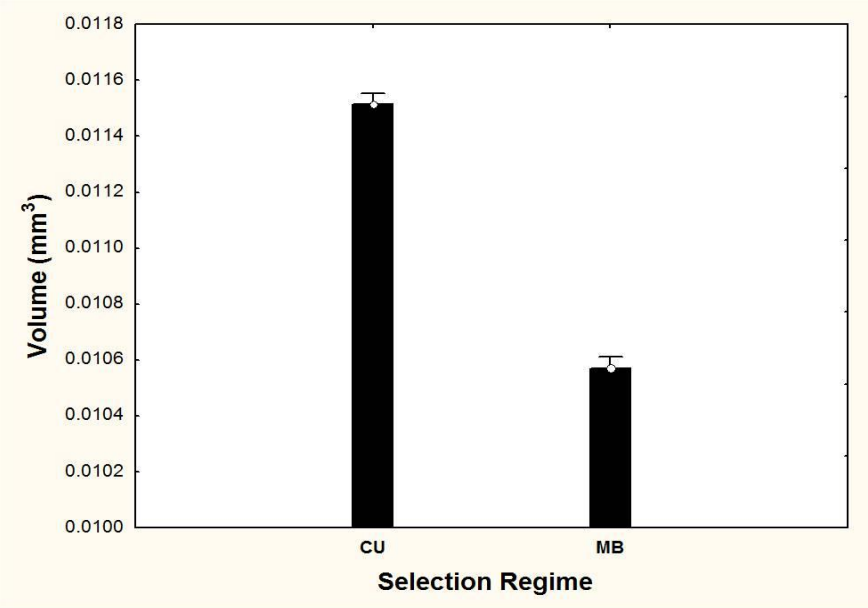

Figure 4.2 MCUs producing larger eggs as compared to their ancestral control MBs.

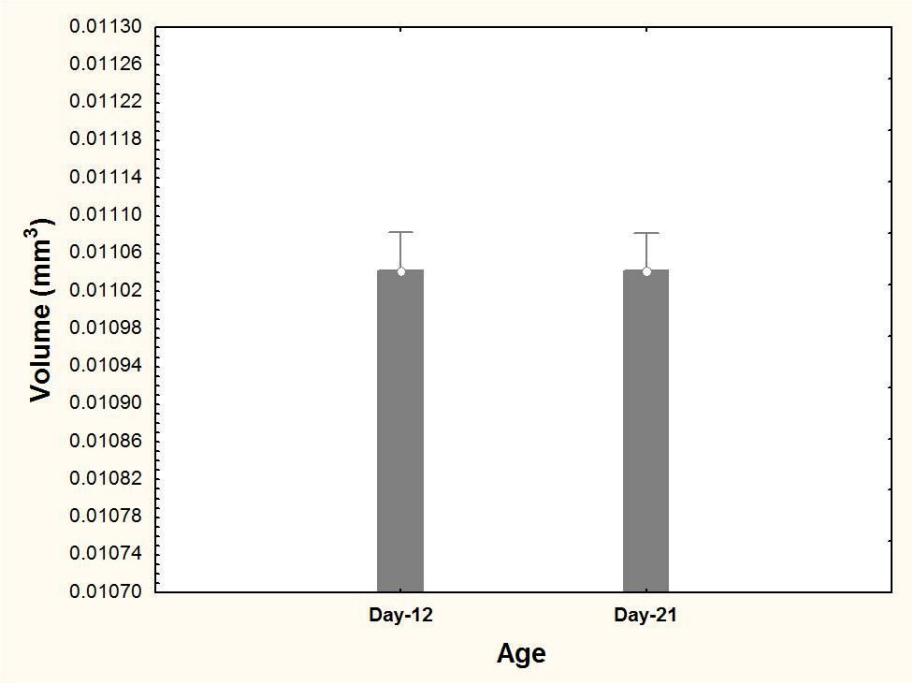

Figure 4.3 Graph showing comparison in egg volume between two different ages

In order to examine the effect of aging on investment, maternal investment was measured at two different time points, Age-I and Age-II. Maternal investment was quantified as the

volume of eggs laid. Age-I denotes the stage when the flies are young adults, whereas Age-II is the period when the flies tend to lay a large number of eggs, hence egg collection for the next generation is done at this stage. Analysis for the two different age classes showed that investment at two different ages was not significantly different (figure 4.3) indicating that ageing has very little effect on maternal investment.

4.1. B Effect of larval density on body-size

Values were obtained from multi-variable mixed model analysis of variance (ANOVA) using selection, larval density as fixed variables and block as a random factor.

Table 4.2 Data-table for thorax length obtained from JMP.

Effect	Degree of Freedom	MS	Den. Syn. Error df	Den. Syn. Error MS	F	p
Selection	1	0.016	2	0.001	26.94	**.035***
Block	2	0.107	3.6	0.016	6.92	0.058
Density	2	0.721	4	0.016	44.31	**.002***
Selection × Block	2	0.001	4	0.001	0.44	0.673
Selection × Density	2	0.013	4	0.001	9.2	**.032***
Block × Density	4	0.016	4	0.001	11.96	.017*
Selection × Block × Density	4	0.001	522	0.001	0.99	0.415

Maternal investment was quantified through egg volume measurement. To normalize the data for egg volume, body-size measurements were also done. Females from all the three larval density treatments were taken and their thorax length was measured as an indicator of body size. Graph (Figure 4.4) showing effect of selection regime on body-size (quantified through thorax length).MCUs are significantly smaller in body-size than MBs with a p value of 0.035. The graph includes females from all three treatments.

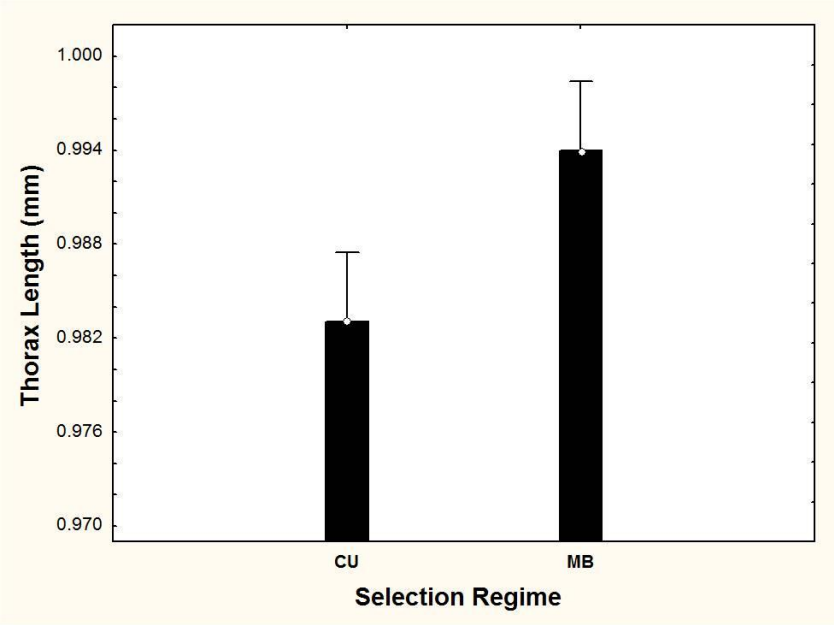

Figure 4.4 Graph between selection regime and thorax length.

Graph (fig: 4.5) showing body-size across different density treatment had significant p value (p=0.002). Body-sizes of females from all three density treatment were significantly different from each other. Females from treatment A had the highest body size when compared to females from treatments C and D, females from treatment C had a higher body size when compared to D, but a lower value with respect to A. All the differences in values across the three treatments were found to be significant.

Analysis revealed that females from higher larval density treatment have smaller body-size than females from low larval density treatment across both the populations.

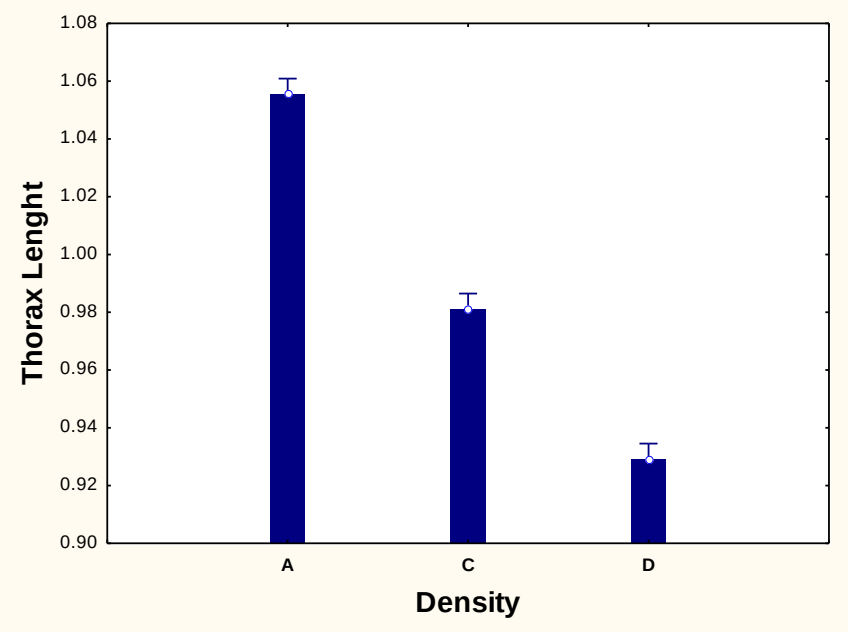

Figure 4.5 Graph for thorax length and larval density treatments.

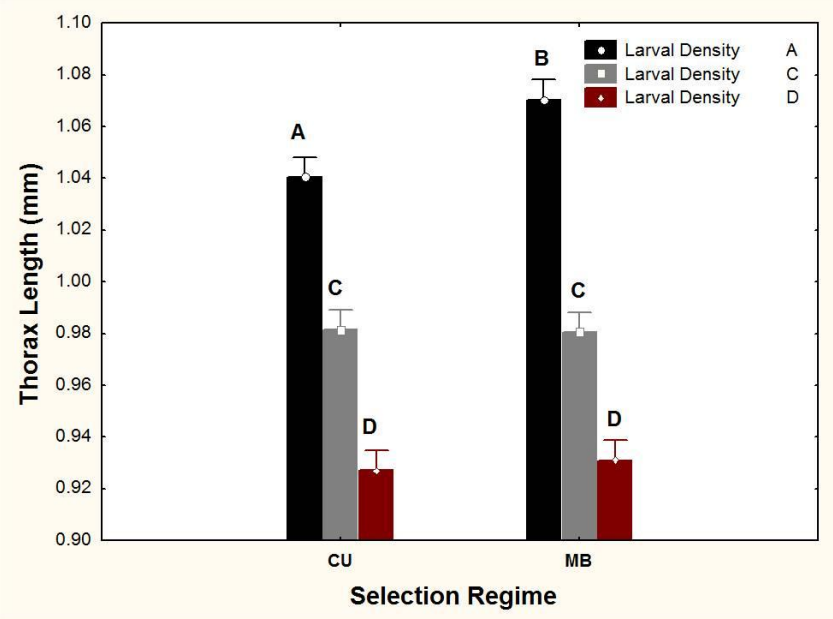

Figure 4.6 Three way interaction graph for thorax length on y-axis, selection regime and density treatments on x-axis. Levels not connected by same letter are significantly different

This graph (fig: 4.6) is an extended combination of graphs 4.5 and 4.6. This figure shows that MCUs were significantly smaller than MBs only in density treatment A (60 eggs/vial). In rest of the treatments MCUs were not significantly different than MBs.

4.2 Experiment 2: *Effect of larval density and food on maternal reproductive investment and of yeast as adult food on maternal investment.*

Table 4.3 data-table obtained by multi-variable mixed model analysis of variance (ANOVA)

Source	SS	MS Num	DF Num	F ratio	prob > F
Selection Regime	0.00036747	0.00036747	1	25.0197381	**0.03772143***
Block	6.29E-06	3.14E-06	2	0.195285	0.83523482
Treatment	1.18E-06	5.88E-07	2	0.15998521	0.85735056
Yeast	1.29E-05	1.29E-05	1	8.51388124	**0.0242485***
Selection × Block	2.94E-05	1.47E-05	2	4.39069957	0.10418832
Selection × Treatment	5.57E-06	2.79E-06	2	1.01713499	0.43941058
Selection × Yeast	5.78E-07	5.78E-07	1	0.41747433	0.58443979
Block × Treatment	1.47E-05	3.68E-06	4	1.53091124	0.38784759
Block × Yeast	3.04E-06	1.52E-06	2	1.44980061	0.51479706
Treatment × Yeast	1.16E-07	5.79E-08	2	0.13101146	0.88082255
Selection × Block × Treatment	1.10E-05	2.74E-06	4	3.51793936	0.12528652
Selection × Block × Yeast	2.77E-06	1.38E-06	2	1.77830064	**0.04419925***
Selection × Treatment × Yeast	1.02E-05	5.09E-06	2	6.54296252	0.05480788
Block × Treatment × Yeast	1.77E-06	4.42E-07	4	0.56790493	0.70145804
Selection × Block × Treatment × Yeast	3.11E-06	7.79E-07	4	1.18329697	0.31629598

4.2. A. *Maternal investment with respect to different larval density and food quantity*

Results were obtained from multi-variable mixed model analysis of variance (ANOVA) using selection, treatment (larval density and food quantity) and yeast as fixed variables and block as a random factor.

When all the larval density treatments and food quantity treatments were included in the analysis, MCUs, the population selected for adaptation to larval crowding were found to be laying larger eggs compared to their ancestral control, MB. These results are consistent with the previous results where we found that MCUs were laying larger eggs compared to MBs in all the density treatments.

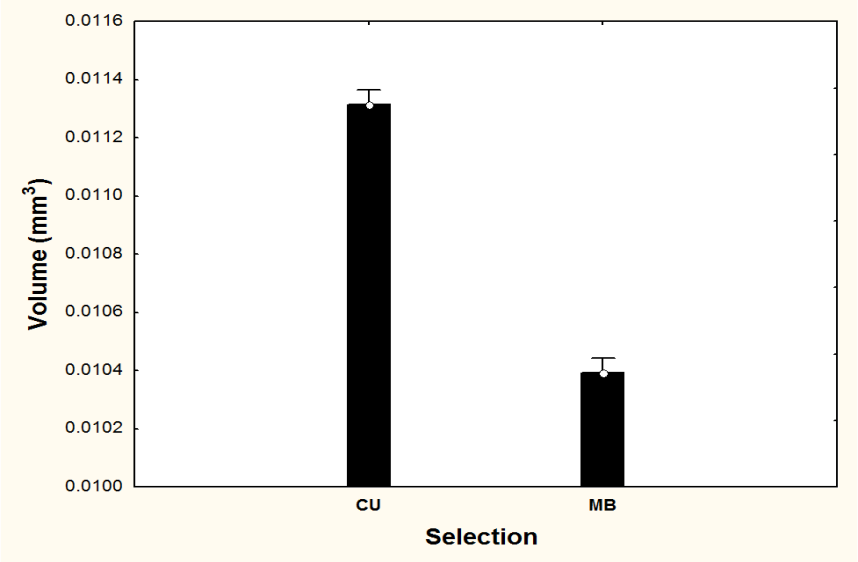

Figure 4.7 Graph between egg and selection regime (MCU and MB)

4.2. B. Effect of yeast on maternal reproductive investment

Table 4.4 Data-table obtained by multi-variable mixed model analysis of variance (ANOVA) using JMP analysis

Effect	Least Sq Mean	Std Error	Volume
Yeasted	0.0107524	3.39E-05	0.0107524
Non-Yeasted	0.0109559	3.39E-05	0.0109559

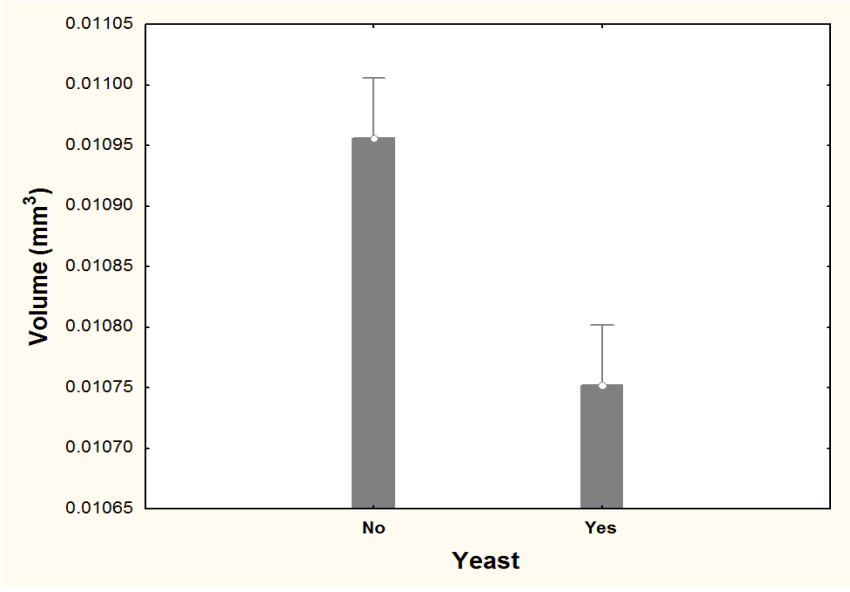

Figure 4.8 Graph between egg volume and yeasting condition.

Yeast was provided to both the populations prior to egg collection, following standard maintenance regime. It was observed that there is an exponential increment in the number of eggs laid by females when food was supplemented with yeast. To assess the effect of yeast on maternal investment, egg volume was measured. Females were housed under two different conditions, one in which food was supplemented with yeast and the other without yeast. Eggs were then collected and their volume measured. Females raised on food without yeast seemed to be laying eggs of larger volume compared to females raised on food with yeast.

CHAPTER-5

DISCUSSION

Investment in reproduction is one of the most important fitness traits for any organism. Investment is likely to be affected by multiple factors such as -

a) Availability of resources: As resources are finite and constantly depleting, a trade-off exists amongst maternal investment into the offspring and other life-history traits.

b) Environmental effects: Besides genetic components, the size of gametes (eggs and seeds), simultaneously a parental and progeny character, can mediate environmental condition experienced by a parent. In both animals and arthropods, mothers are known to reduce their egg mass depending on their nutritional condition (Yanagi and Tuda 2009). But interestingly, the females in our study which were raised in poorer environment (lack of yeast) laid larger eggs.

In promiscuous species like Drosophila, the strategy of maximizing fitness and investment in progeny varies across the sexes. Males get highly benefitted by having higher number of matings. The males also get benefitted by higher maternal investment in the offspring, as this allows the former to preserve more energy and invest in additional matings. Even though the females also are promiscuous, they still have to invest a higher amount of time as well as resources than males in any scenario. It can also prove to be detrimental to the female if too much maternal investment is involved per progeny.

At this point of time, we are unable to predict whether the egg volume directly correlates to the egg mass in the selected populations used in our study. In the light of present findings, it would be interesting to see if measurements of the wet egg mass, which has been used by earlier studies as a proxy for egg volume (Prasad et al, 2003) still hold and can be used interchangeably. There is a possibility that mass and volume can be partially decoupled, i.e. even though the eggs are bigger in size, they can become lighter. Given the harsh larval condition of selection, one can also expect that the females of the selected population also can evolve to manufacture bigger, lighter eggs but with a thicker outer cuticle, so as to

combat the desiccant and food-limiting environment during the development of the eggs. This means that although investment per egg has changed, energy reserves remain constant for the larva that hatches from the egg; and also that maternal investment is being channelized to make the egg survive the initial stages, before hatching. If such an assumption is valid, it also poses another question: Are the larvae from the selected population heavier than those from the baseline populations?

In this study we are able to show that phenotypic plastic behavior exists in terms of body-size with respect to selection as well as larval density treatment. MCUs are smaller in body-size than MBs. In support to Noordwijk et al (1986) a study done by Christopher A. Brown (2003) on a species of scorpion, *Centruroides vittatus* showed a negative correlation between offspring size and number within a population with the ratio of allocation variance to investment variance. Similar negative correlation was also observed in our populations, the MCUs in spite of their smaller body size laid eggs of larger volume; their investment per egg was more.

Larval crowding can potentially have trans-generational effects on progeny production. The amount of food per larva is limited in such a condition. This nutritional deficits and added environmental effects like desiccation during development. This in turn causes the emergence of stressed adults. Such adults partition more of their resources towards the survival than reproduction (Mueller et al, 1988). In a study by Shenoi (unpublished data) on the same population showed that adaptation to larval crowding has resulted in the evolution of multiple adult traits like longevity, mating behavior etc. Such a condition induces plastic response in adults to adjust their investment in reproduction.

In the present study, we report that the maternal investment per egg has evolved as a response to selection to larval crowding. But interestingly, density by itself did not have an effect, nor was there any selection x density interaction. The effects seen in our experiment can be direct or a correlated response to selection.

Recent studies in Drosophila melanogaster (Pischedda et al, 2010) have shown that male type can influence or manipulate the behavior of the females in maternal investment. Studies in other species have also shown interaction effects among maternal environment, maternal

investment and progeny genotype on life history traits (Yanagi and Tuda 2009) and hence the investment strategies can evolve rapidly under environmental variables. Our study was not able to show any *measurable* phenotypic plasticity in maternal investment. This study also does not differentiate male and female effects separately.

It has been previously reported that supplementation of yeast influences maternal investment. Yeast is known to hugely affect the number of eggs laid by the females (Chippindale K. et al 2002). We have found that selected females which were raised on food without yeast laid larger eggs. This is in contrast to the common assumption that eggs laid by females raised under protein supplement (yeast) might be bigger and/or more in number.

The selected populations in this study, the MCUs in spite of their smaller body-size, invested more resources per egg than their ancestral controls, MBs. It was also found that larval density does not have any effect on egg volume. In short, we can say that even though phenotypic plasticity was not observed in the investment with respect to larval density, we found plasticity in body size with respect to larval density and selection.

References

Adam K. Chippindale, K., Armand Leroi M. Sung Kim B. and Rose M., Phenotypic plasticity and selection in Drosophila life-history evolution. I. DEC 2002, Nutrition and the cost of reproduction, 11 DOI: 10.1046/j.1420-9101.1993.6020171.x.

Christopher A. Brown (2003), offspring size –number trade-off in scorpions: an empirical test of the Van Noordwijk and De Jong model, 2003, Evolution 57(9):2184-2190. doi: http://dx.doi.org/10.1554/03-014.

Houle, D *The character problem in life history evolution. The Character Concept in Evolutionary Biology*, 109-140, eds G. P. Wagner.

Joshi, A. and L. D. Mueller. Evolution of higher feeding rate in *Drosophila* due to density-dependent natural selection. 1988, *Evolution* 42: 1090-1092.

Law, R. Optimal life histories under age-specific predation. 1979, *American Naturalist* 114, 399-417.

Noordwijk V., A. J. & Jong de, G. Acquisition and allocation of resources: their influence on variation in life history tactics. 1986, Am. Nat. 128, 137–142. (doi:10. 1086/284547).

Pischedda A., Stewart A.D., Little M.K. & Rice W.R. Male genotype influences female reproductive investment in *Drosophila melanogaster*. 2011, Proceedings of the Royal Society B 278: 2165-2172.

Prasad N. G. and Joshi A., What have two decades of laboratory life-history evolution studies on Drosophila melanogaster taught us?, 2003, Journal of Genetics, 82, 45 – 76.

Roff, D. A. *The Evolution of Life Histories. Theory and Analysis*. 1992, New York: Chapman and Hall.

Rose M. R. Laboratory evolution of postponed senescence in *Drosophila melanogaster*. 1984, Evolution 38, 1004–1010.

Stearns, S. C. *The evolution of life histories*. Oxford: Oxford University Press, 1992.

Stearns, S.C. Life history evolution: successes, limitations, and prospects. N*aturwissenschaften* 87, 476-486 (2000).

Tatar, M. & Carey, J. R. 1995 Nutrition mediates reproductive trade-offs with age-specific mortality in the beetle Callosobruchus maculatus. Ecology 76, 2066–2073. (doi:10.2307/1941681).

Worley Anne, C., Houle, D. & Barrett Spencer, C. 2003. Consequences of hierarchical allocation for the evolution of life history traits. Am. Nat. 161, 153–167. (doi:10. 1086/345461).

Zera, A. J. & Harshman, L. G. 2001 The physiology of life history trade-offs in animals. Annu. Rev. Ecol. Syst. 32, 95–126. (doi:10.1146/annurev.ecolsys.32.081501.114006).