Change in the size of the accessory gland and testis with mating status in a population of *Drosophila melanogaster* adapted to larval crowing.

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

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Certificate of Examination

This is to certify that the dissertation titled "Change in the size of the accessory gland and testis with mating status in population Drosophila melanogaster adapted to larval crowding" submitted by Ms. Neeraj Meena (Reg. No. MS14096) 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 is accepted.

Dr. Manjari Jain Dr. Rhitoban Ray Choudhury Dr. N.G. Prasad

(Supervisor)

Dated: April 26th, 2019

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 the contribution of others are involved, every effort is made to indicate this clearly, with due acknowledgment 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.

Neeraj Meena

(candidate)

Dated: April 26, 2019

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

Dr. N.G.Prasad

(supervisor)

Date :

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ABSTRACT

In promiscuous species, the male`s ejaculate plays an important role in its fitness. Due to high male-male competition, the quality and quantity of ejaculate determines the male`s reproductive success. In a holometabolous insect like *Drosophila melanogaster*, the investment in reproductive tissues is highly dependent on its larval environment. In a larval-crowding like condition, which is possible in these species because of confined foraging ground for larvae, the allocation of resources to different adult reproductive tissues changes (shown in previous studies). The aim of this study is to investigate the evolution of ejaculate depletion pattern as a result of adaptation to larval crowding.

Males of Selected and Control populations were provided with three females for three consecutive matings. After three matings the decrease in size of male reproductive organs (testis and accessory gland size) was measure (as compared to virgin males), to obtain a measure of investment in various components of the ejaculate (sperm and ACPs) in Selected versus Control populations.

Chapter 1

Introduction

Density-Dependent natural selection :

Density is an important ecological stressor that maintains the life history of an organism. Due to trade-offs of traits across densities , alternative life histories can evolve across different densities . Fitness of an organism depends on its ability to survive and reproduce in the given environment . The Pre adult nutritional conditions affects the life history of an organism. Nutrition is a major determinant of body condition.

Density-Dependent selection has been studied in detail by two sets of experimental evolution studies: r and K populations (Mueller and Ayala, 1981)and CU and UU populations (Mueller et al., 1993). They showed that in laboratory populations of *Drosophila melanogaster*, adaptation to high population density led to an increase in population growth rate and decrease in their growth rate at low density. Change in population growth rate affect the population behavior and feeding of larvae. The populations evolved under high population density have a higher feeding rate and are less likely to pupate on or near the food surface than the population at low density. These changes in behavior led to an increase in the competitive ability of larvae for limited food and reduce mortality under crowded condition during the pupal stage of development.

However, these studies did not show the evolution of adult traits in response to adaptation to larval crowding. Larval crowding is also known to affect the adult traits like: body size, lifespan, fecundity, mating success, lipid content, etc . Vinesh Shenoy et al. looked at the evolution of adult traits of Drosophila melanogaster population adapted to larval crowding. These are - Adult life span, Pre-copulatory sexual behavior, Body size, Fluctuating asymmetry, Investment in reproductive tissues, Desiccation and starvation resistance.

Previous studies have shown that post-copulatory sexual selection can influence testis size and seminal receptacle. Post-copulatory sexual selection and fecundity selection are affected by the adult body size. We know that MCUs are cultured at very high larval densities every generation, so adults are extremely small.

So they are under selection for increased fitness at smaller body size. So Vinesh Shenoy found that adaption to high larval density in MCUs leads to the evolution of significantly larger testis as compared to. MCUs have evolved higher lifespan, and lower body weight (Shenoi et a1.,2015) and lifespan do not trade-off with increased courtship activity in MCUs.

Previous studies have show that absolute testis size depends upon the type of mating system with the male of the species in which females are promiscuous have larger testis for their body size than do male of monogamous species (review Pitnick 1996; Kappeler 1997; Stockley et a1., 1997) so they can produce more sperms and have greater chances of fertilization in sperm competition. In Drosophila, testis size evolution may not direct effect of body size or several sperm produced. Testis size might be affected by sperm length – body size. Vinesh Shenoi N found that density negativity affects male wing length, flies grown in high density have shorter wings and flies grown in low density have larger wings. He did not find any selection of × density. He showed that increased longevity of MCU male had not rade off with their courtship activity. Increased longevity in MCU females had no trade-off with their number of mating (Shenoi et a1.,). He found that MCU males have evolved larger relative testis size.

Nutrition is a major determinant of body condition. Individuals from low nutrition condition show lower reproductive success and individual with high nutrition condition show high reproductive success(droney 1998; Kotiaho 2001). So MCU population faced with low larval nutrition condition.

Theory predicts that when the sperm competition is high, and mating opportunities are rare, the male should increase investment in their few mating. This often leads to a relative increase in sperm number. Correlation studies both within and across the species indicate that increased testis size result in the production of more sperms (Harcourt et al. 1981; Kenagy&Trombulak 1986; Gage 1994; Hosken 1997; Stockley et al. 1997; Simmons et al. 1999)

In Drosophila, male gonad not only consists of a pair of testis but along with that they contain a pair of the accessory gland. In many species, mating success is tightly linked with the size of the accessory gland (AG)

Accessory Gland proteins (ACPs)

Accessory glands produce more than 100 different kinds of proteins which transferred along with sperms to the female during copulation. In mated females, ACPs enhance egg production, augment sperm storage, induce refractory mating behavior and effects female's longevity(Ravi Ram K1, Ji S, Wolfner MF. 2005) Early reproductive success in Drosophila males is dependent on maturity of the accessory gland (Hanna Ruhmann Kristina U. Wensing Nicole Neuhalfen Jan-Hendrick SpeckerClaudia Fricke 01 November-

December 2016) In postcopulatory sexual selection , accessory gland proteins (ACPs) play an important role in sperm competition. Along with facilitating sperm transfer, ACPs effects on female reproductive activity and they improve the male's chances of siring a significant proportion of the female's offspring.

Testis

The number of first spermatocytes is correlated with sperm length, which is inversely related to sperm production (Lukas Schärer, Jean-Luc Da Lage and Dominique Joly, 2008). Males of larger bodies Drosophila species make a proportionately greater energetic investment in testis than do males of smaller body species (Scott Pitnick, 1995b).

Chapter 2

2.1Experimental system

2.1.1Fly population used

We carried out all experiments on two sets of Drosophila melanogaster four selected for adaption crowding(MCU 1-4) and its baseline (MB 1-4). And PJBW population. The MB and MCU population derived in the lab of Prof.Amitabh Joshi at the evolutionary and organismal unit, Jawaharlal Nehru Centre for Advance Scientific Research(JNCASR), where they underwent 75 generations of selection. The MB and MCU populations are derived from UU populations used by Mueller(1998,1990)

Both populations MB 1-4 and MCU 1-4 were derived from the long term laboratory population of D. melanogaster called JB population. In 2006 four JB population were mixed to form a single large population called Melanogaster Baseline(MB). Both populations are maintained at standard laboratory condition,i.e., 25' C, 95% relative humidity, standard corn – meal charcoal food; 24-hour light cycle. After ten generations, the single MB population was split into four replicate populations called MB(1-4).MCU(1-4) were derived from each of MB population(i.e., MCU 1 was derived from MB 1 and so on).

2.2Maintenance

2.2.1MB (melanogaster Baseline)

eggs are collected from 21days post –eclosion from the previous generation maintaining 21-day discrete generation cycle. After egg collection, collected eggs are transferred into glass vials (25mm diameter × 90 mm height). Each block forty such replicate vials are collected. Vials containing cornmeal-charcoal food (6-8ml) at 60-80 eggs/vial density. At 12th day all the adult flies have eclosed. On the same day, these flies are transferred into plexiglass cages (24 cm × 19 cm × 14 cm). Cages are containing a large petri plate of cornmeal-charcoal food and wet cotton to maintain relative humidity. The plates and cotton were changed every alternative day.these cages were maintained until both stock and backup collection was over. On

18th-day post-eclosion fresh plate with live yeast paste provided to cages and after two days of yeast supplement, i.e., post eclosion cut plate were given to the cages .there is 18- hour window for egg laying. Then next day egg collection was done for the next generation.

2.2.2 MCU (melanogaster crowed as larvae, uncrowded as adult)

MBs were maintained in the lab for 15 generations according to the regime. After 15 generations, MCU (1-4) were derived from MB (1-4). The MCU populations are also maintained on 21- day discrete cycle at 25° C temperature, 90% RH and constant light. Eggs laid by 12th-day old females are dispensed in glass vials containing 1.5 ml of corn meal-charcoal food of 800 eggs/ vial. Flies start eclosing from 8th-day post egg collection; their eclosion pattern is spread out in comparison to that of MBs.

2.2.3 PJBW

PJBW also derived from the JB population. And JB population derived from UU population. We used females from PJBW population because PJBW is a common ancestor of both populations MCUs and MBs.

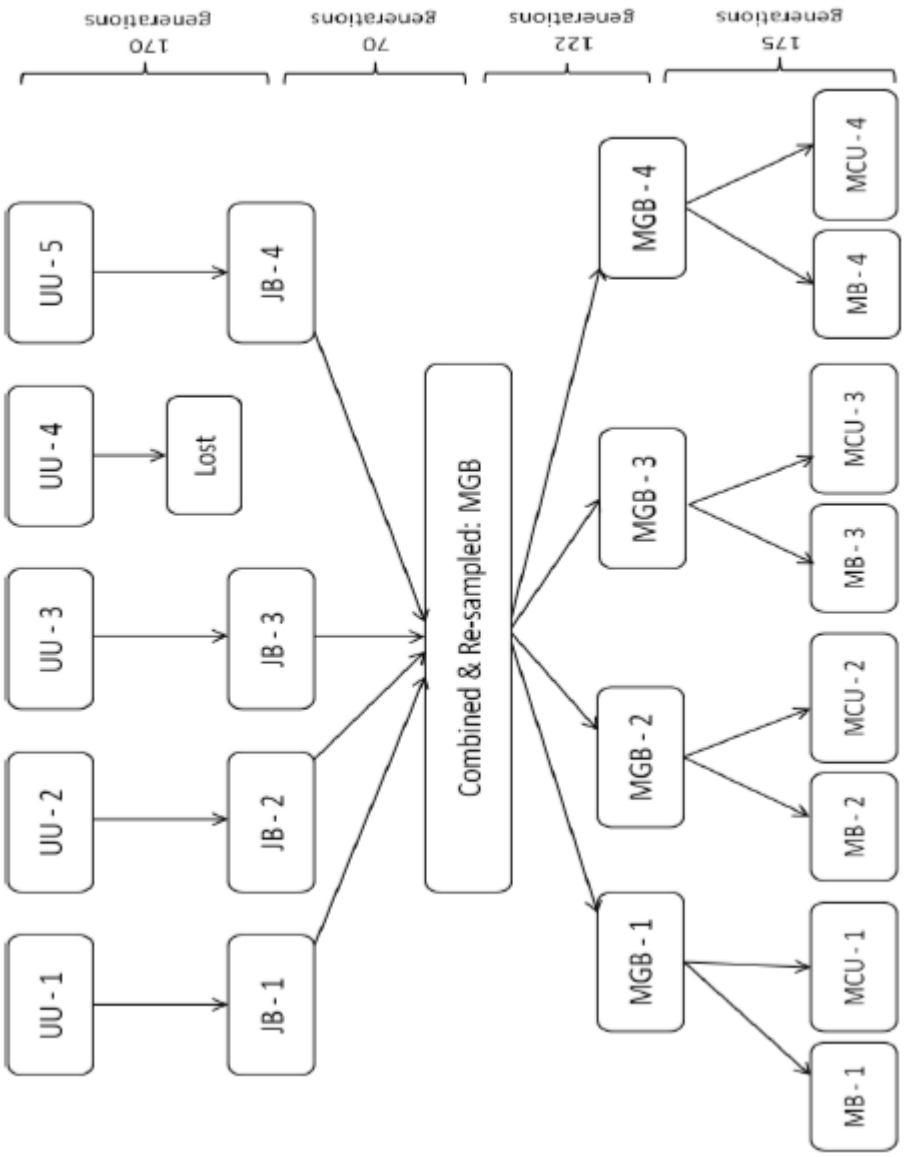


Figure 1. Lineage of MCU / MB population

2.3 Standardization of flies for experiments:

MBs and MCU are reared in similar condition to account for nongenetic paternal effects. For the experiment eggs were collected from backup cages. Backup egg collection was done from the same cages from which stock population. On 22th day cut plate is given to stock cages for 18-hour window. Eggs collected from these cut plate with 350-400 eggs/bottle density with ad libitum amount of food.these backup is dumped into cages on 12th-day post egg collection . after egg collection was done from backup cages for the experiment.

2.4Egg collection for the experiment:

There are two different treatments – high density and low density.

The experiment was carried out on different ages as the selection of 21 days cycle. A fresh plate with yeast plate was given a day before egg collection, and the cut plate was given for 6 hours before egg collection.eggs were collected at different days for HD and LD for MB and CUs.

DAY 0 : PJBw , MB , CU (high density) egg collection

DAY 3 : CU high density egg collection

DAY 6 : PJBw , MB , CU(low density) egg collection

DAY 9 – 19 : virgin collection

2.4.1High density(HD)

For high-density treatment, eggs were collected at a density of 600 eggs/vial for MB, MCU, and PJBw in 2 ml charcoal food. Ten vials were collected for MCU and MBs for every block and 20 vials collected for PJBw population in charcoal food. MCU is selected for adaption to larval crowding, so they show more spread out peak of eclosion, so they eclose a day before MBs. So MCU was collected on 3 rd day and MCU were collected the day after MBs egg collection for the match the peak of eclosion.

2.4.2 Low Density(LD)

For Low-density treatment for MBs, MCU is collected at a density of 60 eggs/ vial in 5 ml charcoal food and PJBW are collected in banana food with the same density. Ten vials were collected for every block for MCUs and MBs, and 20 vials were collected of PJBw population. For low-density eggs were collected for MBs, MCU, and PJBw on the same day.

2.5Dissection and Imaging

Flies from every treatment were frozen at -20 °C in a vial with 5 ml of banana jaggery food after mating over and dissect the testes, accessory gland, and wing for both treatment(HD and LD) for MB and MCU of individuals. Before After the dissection, took the image of testis, accessory gland, and wing and analyzed using ImageJ for each block.Wing length was used as proxy for body size. All imaging was done at 40x zoom using Leica compound microscope.

Image Analysis

All the image of Accesory gland , testes and wings were analyzed using Image J softwere. The wing length was calculated using length tool.two fixed point were selected and length was measured between those points for each wing.

Cross section area of testes and accessory gland, a combination of different tools was required:

(a) The brightness and contrast value for each image were adjusted to bring out the subject of picture (Testes and accessory gland) from the background.
(b) Using the Thershold adjustment, the area of the

2.6Statistical Analysis

Three factor mixed model ANOVA was done to analyze Realtive testis size and relative accessory gland size using selection regime, density, mating factor as fixed factors and block as a random factor.Statistical analysis was performed using JMP statistical software.

Chapter 3

3.1Experimental set-up and Procedure

Experiment: Change in size of accessory gland and testis with mating Status in population of *Drosophila melanogaster* adapted Larval crowing.

3.1.1Experimental set-up

We carried out the experiment using virgin females (PJBW) transferred them into fresh vials in a set of three vials before the experiment and virgin males were also from two different regimes (MBS and MCU). In high-density treatment (HD), flies were collected in 1.5ml charcoal food with egg density of 600eggs/vial.PJBW females for HD treatment also collected in charcoal food with the same density and in low-density treatment, flies were collected in 5 ml charcoal food with egg density of 60 eggs/vial. Low-density female were collected in banana food with the same density. We collected ten such replicates for MCUs and MBs of the vial and twenty replicates of the vial for PJBW population. We collected eggs different days in the case of HD for MCU and MBs. In the case of HD for PJBW and MBS, eggs were collected on the same day. Then we collected adult male-female as virgin during 9-10 the day post egg collection. We dumped flies to a conical after 6 hours starting on nine the –day post egg collection till 19 the day. We sorted flies under mild CO2 anesthesia and held them in the group of 10 in a glass vial (25 mm diameter × 90 mm height) till day 12 post day egg collection, i.e., 48 hours. In total we collected ten vials for male (MBs and MCU) for HD and LD treatment and PJBW , we collected 50 vials for HD and LD treatment. These vials were randomly selected for 21 and 13- day post egg collection.

On the day of the experiment, females aspirated in the fresh vial with medium pore banana food for both treatment(high density and low density) before the observation started. Female were kept separately in each vial with banana food. we did the same thing for both treatment. When the observation started, male (MCU and MBs) transferred into that vials in which females were kept and cotton plug was pushed inside, so there is a gap of 30 mm between the food and plug. After the first mating got over, males were transferred into another fresh vial with a virgin female. We did the same thing three times. So we took single MCU and MB male and mated with three females sequentially . During the time of observations, vials were laid horizontally and mating latency was observed also.twenty sample for each treatment (CULD,CUHD, MBHD,MBLD). After the 3 rd mating over, males were frozen in -20° C. observer noted down the vial identity, mating start time, mating end time for each female. We froze virgin male for each treatment also..

After the freezing males, we dissected male and dissect testis, accessory gland, and wings from the male.wing is proxy for body size.

3.2Dissections:

Frozen flies were taken out for dissection. Accesory Gland , Testis and wings of both mated and virgin males were dissected out and images were taken. 15 males were dissected out in total per treatment.

.

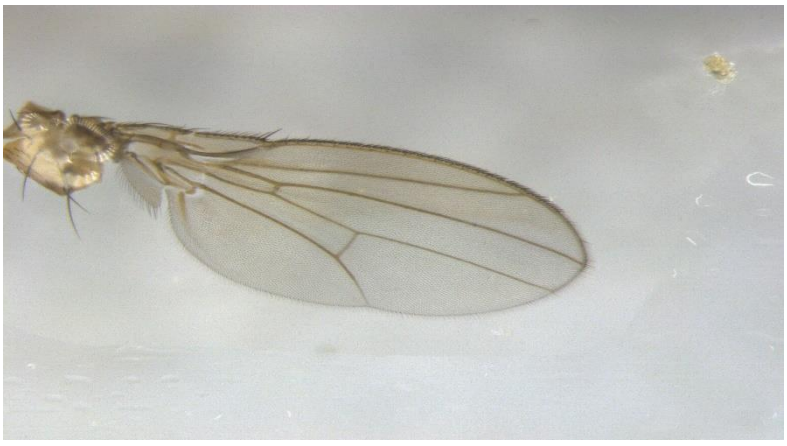


Figure 2 Image of wing taken using LEICA M205 C microscope

Accessory Gland

We dissect out Accessory Gland of virgin males and mated males.Picture of Accessory Gland were captured using LEICA M205 C of both mated and virgin male.

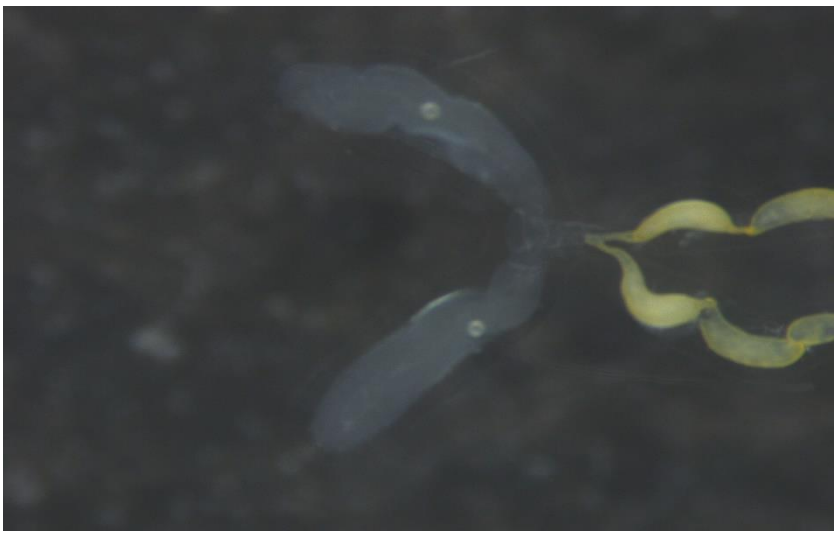


Figure 3 Image of Accesory Gland using LEICA M205 C microscope

Testis

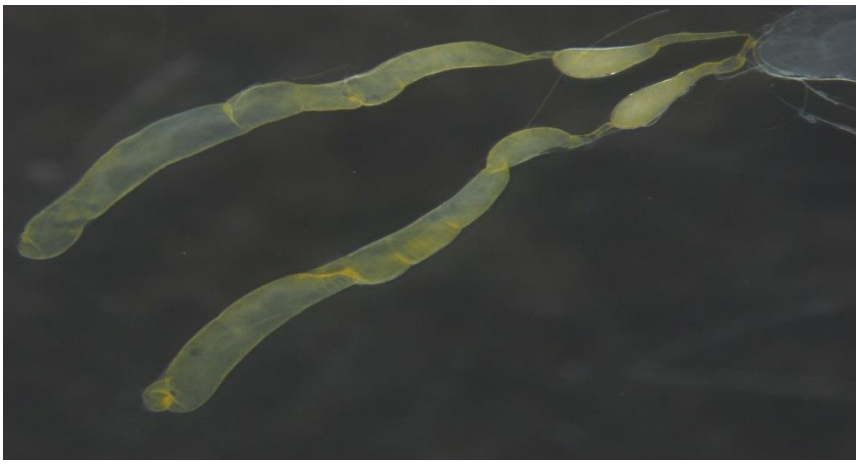


Figure 4. image of testes taken using LEICA M205 C microscope

Chapter 4

Observation :

Experiment was done for block 1, 2, and 4.

4.1Block- 1

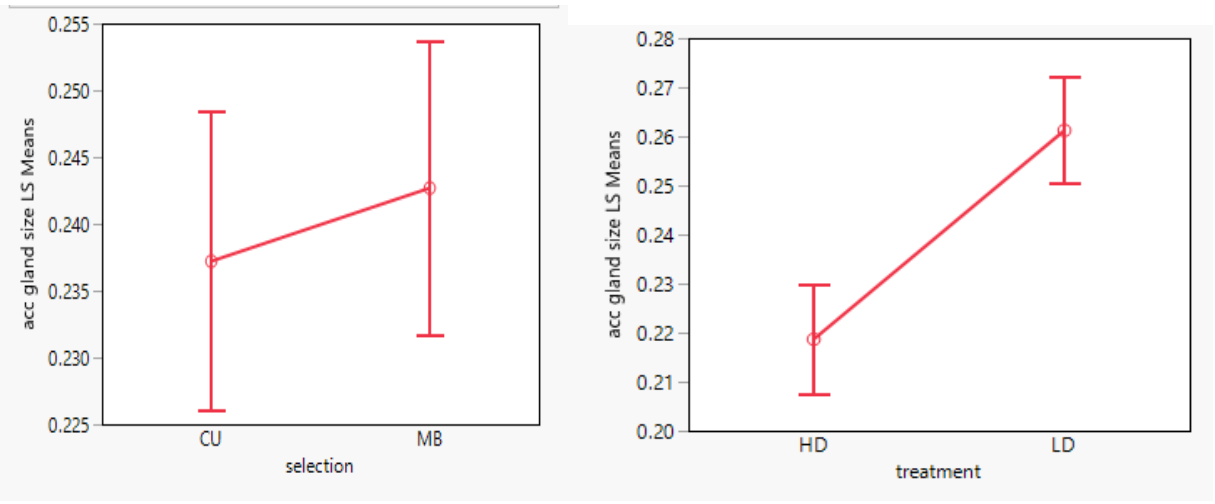
Accessory Gland

Table 1: Accessory Gland

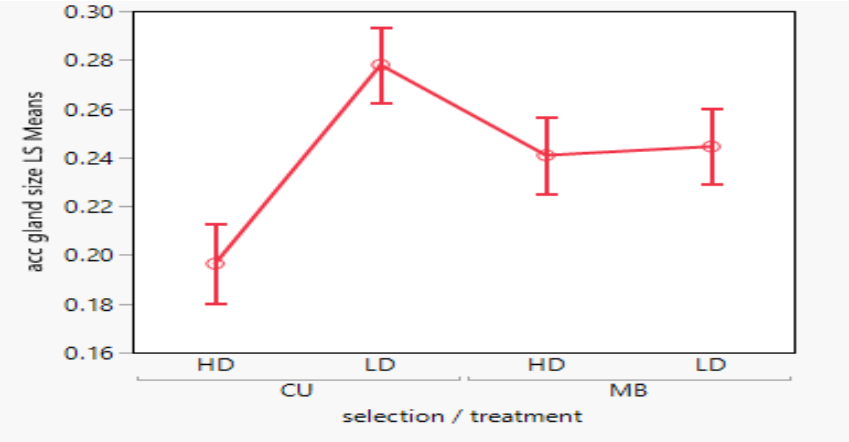
Anova table showing significant effect of treatment , selection*treatment , status on the Accessory Gland.

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
selection	1	1	0.00086479	0.4801	0.4899
treatment	1	1	0.05222118	28.9921	<.0001*
selection*treatment	1	1	0.04377567	24.3033	<.0001*
status	1	1	0.58753357	326.1865	<.0001*
selection*status	1	1	0.00168576	0.9359	0.3355
treatment*status	1	1	0.00440794	2.4472	0.1207
selection*treatment*status	1	1	0.01208141	6.7074	0.0109*



(a) (b).



(c)

Figure 5: accessory gland

(a) Y- axis showing Accessory Gland and X- axis showing selection (population MCU ans MB) MB's Accessory gland size larger as compare to CUs. (b) Y- axis showing Accessory Gland and X- axis showing treatment(High density and Low Density).Low density treatment have larger Accessory gland as compare to High density treatment. (c) X- axis showing selection* treatment and Y-axis showing Accessory Gland size.there is significant effect of selection*treatment on CUs.

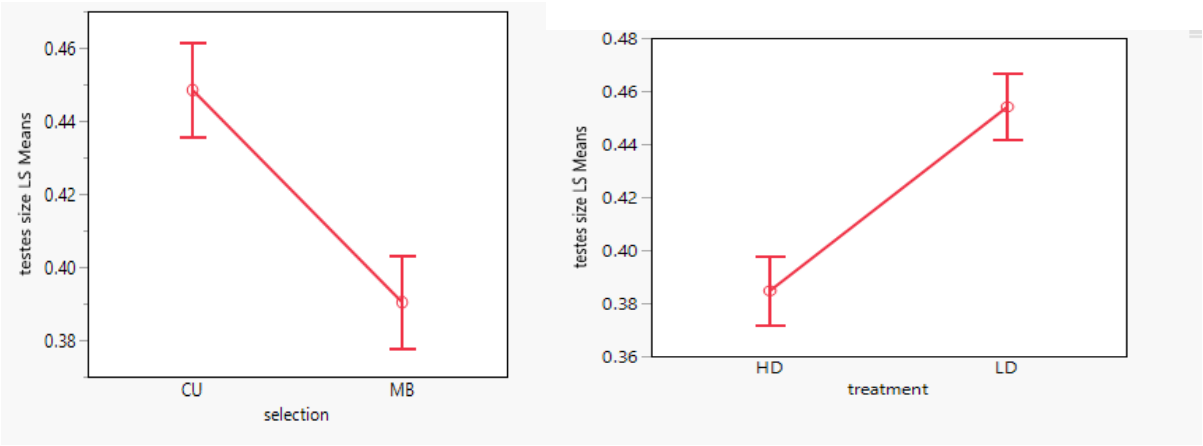
BLOCK- 1

TESTIS

Table :2 Testis

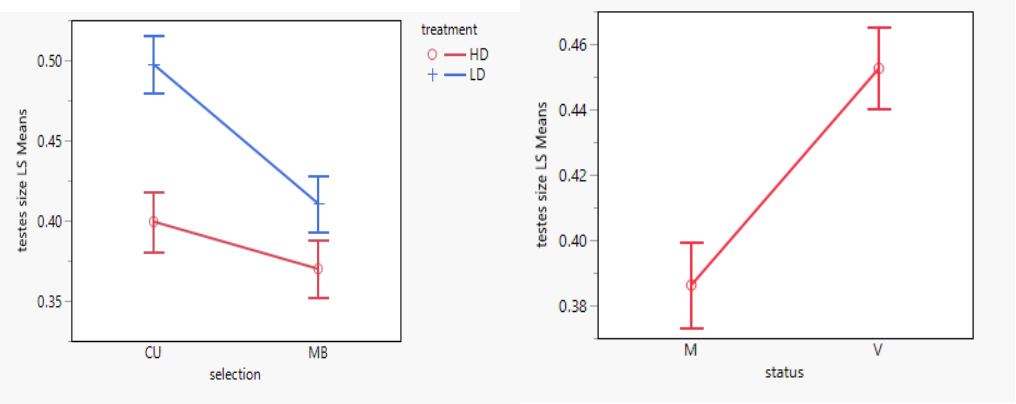
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.4194104	0.004527	92.64	<.0001*
selection[CU]	0.029045	0.004527	6.42	<.0001*
treatment[HD]	-0.034692	0.004527	-7.66	<.0001*
selection[CU]*treatment[HD]	-0.014341	0.004527	-3.17	0.0020*
status[M]	-0.033156	0.004527	-7.32	<.0001*
selection[CU]*status[M]	-0.003431	0.004527	-0.76	0.4501
treatment[HD]*status[M]	0.0072293	0.004527	1.60	0.1132
selection[CU]*treatment[HD]*status[M]	0.016152	0.004527	3.57	0.0005*

Anova Table showing significant effect of selection , treatment , selection*treatment status on testis size.



(a) (b)

(c)



(d)

Figure 6 : Testis size

(a) X-axis showing selection (population CUs and MBs) and Y- axis showing Testis Size. The significant effect of selection on testis size. CU's testis size larger as compare to MBs as well. (b) X- axis showing treatment (High Density and Low density) and Y- axis showing Testis size. There is Significant effect of treatment on testis size .Size of Testis have larger for low density treatment as compare to High density treatment. (c) X-axis showing selection (population MB and MCUs) and Y-axis showing testis size. CUs have significant difference but MBs have not. (d) X-axis showing mating status (mated and Virgin) and Y- axis showing Testis size. There is significant difference that mated testis size is small as compare to virgin male. There is no significant difference of size of testis of virgin and mated males.

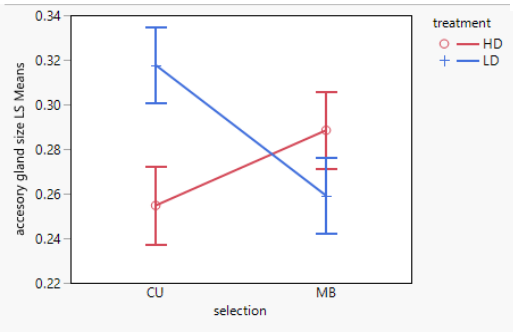
4.2BLOCK-2

Accessory Gland

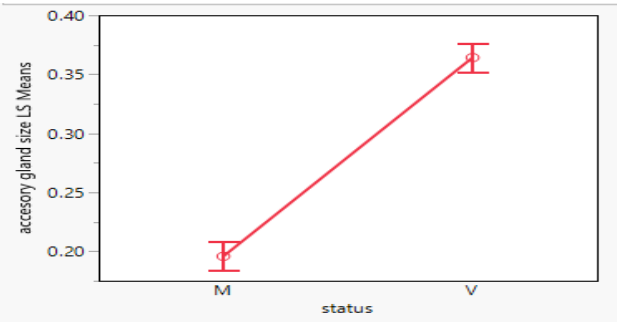
Table 3 : Accessory Gland

Anova table showing the significant difference of selection*treatment , status, selection*status, treatment*status on Accessory Gland

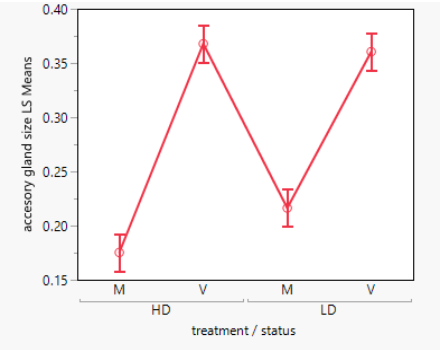
Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.2800217	0.004318	64.85	<.0001*
selection[CU]	0.0062038	0.004318	1.44	0.1536
treatment[HD]	-0.008409	0.004318	-1.95	0.0540
selection[CU]*treatment[HD]	-0.023058	0.004318	-5.34	<.0001*
status[M]	-0.084193	0.004318	-19.50	<.0001*
selection[CU]*status[M]	0.011466	0.004318	2.66	0.0091*
treatment[HD]*status[M]	-0.012086	0.004318	-2.80	0.0060*
selection[CU]*treatment[HD]*status[M]	0.0091293	0.004318	2.11	0.0367*

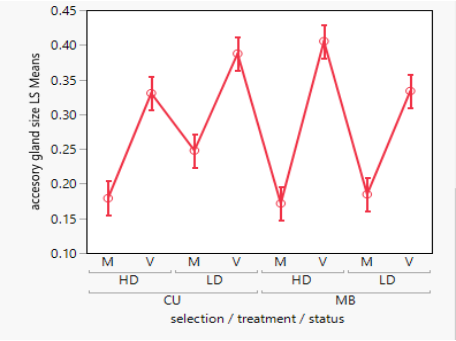


(a)



(b)





(c) **(d)**

Figure 7: Accessory Gland

(a) X-axis showing selection(population) and Y-axis showing Accessory Gland.There is significant difference in Accessory Gland Size of MBs. (b) X-axis showing mating status (mated and virgin) and Y- axis showing Accessory Gland Size. There is significant effect that mated have smaller Accessory Gland as compare to virgin. (c) X- axis showing treatment*status and Y- axis showing Accessory gland size. There is significant difference in size of Accessory Gland of mated and virgin. (d) X- axis showing selection*treatment*status and Y- axis showing Accessory Gland size. There is significant difference in size of CUs Accessory Gland size.

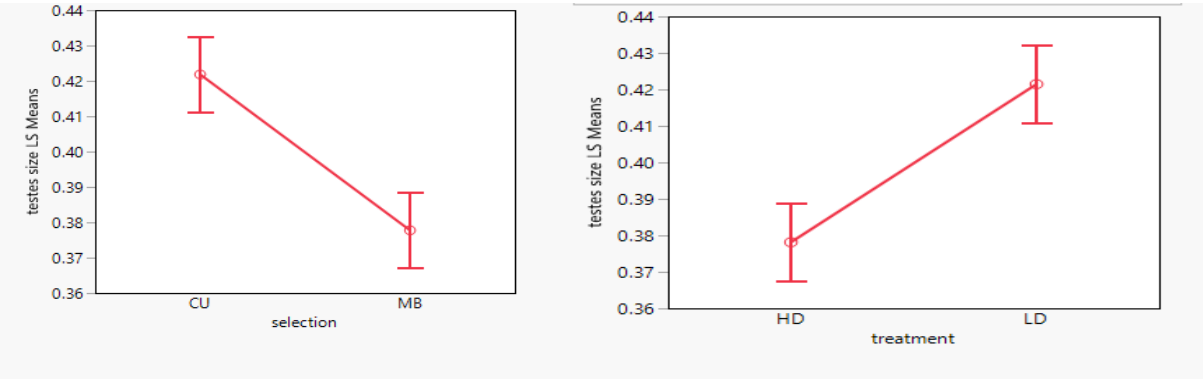
Block- 2

Testes

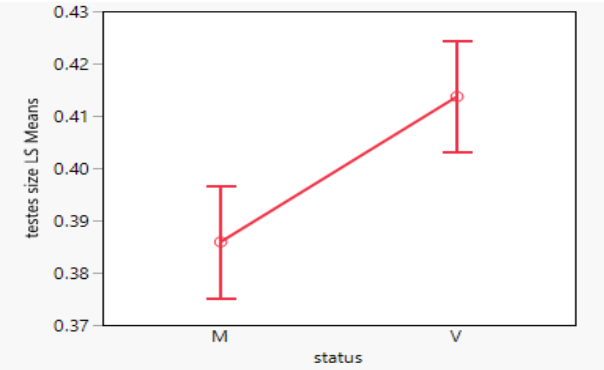
Table 4 : testis

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.3998059	0.003828	104.44	<.0001*
selection[CU]	0.0220375	0.003828	5.76	<.0001*
treatment[HD]	-0.021662	0.003828	-5.66	<.0001*
selection[CU]*treatment[HD]	0.0046102	0.003828	1.20	0.2310
status[M]	-0.013895	0.003828	-3.63	0.0004*
selection[CU]*status[M]	0.0013072	0.003828	0.34	0.7334
treatment[HD]*status[M]	0.0063341	0.003828	1.65	0.1008
selection[CU]*treatment[HD]*status[M]	0.0055145	0.003828	1.44	0.1525

Anova table showing the significant difference of selection, treatment, status on testis Size.



(a) (b)



(c)

Figure 8: testis

(a) X-axis showing selection (population) and Y-axis showing testis size. There is significant difference of selection on testis size. CUs have larger testis size as compare to MBs. (b) X-axis showing treatment (high density and low density) and Y-axis showing testis size. There is significant difference of treatment on Accessory Gland size. Low density treatment have larger testis size as compare to High density treatment. (c) X-axis showing mating status (mated and virgin) and Y- axis showing testis size.virgin males have larger testis as compare to mated male. There is no significant difference of size of testis of mated and virgin males.

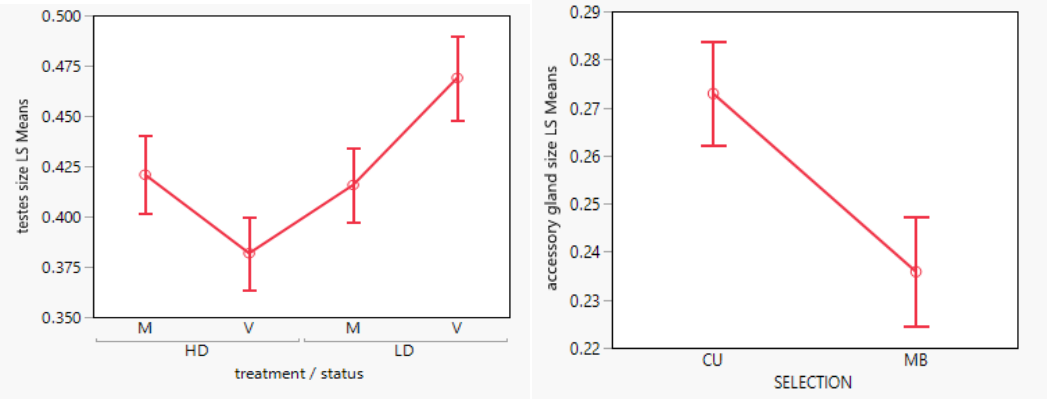
4.3BLOCK 4

Accessory Gland

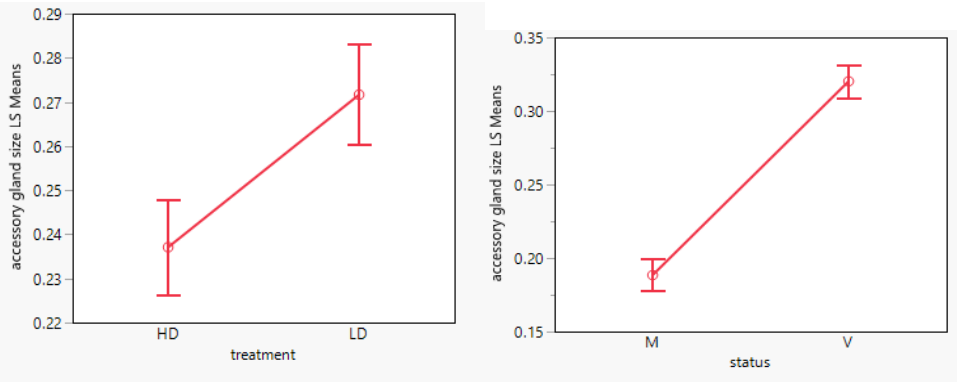
Table 5 : Accessory Gland

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.25438	0.003973	64.03	<.0001*
selection[CU]	0.0185307	0.003973	4.66	<.0001*
treatment[HD]	-0.017277	0.003973	-4.35	<.0001*
selection[CU]*treatment[HD]	0.0005161	0.003973	0.13	0.8969
status[M]	-0.065889	0.003973	-16.58	<.0001*
selection[CU]*status[M]	0.0052967	0.003973	1.33	0.1855
treatment[HD]*status[M]	0.0196352	0.003973	4.94	<.0001*
selection[CU]*treatment[HD]*status[M]	-3.067e-5	0.003973	-0.01	0.9939

Anova table showing the significant effect of selection, treatment, status and treatment*status on Accessory gland size.



(a) (b)



(c) (d)

Figure 9 : Accessory Gland

(a) X-axis showing treatment*status and Y- axis showing Accessory Gland Size. There is significant difference of treatment*status on Accessory Gland. (b) X- axis showing selection (population CUs and MBs) and Y- axis showing Accessory Gland size. CUs have larger accessory gland as compare to MBs. There is significant difference in CUs. (c) X- axis showing treatment (high density and low density treatment) and Y-axis showing Accessory Gland size. Low density males have larger Accessory Gland as compare to MBs. (d) X- axis showing mating status (mated and virgin male) and Y- axis showing Accessory Gland size. Virgin male have larger Accessory Gland Size as compare to mated male.

Block-4

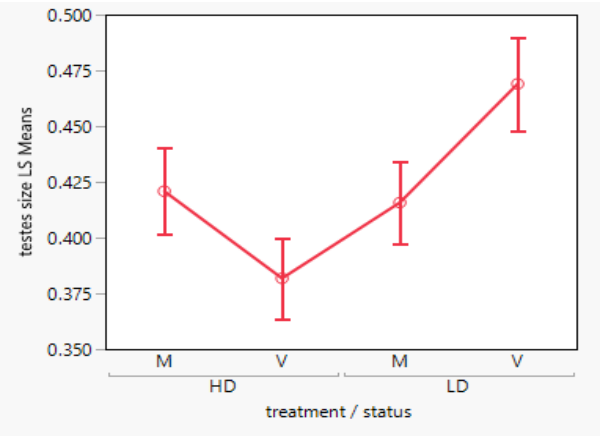
Testes

Table 6 : Testis

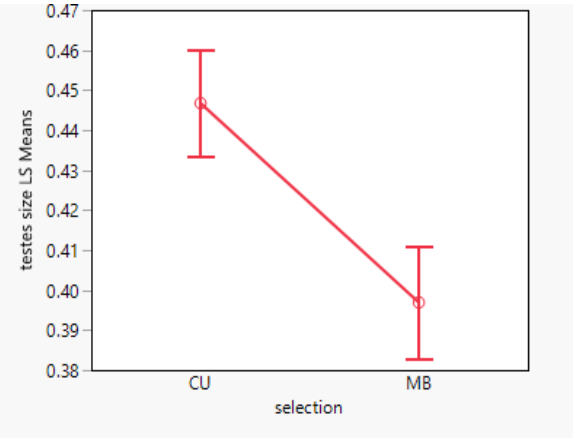
Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.4218516	0.004862	86.76	<.0001*
selection[CU]	0.0249101	0.004862	5.12	<.0001*
treatment[HD]	-0.020524	0.004862	-4.22	<.0001*
selection[CU]*treatment[HD]	0.0092636	0.004862	1.91	0.0596
status[M]	-0.003546	0.004862	-0.73	0.4675
selection[CU]*status[M]	-0.004239	0.004862	-0.87	0.3854
treatment[HD]*status[M]	0.0230733	0.004862	4.75	<.0001*
selection[CU]*treatment[HD]*status[M]	0.0013216	0.004862	0.27	0.7863

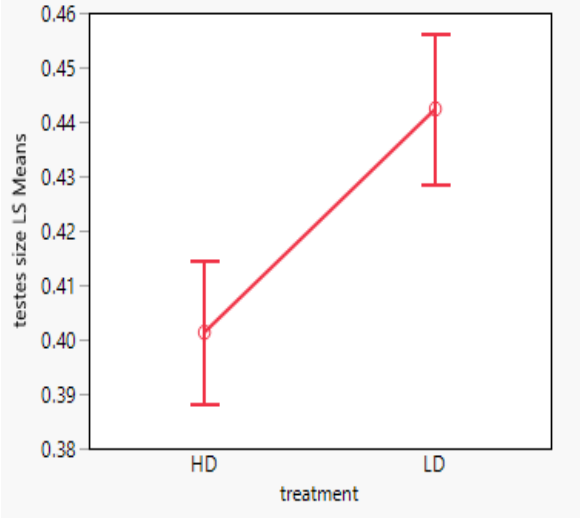
Anova Table showing the significant difference of selection, treatment, and treatment*status on testis size



(a)



(b)



(c)

Figure 9: Testis

(a) X-axis showing treatment*status and Y-axis showing testis size. There is significant difference of treatment*status on testis size. (b) X-axis showing selection (population CUs and MBs) and Y- axis showing testis size. CUs have larger testis size as compare to MBs. (c) X-axis showing treatment (high density and low density) and Y- axis showing testis size. Low density males have larger testis size as compare to High density males. There is no significant difference in size of testis of mated and virgin males.

Chapter 4

Results:

We did the experinment in three Blocks – Block 1, Block 2, and Block 4

Block-1

CUs male have larger testis as compare to MBs males as well. There is no difference in testis size of mated male and virgin male.The size of testis of MBLD males is equal to CUHD males. Effect of selection*status is not significant.

In case of accessory gland, virgin males have larger Accessory gland as compare to mated male.size of Accesory Gland decrease after three matings.this is significant. The size of accessory gland drop more as compare to CUs. The effect of selection is not significant.

Block-2

In case of testis results are same as block 1. CUs males have larger testis as compare to MBs males. Low Density males's testis size is larger as compare to High Density males. And there is no difference in size of testis of mated male and virgin male. The size of testis of MBLD males is equal to CUHD males. Effect of selection*status is not significant. The effect of selection*status is not significant for testis size.

In case of Accessory Gland, the effect of selection*treatment is significant for Accessory Gland but the effect of selection and treatment are not significant. Virgin males have larger Accessory Gland than mated males. There is more drop in size of Accessory Glands for MBs males is more as compare to CUs males

Block-4

In case of testis, there is no difference in size of testis of mated male and virgin male. The effect of selection is significant. CUs have larger testis as compare to MBs. Treatment effects also

significant. The size of testis of MBLD males is equal to CUHD males also same as other Blocks Low Density males have higher testis than the High Density male.

In case of Accessory Gland, the effect of selection*treatment is significant for Accessory Gland. The size of Accessory Gland of CU High Density males equal to MB Low density males. Mated males have small Accessory Gland than the virgin males. Low Density males have larger Accessory Gland than High Density males.

Chapter 5

Discussion

Earlier studies have shown how size of Accessory glands and Testis are affected by mating status of males (chapmann 2007) and body size (pitnik 1996). We know from earlier studies that adaptation to larval crowding can affect the body sizes of males (kappeler 1997, pitnick 1996).

In this study we have reestablished the previous findings of earlier studies that adaptation to larval crowding can lead to evolution of investment in reproductive tissues in males of *D. melanogaster*. As we have seen in all three blocks (Block 1, Block 2, Block 4) there is a significant effect of selection on testis size of males. MCUs always have a bigger testis size than MBs. Stating the fact that adaptation to larval crowding can lead to increased investment in testis size of males. There was a significant effect of Treatment on testis size, such that males growing in low larval densities had a significantly bigger testis size than males of high larval densities. In all the three blocks there was a significant selection cross treatment effect in testis size, most striking of which was, MB low density males had almost similar testis size as of MCUs high densities, suggesting the magnitude at which investment is testis have gone up in MCU population under high densities. We did not find any significant affect of mating status on testis size. The drop in size of testis was similar for both MCUs and MBs across virgin and mated treatment. For accessory gland sizes, there is a significant affect of treatment in block 1 and 4 i.e, low density males had a bigger accessory gland size than high density males. Surprisingly only block 4 shows a significant affect of selection, with MCUs having a bigger accessory gland size than MBs. The effect was similar but not significant in other two blocks. That could be because of mating status cross selection interaction because the size drop in MBs after 3 mattings is significant and quite drastic.

Results here suggests that after 3 matings the drop in testis size is similar in both MCUs and MBs but there is a more significant drop in accessory gland size of MB males. This could be because of two reasons:
1) MB males are throwing out more accessory gland proteins per mating as compared to MCU males .

2) MCU males are continuously producing more protein replenishing the depleted protein bank such that overall change in size of accessory glang is negligible.

Furthers experiments of sperm competition after sperm depletion, accessory gland protein estimation, sperm number counting are needed to be done in order to get a more clear picture of how has ejaculate depletion pattern evolved in MCU population as a result of adaptation to larval crowding.

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