GENETIC VARIATION IN TOLERANCE OF AMMONIUM CHLORIDE IN *AEDES AEGYPTI*

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ABSTRACT. Variation in tolerance to ammonium chloride among four strains of *Aedes aegypti* was investigated. Significant heterogeneity between strains was found and it was possible to more than double the tolerance of one strain by three generations of selection. A reduction in pupal size was observed in the survivors of exposure to ammonium chloride, the effect being more marked in the more susceptible strains. The evidence for a genetic component of tolerance is discussed in relation to possible adaptation to sewage-polluted waters.

INTRODUCTION

Pope and Wood (1981) found significant heterogeneity among eight laboratory strains of *Aedes aegypti* (Linn.) in tolerance to "synthetic sewage" (Stoveland et al. 1979) and they suggested that this species, which is recognized as a clean water breeder, may have the potential to become tolerant to sewage-contaminated water. Lydiard[1] identified ammonium chloride as the major toxic component of the synthetic sewage. In this paper we report the results of a comparative study of tolerance to ammonium chloride solution in four of these strains.

MATERIALS AND METHODS

The synthetic sewage used in previous studies included 4.32 g/liter ammonium chloride as one of its components (Pope and Wood 1981). To make the work described in the present paper comparable, a stock solution of ammonium chloride was made to the same concentration.

A series of dilutions was produced by adding different quantities (50–90) ml of stock solution to tap water and making up to 1 liter. The concentrations of ammonium chloride so produced ranged from 216 to 389 mg/liter. Larvae were reared in 750 ml of the appropriate concentration in 18–20 cm diameter bowls and were thus exposed for their complete larval life. The concentration was not regulated further. There were also control bowls to which no ammonium chloride was added.

Eggs were hatched in a hay infusion. After 2–3 hr the newly hatched larvae were counted, added in batches of 100 to each bowl and fed with ground dog biscuit. Any bacterial scum forming on the surface was removed daily.

In the selection experiment on the Isla Verde strain, 16–80 replicates of 100 larvae were exposed to ammonium chloride in each generation at a range of concentrations down to 21.6 mg/liter but only concentrations above 129.6 mg/liter produced mortality higher than that observed in the controls (no ammonium chloride added). The surviving pupae from bowls in which larvae had suffered at least 75% mortality (14 replicates in generation 1, 5 replicates in generation 2, 6 in generation 3) were transferred to clean tap water. The number of adults finally emerging to form each generation was more than 100 in generation 1 and more than 60 in generations 2 and 3.

The adults were provided with saturated sugar solutions and the females given a blood meal using 10-day old anaesthetized mice. Eggs were collected and the procedure repeated in the next and following generations. During all experiments the air temperature was maintained at 28±1°C and the relative humidity at 75±5%.

In order to determine pupal size, measurements were made of cephalothorax length (c.l.) and cephalothorax width (c.w.), taking the measurements at the points of maximum distance. Dose/response data were analysed using a computer program written in BASIC based on the method of analysis adopted by Litchfield and Wilcoxon (1949). The program in its original form was published by Tallarida and Murray (1981) but was modified by Dr. L. M. Cook (Manchester University) using algorithms given by White and White (1981).

Details of three of the strains investigated (Isla Verde, Enugu and Sri Lanka) are given by Pope and Wood (1981). The fourth strain was Mayaguez which was collected in Puerto Rico on January 23, 1975 and sent by Dr. M. E. Tinker. It was colonized in Manchester on June 12, 1975. The study was carried out in 1982–83.

RESULTS

Four strains were compared for tolerance to a range of concentrations. Because of wide varia-

[1] Lydiard, T. 1981. A mass selection programme for increased tolerance to synthetic sewage in the yellow fever mosquito *Aedes aegypti*. Unpublished M.Sc. Thesis, Pollution Unit, Manchester University.

tion among bowls it was necessary to replicate each test. In a comparison of four strains this meant 24 replicates for a single concentration (to include four tests and two controls for each strain). The number of larvae required was large and it was therefore decided to test different concentrations in successive weeks.

The results of tests over a range of concentrations (216–389 mg ammonium chloride per liter of rearing medium) are given in Table 1 which also presents an analysis of variance of these data. From the analysis it is evident that there was significant heterogeneity between strains ($F = 4.29$) and also between concentrations/weeks ($F = 7.60$).

The Sri Lanka strain was consistently the least tolerant and the Mayaguez strain tended to be the most tolerant strain. The other two strains were intermediate (Table 1). A similar analysis of variance on mortality in control bowls, without added ammonium chloride, showed no significant heterogeneity either between strains ($F = 0.31$) or between weeks ($F = 1.81$).

The selection experiment with the Isla Verde strain produced a response within one generation which increased by generation three to the extent that tolerance was more than doubled at L.C. 50 (Table 2) from 191 to 642 mg/liter.

Larvae of the various strains surviving 216 mg/liter ammonium chloride were reared to pupae and later assessed for size to compare with exposed controls. The results are presented in Table 3 from which it is clear that the pupae from larvae exposed to ammonium chloride were usually significantly smaller than the unexposed controls (the only exception being Mayaguez males). Moreover there was a direct relationship between size and percentage survival, the correlation coefficients (r) for cephalothorax length (c.l.) and cephalothorax width (c.w.) being significant in both sexes. [r (c.l.) $= 0.92$, $p < 0.05$; r (c.l.) $= 0.98$, $p < 0.01$; r (c.w.) $= 0.99$, $p < 0.01$; r (c.w.) $= 0.98$, $p < 0.01$], ie., the greater the survival, the larger the pupae.

DISCUSSION

There was significant heterogeneity among strains in response to ammonium chloride, just as there was in the study by Pope and Wood (1981) in tolerance to synthetic sewage, of which ammonium chloride was one constituent. A genetic component to variation in tolerance to ammonium chloride was also indicated by the response to genetic selection observed. A significant response to selection with ammonium chloride occurred within a single generation and there was a further increase in tolerance by F3. This was in contrast to the selection experiments of Lydiard (footnote) using whole synthetic sewage and the same Isla Verde strain (and also in a separate experiment on the Accra strain), who found no significant increase in tolerance even after eight generations of selection. It is possible that synthetic sewage is too complex a mixture to exert a directional force, taking into account all the many influences on mortality. Whether real sewage could exert di-

Table 1. Mortality (%) ± standard error at a range of concentrations of ammonium chloride, compared with control mortality, in four strains of *Aedes aegypti*. Different concentrations were tested on successive weeks. Analysis of variance was carried out on mortality data which had been corrected to take into account control mortality.

			Week				
Strain	Treatment	Concentration mg/liter mM NH_4	1 216 4.04	2 159 4.04	3 302 5.65	4 346 6.47	5 389 7.27
Mayaguez	Test		17.5 ± 11.9	1.8 ± 0.9	64.8 ± 11.2	90.5 ± 4.0	58.3 ± 15.0
	Control		33.0 ± 16.0	0	3.0	13.5 ± 9.5	2.0 ± 2.0
Enugu	Test		67.5 ± 5.0	35.0 ± 17.4	85.3 ± 9.2	79.3 ± 16.9	35.0 ± 12.8
	Control		13.0 ± 5.0	8.5 ± 1.5	4.0 ± 2.0	7.5 ± 2.5	3.0 ± 2.0
Isle Verde	Test		38.8 ± 16.4	37.3 ± 12.7	76.0 ± 11.0	97.3 ± 2.8	72.3 ± 7.8
	Control		4.5	5.0 ± 1.0	9.5 ± 4.5	1.0 ± 1.0	10.5 ± 0.5
Sri Lanka	Test		84.5 ± 3.6	73.4 ± 8.6	94.5 ± 0.5	99.5 ± 0.5	72.3 ± 12.4
	Control		3.0 ± 2.0	11.5 ± 4.5	6 ± 3.5	9.0 ± 1.0	16.5 ± 6.5

	S.S	d.f.	M.S.	F
Total	14669.28	19		
Strains	3418.16	3	1139.39	4.29
Concentrations (Weeks)	8065.90	4	2016.47	7.60
Error	3185.22	12	265.43	

Table 2. Mortality (%) ± standard error to a range of concentrations of ammonium chloride during three generations of selection of the Isla Verda strain of *Aedes aegypti.* Larvae used to start the selected generations (F_1-F_3) were taken from individual bowls showing 75% mortality or more. These were selected from the boxed area of the table.

	mg/liter ammonium chloride									
Generation	0 (Control)	130	173	216	259	302	346	389	432	L.C.50*
Unselected[a]	8.1 ±1.6	18.8 ±5.9	31.8 ±14.3	60.1 ±13.8	76.8 ±8.7	80.1 ±7.5	92.2 ±4.1	57.9 ±10.7	99.3 ±0.3	191.3 ±16.5
Selected F_1	6.0 ±2.1				22.3 ±15.4	34.0 ±11.1	59.5 ±19.8	89.7 ±5.7		321.3 ±9.7
Selected F_2	9.7 ±2.9					60.2 ±18.9	50.2 ±22.7	79.7 ±20.8	69.2 ±17.3	294.5 ±29.2
Selected F_3	15.3 ±5.2					41.5 ±13.5	30.7 ±11.4	43.0 ±12.7	49.0 ±15.2	642.4 ±96.1
Unselected[b]	5.7 ±1.7				68.7 ±23.8	84.2 ±9.1	86.7 ±7.9	93.7 ±3.9		213.8 ±17.0

[a] Beginning of experiment.
[b] End of experiment.
* L.C.50's are based on calculated lc-p lines, making allowance for control mortality (5.7 – 15.3%).

Table 3. Comparison of mean cephalothorax length and width in pupae of four strains of *Aedes aegypti.* Samples which had been exposed from the first instar larvae onwards to 216 mg/liter ammonium chloride (Test) are compared with unexposed controls. (Mortalities in the test and control groups are given in Table 1).

Strain	Sex	Treatment	No. measured	Mean length (mm) ± S.D.	Mean width (mm) ± S.D.
Mayaquez	♂	Test	34	1.96 ± 0.09	1.57 ± 0.08
		Control	36	1.96 ± 0.08[Δ]	1.56 ± 0.05[Δ]
	♀	Test	45	2.35 ± 0.09	1.82 ± 0.08
		Control	36	2.42 ± 0.08*	1.88 ± 0.07*
Enugu	♂	Test	45	1.79 ± 0.16	1.39 ± 0.14
		Control	41	1.96 ± 0.11*	1.54 ± 0.07*
	♀	Test	30	2.17 ± 0.18	1.64 ± 0.15
		Control	45	2.34 ± 0.11*	1.80 ± 0.08*
Isla Verde	♂	Test	51	1.85 ± 0.05	1.49 ± 0.07
		Control	36	1.89 ± 0.05*	1.54 ± 0.05*
	♀	Test	49	2.16 ± 0.08	1.72 ± 0.10
		Control	43	2.32 ± 0.06*	1.85 ± 0.07*
Sri Lanka	♂	Test	43	1.75 ± 0.08	1.38 ± 0.07
		Control	48	1.97 ± 0.05*	1.56 ± 0.03*
	♀	Test	16	1.98 ± 0.15	1.59 ± 0.08
		Control	35	2.40 ± 0.07*	1.89 ± 0.07*

[Δ] $P > 0.05$, * $P < 0.001$ (Student's *t* test).

rectional selection for tolerance is a matter yet to be determined but one of great practical interest in view of the observation by Chinery (1969) that *Ae. aegypti* in Accra was breeding in septic tanks and soakaways and by Babu et al. (1983) that in Pondicherry, South India, it was found in septic tanks, whereas the species is normally thought to be a clean water breeder.

Studies on the survivors of larvae exposed to ammonium chloride showed that the size of the pupal cephalothorax was usually significantly smaller than the controls. Moreover, the amount that any concentration of ammonium chloride affected the size depended on the tolerance of the strain. The more tolerant the strain the less the effect. It appears therefore that one aspect of ammonium chloride toxicity was an inhibition of growth.

Evidence from studies obtained mainly on fish and crustacea suggests that in freshwater animals excretion of ammonia by the gill is a physiological function linked to the maintenance of acid-base balance and to the cation (Na^+) absorption mechanisms, (Garcia-Romeu and Maetz 1964, Maetz 1973), the influx of Na^+ seeming to be inhibited by external NH_4^+ (Shaw 1960, Maetz 1973).

However in uricotelic animals, e.g., larvae of *Ae. aegypti* which actively absorb sodium through anal papillae, Na^+ ions are exchanged mainly for H^+ ions rather than NH^+ ions (Stobbart 1971a, 1971b). The effect of NH_4^+ on Na^+ influx in *Ae. aegypti* larvae is therefore less certain but any interference by NH_4 on Na influx could affect ionic regulation. Future work on the physiological effects of ammonium salts may elucidate the possible mechanism of ammonium chloride toxicity in relation to the inhibitor of growth observed in the present study.

Moreover it is intended to experiment further on the potential for this species to increase in tolerance to ammonium chloride and to investigate the effects of other ammonium salts on this and other species of mosquito especially those such as *Culex quinquefasciatus* Say known to breed in sewage-polluted water. Free ammonia and lesser amounts of some ammonium salts are commonly found in organically polluted water (Reid 1961).

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