MODE OF INHERITANCE OF MALATHION RESISTANCE IN *ANOPHELES STEPHENSI* LISTON

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ABSTRACT. Discriminating doses of 5% and 10% malathion for susceptible ($M1^s/M1^s$), resistant ($M1^r/M1^r$) and heterozygotes ($M1^r/M1^s$) have been obtained on colonized susceptible and resistant strains of *Anopheles stephensi*. Malathion resistance in this species is suggested to be under the control of a single locus on 1 of the autosomes. The alleles on the locus are codominant.

INTRODUCTION

Anopheles stephensi known to be resistant to DDT, dieldrin/BHC in Afghanistan, Iran, Iraq, Saudi Arabia, India, Pakistan (Davidson and Mason 1963, WHO 1976) has also become resistant to malathion in Iran (Manouchehri et al. 1975, Eshghy 1978). In a recent survey of Punjab Pakistan (Rathor et al. 1980a) malathion resistance has been discovered in *An. stephensi*. *An. culicifacies* the other important malaria vector in the Indo-Pakistan subcontinent is resistant to DDT, dieldrin (WHO 1976) and malathion (Rajagopal 1977). In spite of the fact that both species are the major malaria vectors in Indo-Pak subcontinent, very little information is available on the genetics of insecticide resistance of the species. The present paper reports on the mode of inheritance of malathion resistance in *An. stephensi*.

MATERIALS AND METHODS

The following strains were used in the study:—

1) LT. A strain originally colonized in 1975 from the village (Leti) on Talagang-Mianwali Road, 26 miles west of Tehsil Talagan, District Attock of Punjab Province Pakistan. The strain is homozygous for malathion susceptibility ($M1^s/M1^s$). All adults are killed by 1 hr exposure to 5% malathion.

2) KHR. A strain originally colonized in 1978 from the village of Khano Harni, 20 miles southeast of Lahore. The strain was selected with malathion for 4 generations. The adults survive 10 hr exposure to 10% malathion.

Larvae were reared in enamel pans measuring 45 cm × 22 cm filled to 1 cm depth of water and fed on liver powder. Adults were maintained at 28 ± 1°C and RH 75 ± 5%. Both males and females were given 3% sugar solution and restrained mice for a blood meal. Insectaries were illuminated with fluorescent and incandescent lighting. An artificial dawn and dusk was produced at 05.00 and 21.30 hours for 80 min.

Resistant and susceptible strains were reciprocally crossed *en masse* in 1 gal cylindrical carton cages. The F_1 adults obtained from the cross between the resistant and susceptible strains were exposed to 5% malathion for 1 hr to eliminate susceptible individuals. Surviving F_1 adults were reciprocally backcrossed to their susceptible parents (Crow 1957), and resulting offspring were exposed to 5% malathion-impregnated papers for 1 hr and held for 24 hr before mortalities were counted. In this way 4 backcrosses were made.

TESTING. Adults less than 24 hr old were tested using WHO adult test kits with insecticide papers prepared by the method described by Georghiou and Metcalf (1961) and Rathor and Toqir (1980b), with some modifications; 0.7 ml of 5% malathion solution instead of 1.0 ml in acetone (W/V) was added to 2.3 ml acetone, the 3 ml solution thus obtained was spread on each filter paper (12 × 15 cm). Individuals were tested with diag-

Table 1. Summary of results of tests made with different doses (in time) of malathion on malathion-resistant, susceptible strains and their reciprocal crosses.

Cross No.		5% Malathion				10% Malathion				X^2 1:1 mortality ratio ♀:♂	P
		E^t	N	M	M%	E^t	N	M	M%		
1	$\frac{S}{S}\frac{X}{X} \times \frac{S}{S}\frac{X}{Y}$	1h	♂ 100 ♀ 100	100 100	100% 100%	—	—	—	—	—	—
2	$\frac{R}{R}\frac{X}{X} \times \frac{R}{R}\frac{X}{Y}$	1h	♂ 102 ♀ 102	0 0	0.0% 0.0%	—	—	—	—	—	—
	" " × " "	—	—	—	—	10h	♂ 117 ♀ 117	0 0	0.0% 0.0%	—	—
3	$\frac{R}{R}\frac{X}{X} \times \frac{S}{S}\frac{X}{Y}$	1h	♂ 20 ♀ 20	0 0	0.0% 0.0%	—	—	—	—	—	—
	" " × " "	3h	♂ 20 ♀ 20	0 0	0.0% 0.0%	—	—	—	—	—	—
	" " × " "	5h	♂ 25 ♀ 25	11 12	44.0% 48.0%	—	—	—	—	0.04	.90>P>.80
	" " × " "	—	—	—	—	6h	♂ 31 ♀ 31	18 21	58.06% 67.74%	0.23	.70>P>.50
						8h	♂ 50 ♀ 50	47 46	94.00% 92.00%	0.01	.95>P>.90
	" " × " "	—	—	—	—	10h	♂ 54 ♀ 54	54 53	100.00% 98.15%	0.01	.95>P>.90
4	$\frac{S}{S}\frac{X}{X} \times \frac{R}{R}\frac{X}{Y}$	1h	♂ 20 ♀ 20	0 0	0.0% 0.0%	—	—	—	—	—	—
		3h	♂ 20 ♀ 20	0 0	0.0% 0.0%	—	—	—	—	—	—
	" " × " "	5h	♂ 25 ♀ 25	13 13	52.0% 52.0%	—	—	—	—	—	
	" " × " "	—	—	—	—	6h	♂ 31 ♀ 31	12 10	38.71% 32.26%	0.18	.70>P >.50
	" " × " "	—	—	—	—	8h	♂ 50 ♀ 50	49 45	98.0% 90.0%	0.17	.70>P>.50
	" " × " "	—	—	—	—	10h	♂ 54 ♀ 54	54 54	100.00% 100.00%	—	—

E^t = time of exposure, N = total number of insects exposed, ♂ = male, ♀ = female, S = $M1^s$, R = $M1^r$.
M = mortality, M% = percent mortality.

nostic doses: 1 hr exposure to 5% malathion or 10% malathion for 10 hr. Mortalities were recorded after a 24-hr holding period. Dosage/mortality responses were obtained by exposing the adults to 5% and 10% malathion papers continuously for a period to give 100% mortality. The number of insects dead were noted at different intervals. Appropriate controls were run with all experiments. Results of the controls in which no mortalities occurred were not included.

RESULTS AND DISCUSSION

DETERMINATION OF DISCRIMINATING DOSES. Susceptible and resistant parent strains and the F_1 between the susceptible and resistant strains were exposed to 5% malathion for 1 hr. The susceptible strain showed 100% mortality but the resistant strain and the F_1 showed 100% survival (Table 1). Since the susceptible individuals could be separated from resistant and heterozygous individuals with the dose of 1 hr exposure to 5% malathion, this was

Table 2. Responses of exposures, of malathion-resistant and susceptible strains and progeny of reciprocal crosses between them, to diagnostic doses for susceptible (1 hr exposure to 5% malathion) and heterozygotes (10 hr exposure to 10% malathion).

Cross No.	Genotypes	Sex	N	I^c	E^t	A	D	Progeny % Mortality
5	$\frac{S\ X}{S\ X} \times \frac{S\ X}{S\ Y}$	♂	102	5%	1h	0	102	100
		♀	102			0	102	100
6	$\frac{R\ X}{R\ X} \times \frac{R\ X}{R\ Y}$	♂	104	5%	1h	104	0	0.0
		♀	104			104	0	0.0
7	$\frac{R\ X}{R\ X} \times \frac{S\ X}{S\ Y}(F_1{}^a)$	♂	54	5%	1h	54	0	0.0
		♀	54			54	0	0.0
8	$\frac{S\ X}{S\ X} \times \frac{R\ X}{R\ Y}(F_1{}^b)$	♂	54	5%	1h	54	0	0.0
		♀	54			54	0	0.0
9	$\frac{S\ X}{S\ X} \times \frac{S\ X}{S\ Y}$	♂	101	5%	10h	0	101	100
		♀	101			0	101	100
10	$\frac{R\ X}{R\ X} \times \frac{R\ X}{R\ Y}$	♂	100	10%	10h	100	0	0.0
		♀	100			100	0	0.0
11	$\frac{R\ X}{R\ X} \times \frac{S\ X}{S\ Y}$	♂	60	10%	10h	0	60	100
		♀	60			0	60	100
12	$\frac{S\ X}{S\ X} \times \frac{R\ X}{R\ Y}$	♂	60	10%	10h	0	60	100
		♀	60			0	60	100
13	$\frac{S\ X}{S\ X} \times \frac{S\ X}{S\ Y}$	♂	100	c	—	100	0	0.0
		♀	100			100	0	0.0
14	$\frac{R\ X}{R\ X} \times \frac{R\ X}{R\ Y}$	♂	100	c	—	100	0	0.0
		♀	100			100	0	0.0
15	$\frac{R\ X}{R\ X} \times \frac{S\ X}{S\ Y}$	♂	50	c	—	50	0	0.0
		♀	50			50	0	0.0
16	$\frac{S\ X}{S\ X} \times \frac{R\ X}{R\ Y}$	♂	50	c	—	50	0	0.0
		♀	50			50	0	0.0

♂ = male, ♀ = female, N = total tested, I^c = concentration of insecticide, E^t = exposure time, A = alive, D = dead, C = control, S = $M1^s$, R = $M1^r$.

taken as the discriminating dose for the susceptible ($M1^s/M1^s$) individuals. F_1 adults were exposed to 5% malathion for 1, 2, 3 and 5 hr and to 10% malathion for 6, 8 and 10 hr. The dose of 10 hr exposure to 10% malathion gave 100% mortality in F_1 but the resistant parent showed complete survival at this dose, therefore, 10 hr exposure to 10% malathion was taken as the discriminating dose for

Table 3. Results from repeated backcrosses made reciprocally to susceptible parent with selection at each generation to eliminate susceptible individuals.

Cross No.	Genotypes	Total N	Alive	Dead	Expected A—D	X^2 1:1 Segregation A:D	P
17	F_1^a ♀ × $\frac{S}{S}\frac{X}{Y}$ (BC_1^A)	269	141	128	134.5	0.63	.50>P>.30
18	BC_1^A ♀ × $\frac{S}{S}\frac{X}{Y}$ (BC_2^A)	48	18	30	24.0	3.00	.10>P>.50
19	BC_2^A ♀ × $\frac{S}{S}\frac{X}{Y}$ (BC_3^A)	590	283	307	295.0	0.98	.50>P>.30
20	BC_3^A ♀ × $\frac{S}{S}\frac{X}{Y}$ (BC_4^A)	244	116	128	122.0	0.59	.50>P>.30
21	$\frac{S}{S}\frac{X}{X}$ × F_1^a ♂ (BC_1^a)	47	21	26	23.5	0.53	.50>P>.30
22	$\frac{S}{S}\frac{X}{X}$ × BC_1^a ♂ (BC_2^a)	309	145	164	154.5	1.17	.30>P>.20
23	$\frac{S}{S}\frac{X}{X}$ × BC_2^a ♂ (BC_3^a)	870	422	448	435.0	0.78	.50>P>.30
24	$\frac{S}{S}\frac{X}{X}$ × BC_3^a ♂ (BC_4^a)	604	290	314	302.0	0.95	.50>P>.30
25	F_1^b ♀ × $\frac{S}{S}\frac{X}{Y}$ (BC_1^B)	1012	495	517	506.0	0.48	.50>P>.30
26	BC_1^B ♀ × $\frac{S}{S}\frac{X}{Y}$ (BC_2^B)	672	337	335	336.0	0.01	.95>P>.90
27	BC_2^B ♀ × $\frac{S}{S}\frac{X}{Y}$ (BC_3^B)	559	287	272	279.5	0.40	.70>P>.50
28	BC_3^B ♀ × $\frac{S}{S}\frac{X}{Y}$ (BC_4^B)	618	313	305	309.0	0.10	.80>P>.70
29	$\frac{S}{S}\frac{X}{X}$ × F_1^b ♂ (BC_1^b)	308	159	149	154.0	0.32	.70>P>.50
30	$\frac{S}{S}\frac{X}{X}$ × BC_1^b ♂ (BC_2^b)	882	373	509	441.0	20.97	P>.001*
31	$\frac{S}{S}\frac{X}{X}$ × BC_2^b ♂ (BC_3^b)	417	222	195	208.5	1.75	.20>P>.10
32	$\frac{S}{S}\frac{X}{X}$ × BC_3^b ♂ (BC_4^b)	764	357	407	382.0	3.27	.10>P>.05

* = significant value of P. F_1^a and F_1^b = see Table 2. A = alive, D = dead. BC_4 = Fourth backcross. S = $M1^s$. N = total tested.

Table 4. Summary of results obtained by exposing the resistant parent to 5% malathion (continous exposures) to obtain d-m response.

Time (minutes)	Sex	Mortality* (observed)	% Mortality	Expected mortality in ♂ and ♀	X^2 1:1 mortality ratio ♂:♀	P
180	♂	1	0.65	0.50	1.00	.50>P>.30
	♀	0	0.00			
300	♂	1	0.65	1.50	0.33	.70>P>.50
	♀	2	1.30			
360	♂	2	1.30	2.50	0.20	.70>P>.50
	♀	3	1.95			
480	♂	2	1.30	3.50	1.29	.30>P>.20
	♀	5	3.25			
665	♂	2	1.30	4.00	2.00	.20>P>.10
	♀	6	3.90			
730	♂	5	3.25	6.00	0.33	.70>P>.50
	♀	7	4.55			
830	♂	15	9.74	16.50	0.27	.70>P>.50
	♀	18	11.69			
1100	♂	53	34.42	53.00	—	—
	♀	53	34.42			
1257	♂	75	48.70	74.50	—	—
	♀	74	48.05			
1320	♂	99	64.29	93.00	0.77	.50>P>.30
	♀	87	56.49			
1445	♂	106	68.83	101.50	0.40	.70>P>.50
	♀	97	62.99			
1500	♂	112	72.72	109.00	0.17	.70>p>.50
	♀	106	68.83			
1630	♂	152	98.70	149.50	0.08	.80>P>.70
	♀	147	95.45			
1800	♂	154	100.00	153.50	—	—
	♀	153	99.35			

*= In all 308 adults were tested (154 ♂♂ and 154 ♀♀(in a total of 8 replicates of tests.
♂ = male, ♀ = female.

Table 5. Summary of results obtained by exposing the susceptible parent to 5% malathion (continous exposures) to obtain d-m response.

Time (minutes)	Sex	Observed[a] mortality	% Mortality	Expected mortality in ♂ and ♀	X^2 mortality ratio ♂:♀	P
60	♂	11	4.42	68.5	96.53	P<.001*
	♀	126	50.60			
90	♂	127	51.00	165.0	17.50	P<.001*
	♀	203	81.53			
100	♂	204	81.93	219.50	2.19	.20>P>.10
	♀	235	94.38			
120	♀	249	100.00	249.0	—	—
	♀	249	100.00			

*= significant at the P value of 0.05. ♂ = male, ♀ = female.
[a] =In all 498 adults were tested (249 ♂♂ and 249 ♀♀) in a total of 8 replicates of tests.

heterozygotes ($M1^r/M1^s$). There was no overlapping of the discriminating doses.

MODE OF INHERITANCE. The results of exposures with diagnostic doses to resistant and susceptible strains and F_1 hybrids obtained from reciprocal crosses between the 2 strains are summarized in Table 2. The F_1 hybrids survived 1-hr exposure to 5% malathion and were killed by 10 hr exposure to 10% malathion. No significant departure was observed from the 1:1 ratio in mortality and survival of males and females. This was also found to be true at lower doses, 3, 5, 6, 8, and 10 hr exposures to 5% and 10% malathion (P values in Table 1).

From similar results on dieldrin resistance in *An. gambiae* (Davidson 1956), *An.*

Table 6. Summary of results obtained by exposing the reciprocal backcrosses to 5% malathion (continous exposures) to obtain d-m response.

Time (minutes)	Sex	Mortality	% Mortality	Expected mortality in ♂ and ♀	X^2 1:1 mortality ratio ♂:♀	P
F_1^a ($\frac{R}{R}\frac{X}{X} \times \frac{S}{S}\frac{X}{Y}$) . A.						
60	♂	1	1.18	1	0.00	—
	♀	1	1.18			
120	♂	5	5.88	4	0.50	.50>P>.30
	♀	3	3.53			
377	♂	9	10.59	18.50	9.76	.01>P>.001*
	♀	28	32.94			
425	♂	9	10.59	20.0	12.10	P<.001*
	♀	31	36.47			
477	♂	15	17.65	22.50	5.00	.05>P>.02*
	♀	30	35.29			
615	♂	52	61.18	60.50	2.39	0.20>P>0.10
	♀	69	81.18			
F_1^b ($\frac{R}{R}\frac{X}{X} \times \frac{S}{S}\frac{X}{Y}$) . B.						
120	♂	11	11.11	10.50	0.05	.90>P>.80
	♀	10	10.10			
180	♂	15	15.15	14.00	0.14	.80>P>.70
	♀	13	13.13			
360	♂	10	10.10	23.00	14.70	P<.001*
	♀	36	36.36			
420	♂	16	16.16	27.00	8.96	.01>P>.001*
	♀	38	38.38			
480	♂	32	32.32	42.00	4.76	.05>P>.02*
	♀	52	52.53			
510	♂	47	47.47	53.00	1.36	.30>P>.20
	♀	59	59.60			
540	♂	61	61.62	66.50	0.91	.50>P>.30
	♀	72	72.73			
585	♂	77	77.78	80.50	0.38	.70>P>.50
	♀	84	84.85			
600	♂	80	80.81	84.00	0.38	.70>P>.50
	♀	88	88.89			

♂ = male, ♀ = female, * Significant at P value of 0.05.
A = 170 adults were tested (85 ♂♂ and 85 ♀♀) in 4 replicates of tests.
B = In all 198 adults were tested (99 ♂♂ and 99 ♀♀) in a total of 8 replicates of tests.
S = $M1^s$, R = $M1^r$.

stephensi (Davidson and Mason 1963), *An. farauti* (Bryan 1977) and *An. culicifacies* (Sakai et al. 1979) a semi-dominant mode of inheritance was considered to be operative. Thus the present results suggest that malathion resistance in *An. stephensi* is semidominant and is not sex-linked.

The results obtained from repeated backcrosses to the susceptible strain and selection to eliminate susceptible individuals at each generation are summarized in Table 3. Fifty percent mortalities are expected in each backcross if a single semi-dominant gene determines the resistance. No significant deviation was ob-

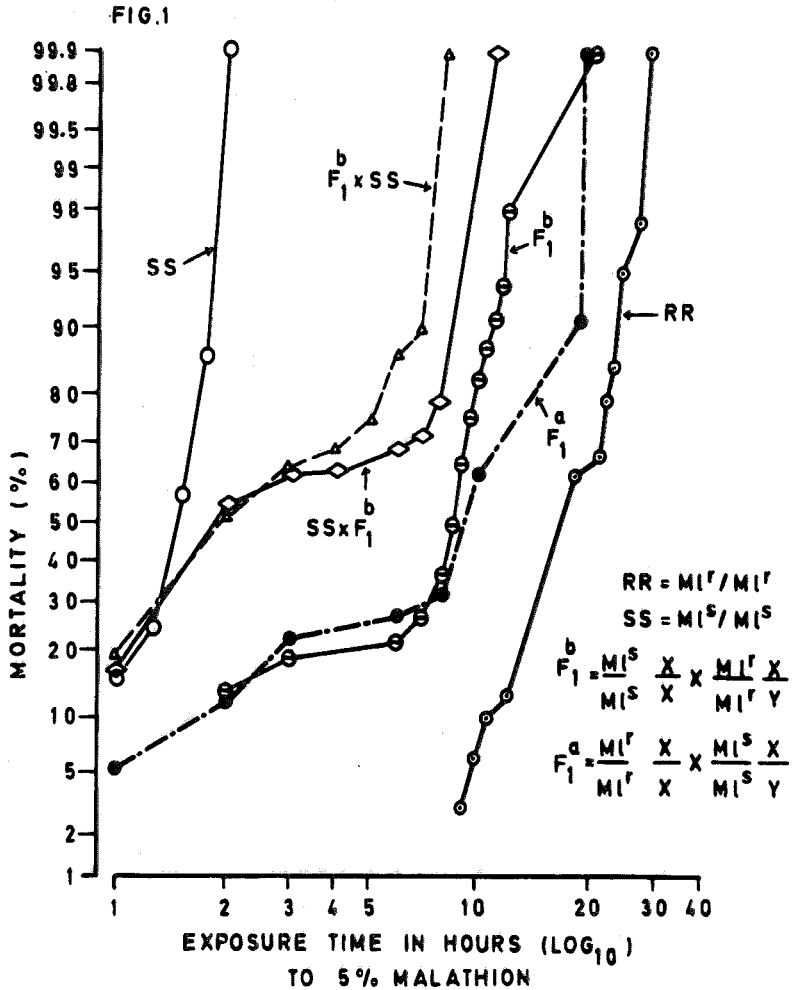

Fig. 1. Dosage in time (log_{10}) percent mortality response lines obtained with 5% malathion on adult *An. stephensi*. Resistant (RR), susceptible (SS) parent strains (F_1 and F_2); reciprocal cross (F_1^a) and backcrosses with the susceptible parent ($F_1^a \times SS$ and $SS \times F_1^a$).

served from a 1:1 ratio in alive: dead individuals except in cross No. 30. These results suggest that malathion resistance in this species is monofactorial.

The dosage mortality (d-m) lines for susceptible ($M1^s/M1^s$) and resistant ($M1^r/M1^r$) strains and their reciprocal crosses (F_1^a and F_1^b) Fig. 1–2, Tables 4, 5 and 6 and backcrosses with the susceptible strain were obtained. The F_1^a and F_1^b were found to be intermediate between the 2 parent strains, F_1 hybrids when backcrossed with the susceptible parental strain, the d-m lines for the backcross off-

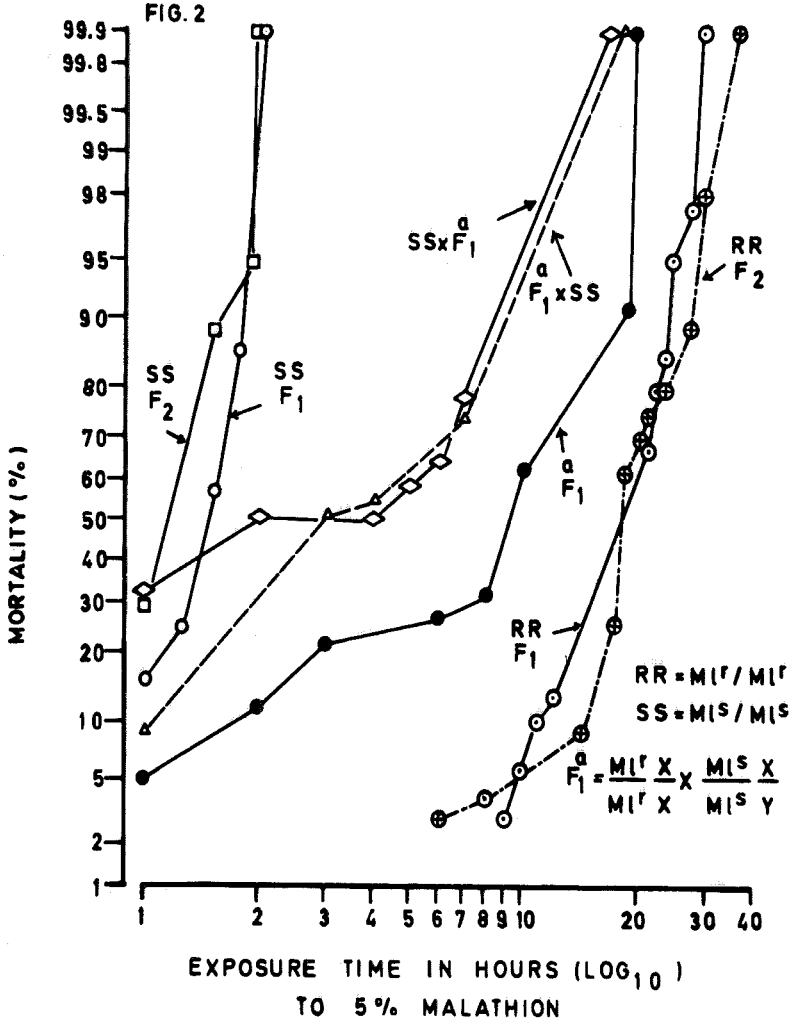

Fig. 2. Dosage in time (log_{10}) percent mortality response lines obtained with 5% malathion on adult *An. stephensi*. Resistant (RR), susceptible (SS) parent strains, reciprocal crosses (F_1^a and F_1^b) and backcrosses with susceptible parent ($F_1^b \times SS$ and $F_1^b \times SS$).

spring showed inflexions at about 50% mortality level. The results are similar to those of Tadano (1969) who, on the basis of similar intermediate d-m lines of F_1 and occurrence of inflexions in d-m lines of backcross at the 50% mortality level, concluded that malathion resistance in *Culex pipiens* was due to a single, incompletely dominant gene.

The results obtained by continuous exposures to obtain d-m lines revealed that susceptible males take longer to be affected by malathion. Table 5 shows that initially there was a significant departure from 1:1 mortality ratio between males and females (females showed significantly higher mortality), but later on the difference disappeared. Similarly the reciprocal crosses (F_1^a and F_1^b, Table 6) showed significantly higher mortality in females between exposure periods of 6 to 8 hr, but at 10 hr exposure the percentage mortality became equal in both sexes. This differential mortality between sexes was not observed in tests where the mosquitoes were exposed to diagnostic doses and mortalities were read after a 24 hr holding period. The resistant strain did not show any significant differential mortality between sexes even when continuous exposures were made. It appears that males possess either some physiological mechanism which delays the onset of symptoms of toxicity or some sex limited genetical modifiers which enable the males to cope with the lower doses of the poison. Further investigations on the mechanism of resistance may provide an answer to these questions.

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