REDUCTION IN THE SUSCEPTIBILITY OF *AEDES AEGYPTI* TO *BRUGIA MALAYI* INFECTION AFTER TREATMENT WITH ETHYL METHANESULFONATE

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ABSTRACT. The susceptibility to *Brugia malayi* infections of F_2 and F_4 progenies of *Aedes aegypti* (Black Eye strain) treated with ethyl methanesulfonate (EMS) was tested. Both 3-day-old males and females were treated with 0.025, 0.050, 0.075 and 0.10% EMS. Control and treated females were then mated with normal or treated males to recover F_1 progeny. F_2 offspring were derived from sibling intercrosses, and 3 lines were established by further intercross matings to generate the F_4. Susceptibility in the 0.025, 0.050, 0.075 and 0.10% EMS F_2s was reduced by 13, 12, 4 and 25%, respectively. The 0.025% and 0.050% EMS F_2 females showed a 29 to 39% decrease in mean L_3 numbers. At 0.075 and 0.10% EMS, mean L_3 numbers decreased by 0.8 and 71%, respectively. The F_4 populations gave overall infections of 65, 56 and 22% for the control, 0.25 and 0.10% EMS lines, respectively. Mean L_3s were reduced by 24 and 77%, respectively, in the 0.025 and 0.10% F_4 EMS-selected populations.

In *Aedes aegypti* (Linn.) susceptibility to the filarial nematode, *Brugia malayi* (Brug), is controlled by a sex-linked recessive gene named f^m (Macdonald 1962b). The f^m gene (Macdonald and Ramachandran 1965) also controls the development of the human parasite, *Wuchereria bancrofti* (Cobbold), and the animal filarid, *B. pahangi* (Buckley and Edeson).

Treatment with ethyl methanesulfonate (EMS) changed the susceptibility to *B. pahangi* in the F_1 progeny of a moderately susceptible *Ae. aegypti* strain (PUGU) (Rodriguez 1985). The present study was conducted to determine whether treatment with EMS alters the expression of the f^m gene for susceptibility to the human filarid. *B. malayi*. The F_2 progeny, derived from EMS-treated parental males and females, and three F_4 populations, established through sibling intercross matings, of *Ae. aegypti* (Black Eye strain) were tested.

Aedes aegypti (Black Eye strain) was obtained from George B. Craig Jr., Vector Biology Laboratory, University of Notre Dame. Mosquitoes were reared, and females infected were maintained in a reach-in incubator at 27 ± 1°C and 85 ± 10% RH. The infection techniques used were described previously (Rodriguez 1973).

Four groups of 50 males and 50 females were allowed to feed freely for 5 days on sugar cubes treated with 0.025, 0.050, 0.075 and 0.10% EMS (Sigma Laboratories, St. Louis, MO) as described earlier (Rodriguez 1985). After treatment with EMS, 2 replicates of 5 groups (1 control and 4 treated) of 10 mosquitoes of each sex were maintained in 1.9-liter (1/2-gal) mosquito cages. The F_2 and F_4 progeny were derived from sibling F_1 and F_3 matings, respectively. Five groups of 10-pair matings with one replicate each were maintained for the F_2 progeny. Three groups of 10-pair matings (control, 0.025 and 0.10% EMS) and one replicate each were made to form the F_4 populations. To ensure maximum egg production, female mosquitoes were given 2 blood meals before mating and at 3-day intervals after mating, on anesthetized mice. Eggs were collected at 7-day intervals over a 21 day period.

Surviving F_2 and F_4 adult females collected from each of the 7-day intervals were tested for changes in filarial nematode susceptibility. Briefly, 3-day-old F_2 and F_4 females from each of the 5 or 3 groups were starved for 24 h then fed simultaneously on 2 jirds (*Meriones unguiculatus*) infected with *Brugia malayi* having a microfilarial density of 108 per 20 mm^3 of blood. A total of 125 F_2 females per group from each week (1, 2 and 3) were exposed to infected jirds. From these only 50 (week 1), 60 (week 2) and 75 (week 3) engorged females per group and week were selected, placed in different 3.8-liter (1-gal) mosquito cages, and held in an incubator 10–14 days for dissection. With the F_4 females only 100 per group were infected and 60 of the engorged females per group (control, 0.025 and 0.10% EMS-tested) were tested for susceptibility. All F_2 and F_4 infected females that remained alive after this 10- to 14-day period were dissected in 2–3 drops of *Aedes* physiological saline. Mosquitoes were classified as susceptible if one or more L_3s per female were present (Rodriguez and Craig 1973). The number of 3rd-stage larvae (L_3) per female for each treatment was compared statistically by analysis of variance (ANOVA).

Table 1 summarizes results from 3 separate experiments wherein F_2 females were infected with *B. malayi* to test for changes in filarial susceptibility over a 3-wk period.

Mosquito mortality during the first week was low. The control group only showed an 11% mortality. The 0.075% EMS-treated group had

Table 1. Susceptibility and variation to *Brugia malayi* in female F_2 *Aedes aegypti* (BLACK EYE) derived from male and female parents treated orally with EMS.

Dose EMS (%)	Week	Females dissected for L_3 larvae: % Infected (*n*)	Mean ± SE
0	1	54 (43)	2.86 ± 0.59
	2	42 (60)	2.83 ± 0.58
	3	32 (75)	1.75 ± 0.45
0.025	1	24 (45)	1.38 ± 0.43
	2	37 (60)	2.08 ± 0.51
	3	27 (75)	1.07 ± 0.26
0.050	1	35 (40)	2.03 ± 0.53
	2	33 (60)	2.07 ± 0.43
	3	25 (75)	1.20 ± 0.30
0.075	1	43 (37)	2.41 ± 0.60
	2	37 (60)	2.43 ± 0.57
	3	36 (75)	2.53 ± 0.50
0.10	1	15 (48)	0.83 ± 0.32
	2	22 (60)	0.82 ± 0.26
	3	15 (75)	0.52 ± 0.17

13%, while at 0.025, 0.050 and 0.10% EMS only gave 5, 10 and 11% mortality, respectively.

Susceptibility in the control population over a 3-wk test period was 43%. At 0.075% EMS susceptibility was 39%. The 0.025% EMS-treated group showed a 29% susceptibility. At 0.050% EMS susceptibility was reduced to 31%, while at 0.10% EMS susceptibility decreased to 17%. Only the 0.10% EMS-treated group showed a statistically significant decrease in susceptibility when compared to the control (Table 1; $\chi^2 = 12.54$; df = 4; $P < 0.05$).

The mean number of 3rd-stage larvae (L_3) present per F_2 female in EMS-treated populations was also significantly reduced with increased doses of EMS (Table 1; F = 2.746; df = 4,883; $P < 0.002$). The control population showed an average of 1.75 L_3s after 3 weeks. At 0.10% EMS, the mean L_3 number was significantly reduced (71% decrease, $P < 0.05$). Two-sample *t*-tests also indicate that differences in mean L_3 numbers between control and 0.10% EMS-treated populations occurred after weeks 1, 2 and 3 ($t = 3.025$, df = 89, $P < 0.01$; $t = 3.162$, df = 118, $P < 0.01$; $t = 2.557$, df = 148, $P < 0.05$; respectively). During week 1, the 0.025% EMS F_2 females also had a 52% decrease in mean L_3s, which proved significant ($t = 2.027$, df = 86, $P < 0.05$).

Table 2 summarizes the results obtained from the 3 lines (control, 0.025 and 0.10% EMS-treated) of F_4 females which were only infected with *B. malayi* over a 3-wk period.

Susceptibility in the F_4 control population over the 3-wk test period was 65%. The 0.025% EMS-selected line gave a slight decrease of 56%, while the 0.10% EMS-selected experimental line was significantly reduced by 43% ($\chi^2 = 28.41$; df = 3; $P < 0.001$).

The mean number of L_3s supported per F_4 female in EMS-selected populations was also significantly reduced (F = 13.377; df = 2.357; $P < 0.00001$). The control line showed an average 3.82 L_3s during 3 wk while the 0.10% EMS line was decreased significantly by 76% ($\bar{X}$ L_3 at 0.10% EMS = 0.91; $t = 5.333$; df = 258; $P < 0.001$). Two-sample *t*-tests also showed mean number L_3 differences between control and 0.10% EMS-selected populations during weeks 1, 2, and 3 ($t = 5.661$, df = 118, $P < 0.001$; $t = 5.055$, df = 118, $P < 0.001$; $t = 5.150$, df = 118, $P < 0.001$, respectively).

Although previous studies with *Aedes aegypti* have been conducted on the susceptibility to vertebrate pathogens, *Plasmodium gallinaceum* (Kilama and Craig 1969) and the filarial nematodes, *Brugia malayi* (Macdonald 1962a, 1962b; Macdonald and Ramachandran 1965) and *B. pahangi* (Rodriguez 1973, Rodriguez 1975, Paige and Craig 1975, Rodriguez and Craig 1973) there have been relatively few reports on the genetic effects of chemically (EMS) induced changes on the susceptibility and development of a pathogen in a vector host (Rodriguez 1985). Genetic (Macdonald 1962b) and environmental factors, such as radiation (Richey and Rodriguez 1976) and temperature (Rodriguez 1975), have been shown to affect filarial development, with the latter possibly modifying the expression of the f^m gene.

The present study indicated that EMS-induced mutations affected the f^m locus for filarial susceptibility in F_2 and F_4 progeny of *Ae. aegypti.*

Table 2. Susceptibility and variation to *Brugia malayi* infections in female F_4 *Aedes aegypti* (BLACK EYE) selected from control and EMS-treated lines.[a]

Dose EMS (%)	Week	Females dissected for L_3 larvae: % Infected (*n*)	Mean ± SE
Control	1	67 (60)	4.67 ± 0.55
	2	67 (60)	4.10 ± 0.53
	3	60 (60)	2.70 ± 0.40
0.025	1	55 (60)	3.27 ± 0.53
	2	62 (60)	3.15 ± 0.46
	3	50 (60)	2.28 ± 0.39
0.10	1	23 (60)	1.15 ± 0.29
	2	25 (60)	1.07 ± 0.28
	3	18 (60)	0.50 ± 0.15

[a] Derived from F_2 and adult male and female parents originally exposed orally to 0, 0.025, and 0.10% EMS, respectively.

Alternatively, the mutations induced by EMS could have altered modifier genes or specific loci that are associated with the f^m gene. This genetic change was manifest by both a reduced susceptibility and number of infective larvae in F_2 females derived from adult parental males and females exposed to EMS.

Other experiments completed in our laboratory with *B pahangi* infections and the same BLACK EYE strain of *Ae. aegypti* have given similar results in F_2 progeny. Adult F_2 females derived from female pupae exposed to 0.025% EMS showed a 21% decrease in filarial nematode susceptibility and 25% fewer L_3s. Although susceptibility was reduced by 18% in 0.075% EMS-treated populations, no significant reductions in mean L_3 numbers were obtained (Larson 1986).[1] Too few F_2 progeny were recovered in 0.10% EMS-treated populations to permit any filarial susceptibility experiments.

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[1] Larson, G. A. 1986. Genetic fitness and filarial susceptibility effects of ethyl methanesulfonate (EMS) and thiotepa on populations of *Aedes aegypti*. M.S. thesis, University of Texas at San Antonio.

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