Effect of male's fitness on male mate choice in *Drosophila melanogaster*

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Dedicated to my parents who stood by me.

Certificate of Examination

This is to certify that the dissertation titled "**Effect of male's fitness on male mate choice in *Drosophila melanogaster***" submitted by **Ms. Sushma Thingujam (Reg. No. MS12088)** 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.

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Dated: April 21, 2017

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.

Sushma Thingujam

Dated: April 21, 2017

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)

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Abstract

Males show male mate choice when the reproductive investment is high and if there is a fitness difference between females. The choice is greater when the fitness difference is higher. If there is a variance in male fitness, there might be a choice difference of the males toward the females. We test this hypothesis using *Drosophila melanogaster* by taking males differing in age and nutritional status. We found out that the males generally prefer the higher quality females and the choice is significant for higher quality males in case of age; but in case of nutritional status, the males show significant preference for the higher quality females when the fitness difference is high and no significant preference when the fitness difference is low.

Contents

Chapter 1

Introduction

1.1 Basic Theory

When there is difference in reproductive investment between males and females, the high investing sex chooses the low investing sex eventually leading to mate choice. Generally, females are considered to be the choosy sex because of higher investment in reproduction by producing large eggs and males get chosen because of lower investment in reproduction by producing small sperms (Darwin 1874, Bateman 1948). The investment of the males is considered to be trivial because of the smaller size of sperms relative to eggs. Also, males increases their fitness with each mating whereas, there is no increase in female fitness as they produce limited number of eggs (Bateman 1948).

Recent studies have found that males pay a non-trivial cost in reproduction in contrast to the age old perception (Trivers 1972, Edward and Chapman 2011). They also invest in the production of ejaculate, courtship and other mating related activities such as copulation duration (Dewsbury 1982, Pitnick and Markow 1994, Cordts and Partridge 1996). Because of the non-trivial investment, males become choosy and they generally choose the higher fitness female and leads to male mate choice as males can get some fitness returns from each mating. The variance in the female quality is thought to be one of the prerequisites for male mate choice. Fecundity of the females is the main describing factor for female fitness and it can be identified through female body size, fatness, gravid or non-gravid condition, etc.

Male mate choice can be described as differential male sexual response to different reproductively mature conspecific females (Bonduriansky 2001). Males show mate choice in the form of mating decisions or post-copulatory events such as differential ejaculate investment. It increases the fitness of the males despite the cost associated.

It has been reported that age and nutritional status of females affect the female fecundity i.e., it affects the fitness of the females. When the males are given a choice between a high and low quality females where young females being higher fit than old females and the High Yeasted females having higher fitness than Low yeasted females, the males generally chooses the high quality females (Nandy et. al., 2012). Also, the choice of the males was greater when the difference in quality of the females was greater.

1.2 Hypothesis

Varying the male quality will have an effect on male mate choice in *Drosophila melanogaster* i.e., males of varying quality or fitness will have an effect on male mate choice and that they will have different preferences of females. The fitness of the males can be varied by using males of different age and nutritional status.

Chapter 2

Materials and Methods

2.1 Experimental system

The experiments were done using an outbred population of *Drosophila melanogaster* (LHst) derived from the base population, LH. The LH population was caught from central California and named after the original founder, Larry Harshman. It is maintained in a 14 day discrete generation cycle with $25°C$ temperature, $60 - 80\%$ relative humidity and 12 hour light, 12 hour dark. They are grown in standard cornmeal molasses food.

Eggs are collected at Juvenile competition vials at a density of 150 eggs/$8 - 10$ ml food per vial. It takes about $10 - 11$ days for the eggs to develop into a fully grown adult fly. The eggs hatch into larvae in about $18 - 24$ hours. The larvae goes through three stages of growth- $1st$ instar larva, $2nd$ instar larva and $3rd$ instar larva which takes about $4 - 5$ days after which it undergoes metamorphosis and becomes a pupa. It remains in the pupa stage for $4 - 5$ days during which it gets matured and the pupa turns from white to brown. The fully grown adult finally ecloses from the pupa on the $10 - 11th$ day after the eggs are collected. On the $12th$ day, the adult flies are mixed across vials and the redistributed into adult competition vials (fresh food vials seeded with limited yeast) where 12 pairs of flies are kept. They were allowed to mate and on the $14th$ day, the females ate transferred into oviposition vials where the females are allowed to oviposit (lay eggs) for about 18 hours in vials having $8 - 10$ ml food. The food are trimmed and kept at a density around 150 eggs per food vial ($8 - 10$ ml) which becomes the juvenile competition vial for the next generation.

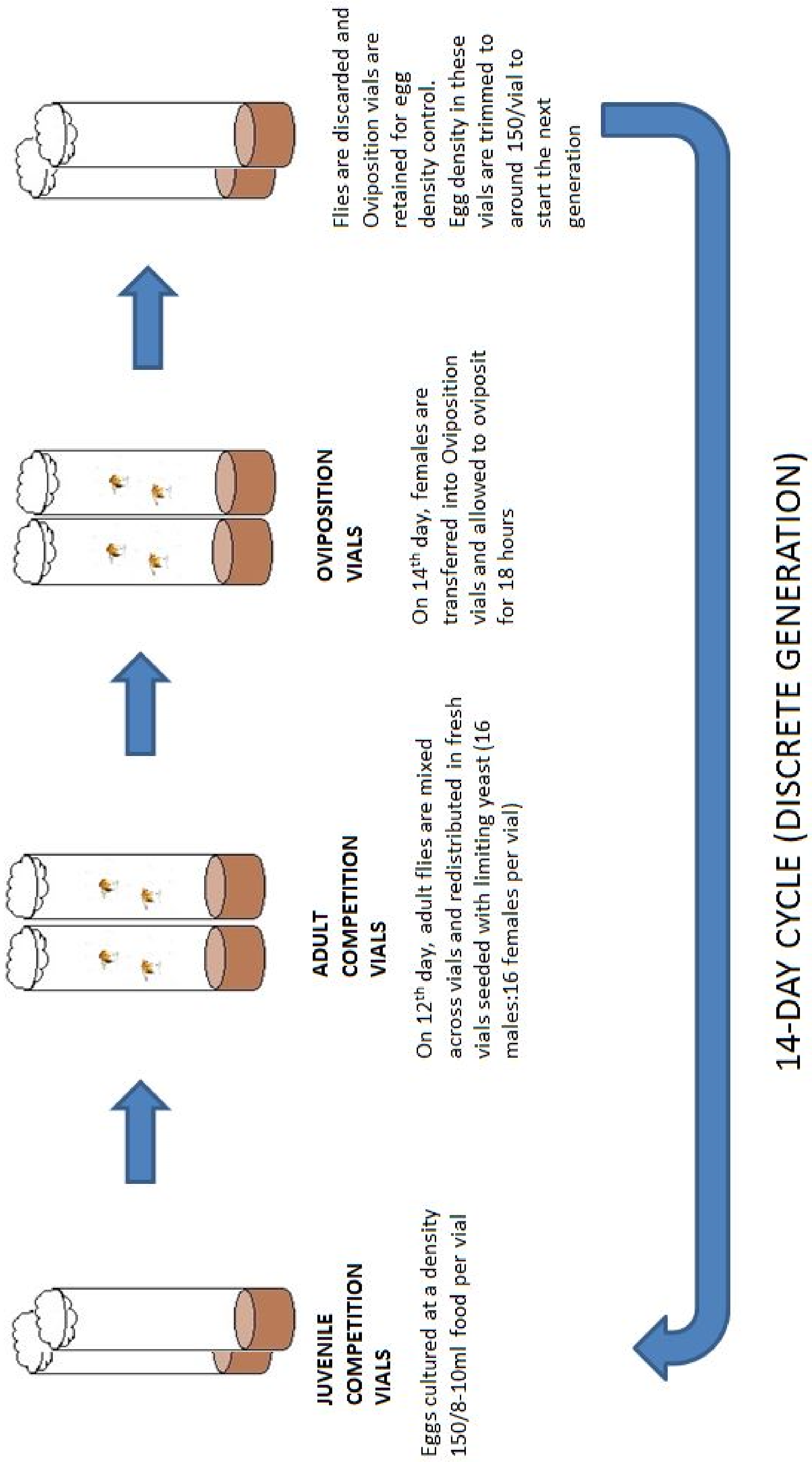

Fig. 2.1: Maintenance protocol for the LHst population.

2.2 Composition of cornmeal food

Sl. No.	Ingredient	Amount (per litre of food)
1	Water (ml)	1100
2	Agar (gm.)	14.8
3	Molasses (ml)	100
4	Baker's Yeast (gm.)	41.2
5	Cornmeal (gm.)	100
6	Propionic acid (ml)	8
7	p-Hydroxymethyl benzoate (gm.)	2.25
8	Ethanol (ml)	22.5

The food is prepared by boiling ingredients $1-5$ to make a thick suspension and the preservatives (ingredients $6-8$) are added.

2.3 Experimental Females

There were 4 types of experimental females: Young High Yeasted (YH), Young Low Yeasted (YL), Old High Yeasted (OH) and Old Low Yeasted (OL). The Young females were 3 day post eclosion and Old females were 13 days post eclosion. The high yeasted females were given $15mg$ yeast / 10 females per vial and low yeasted females were given $5mg$ yeast / 10 females per vial.

Sl. No.	Female type	Age (Days post eclosion)	Nutrition (mg/10 females)
1	YH	3	15
2	YL	3	5
3	OH	13	15
4	OL	13	5

The experimental females were cultured in Cornmeal molasses food. The young females were generated by collecting eggs at a density of 150 eggs / vial. The adult virgin females were collected 10 days later within 6 hours of eclosion which ensures its virginity using light CO_2 anaesthesia. They were kept in single sex vials with a density of 10 females /

vial. On the next day, the flies were distributed into 2 groups and given yeast treatment to generate Young High Yeasted (YH) and Young Low Yeasted (YL) flies by transferring the flies to food vials containing $15mg$ and $5mg$ yeast respectively. The old females were also generated using the same protocol except the eggs were collected 10 days before. They were kept in single sex vials with a density of 10 females / vial till the $11th$ day by changing the food every alternate day. On the $11th$ day after virgin females were collected (same day as the young females were given yeast treatment), the flies were divided into 2 groups and given yeast treatment to generate Old High Yeasted (OH) and Old Low Yeasted (OL) flies by transferring them in food vials containing $15mg$ yeast and $5mg$ yeast respectively.

The sperm limiter females were generated by collecting eggs at a density of 150 eggs / vial, one day before the egg collection for young females. Virgin females were collected by using light CO_2 anaesthesia within 6 hours of eclosion. They were kept in single sex vials at a density of 30 females / vial.

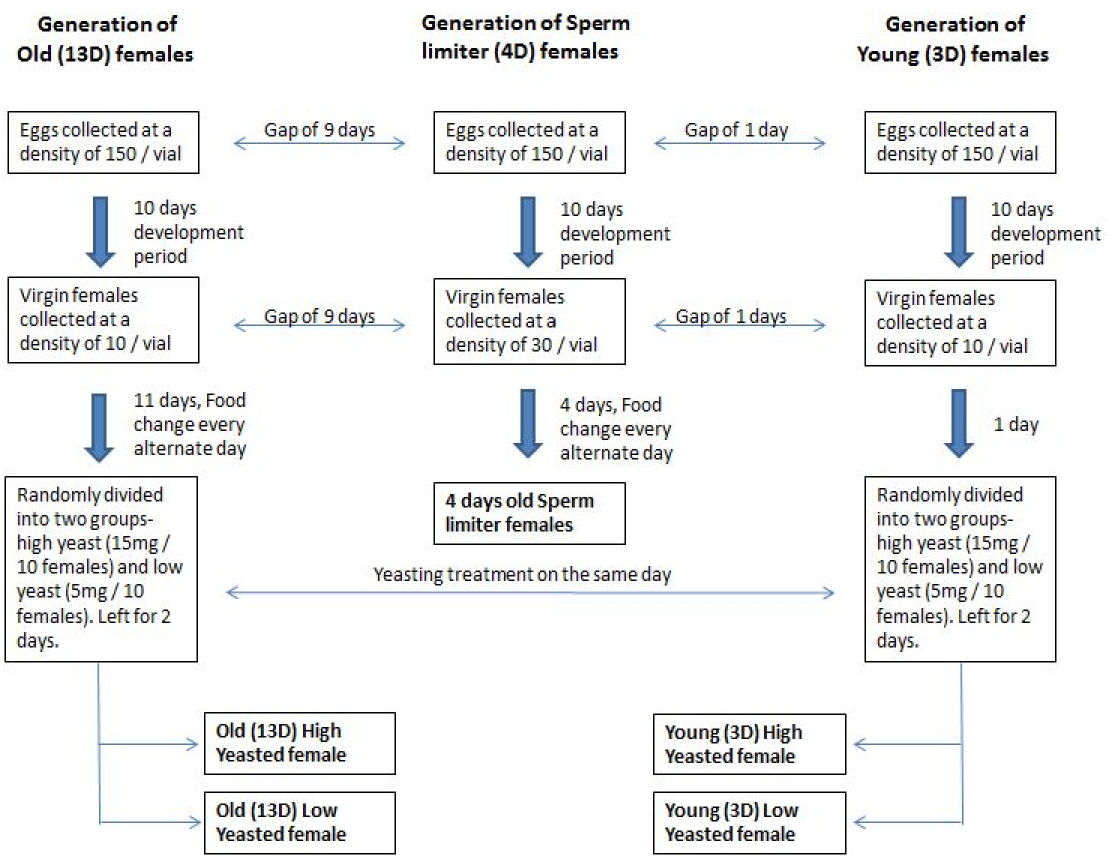

Fig 2.2: Protocol for generation of Experimental females.

2.4 Experimental Males

There were four types of experimental males, two each for male's fitness varying in:

$i)$ Age: Young (YM) and Old (OM) and Young males were 3 days post eclosion and old males were 13 days post eclosion.

The experimental males are cultured in cornmeal molasses food in 12 hour day - 12 hour night cycle with $25°C$ temperature and $60 - 80\%$ relative humidity. To generate the young males, the eggs are collected at a density of 150 eggs / $8 - 10ml$ food per vial. On the $10th$ day the mature virgin males are collected by giving light CO_2 anaesthesia. The eggs for the old males are collected 10 days before the collection of the eggs for the young males. Both the males are collected in single sex vials at a density of 10 males / vial.

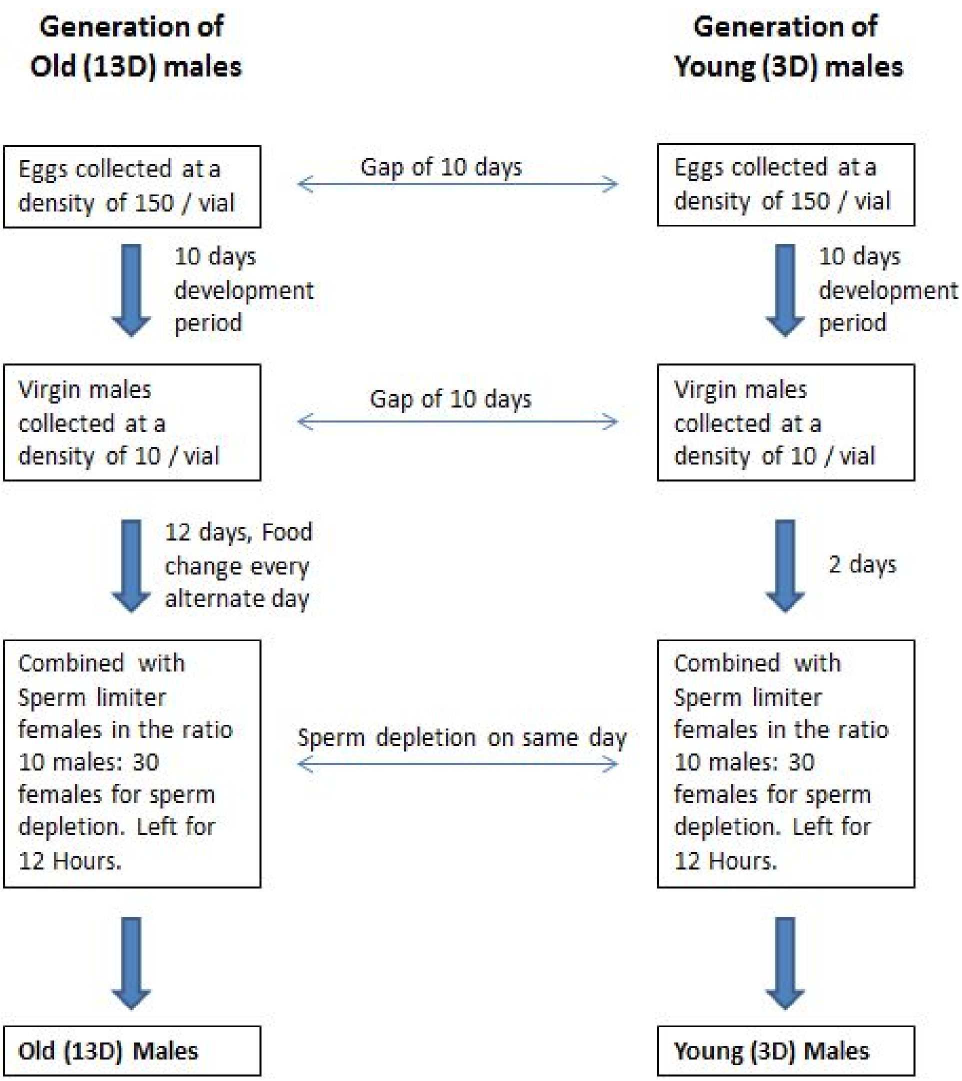

Fig 2.3: Protocol for generation of Young and Old males.

$ii)$ Nutritional status: High quality food (HM) and Low quality food (LM).
High quality food males are kept in cornmeal food after eclosion and Low quality food males are kept in sugar food after eclosion.
To generate the males, the eggs are collected at a density of 150 eggs / vial in cornmeal molasses food in 12 hour day-12 hour night cycle with $25°C$ temperature and $60 - 80\%$ relative humidity. On the $10th$ day, the mature virgin males are collected by giving light CO_2 anaesthesia. The HM males are collected in cornmeal molasses food at a density of 10 males / vial and LM males are collected in sugar food at a density of 10 males / vial. The males are used for experiment 3 days post eclosion.

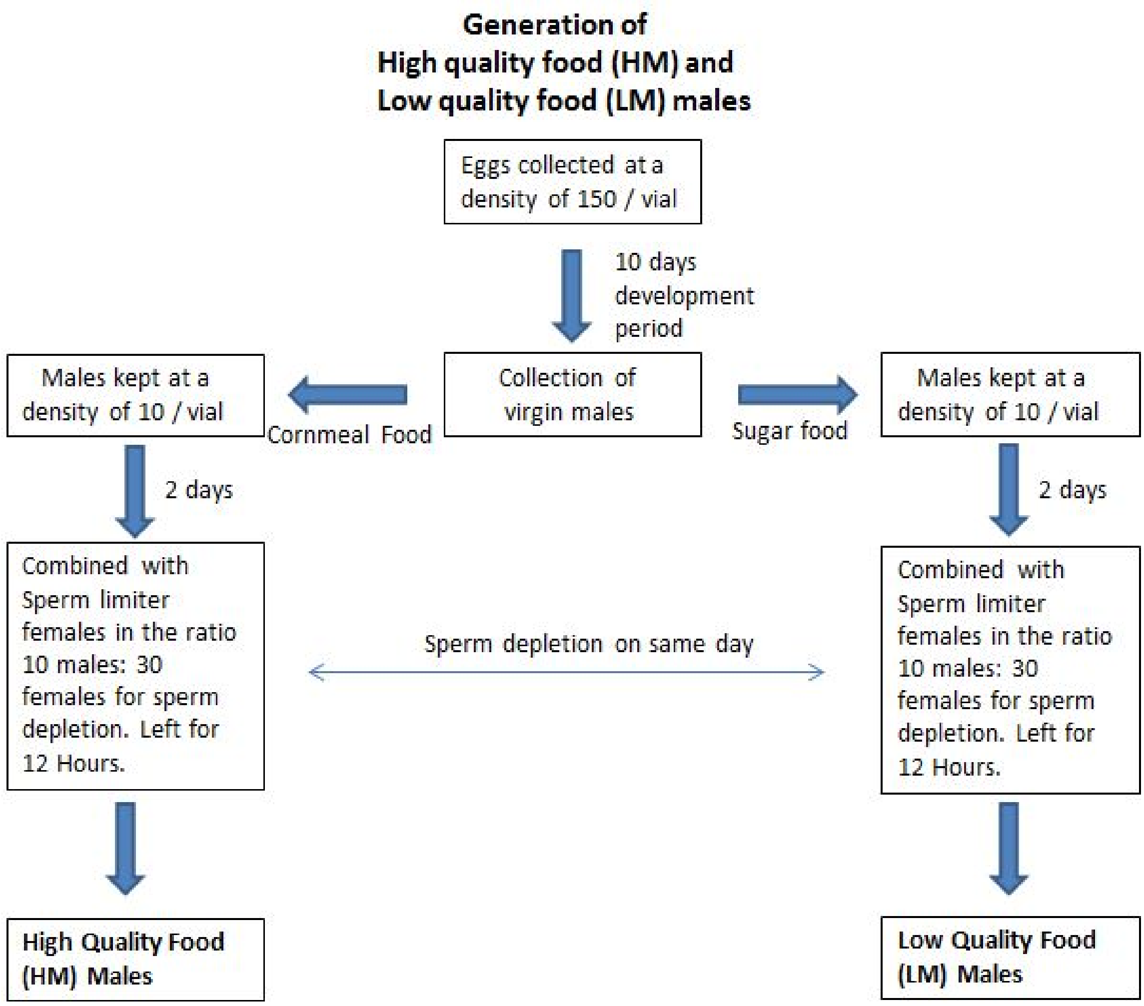

Fig 2.4: Protocol for generation of High quality food (HM) and Low quality food (LM) males.

All four types of males are sperm depleted 12 hours prior to the experiment by keeping them with sperm limiter females mentioned earlier to make them resource limited. They are kept at a ratio of 10 males / 30 sperm limiter females to ensure multiple mating. The males are separated by aspirating out each male and transferred to experimental vials.

2.5 Experiment 1: Effect of male's age on male mate choice

2.5.1 Choice Experiment:

The Young males (YM) and Old males (OM) are given a choice of two females which have different fitness. There were 2 combinations each for the Young males (YM) and Old males (OM). The females were coloured with green and pink food colour 12 hours prior to the experiment so that they can be identified during the experiment. There was also reverse colouration to ensure that the choice of the males is because of the females fitness and not because of the colour.

Sl. No.	Males	Females
1	Young Male (YM)	Young High Yeasted (YH) + Old Low Yeasted (OL)
2	Young Male (YM)	Young Low Yeasted (YL) + Old High Yeasted (OH)
3	Old Male (OM)	Young High Yeasted (YH) + Old Low Yeasted (OL)
4	Old Male (OM)	Young Low Yeasted (YL) + Old High Yeasted (OH)

The males and females were combined in the ratio of 1 male: 2 females in each vial. The males and females were allowed to interact for 1 hour. The female that the male mates is considered as success. The mating latency (time taken to mate from observation start time) and copulation duration (time taken to mate) was noted down.

2.5.2 No choice experiment:

The experiment was performed to check if there was any effect of the females on the mating latency or copulation duration. Both young and old males (YM and OM) are combined with all the four types of females in the ratio 1 male: 1 female. They were allowed to interact for 1 hour and the mating latency and copulation duration was noted down.

Sl. No.	Male	Female
1	Young Male (YM)	Young High Yeasted (YH)
2	Young Male (YM)	Young Low Yeasted (YL)
3	Young Male (YM)	Old High Yeasted (OH)
4	Young Male (YM)	Old Low Yeasted (OL)
5	Old Male (OM)	Young High Yeasted (YH)
6	Old Male (OM)	Young Low Yeasted (YL)
7	Old Male (OM)	Old High Yeasted (OH)
8	Old Male (OM)	Old Low Yeasted (OL)

2.6 Experiment 2: Effect of male's nutritional status on male mate choice

The High quality food (HM) males and Low quality food (LM) males were given a choice of two females same as in Experiment 1. The females were coloured with green and pink food colour. The males and females were allowed to interact for 1 hour. The mating latency and copulation duration was noted down and the mated female is considered success.

Sl. No.	Males	Females
1	High quality food (HM)	Young High Yeasted (YH) + Old Low Yeasted (OL)
2	High quality food (HM)	Young Low Yeasted (YL) + Old High Yeasted (OH)
3	Low quality food (LM)	Young High Yeasted (YH) + Old Low Yeasted (OL)
4	Low quality food (LM)	Young Low Yeasted (YL) + Old High Yeasted (OH)

2.7 Data Analysis

The mating latency and copulation duration was analysed using mixed model ANOVA. Binomial test were done to see the preference of the males for the higher fitness females. The raw data was converted into choice score using the equation:

$$CS = P/(P + Q)$$

Where, P is the proportion of higher fitness females mated and Q is the proportion of lower fitness females mated. Choice score $= 0.5$ is taken to be a no choice scenario.
The choice score was taken to perform a one sample $t - test$ to see the preference of males towards the higher fitness females. Logistic regression was also done to see the preference of young and old males. The analysis was done using $JMP\ 7,\ R$ and $Sigma\ Plot$.

Chapter 3

Results

3.1 Experiment 1

From the choice experiment, it can be deduced that the males generally show the preference for higher fitness females (Fig 3.1). The young females always show the preference for higher fitness female as we see consistent significant effect in both binomial test ($Table$ 3.1) and one sample $t-test$ ($Table$ 3.2). The old males also show a significant preference for the higher fitness female when the fitness difference between the females is high but doesn't show significant preference for the higher fitness females when the fitness difference is low. When performed a logistic regression for the mate choice of young and old males, both males show preference for the higher fitness females in both the combinations (Fig 3.2 and 3.3).

TREATMENTS		Young male YH/OL female	Young male YL/OH female	Old male YH/OL female	Old male YL/OH Female
1	P value	0.0082*	0.0091*	0.1487	0.0011*
	Choice Score	0.649123	0.644068	0.559322	0.6896552
2	P value	0.000026*	0.0011*	0.0026*	0.4999
	Choice Score	0.759259	0.689655	0.672414	0.4915254
3	P value	0.0018*	0.114	0.022*	0.0000095*
	Choice Score	0.677966	0.571429	0.625	0.7627119
Pooled	Choice Score	0.694118	0.632184	0.618497	0.6477273

Table 3.1: Summary of Binomial test and Choice Scores (CS) to see the effect of age (p-values marked with * represents statistically significant values).

	Young male YH/OL female	Young male YL/OH female	Old male YH/OL female	Old male YL/OH female
P ($T_{cal} > T_{crit}$)	0.004059349*	0.0172046*	0.022225792*	0.141813483

Table 3.2: Summary of one sample t-test for the Choice Scores (CS) to see the effect of age (p-values marked with * represents statistically significant values).

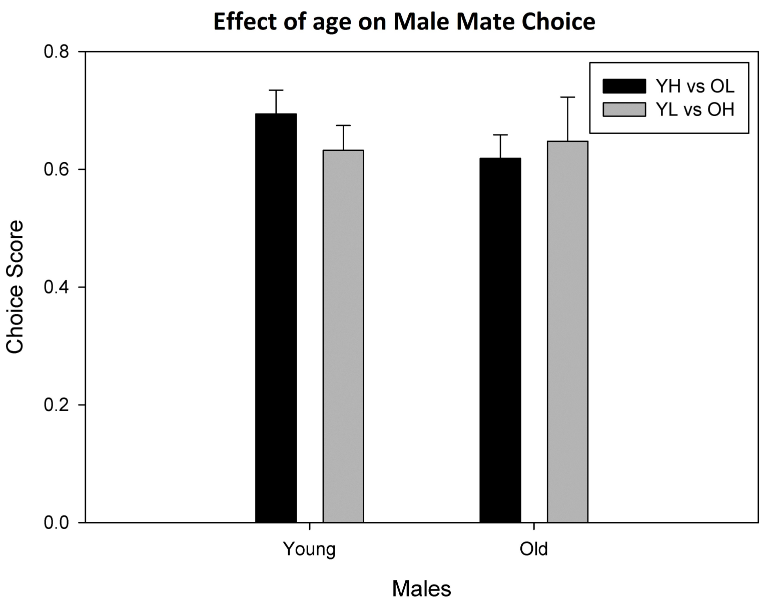

Fig 3.1: Effect of Male's Age on Male Mate Choice

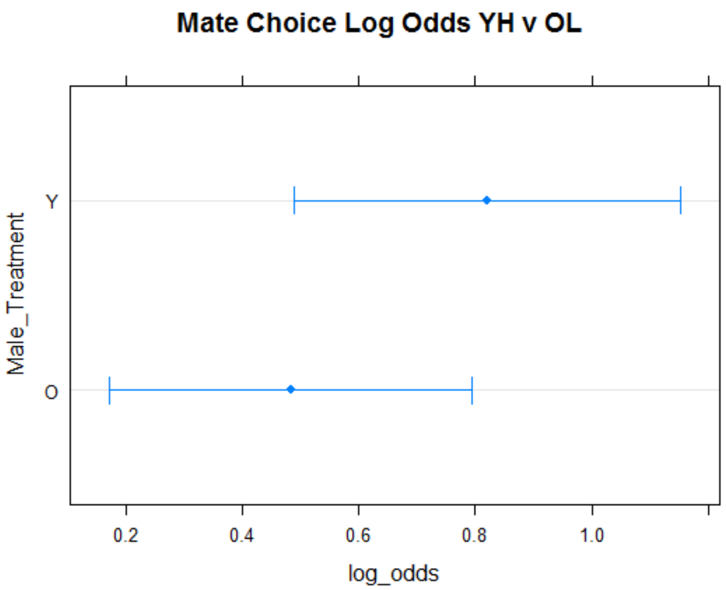

Fig 3.2: Mate choice log odds for young and old males towards YH and OL females.

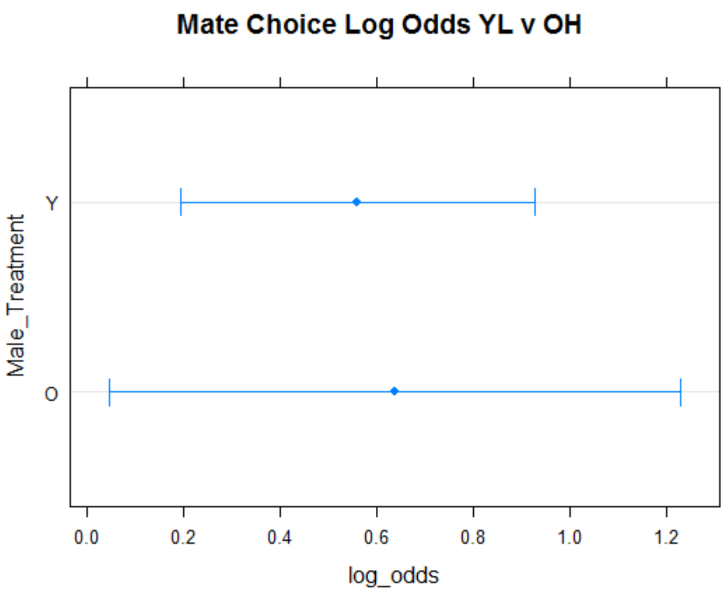

Fig 3.3: Mate choice log odds for young and old males towards YL and OH females.

Neither the males nor females had any effect on the mating latency (time taken to initiate mating) and copulation duration (time for which the copulation lasted) (Table 3.3 and table 3.4).

Source	SS	MS Num	DF Num	F Ratio	Prob > F
Male	118.543	118.543	1	1.7747	0.3142
Female	62.5772	20.8591	3	0.1394	0.9328
Male*Female	116.728	38.9093	3	0.2166	0.8815
Trial & Random	83.0714	41.5357	2	1.0681	0.8158
Male*Trial & Random	113.624	66.8118	2	0.3916	0.6915
Female*Trial & Random	900.204	150.034	6	0.8331	0.5849
Male*Female*Trial & Random	1080.58	180.097	6	3.8412	0.0009*

Table 3.3: Summary of ANOVA for mating latency for effect of age on male mate choice (p-values marked with * represents statistically significant values).

Source	SS	MS Num	DF Num	F Ratio	Prob > F
Male	506.839	506.839	1	8.6324	0.0989
Female	116.069	38.6898	3	0.7997	0.5375
Male*Female	207.675	69.2249	3	1.6042	0.2840
Trial & Random	1020.51	510.253	2	8.0148	0.1328
Male*Trial & Random	117.584	58.7921	2	1.3835	0.3138
Female*Trial & Random	290.606	48.4343	6	1.1214	0.4464
Male*Female*Trial & Random	259.135	43.1892	6	1.2923	0.2584

Table 3.4: Summary of ANOVA for copulation duration for effect of age on male mate choice.

From the no choice experiment, it can be deduced that the females have an effect on the mating latency (Table 3.5) and that the Young High Yeasted (YH) females mate readily than the Old Low Yeasted (OL) females (Fig 3.4).

Source	N parm	DF	SS	F Ratio	Prob > F
Male	1	1	59.1022	0.866979	0.352435
Female	3	3	744.7631	3.64168	0.013017*
Male*Female	3	3	283.2654	1.385087	0.247079

Table 3.5: Summary of ANOVA for mating latency for no choice experiment (p-values marked with * represents statistically significant values).

Source	N parm	DF	SS	F Ratio	Prob > F
Male	1	1	0.000031	0.0000	0.9988
Female	3	3	65.204831	1.4824	0.2190
Male*Female	3	3	33.505774	0.7618	0.5162

Table 3.6: Summary of ANOVA for copulation duration for no choice experiment.

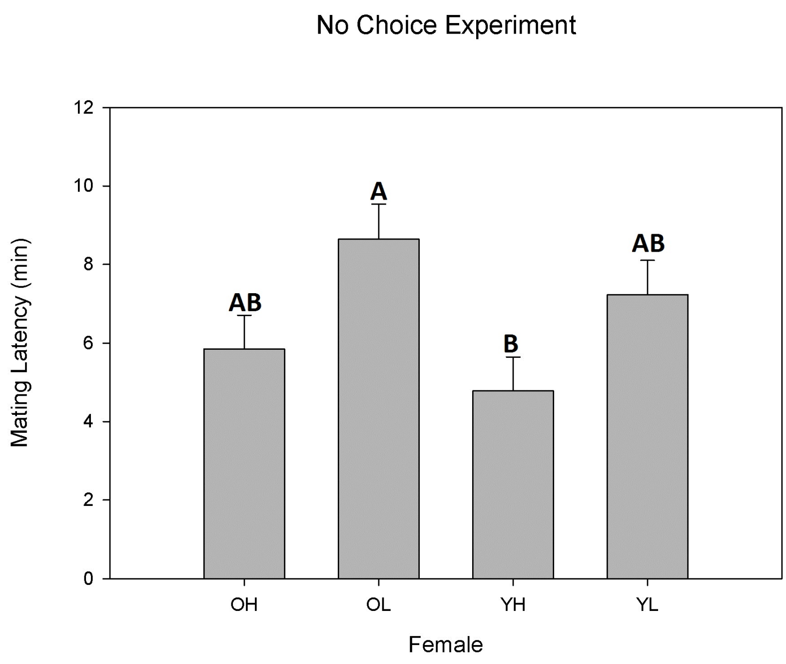

Fig 3.4: Effect of females on the mating latency for a no choice experiment.

3.2 Experiment 2

From the experiment, it can be deduced that both High quality food (HM) males and Low quality food (LM) males prefer the higher quality females ($Figure$ 3.5). The preference is significant when fitness difference between the females is high but the preference isn't significant when the difference in quality between the females is low ($Table$ 3.7).

TREATMENT 1	HM male YH/OL female	HM male YL/OH female	LM male YH/OL female	LM male YH/OL female
P value	0.0379*	0.144	0.00043*	0.347
Choice Score	0.611111	0.561404	0.706897	0.5172414

Table 3.7: Summary of Binomial test and Choice Scores (CS) to see the effect of nutritional status (p-values marked with * represents statistically significant values).

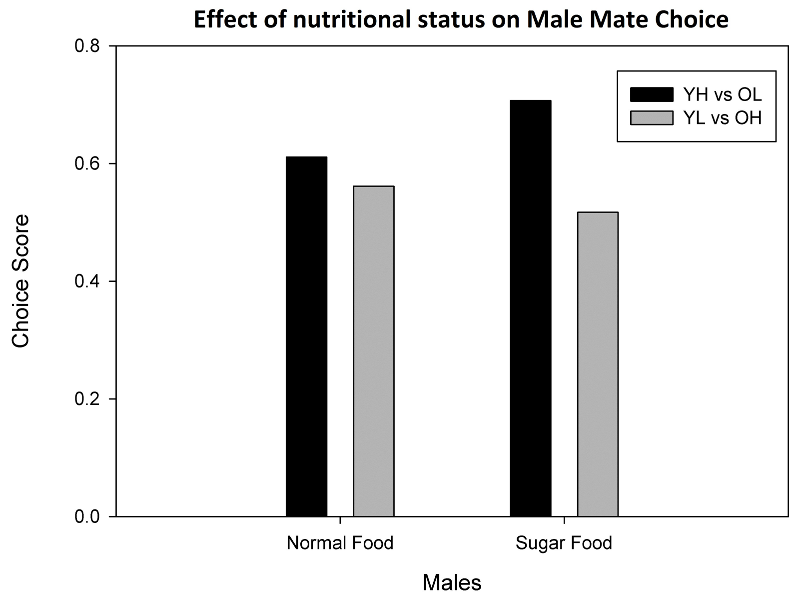

Figure 3.5: Effect of Male's Nutritional Status on Male Mate Choice.

Source	N parm	DF	SS	F Ratio	Prob > F
Male	1	1	4.77187	0.1524	0.6966
Female	3	3	143.84543	1.5312	0.2073
Male*Female	3	3	24.22469	0.2579	0.8557

Table 3.8: Summary of ANOVA for mating latency for effect of nutritional status on male mate choice.

Source	N parm	DF	SS	F Ratio	Prob > F
Male	1	1	3.45415	0.1724	0.6784
Female	3	3	117.12320	1.9483	0.1227
Male*Female	3	3	70.24903	1.1686	0.3226

Table 3.9: Summary of ANOVA for copulation duration for effect of nutritional status on male mate choice.

Chapter 4

Discussion

When the males are kept with different types of female in a no choice condition, the females have an effect on the mating latency with the Young High Yeasted (YH) females mating relatively faster than the other three types of females and Old Low Yeasted (OL) females mating relatively slower than the other females. From the Binomial Test, we can see that Young males show a preference for the higher fitness females in all the three treatments when given a choice between YH and OL females but the preference isn't consistent for all three treatments in case of the YL and OH females. Similarly, there was no consistent preference for the higher fitness females by the Old males in both combinations. Since the preference shown by the Young males can be an effect of the females, we can't say that it is due to male mate choice.

But from the one sample $t - test$, we can see that there was preference for the higher fitness females by the Young males and Old males (when the fitness difference is high). So, we can conclude that the Young males show the preference for the higher fitness females as they show the preference even when the fitness difference between the females are low but it can't be concluded that the Old males show preference for the higher fitness female in YH/OL as it can be female effect.

Also, from the logistic regression data, we can see that all the males show significant preference for the higher fitness females as the Mean $+/-$ CI doesn't include the value 0 in the log odds ratio (if 0 is included, it would mean that there is no preference for the higher fitness female). Taking all the analysis into consideration there is no solid conclusion as the result differs for different analysis.

For the effect of nutritional status on the male mate choice, we can see that both males show preference for higher fitness females when the fitness difference is high but there is no such preference when the fitness difference is low. But, we can't conclude anything as there was only one treatment.

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