Investigating role of mitochondrial DNA in mate harm resistance.

By

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

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Certificate of Examination

This is to certify that the dissertation titled "**Investigating role of mitochondrial DNA in mate harm resistance**" submitted by **Mr. Ankuj Kumar (Reg. No. MS14080)** for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Manjari JainDr. R. R. Choudhury Dr. N. G. Prasad

(Supervisor)

Dated: April 26, 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 contributions of others are involved, every eff ort 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.

Ankuj Kumar

(Candidate)

Dated: April 26, 2019

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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INTRODUCTION

For the longest time, the variations present in the mitochondrial DNA were assumed to be neutral. But recent works have concluded that the variations in mitochondrial DNA are non-neutral and harbor various fitness related variations. Many experiment have shown that these variation affect phenotype and life history traits. In energy metabolism mitochondrial DNA plays an important role, variation in metabolic rate may be associated with variation in lifespan (Sacher, 1977) and in mtDNA genetic variation could certainly affect both metabolism and aging (Beckman B. and Ames N., 1998).

In promiscuous species, the studies have documented that the evolution of male specific adaptation such as persistent courtship, forceful mating, traumatic insemination, mating plug etc, often reduce female fitness as a byproduct, leading to selection by female to evolve "resistance" to such male induced harm(Nandy, 2013). This consistent arms race between male and females is referred to as "interlocus sexual conflict".

Here we attempt to investigate the role of mitochondrial DNA, if any in traits evolved under sexual conflict.

Interlocus sexual conflict is a type of conflict that occur through the interaction of set of antagonistic alleles at two or more different loci in male and female, resulting in the deviation of either or both sexes from the fitness optima for trait.

There is a tug-of war between male and female to achieve a fitness optima due to differential investment in reproduction. Males fitness is depends upon how many mating they can achieve. Therefore males try to increase their fitness by mating successfully with as many females available. Their pursuit to achieve maximum mating number, thus lead to male-male competition for mates and as a byproduct of competition, males became more harming to the females by either physical or chemical mean(Smith and Price, 1973).

Female fitness depends upon no. of eggs they lays, so they develop strategies to overcome this harm. The resistance traits to male induced harm are expected to be costly to female. The evolution to this mate harm resistance is predicted to come at the cost of other life-history traits, such as life span, fecundity etc.

1.1 Laboratory Population

MCF (started by Dr. Bodhisattanandy in Dr. N. G. Prasad's lab) have been evolved under various level of sexual conflict by using a sex ratio bias, where

- M stands for male biased with ratio (3:1) because of which male-male competition is heightened, thus leading to increase in sexual conflict.
- C stands for control with ratio (1:1), so moderate male-male competition and sexual conflict.
- F stands for female biased with ratio (1:3), which means decreased male male competition and sexual conflict.

According to Dr. Nandy's study on evolution of mate harm ability of males in the MCF it shows that M males have evolved over the time to be more mate harming as compared to F males due to the differential availability of mates(Nandy, 2013).

1.2 Introduction to MN (mitochondrial nuclear) lines

We derived MN lines from MCF lines. In these lines mitochondrial DNA from the females of M regime and F regime are expressed in common nuclear background. MN lines have variation in mitochondrial DNA, with MNM mitochondrial DNA coming from M females and for MNF mitochondrial DNA is coming from F females. The main focus of the experiment was to determine the role of mitochondrial DNA.

1.3 Aim

To investigate role of mitochondrial DNA in evolution of mate harm resistance and to see if mitochondrial DNA plays a role in mate harm resistance ability of female since it has implication on female fitness.

EXPERIMENTAL SETUP

2.1 MN selection line

MN lines were established from the MCF selection lines. MCF lines have evolved under different levels of sexual selection, by skewing the sex ratio. M regime has male-biased adult sex ratio (3:1 :: Male:Female), evolving under high sexual conflict. F regime has female biased adult sex ratio (3:1 :: Female:Male) evolving under low level of sexual conflict.

Mitochondrial DNA from these two M and F regime was expressed in the common ancestral LHst nuclear background. To successfully bring about this result, selection line females (M and F) and ancestral base line (LHst) males were crossed and subsequently female progeny was backcrossed with LHst males for 10 generations. In total there are 3 blocks of MCF. For each block 25 families of MN per regime were established. In total there are 150 MN families.

- Block 1: 25 M MN families + 25 F MN families
- Block 2: 25 M MN families + 25 F MN families
- Block 3: 25 M MN families + 25 F MN families
- 75 M MN families + 75 F MN families
- Total: 150 MN families are maintained in the below described maintenance protocol.

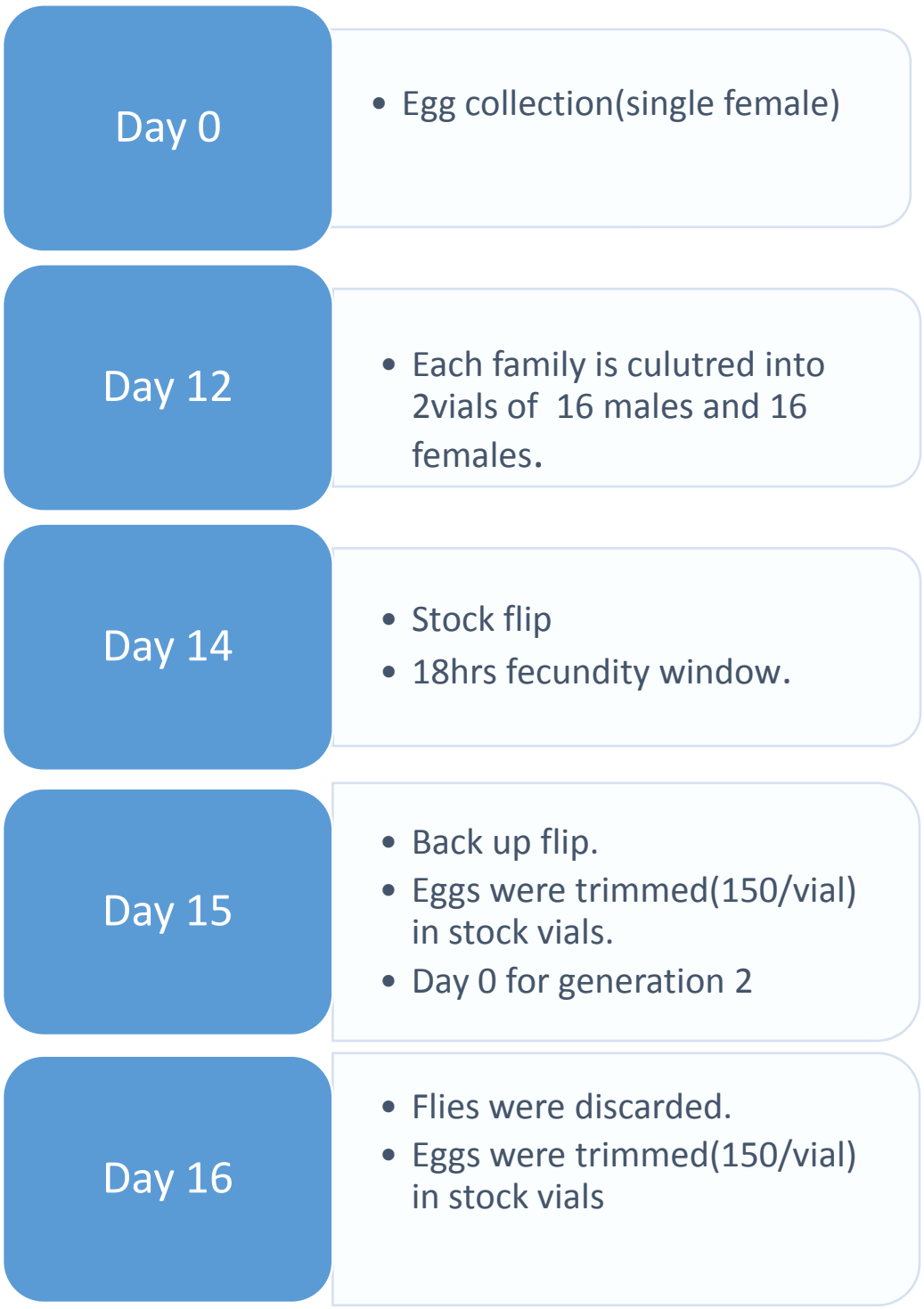


Figure1. Maintenance protocol of MN lines

2.2 Experiment design

Experiment was conduct in laboratory adapted selected population of Drosophila melanogaster, MN Lines, which are derived from MCF. These lines are maintained on cornmeal-molasses food and 12hlight/12hdark circadian, in a discrete 14 day generation cycle.

For this experiment, the eggs were collected from the MN lines and LHst baseline population, at the density of 150 eggs per vial. The egg collection vials were left for development for 8 days. From day 9 onwards, then the pupated larvae, start eclosing as adult flies, virgin females from MN and males from LH population were collected in single sex vials.

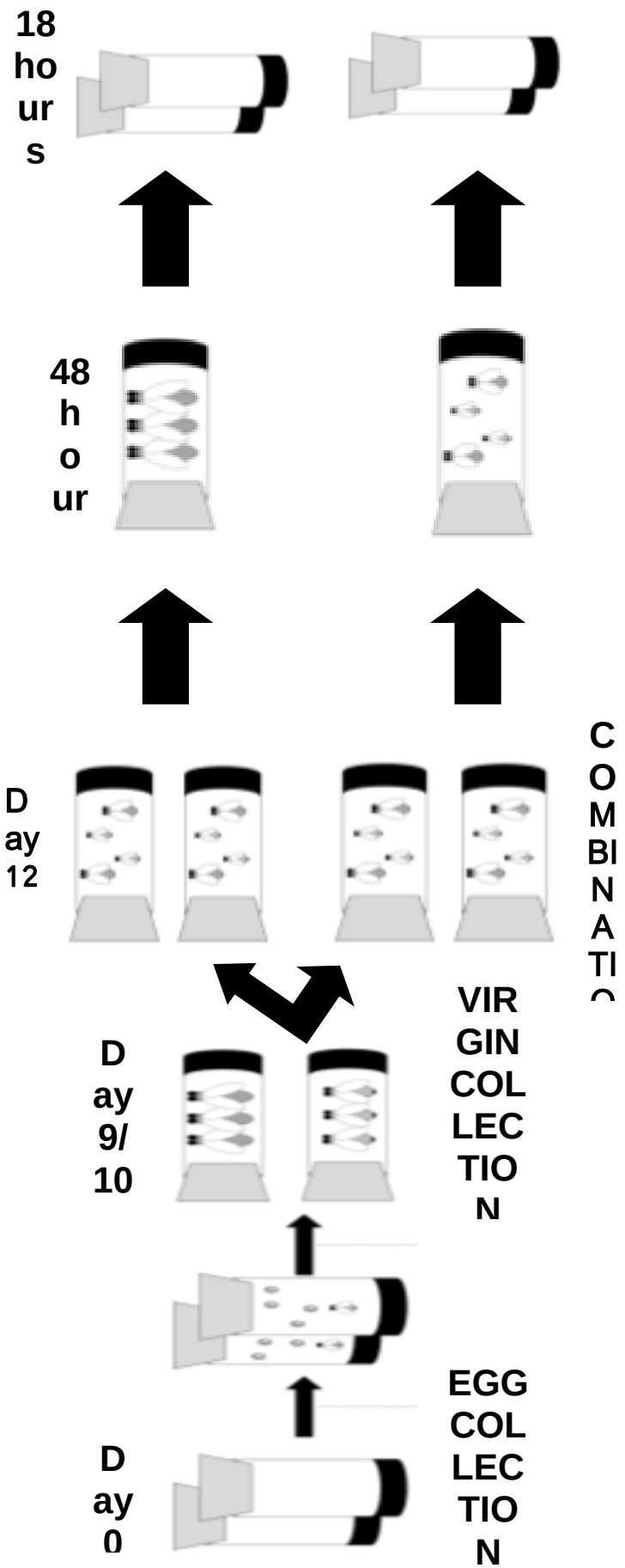
For each block there are total 36 families in 18 families randomly selected from MNM and 18 from MNF. 5 vails with 8 female per vial were collected for each family. So total number of female was 1440, and same number of males were also taken from LHst population. On the 12^{th} day, virginfemales from MN families are combined with virgin LHst males. % such vials are setup for each family. Out of 5 vials randomlytwo vials are selected for Single Mating treatment and 2 for Continuous exposure treatment.

Vials in single mating treatment are observed manually to ensure mating of all the virgin females present in the vial. After the last female in the vial has mated, the males are discarded using mild CO_2 anesthesia.

Vials in the continuous exposure treatment are left undisturbed for next 48 hours, for male and females to interact. After 48 hours, the males from these vials are removed under mild CO_2 anesthesia.

On 14^{th} day (48 hours after combination) females from both the treatments are flipped into oviposition vials and left to lay eggs for 18 hours of duration, after which the females are discarded and the oviposition vials are frozen. Eggs laid in each vial were counted for fecundity count.

The same experimental design was repeated for all the three blocks.



Experimental setup: Virgin females from MN selection lines were combined with LHst males, and divided randomly into two treatments: a) Singly mated, where males are discarded after manually observing single mating b) Continuous exposure, where males are females are allowed to interact for 48 hours. After 48 hours, the females from both the treatments are flipped into oviposition vials to lay eggs for 18 hours. After 18 hours the females are discarded and eggs are frozen for fecundity count.

Figure 2. Experimental Setup

RESULTS

3.1 Data analysis

The fecundity data was analyzed using a mixed model ANOVA, (R packages lme4 and lmerTests) with selection and treatment being fixed factor and line identity as random factor.

3.2 Conclusion

The eggs laid by females from each family of MN lines, in each treatment were counted. We didn't find any significant effect of selection on the number of eggs laid by the females. This suggest that number of eggs laid by females from MNF lines (mitochondrial DNA coming from F regime female) did not differ from number of eggs laid by females from MNM lines (mitochondrial DNA coming from M regime female). In block 1, there is a significant effect of selection, but the treatment and selection x treatment effect are not significant.

The effect of treatment is not significant on the number of eggs laid, suggesting that the females laid almost equal number of eggs in both the singly mated as well as continuous exposure treatment. There is no effect of selection x treatment interaction, suggesting that the selection lines do not differ with respect to the treatment, in terms of number of eggs laid.

Overall, from the results we can conclude that mitochondrial DNA does not play any role in the mate harm resistance ability of the females. MN lines were created from the M and F regime females, where the results have shown M females to be significantly more mate harm resistant than F regime females. But the difference disappears in the mitochondrial DNA from these M and F regimesare expressed in common nuclear background in MN lines.

3.3 ANOVA tables

Block 1	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Selection	30072.4	30072.4	1	33.821	8.227	0.007
Treatment	9076.7	9076.7	1	138.188	2.483	0.117
Selection x Treatment	11530.6	11530.6	1	138.188	3.154	0.077

Block 2	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Selection	1289.8	1289.8	1	38.141	1.403	0.243
Treatment	2943.4	2943.4	1	152.84	3.201	0.075
Selection x Treatment	1421.1	1421.1	1	152.84	1.545	0.215

Block 3	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Selection	4723.8	4723.8	1	34.81	3.529	0.068
Treatment	68.3	68.3	1	146.49	0.051	0.821
Selection x Treatment	2541.3	2541.3	1	146.49	1.898	0.17

Figure 3.ANOVA tables for mate harm resistance assay: fecundity, block 1, 2 and 3.

3.4 Descriptive graphs

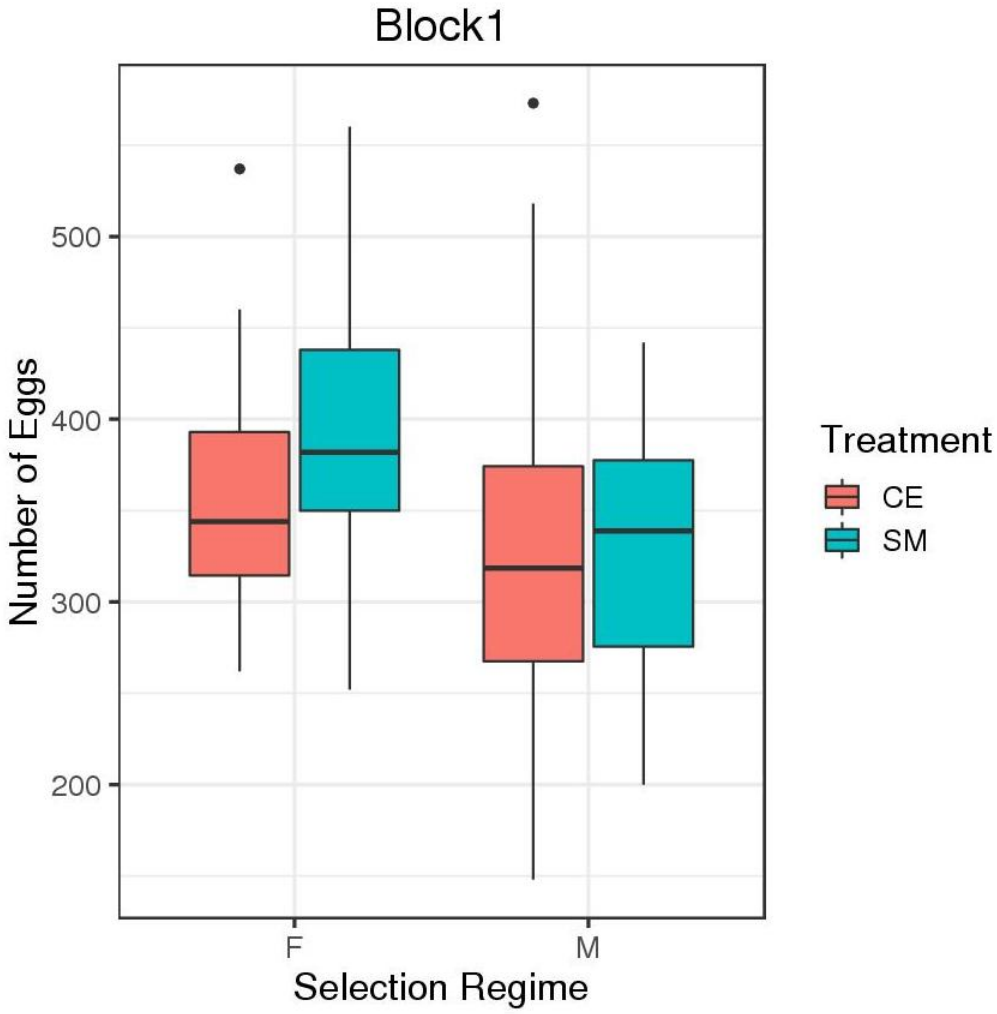


Figure 4.Female fecundity in different selection lines for two selection treatment for block 1

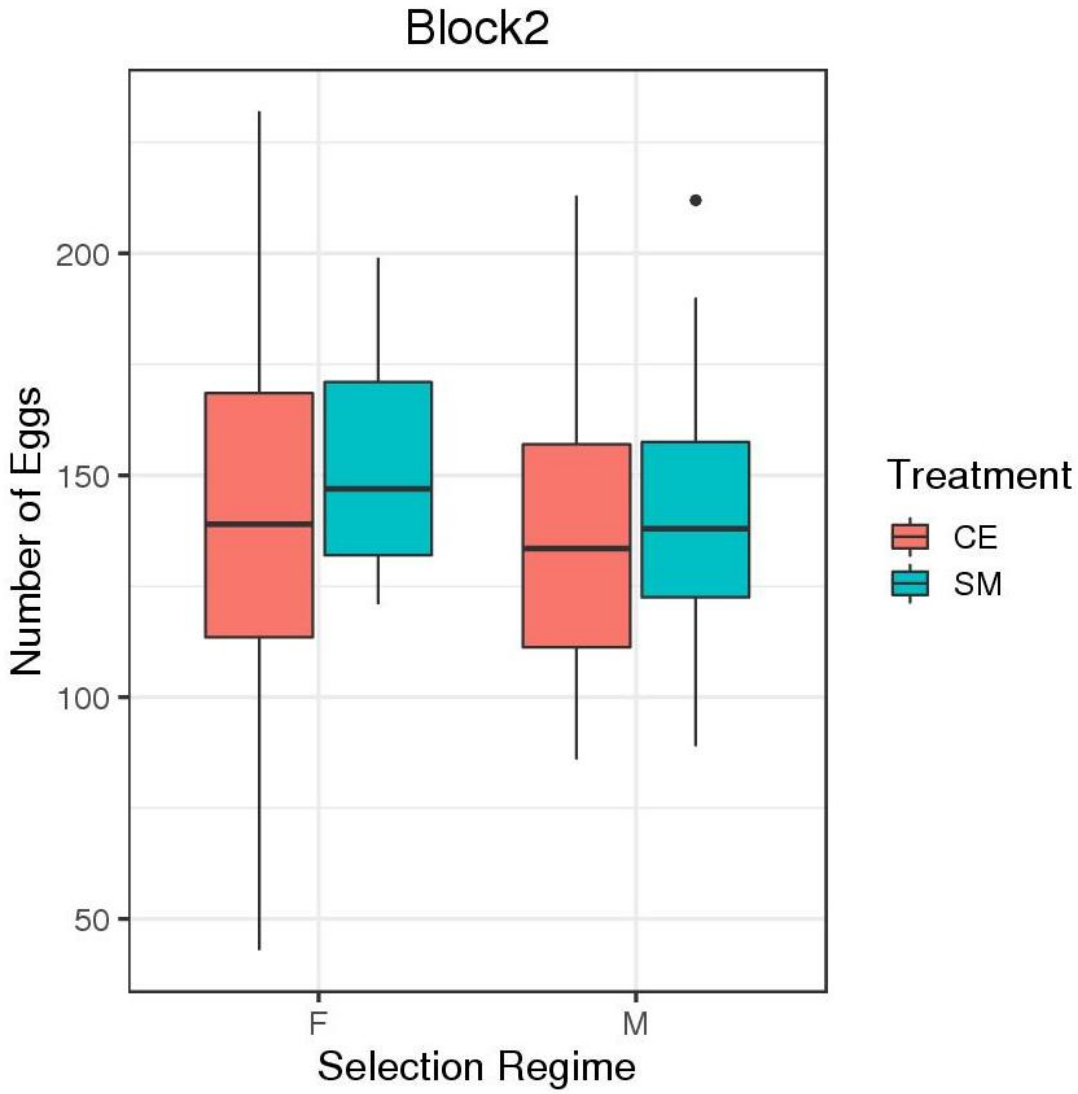


Figure 5.Female fecundity in different selection lines for two selection treatments for block 2

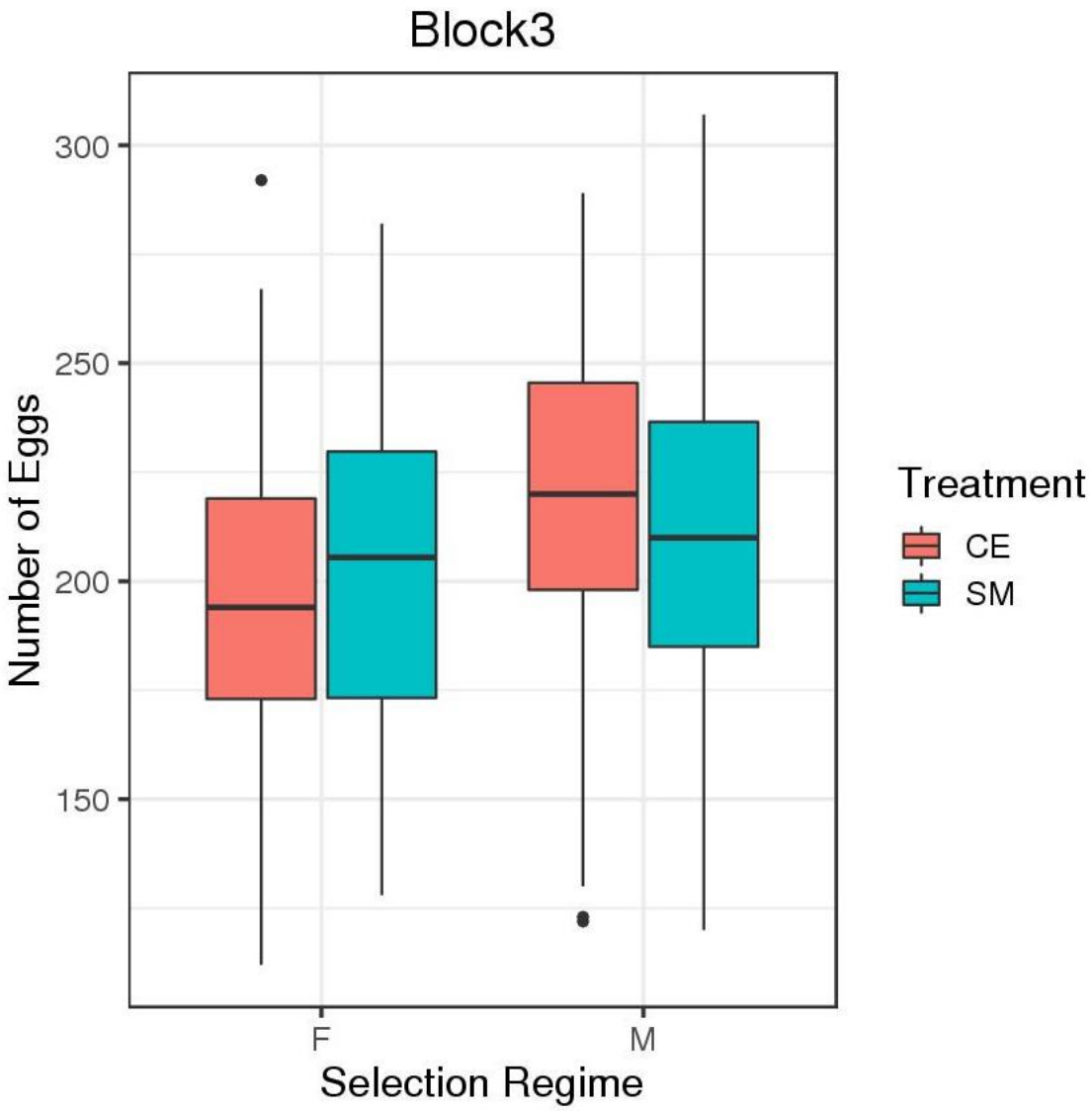


Figure6.Female fecundity in different selection lines for two selection treatmentsfor block 3

DISCUSSION

This study aimed to investigate the role of mitochondrial DNA in mate harm resistance ability of the females.

Using the MN lines, we expressed the mitochondrial DNA from the M and F populations' in common nuclear background. MNM and MNF differ in the source of mitochondrial DNA, everything else being the same. M regime females have been shown to have significantly higher mate harm resistance than the F regime females. But this difference disappear in the MNM and MNF lines, which differ in only the mitochondrial DNA coming from M and F regime females.

Therefore the results suggest that the mitochondrial DNA does not seem to have a role in mate harm resistance ability of the females, measured in terms of fecundity.

The mate harm resistance ability of the females can be measured in terms of fecundity as well as the longevity. The females which have higher mate harm resistance ability would have better longevity when housed with males. Variations in mitochondrial DNA are shown to have effects on metabolism and aging. Therefore the effects of mitochondrial DNA in mate harm resistance, if any could show up there.

Previous results in MN lines suggest that mitochondrial DNA does not have a role in sperm competition and mate harm ability of males. The male fitness related traits does not seem to depend on the mitochondrial DNA. This is the first result for the female fitness related trait, which also does not seem to depend on the mitochondrial DNA.

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