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REDUCTION IN SUSCEPTIBILITY TO *BRUGIA MALAYI* OF F_2 PROGENY OF *AEDES TOGOI* TREATED WITH ETHYL METHANESULFONATE

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ABSTRACT. The susceptibility to *Brugia malayi* infection was tested in F_2 female progeny derived from male and female *Aedes togoi* treated with ethyl methanesulfonate (EMS). Three-day-old males and females were treated with 0.025, 0.050, and 0.075, 0.10, 0.15, or 0.20% EMS by allowing them to feed for 5 days on sugar cubes containing EMS and then mated at random. Percentage of susceptibility and mean number of infective larvae (L_3) in F_2 females were analyzed over a 2-wk period. Reductions in susceptibility were significant in the F_2 populations arising from the 3 highest EMS concentrations. F_2 infections were reduced by 80%, indicating that EMS-induced mutations affect loci associated with filarial nematode susceptibility.

KEY WORDS *Aedes togoi*, EMS-induced mutations, *Brugia malayi*

INTRODUCTION

In *Aedes aegypti* (L.), susceptibility to the filarial nematode *Brugia malayi* (Brug) is controlled by a sex-linked recessive gene, f^m (Macdonald 1962, Kilama and Craig 1969). The f^m gene (Macdonald and Ramachandran 1965) also controls the development of the human parasite *Wuchereria bancrofti* (Cobbold) and the animal filarid *Brugia pahangi* (Buckley and Edeson). Research is under way in our laboratory to extend our information on the genetics of filarial nematode susceptibility in the laboratory model *Ae. aegypti* and to determine whether similar or other mechanisms exist in a natural vector (Ramachandran et al. 1963, Macdonald 1971, Mori et al. 1985) such as *Aedes togoi* (Theobald). At present, the genetic mechanisms involved in the transmission of vector borne diseases are poorly understood, and the mode of inheritance in the natural vector *Ae. togoi* has not been adequately determined. Susceptibility to filarial nematode infection could well be polygenic, with major and minor gene involvement, or may include cytoplasmic factors, as observed in some studies with *Aedes polynesiensis* strains (Tripis at al. 1981).

The mutagenic effects of radiation and various alkylating agents in insects have been reported by several investigators. Parameters include effects on reproduction and development (e.g., Crystal and La Chance 1962, Thompson and Rodriguez 1979) or genetic fitness such as viability, longevity, population size, or productivity (e.g., Wallace 1958, Crenshaw 1965, Ayala 1966). However, relatively few studies have focused on the genetic effects of chemical mutagens on the development of a pathogen in a vector–host system (Rodriguez 1985, Rodriguez, et al. 1992). In mosquitoes, Bertram (1963) described chemosterilization in *Anopheles gambiae* and *Ae. aegypti* with thiotepa. Apholate treatment of *Ae. aegypti* larvae produced both somatic and chromosomal damage (Rai 1964), developmental abnormalities in adult tissue (Sharma and Rai 1969), and pathological changes in male accessory glands (Powell and Craig 1970). Ethyl methanesulfonate (EMS) and thiotepa treatment of adults induce mutations that also affect reproductive potential and development in *Ae. aegypti* (Esparza and Rodriguez 1975, Thompson and Rodriguez 1979). Specific host–parasite interaction experiments in our laboratory showed that treatment with EMS decreased the susceptibility to *B. pahangi* in the F_1 progeny of a moderately susceptible *Ae. aegypti* strain (PUGU) (Rodriguez 1985). Other studies (Rodriguez et al. 1992) have produced similar results for the F_2 progeny of the BLACK EYE Liverpool strain of *Ae. aegypti* with *B. malayi* infection after treatment with EMS. The current study determined whether treatment with EMS also altered the susceptibility of *Ae. togoi*, a natural *B. malayi* vector.

MATERIALS AND METHODS

Mosquitoes originated from the IMR strain of *Ae. togoi* obtained from the National University of Singapore, Republic of Singapore. The original filarid susceptibility of this strain is as high as 65% (Rodriguez, unpublished data), and the F_2 progeny, derived from EMS-treated parental males and females, were tested for development of *B. malayi*. Clawed Mongolian gerbils were used as the vertebrate host of *B. malayi* (Ash and Riley 1970).

Mosquitoes were reared and maintained at 25°C

Table 1. Susceptibility to *Brugia malayi* in female F_2 *Aedes togoi* (IMR) derived from male and female parents treated orally with ethyl methanesulfonate (EMS).

Concentration EMS (%)	Week	Females tested for L_3 larvae: % with L_3 larvae (*n*)	Females tested for L_3 larvae: L_3 per female (Mean ± SE)
0	1	70 (60)	2.87 ± 0.48
	2	50 (60)	1.67 ± 0.22
0.025	1	60 (60)	1.87 ± 0.31
	2	38 (60)	0.70 ± 0.09
0.050	1	60 (55)	1.49 ± 0.21
	2	33 (60)	0.50 ± 0.07
0.075	1	39 (51)	0.55 ± 0.11
	2	33 (60)	0.53 ± 0.07
0.10	1	35 (60)	0.45 ± 0.09
	2	27 (60)	0.40 ± 0.05
0.15	1	24 (51)	0.65 ± 0.20
	2	20 (60)	0.32 ± 0.04
0.20	1	30 (60)	0.58 ± 0.18
	2	22 (60)	0.38 ± 0.05

Table 2. Variation in susceptibility to *Brugia malayi* in female *Aedes togoi* (IMR) derived from male and female parents treated orally with EMS.

Dose EMS (%)	Week	No. L_3 larvae/female: Range	No. L_3 larvae/female: Mean ± SE
0	1	1–19	2.87 ± 0.48
	2	1–8	1.67 ± 0.22
0.025	1	1–10	1.87 ± 0.31
	2	1–9	0.70 ± 0.09
0.050	1	1–5	1.49 ± 0.21
	2	1–3	0.50 ± 0.07
0.075	1	1–2	0.55 ± 0.11
	2	1–8	0.53 ± 0.07
0.10	1	1–3	0.45 ± 0.09
	2	1–3	0.40 ± 0.05
0.15	1	1–6	0.65 ± 0.20
	2	1–4	0.32 ± 0.04
0.20	1	1–4	0.58 ± 0.18
	2	1–4	0.38 ± 0.05

and 80% relative humidity under a 16:8 L:D photoperiod in a reach-in environmental chamber (Scientific Systems, Baton Rouge, LA). Females were infected by techniques similar to those described by Rodriguez (1973). Briefly, males and females were sorted during the pupal stage and kept separated in 3.8-liter paper carton mosquito cages prior to EMS exposure. Four groups of 50 5-day-old males and 50 5-day-old females were allowed to feed freely for 5 days on 4 sugar cubes treated with either 0.025, 0.050, 0.075, 0.10, 0.15, or 0.20% EMS (Sigma Laboratories, St. Louis, MO). Each cube was treated with 250 μl of the EMS solution for a total of 1 ml per experimental cage (Rodriguez 1985). A control group of 50 5-day-old males and 50 5-day-old females received sugar cubes treated with deionized water (solvent).

After exposure to the sugar cubes, 2 replicates of the 7 groups (1 control and 6 EMS concentrations) of 10 mosquitoes of each sex were transferred to 1.9-liter mosquito cages. F_2 progeny were derived from these F_1 matings. Mosquitoes were offered the opportunity to bloodfeed twice before mating and at 3-day intervals thereafter on anesthetized mice (Rodriguez 1985, Rodriguez et al. 1992). Egg collections were made once during each following week.

F_2 adult females collected from the 1-wk and 2-wk periods were used to test for changes in filarial susceptibility. Briefly, 3-day-old F_2 females from each of the 7 groups were starved for 24 h, then fed on 4 jirds infected with *B. malayi* having a microfilarial density of 108–240 microfilaria per 20 mm^3 of blood. Four jirds were placed on 1 cage group, then transferred to each of the other 6 cages. Overall, 100 engorged F_2 females per group per week were selected, placed in different 3.8-liter mosquito cages, and held in an incubator for 12–14 days. All F_2 females that remained alive after this holding period were dissected in 2–3 drops of *Aedes* physiological saline (Hayes 1953). Animal use protocols for both mice and jirds were approved by our institution's Institutional Animal Care and Use (IACUC) and are in compliance with National Institutes of Health regulations.

Mosquitoes were classified as susceptible if infective L_3 larvae were present in the proboscis, head, thorax, or abdomen (Rodriguez and Craig 1973). The numbers of L_3 larvae per female for each treatment and the 2 separate experiments were compared statistically by a χ^2 contingency test, analysis of variance (ANOVA), and Duncan's multiple range test with the GB-Stat statistical package (Dynamic Microsystems 1988).

RESULTS AND DISCUSSION

Table 1 summarizes results from 2 separate experiments in which F_2 females were infected with *B. malayi* to test for changes in filarial nematode susceptibility over the 1- and 2-wk periods. Table 2 and Figure 1 show mean L_3 variation in susceptibility to *B. malayi* infections in F_2 *Ae. togoi.* Susceptibility in the control population over the 2-wk test period was 60%. The 0.025% EMS-treated group showed only an 11% decrease in susceptibility. At 0.050, 0.075, and 0.10% EMS, susceptibility was reduced by as much as 29%. In the 0.15 and 0.20% EMS-treated groups, susceptibility was decreased by 38 and 34%, respectively, when compared with the control (χ^2 = 22.72, df = 6, $P < 0.01$; Table 1).

The mean number of L_3 per F_2 female varied among EMS-treated and control populations (F =

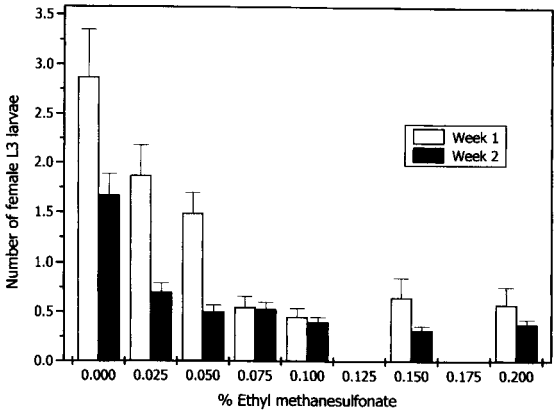

Fig. 1. Variation in mean L_3 larvae (±SEM) to *Brugia malayi* infections in female F_2 *Aedes togoi* (IMR) over a 2-wk period derived from male and female parents initially treated orally with ethyl methanesulfonate (EMS).

18.71, df = 6,810, $P < 0.0001$; Table 1 and Fig. 1). The control population had an average of 2.27 L_3 per infected female after 2 wk. The 0.025, 0.050, and 0.075% EMS F_2 females had a 44–76% decrease in mean L_3 numbers. At 0.10, 0.15, and 0.20% EMS, mean L_3 numbers were reduced by 81, 79, and 79%, respectively (Table 1). Differences between weeks 1 and 2 occurred only in the controls and the 0.025% and 0.050% EMS groups. Multiple range tests (Sokal and Rohlf 1981) also indicated that most significant decreases in mean L_3s per female between control and EMS-treated populations occurred in both weeks 1 and 2 for the highest groups, 0.075–0.20%.

Relatively few investigators have reported the effects of mutagens on susceptibility and development of a pathogen in the mosquito vector (Rodriguez 1985). Genetic (Macdonald 1962) and environmental factors, including radiation (Richey and Rodriguez 1976) and temperature (Rodriguez 1975), can affect filarial susceptibility and development. Filarial susceptibility in progeny of *Ae. aegypti* also was altered with EMS (Rodriguez 1985).

The present study indicates that EMS-induced mutations affect filarial susceptibility in F_2 progeny of *Ae. togoi*. The phenotypic expression itself was manifest by both a reduced susceptibility and number of infective larvae in F_2 *Ae. togoi* derived from adult parents exposed to EMS. Susceptibility to filarial nematode infection could well be multigenic, and EMS exposure could have affected various genes, including viability, longevity, or other genetic fitness alleles. One group of investigators, for example, provided evidence for a complex mode of inheritance of *Plasmodium* susceptibility in *Ae. aegypti* (Thathy et al. 1994). Previous studies with other insect systems produced mutagenic changes affecting viability, productivity, and population size (Wallace 1958, Crenshaw 1965, Ayala 1966, Esparza and Rodriguez 1975). A mutagen study with the flour beetle–cestode system also reduced productivity or genetic fitness upon mutagenic exposures (Rodriguez 1970). Our own studies with the mosquito–filarial nematode system have shown significant differences in filarial larval development between susceptible and refractory strains 2–3 days postinfection (Rodriguez et al. 1984). Related studies also indicated high titers of acid hydrolase enzyme activities in refractory strains of *Ae. aegypti* and an EMS-induced partially refractory F_3 strain of *Ae. togoi* challenged with *B. malayi* infections (Schirf and Rodriguez, unpublished).

Experiments with *B. malayi* infections and the BLACK EYE Liverpool strain of *Ae. aegypti* have given similar results in F_2 and F_4 progeny. These progeny also were derived from parental males and females exposed to EMS (Rodriguez et al. 1992). Susceptibility in 0.10% EMS-selected F_2s, for example, was reduced by 25%, whereas mean L_3 numbers decreased by 71%. F_4-selected populations derived from the 0.10% EMS treatments were 43% less susceptible when compared with the controls, and mean L_3s were reduced by 77%.

Currently, studies are being pursued to determine specific genetic mechanisms for filarial susceptibility in *Ae. togoi*. Partially refractory lines were established, and biochemical procedures are being developed to compare variation in acid hydrolase enzyme titers (Rodriguez et al. 1998) and electrophoretic variants in different filarial worm-susceptible and partially refractory genotypes of *Ae. togoi*. Experiments with another mosquito, *Aedes albopictus*, indicate that EMS and low levels of thiotepa induce dominant lethal mutations and reduce reproductive potential in this species. Reduced levels of enzyme activity or titers of acid hydrolases like acid phosphatase, α-glucosidase, and β-glucuronidase also demonstrated strong correlation with decreases in reproductive potential (Gonzalez, Brown and Rodriguez, unpublished). Perhaps exposure to EMS or other mutagens affects genes as well associated with these acid hydrolases. Acid hydrolases have been proposed as humoral or cell-mediated defenses to immunologic challenge in the snail host (Cheng et. al 1977, Cheng and Garrabrant 1977, Cheng and Dougherty 1989) as well as the mosquito vector and may play an important role in genetic mechanisms related to filarial nematode susceptibility (Soderhall and Smith 1986, Stoffolano 1986, Rodriguez et al. 1998).

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