Loyal or promiscuous: A search for female mate preference in *Drosophila melanogaster*

A thesis submitted towards partial fulfillment of

BS-MS dual degree programme

by

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Certificate

This is to certify that this dissertation entitled **"Loyal or promiscuous: A search for female mate preference in *Drosophila melanogaster*"** towards the partial fulfillment of the BS-MS duel degree programme at the Indian Institute of Science Education and Research Pune, represents original research carried out by **Susheel Kumar** at **Dept of MRDG, Indian Institute of Science, Bangalore,** under the supervision of **Dr. Upendra Nongthomba, Assistant Professor, Dept of MRDG, IISc, Bangalore,** and **Dr. N. K. Subhedar, Professor, IISER, Pune** during the academic year **2010-2011.**

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Abbreviations: **MS** (females remating with the same male);

MD (females remating with a different male);

DNM (females that did not remate);

M_A and M_B (groups of males in loyalty test assay);

F_A and F_B (groups of females in loyalty test assay);

MS^2 (The progeny of MS which remated with the same mate during remating assay);

MD^2 (The progeny of MD which did not remate with the same mate during remating assay); **MS[MD]** (The progeny of MS which did not remat with the same mate during remating assay); **MD[MS]** (The progeny of MD which remated with the same mate during remating assay);

MS^4 (The progeny of MS^2 after two generations);

MD^4 (The progeny of MD^2 after two generations);

MS^5 (The progeny of MS^4 that remated with the same mate during remating assay);

MD^5 (The progeny of MD^4 that did not remate with the same mate during remating assay); **MS^4[MD]** (The progeny of MS^4 that did not remate with the same mate during remating assay); **MD^4[MS]** (The progeny of MD^4 that remated with the same mate during remating assay)

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Abstract.

In *Drosophila*, females are generally known to be promiscuous. Several studies suggest that the phenomenon of polyandry has a selection advantage caused by stress conditions induced by sex-linked meiotic drive and other selfish sex ratio-distorting elements during evolution. In contrast, females are known to recognize and mate with conspecific males from amongst members of different species and races. In this study, assuming a genetic basis for mate preference, using selection studies, we tried to amplify the percentage of monandrous females and establish two separate lines of females that chose to remate with the same male and those that do not. We selected the females which did, or did not, prefer to mate with the same male, for two generations. We then allowed these selected females to inbreed for three more generations and found that though there was an increase in the frequency (from 0.35 to 0.55) of females choosing to remate with the same male in the fifth generation as compared to first generation, no significant difference in the mate preference by females in the fifth generation between the two selected lines was observed. These findings suggest that the selection experiments need to be performed on a greater number of generations to get conclusive results or that there may be no genetic basis for loyalty in females for mate choice in *Drosophila melanogaster*.

Introduction

Ever since Thomas Hunt Morgan discovered the *white* gene, responsible for white eyes in *Drosophila* in 1910, *Drosophila* as a model system has aided in understanding genetic as well as complex biological phenomenon that have been eluding biologists[1]. Many behavioural traits like phototaxis, geotaxis, aggression, mate choice copying etc. have been studied in *Drosophila* [2,3,4,5]. Courtship behaviour has been well studied and documented from the early days of *Drosophila* research [6,7,8,9]. The mating ritual of *Drosophila* involves the male orienting itself in the direction of the female for the visual as well as olfactory stimulus. It then gently taps the female to assess the taste stimulus and later it starts with the courtship song by beating its wings to influence the female with auditory and visual cues. The male then licks the female genitals and then mounts and copulates with the female[10]. The female can thwart and reject a male by ignoring, kicking, walking away from the male, or extruding its ovipositor. It can also decide whether or not to open its vaginal plate for copulation to occur [11,12]. All these signals involved in the courtship indicate the possibility of recognition of a mate by females using one or more of the stimuli before mating with a particular mate.

Females which have been primed to the courtship song of males are more receptive as compared to the un-primed females, suggesting that females can be sensitized and they can learn to distinguish between conspecific males [13,14]. Previous studies show that visual as well as non visual sensory cues could modulate the preference of females to mate with a particular male [4,7].

Cuticular hydrocarbons and accessory gland proteins play an important role in courtship behaviour of the flies by influencing the receptivity of females [15,16,17].

Acoustic and pheromonal tags help to regulate sex and species recognition in *Drosophila* [18]. As individual flies can regulate their hydrocarbon display, these compounds are believed to help in individual recognition in flies [19].

Mating studies on *Drosophila* have suggested sexual isolation between different species, geographic races and mutants [8,20], indicating the capability of flies to recognize and mate with conspecific members. Experiment on *Drosophila simulans* in increasing the percentage of mate preference of females for *ebony* males in a population by selecting in subsequent generations, suggests that the occurrence of a particular mate choice in a population can be amplified by selection [21].

The sexual conflict like cost of mating between the male and the female in insects leads to cycles of antagonistic co-evolution of polyandry and monoandry. Monandry can be caused when female is resistant to remating with other males or due to male imposed monogamy with the help of mating plug, nuptial gifts or the transfer of antiaphrodisiacs. Polyandry can be beneficial to females whenever there is possibility of sperm depletion or genetic incompatibility of males [22]. Monandry has also been a very strong reason for the evolvement of eusociality due to kin selection in many insects like in bees and wasps [23].

Though females of *Drosophila* are generally thought to be polyandrous and promiscuous [24,25,26,27,28], we hypothesised that females can recognise and remate with the same male and we went ahead to look whether this trait of females being loyal to a mate, has a genetic basis to it or is it just an acquired behavior. We also tried to amplify the frequency of these loyal females in the population by subjecting them to selection experiments. We define loyalty in females as a mate

choice trait, wherein the female prefers to remate with the same male when given a choice to mate with either the same male or a new male.

Results

The total sample size of the females that were subjected to loyalty tests for selection is given in Table 1.

The males marked by trimming of the posterior bristles on the scutellum, were not preferred over unmarked males by virgin females. The experiment was performed with 143 virgin females, wherein 66 females chose marked males and 77 chose unmarked males. The χ^2 analysis assuming equal preference of females for both marked and unmarked males showed no significant difference (with $\chi^2 = 0.846$, and $p = 0.3576$).

The results of ANOVA on MS^4 and MD^4 comparing between percent mating frequencies are shown in Fig. 2. (Table. 2.) The mating frequencies were normalized using the $Sin^{-1}[(\text{mating frequency})^{1/2}]$ treatment. The factors involved were the female mate preference for remating: MS (females remating with the same male), MD (females remating with a different male) or DNM (females that did not remate). No significant difference was observed between mate choice of females between MS^4 and MD^4.

The results of ANOVA on MS, MD and DNM comparing percent mating frequencies in the fourth of MS^4 and MD^4 is shown in Fig. 3. (Table. 2.) There was a significant difference observed, after doing Post-hoc using Tukey HSD, between MS, MD and DNM (with $p < 0.03$) suggesting that the females MS line had a greater probability of remating as compared to MD and DNM (Table. 3.).

Discussion

Results from the control experiment suggest that there is no difference in mate preference between marked and unmarked males by the female. This method serves as an advantage, as there is no involvement of hydrocarbons or alcohols like in paints[29], and the use of feeding the flies with different colored food[4] that are widely used to differentiate between different individuals. This method of marking can be used to distinguish between two males without influencing the female's preference for mating.

Time taken for the copulation to occur can also be used a measure for the female mate preference during remating when a single male is introduced along with the female[30]. In our experiment as two males were introduced, the female mate choice preference during remating was confirmed by directly ascertaining the identity of the male under the microscope. Due to experimental constraints the selection pressure applied on females to test loyalty, using only two males to choose from, can be considered as weak. In future studies more number of males can be introduced during remating experiments for the female to choose from.

An increase in frequency of females mating with the same mate in MS^4 (0.55) as compared to those in Generation 1 (0.35) was observed. This result is similar to the study by Sharma M D *et al.* showing the increase in the frequency of the females mate choice towards a particular mate (ebony females choosing to mate with ebony males) in a population[21]. This result suggests a possibility that the frequency of these loyal females could be increased in a population by applying selection pressure on a greater number of generations. There was also an increase of frequency of

females preferring to remate with the same male in MD^4, for which we could not find a conclusive answer.

The non-significant difference between mate preference of females of MS^4 and MD^4 lines (Fig. 2) suggests that either the selection experiments on generations have to be conducted on a greater number of generations to have two separate lines of females or that there is no genetic correlation for the loyalty factor that we tested. The significant difference in the remating behavior of females among MS, MD and DNM group of MS^4 and MD^4 suggests that females of MS have higher remating probability.

As the present study was a pilot experiment and included many constraints. The relaxation of selection on the third and fourth generations was due to technical difficulties that arose while performing the experiment. So the flies were allowed to inbreed and selection experiments not performed on them. There needs to be an increase in the sample sizes when the selection experiments are done on larger generations to rule out the discrepancies that might arise due to genetic drift while sampling. As only the females of a particular phenotype (MS or MD) are used to populate and generate the two different lines, one can expect that the genetic drift as well as founder effect arising due to lower sample sizes. There can also be reverse evolution of selection due to relaxation of selection pressure [31,32].

Materials and Methods

***Drosophila* Strains.** The wild type strain of *Drosophila melanogaster*, Canton S, were cultured at 25° C room, 12 hours Light: 12 hours Dark cycle, using Yeast-agar-semolina-jaggery media along with propionic acid to retard the growth of mold.

Marking. Virgin males and females were isolated from the parent population of *Drosophila melanogaster* in the pupal stage**.** The males were divided into two groups. Males from one group were marked for distinction, by trimming the posterior bristles on the scutellum, on the second day post-eclosion under the influence of ether. The other group of males was also given ether treatment. The males and females were allowed to mature for four days. Four days post eclosion, two males, one from each group, was introduced into a vial along with a female. The female preference for the mate choice was checked by looking at the male it mates.

Loyalty test assay. (Fig . 4) Males and females were collected in separate vials in the pupal stage from the parent stock, P. Two days post-eclosion males and females were divided into two groups each: M_A and M_B; F_A and F_B. Male flies from one group, M_A, were marked for distinction by trimming the posterior bristles on the scutellum. Ether was used to anaesthetise the males before trimming. Both groups of males received ether treatment. Flies were allowed to mature for 3-4 days post-eclosion. On the fourth day, single pair mating of M_{Ai} X F_{Ai} (where i= 1 to n) was carried out by their introduction into a food vial, whose volume was decreased with the help of cotton plug. They were allowed to mate and were kept together. After 12-14 hours the flies were aspirated out and were individually maintained in separate vials for the next 10 days to maximize the frequency of remating [30,33,34], by transferring them into new vials every 3 days. The single pair mating experiment was carried out for M_{Bi} X F_{Bi} flies too. After 10 days of the first round of mating, females of either A or B, were

chosen, and the females from other group were discarded. The female were then introduced in a fresh food vial with the mate it mated with before along with a male from the other group F_{Ai} X M_{Ai} X M_{Bi}. The mate choice of the female was checked during the remating by aspirating the male it mated with and examining its marking under a microscope. The females that chose the same mate, which they mated with before and were kept together, they were collected and labelled MS and those that chose a different mate were labelled MD.

Selection lines. Parent population of wild type strain of *Drosophila melanogaster*, Canton S, flies were allowed to populate and were subjected to the loyalty test. The MS and MD lines were obtained from the parent population. The progeny from MS and MD lines were subjected to loyalty tests. The progeny of MS which remated with the same mate during remating assay were termed MS^2, and those that didn't remate with the same mate were labelled MS[MD]. Similarly the progeny of MD that mated with a different mate during the remating were labelled MD^2 and those that remated with the same mate were labelled MD[MS]. MS^2 and MD^2 were allowed to populate for another two generations there were no selection experiments done on them. After two generations, the MS^4 and MD^4 were subjected to modified loyalty test of females. These females were given a choice during remating to mate either with the male it mated before or with a male from the parent population P. The progeny of MS^4 that remated with the same mate were labelled MS^5 and those that chose a different mate were labelled MS^4[MD]. The progeny of MD^4 that remated with the same male they mated before were labelled MD^4[MS] and those that mate with a different male were labelled MD^5.

Statistical analysis. ANOVA was done using STATISTICA 8.0, copyright Stat Soft, Inc. ANOVA was done at two levels for selected lines, MS^4 and MD^4, and three

levels for mating type, MS, MD and DNM. Both selected lines and mating type were fixed.

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Figure legends.

Fig. 1. The mating frequency* distribution of the flies during subsequent generations

* The mating frequencies were normalized to the interval [0, 1].

Fig. 2. ANOVA on MS^4 and MD^4

*The mating frequencies were normalised to the interval [0, 1] and $Sin^{-1}[(\text{mating frequency})^{1/2}]$ treatment.

Fig. 3. ANOVA on MS, MD and DNM of the MS^4 and MD^4

*The mating frequencies were normalised to the interval [0, 1] and $Sin^{-1}[(\text{mating frequency})^{1/2}]$ treatment.

Fig . 4. Schematic representation of the Loyalty test.

Tables.

Table. 1. Sample size of the females*

	MS (Remated same)	MD (Remated different)	DNM (Disnot remate)	Total females
Generation 1	47	48	36	131
Generation 2 MS	20	15	22	57
Generation 2 MD	9	12	4	25
Generation 5 MS^4	21	8	9	38
Generation 5 MD^4	20	9	5	34

* Generation 1 with four replicates, generation 2 MS with two, generation 2 MD with only one, generation 5 $MS^4$and MD^4 with 2 replicates each. The replicates were of unequal sizes.

Table. 2. ANOVA table of MS, MD and DNM of the MS^4 and MD^4

Effect	*Mean square*	*Degrees of freedom*	*F-value*	P
Selected lines (MS^4 and MD^4)	0	1	0.017	0.889
Mating type (MS, MD and DNM)	0.152	2	8.887	.016*
Selected lines X Mating type	0.027	2	1.589	0.279

* The mating frequencies were normalised to the interval [0,1] and the given $Sin^{-1}[(\text{mating frequency})^{1/2}]$ treatment for conducting ANOVA

Table. 3. ANOVA table for MS, MD and DNM of the MS^4 and MD^4 after conducting Post-hoc using Tukey HSD test

Tukey HSD test; variable Var3 (Spreadsheet5) Approximate Probabilities for Post Hoc Tests
Error: Between MSE = .01708, df = 6.0000

	Var2	{MS} - .82745	{MD} - .48727	{DNM} - .49294
1	MS		0.024149	0.025926
2	MD	0.024149		0.998000
3	DNM	0.025926	0.998000	

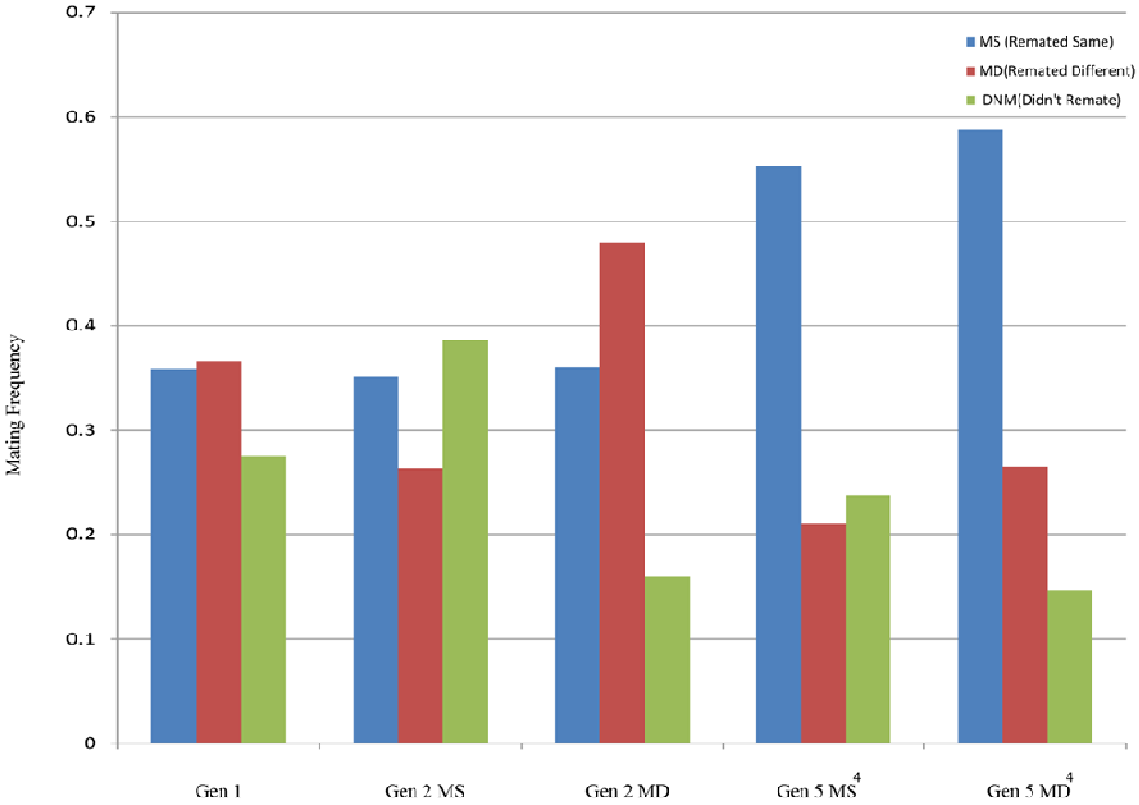
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Mating Frequency
MS (Remated Same)
MD(Remated Different)
DNM(Didn't Remate)
Gen 1
Gen 2 MS
Gen 2 MD
Gen 5 MS4
Gen 5 MD4

Fig. 1.

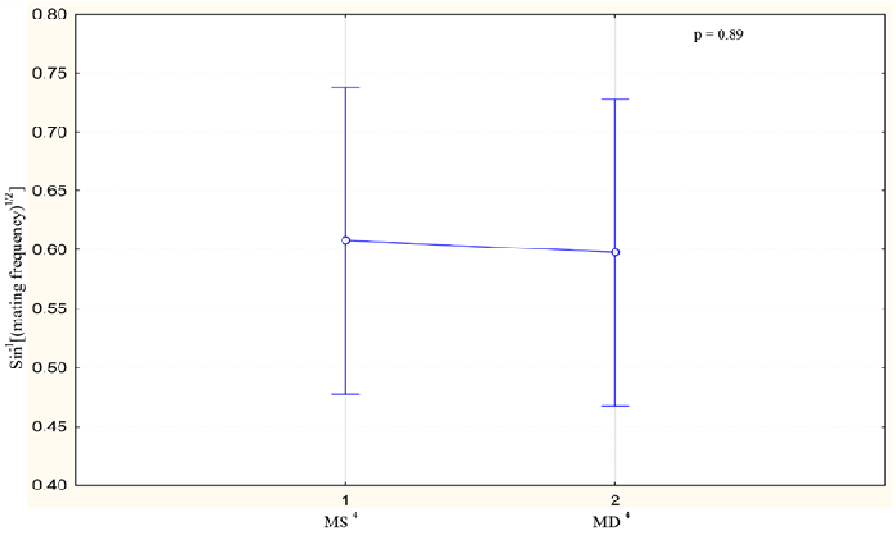
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
p = 0.89
Sin[(mating frequency)1/2]
1
MS 4
2
MD 4

Fig. 2.

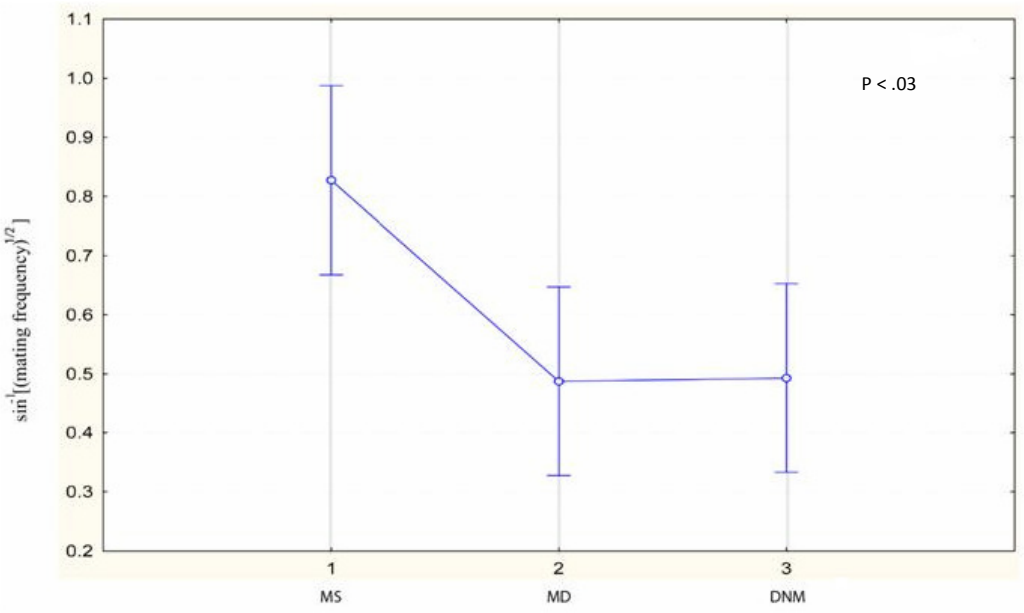
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
sin-1[(mating frequency)1/2]
P < .03
1
MS
2
MD
3
DNM

Fig. 3.

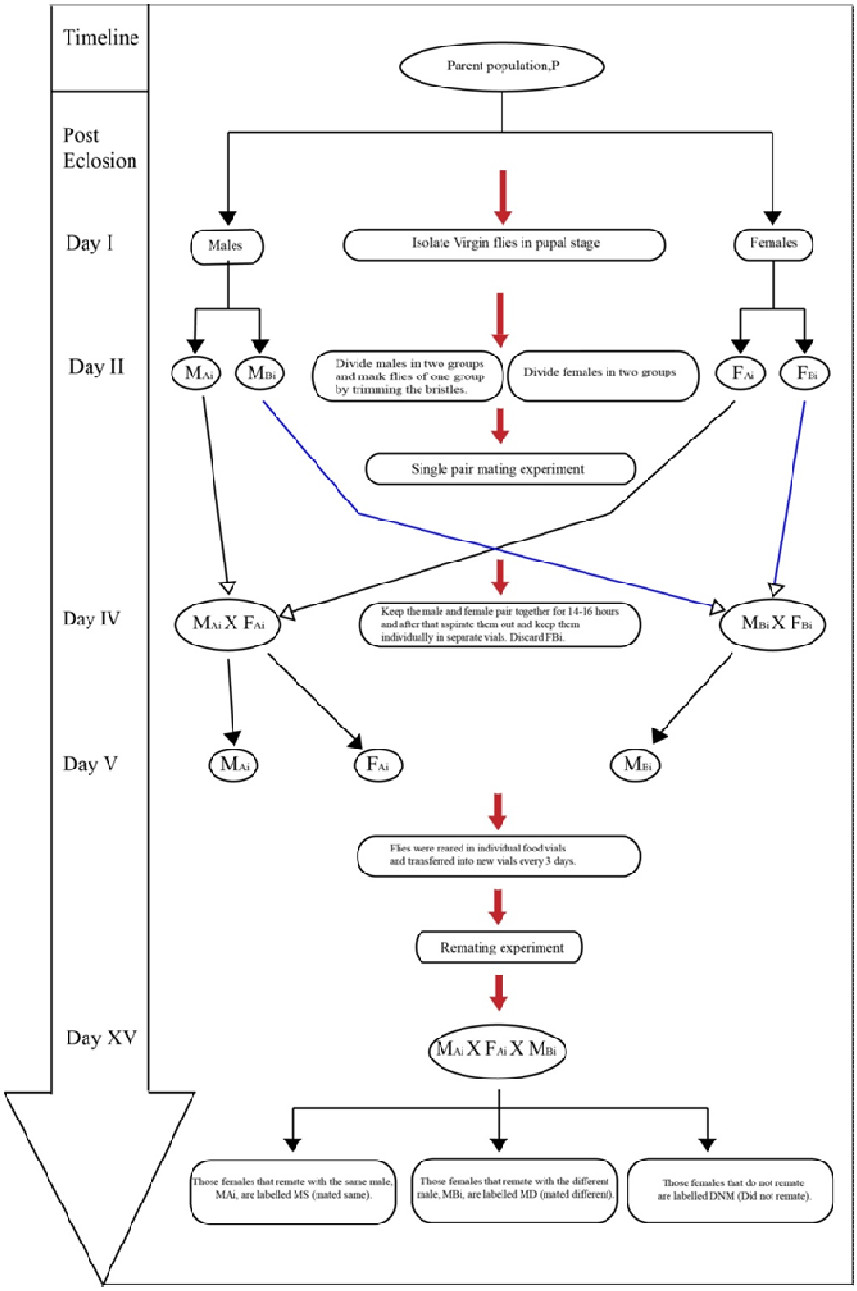
Timeline
Post Eclosion
Day I
Day II
Day IV
Day V
Day XV
Parent population,P
Males
Isolate Virgin flies in papal stage
Females
MAi
MBi
Divide males in two groups and mark flies of one group by trimming the bristles.
Divide females in two groups
FAi
FBi
Single pair mating experiment
MAi X FAi
Keep the male and female pair together for 14-16 hours and after that aspirate them out and keep them individually in separate vials. Discard FBi.
MBi X FBi
MAi
FAi
MBi
Flies were reared in individual food vials and transferred into new vials every 3 days.
Remating experiment
MAi X FAi X MBi
Those females that remate with the same male, MAi, are labelled MS (mated same).
Those females that remate with the different male, MBi, are labelled MD (mated different).
Those females that do not remate are labelled DNM (Did not remate).

Fig. 4.