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TOLERANCE OF *AEDES AEGYPTI* LARVAE TO SYNTHETIC SEWAGE

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ABSTRACT. Tolerance of *Aedes aegypti* larvae to synthetic sewage was investigated in 8 strains of the mosquito. First instar larvae, 4 hr after hatching, were raised in a range of concentrations of sewage, mortality being scored on day 4. There was significant heterogeneity between strains. Tolerance was particularly low in 2 longstanding laboratory strains suggesting laboratory selection for clean water breeders. Tolerance also seemed partly related to the geographical origins of strains, being consistently high in 3 from Africa.

The sex ratio in survivors of exposed individuals did not differ significantly. The tests are discussed in relation to reported cases of breeding by *Ae. aegypti* in soakage pits, drains and similar sites.

INTRODUCTION

Aedes aegypti (Linnaeus) is generally considered to be a clean water breeder (Christophers 1960). However, some observers have found *Ae. aegypti* in dirty water, larvae having been found by Boyce (1910) in drains, by MacFie and Ingram (1916) thriving in liquids rich in animal and vegetable debris, by Reed, Carroll and Agramonte (1901, quoted by Horsfall 1955), in pools and drop buckets of privies, and by Chinery (1969) in drains, latrines and septic tanks. Recently, Curtis (1980) recovered *Ae. aegypti* adults from a soakage pit in Dar es Salaam, Tanzania.

This paper is a report of preliminary experiments to compare strains of *Ae. aegypti* from various parts of the world for tolerance to synthetic sewage (Stoveland et al. 1979) in the laboratory.

METHODS AND MATERIALS

A stock solution of synthetic sewage was made up according to the method of Darwall (1979), modified after Stoveland et al. (1979), the constituents of which are given in Table 1, and diluted with tap water to known concentrations. The units of concentration are given as ml liter of concentrate, e.g., 40 ml liter represents 40 ml of concentrate in 1 liter of water.

Table 1. Composition of synthetic sewage. Amounts to make 1 liter of stock solution.

Constituent	gm
Neutralized Bacteriological Peptone (Oxoid, L34)	14.04
Lab Lemco (meat extract; Oxoid, L29)	9.36
Sodium chloride	0.57
Calcium chloride	0.29
Magnesium sulphate	0.09
Ammonium chloride	4.32
Potassium dihydrogen orthophosphate	0.39

A summary of information on the various strains used in these experiments is given in Table 2. The eggs, taken from laboratory stocks, were hatched in an infusion of hay. Three to 4 hrs later the first instar larvae were transferred to a range of synthetic sewage concentrations. Normally, 100 larvae were tested at each concentration. The tests were carried out in 7–8″ diameter white porcelain bowls, using 500 ml of solution. Controls were raised in tap water and fed with powdered dog biscuit from the first day. The air temperature for all investigations was 28±1°C, with a relative humidity of 75±5%.

Very few larvae (no more than 2 or 3 per bowl) died within 24 hrs. The weekend intervened, and mortality was scored on day 4. Percentage mortality was plotted in probits against log concentration (Finney 1971), and the LC50 estimated visually.

RESULTS

Percentage larval mortality on day 4 is shown in Table 3. Means of replicates and standard errors were calculated for each strain at 0, 20, 30, 40, and 60 ml liter. There were 2 or more replicates of 100 larvae tested at each concentration. The standard errors were often high reflecting wide variation between replicates. An analysis of variance was conducted on the arc sin transformed mortalities at each concentration using 2 replicates for each strain, tested on different days. The variance between strains was highly significant ($F = 4.3$, $P < 0.01$, Table 4). Accra, Enugu, Kwa Dzivo and San Salvador were the most tolerant strains and Bangalore, Sri Lanka, Penang and Isla Verde the least tolerant.

A proportion of the larvae surviving

Table 2. Information on strains of *Aedes aegypti* used in these experiments.

Strains	Information	Laboratory age (years)
San Salvador	El Salvador. Sent by Dr. M. E. Tinker	5
Isla Verde	Puerto Rico. Collected from airport sent by Dr. I. Fox	22
Accra	Ghana. Larvae collected in wild, and eggs sent by Dr. W. Z. Coker	7
Enugu	Nigeria. Larvae collected in wild, eggs sent by Dr. F. Lambrecht	5
Kwa Dzivo	Kenya. Ovitraps inside houses. Eggs sent by Dr. J. L. Petersen	5
Bangalore	India. Eggs laid in captivity by females inseminated in wild. Sent by Dr. C. F. Curtis	7
Sri Lanka	Sri Lanka. Eggs sent by Dr. W. A. Samarawickrema	5
Penang	Penang Island, Malaysia. Eggs from laboratory stocks sent by Prof. A. W. A. Brown	24+

Table 3. Mean percentage larval mortality ± standard error, scored on day 4 at a range of concentrations of synthetic sewage. Mean percentage mortality has been taken to the nearest whole number ± standard error corrected on the same basis except when less than unity.

	Strains	Control	ml/liter 20	30	40	50	60	LC50
America	San Salvador	1 ± 0.5	23 ± 16	27 ± 19	56 ± 13	96 ± 1		33
	Isla Verde	6 ± 1	51 ± 9	66 ± 11	93 ± 2	94 ± 2	99 ± 1	20
Africa	Accra	1 ± 0.4	29 ± 14	17 ± 6	61 ± 15	77 ± 8		34
	Enugu	6 ± 2	44 ± 9	41 ± 10	62 ± 18	75 ± 11	93 ± 3	34
	Kwa Dzivo	0	22 ± 6	47 ± 30	72 ± 20	80 ± 14		34
Asia	Banglore	6 ± 1	37 ± 12	54 ± 12	79 ± 15	95 ± 3	98 ± 1	25
	Sri Lanka	8 ± 5	48 ± 6	71 ± 3	90 ± 7	80 ± 14	93 ± 5	20
	Penang	12 ± 4	51 ± 4	86 ± 2	75 ± 11	96 ± 2	97 ± 0.8	20

Table 4. Analysis of variance in the response of larvae of 8 strains of *Ae. aegypti* to 5 concentrations of synthetic sewage.

Source	D.F.	SS	MS	F
Concentrations	3	14,131.53	4,710.51	18.494
Strains	7	7,743.81	1,106.26	4.343
Concentrations × Strains	21	4,148.81	197.56	0.776
Replicates	32	8,150.30	254.70	
Total	63	34,174.45		

to day 4 pupated. Data for 3 strains are given in Table 5. Bangalore continued to be very susceptible but Isla Verde and Enugu were more tolerant. However, many of the pupae which survived were undersized and gave rise to inviable adults which drowned on emergence. A higher proportion of viable adults was obtained if larvae were fed after day 4 with dog biscuit. The sex ratio was normal in all strains. (χ^2 = 0.17−3.54).

DISCUSSION

Larvae of all strains were found to have some degree of tolerance to synthetic sewage. The maximum concentration which would allow minimal larval survival was 70 ml/liter. Comparing strains, it was shown that Accra, San Salvador, Enugu and Kwa Dzivo were more tolerant of synthetic sewage than the remaining strains.

The 2 strains (Isla Verde and Penang) with the longest history of laboratory culture (22, 24+ yr) were alike in showing particularly low tolerance. This suggests that the laboratory environment may tend to select for clean water breeders. It is all the more interesting therefore, that,

Table 5. Percentage of larvae surviving on day 4 that went on to pupate, at a range of concentrations of synthetic sewage. Sample size is given in parentheses.

Strains	Control	ml/liter 20	25	30	35	50
Isla Verde	100 (92)	71 (55)	89 (28)	42 (12)	63 (8)	0 (1)
Enugu	80 (92)	77 (73)	79 (56)	82 (60)	65 (51)	57 (63)
Bangalore	98 (92)	20 (96)	34 (86)	27 (82)	30 (80)	—

when comparing all strains together, none of which had less than 5 yr in the laboratory, we should find a significant genetic diversity between them. It is concluded that tolerance to synthetic sewage is rather stable even in the face of laboratory selection.

Tolerance seems possibly related to geographical distribution, the most tolerant strains being of African origin. The San Salvador strain was also very tolerant. The only cases reported in the literature of tolerance to sewage are for African (Chinery 1969, Curtis 1980) and American (Horsfall 1955) strains. Observations from as early as 1910 (Boyce) indicate that tolerance to polluted water existed at that time. However, changes in the environment such as the use of insecticides in clean water breeding sites, and the relative abundance of polluted water in some cities may favor the selection of strains tolerant of water contaminated with human waste and other organic matter, particularly if alternative breeding sites are not available. Consideration should be given to this possibility when planning excreta disposal systems.

It is relevant to consider which constituents of synthetic sewage might cause mortality in mosquito larvae. It seems unlikely that salts are a significant factor, as the concentration in the synthetic sewage was very low. In the wild, larvae have been found in total chloride concentrations of up to 0.033%, (although they will survive higher concentrations experimentally), while in our experiments, the maximum was 0.025%. A 2% sodium chloride solution is fatal to *Ae. aegypti* (MacFie 1916), but this far exceeded our maximum of 0.006%, and this concentration should not deter oviposition, since Wallis (1954) found that 0.25% NaCl was preferred over distilled water.

Aedes aegypti tolerates a wide pH range being found naturally in pH 5.8–8.6 (Christophers 1960). The synthetic sewage contained a neutralized peptone, and the meat extract was of pH 7.0, so this factor is not likely to be responsible for larval mortality. Of other factors which may cause larval death, all the amino acids essential for *Ae. aegypti* (as determined by Singh and Brown 1957) were present. Media containing only amino acids are inferior to those with an alternative protein source (Akov 1962), but since the synthetic sewage contained protein in the form of meat extract, this should have presented no problem.

These investigations have been limited to the survival of larvae and pupae in synthetic sewage. We have yet to investigate the ability of eggs to hatch successfully in this medium, and to conduct oviposition experiments.

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ATTEMPTED SUPPRESSION OF A SEMI-ISOLATED POPULATION OF *CULEX TARSALIS* BY RELEASE OF IRRADIATED MALES

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ABSTRACT. During the summer of 1980, an attempt was made to numerically suppress a semi-isolated population of *Culex tarsalis* by releasing radiosterilized males. A total of 71,016 males was collected as pupae from a productive source, Poso West, sterilized by exposure to 6 KR of gamma radiation within 24 hr of emergence and released at Breckenridge, 12.5 km east of Bakersfield in Kern County, California. The incidence of sterility in egg rafts oviposited by females collected in CO_2-augmented light traps increased significantly from 2.9% prior to sterile male releases to 9.2% during the release period. The mating competitiveness of the sterile males was estimated to be 1.1 based on the proportions of sterile males among all males and sterile egg rafts among all egg rafts. Even though the radiosterilized males mated competitively, the numbers released were insufficient to numerically suppress the target population.

Feasibility trials towards the genetic control of *Culex tarsalis* Coquillett were initiated in 1977 and 1978 at a semi-isolated site in Kern County, California by the release of males carrying a sex-linked double heterozygous translocation (Asman et al. 1979, Milby et al. 1980). The target population was not suppressed numerically which was attributed, in part, to the low mating competitiveness of the laboratory-adapted genetically altered males (Milby et al. 1980). Release of *Cx. tritaeniorhynchus* Giles males that carried a similar genetic control system yielded comparable results in Pakistan (Baker et al. 1979). These studies suggested that the colonization of *Culex* mosquitoes may rapidly alter mating behavior, resulting in a competitive disadvantage for mating when such males are released back into a field population (Reisen et al. 1980).

In a subsequent pilot study, 13,500 *Cx.*