

HATCHABILITY STUDIES OF THE EGGS OF  
CULEX QUINQUEFASCIATUS, SAY (CULEX FATIGANS) AFTER TREATMENTS



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The work described in this thesis was carried out at the Department of Biological Sciences of the University of Sri Jayewardenepura under the supervision of Professor V. L. Rajapala and is reported in this thesis. It is not being submitted to any university for another degree.

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A B S T R A C T

1. Culex quinquefasciatus, Say (Culex pipiens fatigans, Wiedemann) the vector of urban filariasis in Sri Lanka was studied with special reference to its mutational response to three known mutagenic agents; Gamma-radiation (physical agent), Formaldehyde (non-alkylating chemical mutagen) and Nitrogen Mustard (alkylating chemical mutagen). Experiments were carried out in the laboratory using a colony of C. quinquefasciatus maintained at room temperature. The mutational response of the mosquito was assayed by obtaining the percentages of dominant lethal mutations induced in different male germ cells. The percentages of unhatched eggs laid by females that had been inseminated by treated males were used as a measure to estimate the induced dominant lethality in the treated male germ cells.
2. In order to study the differential response of various germ cells in the male germ tract of C. quinquefasciatus a brood technique was employed. At least three broods were analysed in each of the treated males by mating them individually to successive batches of untreated virgin females. The mating protocol in most of the experiments was one male to three females for three days followed by a rest of two days for the male between each mating period. The resulting egg



rafts of each of the above broods was screened to obtain the percentage of unhatched eggs from which was estimated the induced percentage of dominant lethal mutations.

3. The effect of gamma-irradiation was studied separately in all four stages of the male mosquito namely, eggs, larvae, pupae and adults. The effect of formaldehyde was only studied for the larval stages and the effect of N-mustard was studied both in the adult males and in male larvae.

4. In the adult male-irradiation studies, batches of two-day old males were irradiated with gamma-rays ranging from 500R to 10,000R and the treated males were screened after two days

for dominant lethals in three successive broods. The results showed that the dominant lethal frequency increased linearly with dose in all the three broods upto a dose of 8,000R.

In the third brood the gradient was found to be half that for the first two broods. This indicates that 50% of the radiation damage was restituted in the third brood.

5. Pupal irradiation studies were carried out as in the case of adult treatment and the males emerging from irradiated pupae were subjected to the brood analysis. Here, too, the results showed a linear relationship between the gamma-ray dose and the dominant lethal frequency. In the pupae the

restoration of dominant lethal damage was so prominent that in the third brood a 100% recovery was observed.

6. Larval irradiation studies, too, were carried out as in the cases of pupal and adult screenings. The results showed that the males emerging from treated larvae (third instar) did not show the induction of dominant lethality in any of the broods screened. However, it should be noted that the larvae cannot tolerate irradiation doses above 3,000R and thus it was only possible to screen males emerging from larvae treated with gamma-ray doses less than 3,000R, whereas, in adults and pupae the irradiation doses were upto 8,000R.
7. Eggs of C. quinquefasciatus, too, were, very sensitive to gamma-irradiation producing about 100% mortality at doses around 2,000R. The screening of adult males emerging from treated eggs too did not show any significant mutation rate at the range of doses that could be tested.
8. The results obtained by irradiating different life stages of the mosquito C. quinquefasciatus can be discussed by comparing them with those of Drosophila melanogaster. It has been found in D. melanogaster that the most radiosensitive stage in spermatogenesis is the late meiotic stage, and the least is the spermatogonial stage. The results obtained in the present



study with C. quinquefasciatus parallel closely with those obtained for D. melanogaster, in that, in the irradiated adults the first two broods are most radiosensitive whilst the third brood is half as sensitive. The fact that sensitivity is least in the third brood would suggest that this brood has resulted from irradiated spermatogonia, as in the case of D. melanogaster. The results of pupal irradiation too agree with the above suggestion in that the late broods have resulted from less sensitive irradiated spermatogonia or even perhaps stem cells. The total absence of dominant lethal mutations in irradiated larvae and eggs suggest that the gonads found in eggs and larvae do not bear mature germ cell stages.

However, in D. melanogaster irradiated larvae were treated with

9. In the cases where adult males and pupae were irradiated with high gamma doses, as much as 8,000R and 10,000R, both the irradiated males and the males emerging from irradiated pupae did not show the late recovery from dominant lethal damage as shown by them with relatively lower doses of gamma-irradiation. However, it should be mentioned that these males rarely mated in the third mating period corresponding to the third brood.
10. Twenty nine semi-sterile lines were isolated from  $F_1$  males of adult irradiation experiments by successive outcrossing

into a few generations. In these lines fifteen of them carried the semisterility character closely linked to the male determining factor (M).

11. The studies with formaldehyde was carried out by treating larval stages with formaldehyde. The formaldehyde treatment was given by supplementing food media (dried yeast) with varying concentrations of formaldehyde where known quantities of formaldehyde were added to known volumes of water. The results showed that the males emerging from treated larvae did not significantly produce dominant lethal mutations. Even with doses that produced high mortality the mutation rate was insignificant. However, in D. melanogaster when larvae were treated with formaldehyde treated food highly significant increases in ~~recessive~~ lethal frequencies had been observed.
12. The negative results with mosquitoes could be adduced to many reasons. In contrast to formaldehyde studies with D. melanogaster, the treatment in the present study differs in that, firstly in mosquitoes the treatment was to immerse the entire larvae in water to which formaldehyde food had been added and secondly, after the formaldehyde food was introduced it soon becomes amply diluted (about 250 fold). By comparison in the Drosophila studies larvae only move on the surface of the treatment medium for feeding and their



entire bodies do not come into contact with formaldehyde, and also, the treatment medium in no way becomes diluted. It can also be mentioned here that the mutagenicity of formaldehyde is very peculiar in many respects when compared to X-rays. In D. melanogaster the mutagenic action of formaldehyde was only demonstrated in a restricted region of the germ tract of male larvae. It has also been observed that formaldehyde exerts its mutagenic activity by reacting with some constituent of food. Therefore, because of the above differences in treatment and the peculiarity of formaldehyde as a mutagen it is difficult to comment on the disappointing results obtained in the present study. Perhaps, also, the mosquito testis is tolerant to formaldehyde just as much as the Drosophila ovary is.

12. The experiments carried out with Nitrogen Mustard were also very disappointing as no clearcut patterns of mutagenicity could be discerned. N-mustard was administered to adult males by employing a micro-injection technique and the larvae were treated by immersing in N-mustard solutions. In the case of adult treatments the treated males were screened for four broods. In none of the above four broods was an overall effect of the treatment observed.

The adult males emerging from treated larvae were screened for three broods, and here, too, a treatment effect was not shown in any of the broods. However, in one of the adult experiments (fourth experiment) carried out by injecting adult males with a dose of 10 µg per mg body weight a certain amount of dominant lethals (10% - 20%) were observed.



## C O N T E N T S

ACKNOWLEDGEMENTS	i
ABSTRACT	iv
CHAPTER 1 - INTRODUCTION	1
1.1 Importance of studying genetics of mosquitoes	1
1.2 <u>Culex quinquefasciatus, Say</u> , as the chief vector of urban filariasis in Sri Lanka and its genetic studies	2
1.3 Status of genetic control in <u>Culex quinquefasciatus, Say</u>	7
1.3.1 Sterile male technique (Sterile Insect Release Method-SIRM)	8
1.3.2. Inherited semisterility for control of <u>C. quinquefasciatus</u>	12
1.3.3 Cytoplasmic incompatibility	14
1.3.4 Integrated mechanisms	16
1.3.5 Other genetic mechanisms	17
1.4 Objectives of the study	18
1.5 Mutagenicity of gamma-radiation	22
1.6 Mutagenicity of formaldehyde	30
1.7 Mutagenicity of Nitrogen Mustard	33
CHAPTER 2 - MATERIALS AND METHODS	38
2.1 The life cycle of the mosquito <u>Culex quinquefasciatus, Say</u>	38
2.1.1 The egg	38
2.1.2 The larva	40
2.1.3 The pupa	43
2.1.4 The adult	45

contd..

	<u>page</u>
2.2 Culture of <u>C. quinquefasciatus</u>	50
2.2.1 Procedures related to egg rafts	51
2.2.2 Larval rearing	52
2.2.3 Pupal rearing	53
2.2.4 Adult rearing	53
2.2.5 Inbreeding depression	55
2.3 Methods of treatments	56
2.3.1 Gamma-irradiation	57
2.3.2 Chemical Treatments	61
2.4 Screening procedure	67
2.5 Experimental procedure	72
2.5.1 Gamma-irradiation experiments	72
2.5.2 Formaldehyde treatment experiments	74
2.5.3 N-mustard treatments	75
2.6 Isolation of semisterile lines	84
CHAPTER 3 - RESULTS	87
3.1 Gamma-ray mutagenesis in <u>Culex quinquefasciatus, Say</u>	87
3.1.1 Brood analyses of gamma-irradiated adult males of <u>Culex quinquefasciatus, Say</u>	88
3.1.2 Brood analyses of gamma-irradiated pupae of <u>Culex quinquefasciatus, Say</u>	99
3.1.3 Brood analyses of gamma-irradiated third instar-larvae of <u>C. quinquefasciatus</u>	108
3.1.4 Brood analyses of gamma-irradiated eggs of <u>Culex quinquefasciatus, Say</u>	115
3.2 3.2.1 Effect of formaldehyde on second instar-larvae of <u>Culex quinquefasciatus, Say</u>	128
3.2.2 Effect of formaldehyde on third instar-larvae of <u>Culex quinquefasciatus, Say</u>	136
3.3 Attempts to induce dominant lethals with Nitrogen-Mustard in <u>C. quinquefasciatus, Say</u>	147



	3.3.1	Effect of Nitrogen Mustard on adult males of <u>Culex quinquefasciatus</u> , Say	147
	3.3.2	Effect of Nitrogen Mustard on larvae of <u>Culex quinquefasciatus</u> , Say	157
	3.4	Isolation of semisterile lines of <u>Culex quinquefasciatus</u> , Say	164
W.E. Ratnayake,			
Jayewardene			
CHAPTER 4	-	DISCUSSION	168
this research	4.1	Gametogenesis	169
gratitude to	4.2	Differential sensitivity of various germ cell stages to physical and chemical mutagens in <i>Drosophila</i> males	172
given to me	4.3	Sensitivity of <u>Culex quinquefasciatus</u> , Say testis to Gamma-rays	176
research and	4.4	Effect of formaldehyde on larvae of <u>Culex quinquefasciatus</u> , Say	186
	4.5	Effect of Nitrogen Mustard on larvae and adults of <u>Culex quinquefasciatus</u> , Say	190
A.C.J. Weer	4.6	Semisterile lines	193
Jayewardene			
advice given			
REFERENCES			194

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