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DDT-Resistance in *Anopheles stephensi*

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In view of the increasing number of reports from different parts of the world indicating resistance to DDT in both adults and larvae of Anopheles stephensi, *an important malaria vector, a series of laboratory studies has been carried out on the degree, the pattern and the mode of inheritance of resistance in this species. A DDT-resistant strain from Iraq and a susceptible strain from India were used.*

In four sets of observations made in the course of tests on both adults and larvae a monofactorial type of inheritance was indicated, and the factor involved was shown to be dependent for its expression on the genetic background.

DDT-resistance in A. stephensi *appears to be similar in most respects to that in* A. sundaicus.

The first indications that DDT was failing to control malaria transmitted by *Anopheles stephensi* were given by epidemiological observations made by Daggy (1957) in Saudi Arabia. The testing of adult mosquitos found in DDT-sprayed houses in this area confirmed that they were resistant to the insecticide (Davidson, 1958; Peffly, 1959). Later, a similar resistance appeared in the same species in Iraq (Gramiccia et al., 1958) and in Iran,[3] and indications of larval resistance were seen in Erode, South India (Rajagopalan et al., 1956). In all these areas DDT had been used for several years before resistance became apparent.

In Saudi Arabia a change of insecticide from DDT to dieldrin has resulted in the successful control of the DDT-resistant *A. stephensi*, though other species have become resistant to dieldrin (Peffly, 1959). In Iran a similar change has resulted in the appearance of *A. stephensi* resistant to dieldrin as well as to DDT.[4]

Laboratory investigations on resistance to insecticides in mosquitos started in the Ross Institute of Tropical Hygiene in 1955 with a study of the effect of selection with DDT of both larvae and adults of a strain of *A. stephensi* from Delhi, India, which had never been subjected to selection by insecticides in the field. The result of prolonged selection was a slight increase in resistance to DDT which was concluded to be of the "vigour tolerant" type as distinct from true specific resistance to DDT (Davidson, 1958). This Indian strain of *A. stephensi* was therefore taken to be a homozygous susceptible strain and the unselected stock used as such in the first crossings with the true resistant strain later acquired.

Towards the end of 1957, Dr Gramiccia of the World Health Organization kindly supplied us with a strain of *A. stephensi* from Moawiya, Iraq, where field-testing had established the presence of DDT-resistance. Preliminary testing of adult mosquitos of this strain showed a low mortality on that dosage of DDT giving a high mortality of the unselected Indian strain. A single selection of the Iraq strain was therefore made, saving only the survivors of a dosage which completely killed the Indian strain. However, the offspring of these survivors continued to show the low mortality they had shown before this selection, and we concluded that we were dealing with a pure DDT-resistant strain.

Crosses were then made between the resistant and susceptible strains and attempts made to work out the mode of inheritance of resistance along similar lines to those of Coker (1958) for a species in which the degree of resistance is low and in which complete discrimination of phenotypes is impossible.

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[3] Mofidi, C., Samimi, B., Eshghi, N. & Ghiassedine, M. (1957) In: *Information circular on the resistance problem, No. 11* (unpublished WHO document).

[4] Mofidi, C. et al. (1959) In: *Information circular on insecticide resistance, No. 20* (unpublished WHO document).

The results were confusing, and only a faint hint of a monofactorial pattern of inheritance was given.

During these observations it was frequently noticed that for any given population of insects exposed to the same dosage of insecticide and for the same exposure time large variations in mortality occurred. It was therefore decided to standardize the age of the insects at the time of testing and their method of rearing as far as possible. At the same time a further selection of the resistant strain was made as well as an attempt to select a more susceptible strain from the existing Indian strain. The results here recorded stem, for the most part, from observations after these last selections were made and after careful attention had been paid to standardization of the insects.

Egg measurements of the two strains showed little difference between the two. The measurements resembled those recorded by Sweet et al. (1938) for *A. stephensi mysorensis* (Table 1).

TABLE 1

MEAN EGG MEASUREMENTS (IN MICRONS) OF DDT-RESISTANT *A. STEPHENSI* FROM IRAQ AND SUSCEPTIBLE *A. STEPHENSI* FROM INDIA COMPARED WITH MEASUREMENTS RECORDED BY SWEET ET AL. (1938) FOR *A. STEPHENSI* TYPE FORM AND *A. STEPHENSI* VAR. *MYSORENSIS* [a]

Strain	Length	Width including floats	Length of float	Number of float ridges	Proportion of length covered by float
Type form	555	204	294	18	0.53
Var. *mysorensis*	476	160	218	13	0.46
Resistant (Iraq)	530(20)	176(20)	253(70)	14(50)	0.48
Susceptible (India)	506(20)	165(20)	221(70)	13(50)	0.44

[a] The figures in parentheses represent the number of eggs examined for a particular measurement.

TESTING TECHNIQUES

The susceptibility of adult mosquitos was assessed for the most part by the Busvine & Nash (1953) test; comparatively few tests were made with the World Health Organization adult susceptibility test kit. Both males and females were used, though the results for each sex were recorded separately. Except where otherwise stated, these were one-day-old mosquitos in an unfed state. Unwaxed paper cups with netting tops were used as recovery vessels.

Larval susceptibilities were assessed by a modification of the short larval test of Elliott (1958). Fourth-stage larvae were exposed for one hour to alcohol-water mixtures of insecticide; 25 larvae in 70 ml of the mixture in a small paper cup. At the end of the hour, the larvae were transferred to clean water in larger paper cups of approximately 220-ml capacity and given food. After a recovery period of five hours larvae capable of swimming to the surface and remaining there were counted as alive, the remainder as dead. The cups were placed under a mirror inclined at an angle of 45° to facilitate counting. Results could then be recorded by looking in the mirror without disturbing the larvae by casting shadows. Both exposure and recovery vessels were used once only, thus avoiding contamination hazards. The standard solution used for these tests was a 1% solution of DDT in absolute ethanol, 1 ml of this solution in 100 ml of water being taken as a dilution of 100 parts per million of DDT. A similar amount of alcohol in water was used as a control or check.

As will be noted later other dilutions were also used, as were different exposure and recovery times, though the total period of the test remained six hours throughout. In some cases, also, mass exposures were made in cylindrical glass vessels 9 inches (22.5 cm) in diameter and 5 inches (12.5 cm) deep. Lots of 350 larvae were exposed in these vessels in 1 litre of insecticide mixture, the equivalent in numbers of larvae and volume of test mixture to 14 paper cup exposures.

Knockdown rates of adult mosquitos were in some cases recorded at a given time after removal from the exposure tubes. More accurate results were given, however, from thistle-funnel tests in which the 3-inch × 1-inch (7.5 × 2.5 cm) exposure tube of the Busvine & Nash test, without its cork, was inverted in the thistle part of the funnel. Ten mosquitos were blown from a sucking-tube down the stem of the funnel into the exposure tube and a small wad of cotton wool pushed to within ¼ inch (6 mm) of the base of the funnel. The whole was then set upright in a test-tube rack and the rate of knockdown recorded.

Knockdown rates in larvae were observed in a 5-inch (12.5 cm) diameter glass funnel, the stem of which had been removed and the hole sealed. A glass tube running down the side of the funnel to this sealed, pointed, end was connected to a vacuum pump so that knocked down larvae could be removed at intervals. As a standard for this test,

50 p.p.m. of DDT in an alcohol-water mixture, made by adding 0.5 ml of a 1% DDT solution in ethanol to 100 ml of water, were used; and either 100 fourth-stage larvae in 300 ml of insecticide mixture or 200 larvae in 500 ml were observed for varying times.

STRAIN CHARACTERISTICS

In the adult stage the susceptible strain of *A. stephensi* from India shows a higher LC_{50} for DDT (1.6% for males and 2.8% for unfed females) than most anopheline species and shows an incomplete mortality even on saturated DDT (4%) in Risella oil after one hour's exposure. Two hours' exposure to this dosage kills all, however. One-hour exposures to this dosage produce a high knockdown with a low recovery rate during the ensuing 24 hours (Table 2). Using the thistle-funnel technique, which makes no allowance for recovery from knockdown, the unselected susceptible strain showed a complete knockdown in 35 minutes when exposed to 4% DDT in Risella oil (Table 3).

Using the short larval test of Elliott (1958) the susceptible strain shows an LC_{50} of about 30 p.p.m. DDT and an almost complete mortality at 100 p.p.m. The larval funnel test described shows a 50% knockdown in one hour in 50 p.p.m. DDT. An attempt at selection for increased susceptibility was made using this funnel test and keeping the first 20%-30% of the larvae knocked down. Most of these recovered in clean water and produced adults from which eggs could be obtained. This was done in three successive generations but little difference in the time to produce 50% knockdown was apparent, and although the third selection was adopted as the susceptible strain which was used later in studies on the inheritance of resistance, it is considered very little, if at all, different from the original strain.

Adults of the DDT-resistant strain from Iraq show a small but significant mortality when exposed to 4% DDT in Risella oil for one hour, but little or no mortality below this dosage. As in the sus-

TABLE 2

A COMPARISON OF PERCENTAGE KNOCKDOWN (SOME 4 HOURS AFTER REMOVAL FROM THE EXPOSURE TUBE) AND 24-HOUR MORTALITY AMONG MALE AND UNFED FEMALE SUSCEPTIBLE (SS), HYBRID (RS) [a] AND RESISTANT (RR) *A. STEPHENSI* WHEN EXPOSED FOR 1 HOUR TO 4% DDT IN RISELLA OIL

Strain	Number of mosquitos	Percentage knockdown	Percentage mortality
	Males		
SS	122	99	97
RS [a]	421	66	61
RR	314	10	15
	Females		
SS	128	84	81
RS [a]	432	53	45
RR	197	9	14

[a] Resistant male × susceptible female.

TABLE 3

A COMPARISON OF KNOCKDOWN TIMES OF MALE AND UNFED FEMALE SUSCEPTIBLE (SS), HYBRID (SR) [a] AND RESISTANT (RR) *A. STEPHENSI* WHEN EXPOSED TO 4% DDT IN RISELLA OIL USING THE THISTLE-FUNNEL APPARATUS

Time in minutes	Susceptible (SS)		Hybrid (SR) [a]		Resistant (RR)	
	Number of mosquitos	Percentage knockdown	Number of mosquitos	Percentage knockdown	Number of mosquitos	Percentage knockdown
	4% DDT					
35	36	100	27	82	54	6
60			27	100	54	9
	Check (Control)					
60	16	0	8	0	19	5

[d] Susceptible male × resistant female.

ceptible strain, the males are more susceptible than the females. Two hours' exposure to this dosage produces a 29% mortality in unfed females and a 51% mortality in males. Unlike the susceptible strain, the knockdown shown by the resistant strain shortly after removal from the DDT is slightly lower than the 24-hour mortality (Table 2). Using the thistle-funnel test, only 6% resistant mosquitos were knocked down in 35 minutes when exposed to 4% DDT in Risella oil, and in 60 minutes only 9%. These figures were only a little higher than the control (Table 3).

One hour's exposure of fourth-instar larvae of this resistant strain to 100 p.p.m. DDT, followed by 5 hours' recovery in clean water, produces a very low mortality while an increase in exposure time to two hours, with a 4-hour recovery period, produces a significant mortality (some 45%). The larval funnel test showed a 31% knockdown in two hours in 50 p.p.m. DDT. Selection was carried out by this method, saving and rearing from the survivors at 100-125 minutes in the first instance. The offspring of these survivors showed a 41% knockdown in 3 hours. The offspring produced by the remaining 59% showed only a 19% knockdown in 180-215 minutes, and 41% in 320-385 minutes. Survivors of these last exposure times were used to produce the resistant strain used later for crossing experiments. Here selection had quite definitely altered the tolerance of the strain.

TABLE 4

DEGREE OF RESISTANCE OF *A. STEPHENSI* ADULTS, EXPRESSED AS THE RESULTS OF EXPOSURE OF ONE-DAY-OLD, UNFED, FEMALE AND MALE *A. STEPHENSI* FROM THE IRAQ AND INDIAN STRAINS TO VARIOUS DOSAGES OF DDT IN RISELLA OIL FOR 2 HOURS USING THE BUSVINE & NASH (1943) ADULT SUSCEPTIBILITY TEST

Strain	DDT dosage (%)	Females		Males	
		Total mosquitos	Percentage mortality	Total mosquitos	Percentage mortality
Susceptible (India)	0.1	21	5	23	0
	0.25	75	11	85	32
	0.5	117	50	86	74
	1.0	38	95	12	100
DDT-resistant (Iraq)	4.0	256	29	411	51

TABLE 5

DEGREE OF RESISTANCE OF *A. STEPHENSI* LARVAE, EXPRESSED AS THE RESULTS OF EXPOSURE OF 4TH-STAGE LARVAE OF IRAQ AND INDIAN STRAINS OF *A. STEPHENSI* TO VARIOUS CONCENTRATIONS OF DDT IN ALCOHOL-WATER MIXTURES FOR 2 HOURS FOLLOWED BY A 4-HOUR RECOVERY PERIOD IN CLEAN WATER [a]

Concentration of DDT (p.p.m.)	Total larvae exposed	Percentage mortality
Susceptible Indian strain		
3	299	13
5	499	42
10	200	66
20	200	87
DDT-resistant Iraq strain		
100	200	45

[a] Larvae 8-13 days old; less than 10% pupation during test.

DEGREE OF RESISTANCE

The exposure of one-day-old males and females of the funnel-test larval-selected susceptible and resistant strains to varying dosages of DDT in Risella oil for two hours, followed by a 24-hour recovery period, shows the degree of resistance of the Iraq strain to be of the order of 12 to 13 times in the adult stage (Table 4). Exposures of larvae for two hours, followed by a 4-hour recovery period, indicate a degree of resistance of some 20 times (Table 5). These low orders of resistance are even lower than that recorded for DDT-resistance in *A. sundaicus* (some 40 times in the adult stage) and very much lower than the degree of dieldrin-resistance shown by *A. gambiae* (some 800 times in the adult stage) (Davidson, 1958).

CROSS-RESISTANCE

DDT-resistant *A. stephensi*, like DDT-resistant *A. sundaicus*, show cross-resistance to DDT analogues. Table 6 shows comparative mortalities of the unselected susceptible and resistant strains at specific dosages of methoxychlor, dichloro-diphenyl-dichloroethane and diethyl-diphenyl-dichloroethane. Both strains are equally susceptible to dieldrin, however. To malathion the larvae of the two strains are equally susceptible (the LC_{50} using the Elliott larval test is approximately 10 p.p.m.). With the

adults, however, the susceptible strain is some 2½ times more tolerant to malathion than the resistant strain (LC_{50}'s are 1.5% and 0.6% respectively using malathion dissolved in olive oil).

INHERITANCE OF RESISTANCE

No difficulty was experienced in crossing the Iraq and India strains and normal hybrids were produced from reciprocal crosses. Both hybrids appeared intermediate in their resistance to DDT. Adult testing showed a significant difference in tolerance between the hybrid produced by crossing the resistant male with the susceptible female, and its reciprocal; the former was the more resistant. Little difference was evident in the larvae of the two hybrids, however.

Knockdowns recorded among adult hybrids shortly after removal from the treated surface showed these to be higher than final 24-hour mortalities as in the susceptible strain (Table 2). The more exact thistle-funnel method of recording rates of knockdown showed the hybrid to be much closer to the susceptible strain than to the resistant one. In the time that it took to knock down the susceptible strain completely (36 minutes), 82% of the hybrids had succumbed, and in one hour, when very few of the resistant strain had been knocked down, all the hybrids had succumbed (Table 3). Also, when susceptible, resistant and hybrid adults were exposed to mud blocks sprayed with a DDT wettable powder at a dosage of 200 mg DDT per square foot (2 g/m²), the hybrid was found to resemble the susceptible strain much more closely than the resistant one in the resulting mortalities, in the first week after treatment at any rate (see Table 11 below).

The fact that, by the adult (Busvine & Nash, 1953) and larval (Elliott, 1958) testing techniques used, the hybrid was intermediate in its resistance and that the degree of resistance was low made the absolute discrimination of phenotypes (as was possible with *A. gambiae*; Davidson, 1958) impossible with *A. stephensi*. Resort was had to the accurate recording of mortalities in the three phenotypes at one specific dosage for each test. This involved the standardization of age and rearing of the mosquitos tested as far as possible. Larvae were tested 7-10 days from their birth and only those tests in which there was less than 10% pupation have been included in the results. The dosage of insecticide used was 100 p.p.m. DDT for an hour followed by a 5-hour recovery period in clean water. This was the dosage killing almost all of the susceptible strain and only a small percentage of the resistant one. Adults were tested within 24 hours of their emergence from pupae, in an unfed state. The exposure given was to 4% DDT in Risella oil for one hour followed by a 24-hour recovery period. This exposure gave a high kill of the susceptible strain and a low kill of the resistant one.

Hybrids were interbred and also backcrossed to the parent strains. The offspring of these crosses were tested on the above specific dosages and the mortalities compared with the expected values assuming a genetic factor to be involved. As an example of the method of calculation of the expected value some of the larval mortality figures may be taken. For the susceptible, resistant and combined F_1 generations these figures are 99%, 7% and 51% respectively. The expected combined F_2 generation thus should be as follows:

25% of 99 = 24.75%
50% of 51 = 25.5 %
25% of 7 = 1.75%
Total = 52.0 %

The actual mortality in the combined F_2 generations was 55%.

Table 7 gives the observed and expected values for adult mosquitos of all but one of the possible

TABLE 6

SPECTRUM OF RESISTANCE IN *A. STEPHENSI*, EXPRESSED AS THE PERCENTAGE MORTALITIES OF ADULT MOSQUITOS (MALES AND FEMALES COMBINED, UP TO 4 DAYS OLD) AFTER EXPOSURE FOR 1 HOUR TO VARIOUS DOSAGES OF VARIOUS INSECTICIDES USING THE BUSVINE & NASH (1943) ADULT SUSCEPTIBILITY TEST [a]

Dosage and insecticide	Susceptible strain (India)	Resistant strain (Iraq)
1% Methoxychlor	77 (86)	14 (77)
2% Dichloro-diphenyl-dichloroethane	92 (101)	17 (78)
4% Diethyl-diphenyl-dichloroethane	100 (92)	33 (86)
0.1% Dieldrin	76 (243)	86 (117)
0.2% Dieldrin	97 (75)	100 (58)
1.5% Malathion	50 (102)	100 (67)

[a] The numbers of mosquitos tested are shown in parentheses.

TABLE 7

MODE OF INHERITANCE OF RESISTANCE IN ADULT *A. STEPHENSI*, EXPRESSED AS THE 24-HOUR MORTALITIES AMONG ONE-DAY-OLD MALE AND UNFED FEMALE ADULTS OF *A. STEPHENSI* EXPOSED TO 4% DDT IN RISELLA OIL FOR 1 HOUR

Parents [a]			Offspring					
			Males			Females		
	♂	♀	Total tested	Percentage killed	Expected percentage mortality	Total tested	Percentage killed	Expected percentage mortality
Stocks	SS	SS	440	97		258	93	
	RR	RR	487	18		336	9	
F_1 Hybrids	RR	SS	855	75		464	52	
	SS	RR	301	83		257	70	
F_2	RS	RS	317	65	66	263	51	51.5
	SR	SR	136	75	70	127	67	60.5
Backcross to RR stock	RR	RS	329	47	46.5	326	27	30.5
	RR	SR	172	47	50.5	142	31	39.5
	SR	RR	95	48	50.5	70	49	39.5
Backcross to SS stock	SS	RS	284	82	86	231	65	72.5
	RS	SS	81	75	86	91	70	72.5
	SS	SR	248	87	90	140	74	81.5
	SR	SS	136	82	90	108	65	81.5

[a] S = factor for susceptibility;
R = factor for resistance;
RS = resistant ♂ × susceptible ♀;
SR = susceptible ♂ × resistant ♀.

crosses and backcrosses, and Table 8 records these values for the larvae of the complete range of crosses. As can be seen there are strong indications of a monofactorial pattern of inheritance.

Monofactorial inheritance has been confirmed in a number of ways. Firstly, from the exposure of the larval offspring of the backcross involving the susceptible male and the hybrid female to the dosage known to kill susceptibles, the survivors were kept and reared to the adult stage, when the females were again crossed to susceptible males. This procedure was repeated through six generations in the case of the cross using the hybrid produced from mating the susceptible male with the resistant female (the Sel. SR series in Table 8). If resistance is due to many additive factors, then repeated backcrosses to the susceptible strain should result in more and more susceptible individuals in each successive generation. Starting with a mortality in the first backcross of 50%, this mortality would increase to 63%, 67% and 69% in the second, third and fourth generations where two factors are involved, and to 69%, 73% and 74% in similar generations where three factors are involved.

None of the observed mortalities in the Sel. SR series (Table 8) exceeded the figure for the original backcross (78%) and most were lower than the expected value of 74%. This could be due to the selection of the multiple factors which contribute to " vigour tolerance ", thus giving a more favourable background for the expression of the resistance factor.

In addition to this repeated backcrossing with selection, some of the survivors after selection were allowed to interbreed to produce a selected F_2 generation. This, in its mortality, was not unlike the ordinary F_2 generation and this again provided confirmation of monofactorial inheritance.

Eventually a dosage and exposure time was found which killed all hybrid larvae but from which a significant proportion of homozygous resistant larvae survived. This was 100 p.p.m. DDT for

TABLE 8

MODE OF INHERITANCE OF RESISTANCE IN *A. STEPHENSI* LARVAE, EXPRESSED AS THE PERCENTAGE MORTALITIES AMONG 7-10-DAY-OLD FOURTH-STAGE LARVAE EXPOSED TO 100 P.P.M. DDT FOR 1 HOUR FOLLOWED BY A 5-HOUR RECOVERY PERIOD IN CLEAN WATER

Parents [a]			Offspring		
	♂	♀	Total tested	Percentage killed	Expected Percentage mortality
Stocks	SS	SS	449	99.8	
	RR	RR	323	7	
F^1 Hybrids	RR	SS	1545	54	
	SS	RR	1951	48	
F^2	RS	RS	589	49	54
	SR	SR	2210	57	51
"Selected" F^2	Sel. RS	Sel. RS	1781	61	54
	2 Sel. RS	2 Sel. RS	243	56	54
	Sel. SR	Sel. SR	675	45	51
	2 Sel. SR	2 Sel. SR	1443	41	51
	3 Sel. SR	3 Sel. SR	2106	32	51
Backcross to RR stock	RR	RS	1195	34	30.5
	RS	RR	598	30	30.5
	RR	SR	786	36	27.5
	SR	RR	1041	35	27.5
Backcross to SS stock	SS	RS	1641	69	77
	RS	SS	799	71	77
	SS	SR	1414	78	74
	SR	SS	1604	80	74
Repeated backcross to selected hybrid females	SS	Sel. RS	550	85	77
	SS	Sel. SR	775	61	74
	SS	2 Sel. SR	899	74	74
	SS	3 Sel. SR	217	69	74
	SS	4 Sel. SR	643	66	74
	SS	5 Sel. SR	249	68	74
	SS	6 Sel. SR	497	72	74

[a] S = factor for susceptibility; R = factor for resistance; RS = resistant ♂ × susceptible ♀; SR = susceptible ♂ × resistant ♀; Sel. = selected as a survivor from former backcross to SS; 2 Sel. = second selected generation; 3 Sel. = third selected generation; etc.

4 hours followed by a 2-hour recovery period. All of 609 hybrid larvae exposed in this way died. Of 2971 resistant larvae so exposed, 64% died. An F_2 generation was exposed to this dosage and a 95% mortality resulted (6225 larvae exposed). The expected mortality was 91%. The survivors were reared from and their larvae were found to be more resistant than the parent resistant strain, in fact comparable in resistance with this strain after it had been selected through two generations by a similar dosage and exposure time to that used in this selection of the F_2 generation (Table 9).

This higher tolerance appeared, however, to be confined to the larvae and much of it was lost in 8-10 generations after selection. Little difference was observed between the adults of the two strains (Table 10), suggesting perhaps that the factors responsible for "vigour tolerance" are not the same in larvae and in adults.

DISCUSSION

These facts suggest that DDT-resistance in *A. stephensi* has a monofactorial type of inheritance, the gene expression being very dependent upon the genetic background. Both stocks are capable of producing "vigour tolerant" strains which revert to the original level of susceptibility in the absence of selection and probably contain many minor factors for this tolerance. DDT-resistance in *A. stephensi* and *A. sundaicus* shows many similarities. It is of a low order in both species, is monofactorial in inheritance-pattern and imparts cross-resistance to DDT-analogues but not to dieldrin. The slightly

TABLE 9

"VIGOUR TOLERANCE" IN DDT-RESISTANT *A. STEPHENSI* LARVAE, EXPRESSED AS THE LARVAL MORTALITIES BY GENERATIONS IN A RESISTANT STRAIN SELECTED FROM AN F_2 GENERATION COMPARED WITH SIMILAR MORTALITIES IN THE PARENT RESISTANT STRAIN BEFORE AND AFTER SELECTION [a]

F_2-selected strain			Parent resistant strain	
Generation	Exposure period [b]/ Recovery period (hours)	Percentage mortality	Before selection (percentage mortality)	After selection (percentage mortality)
1	1/5	0 (73)		
2	1/5	4 (300)	7 (323)	4 (535) [d]
13	1/5	16 (97)		
4	2/4	26 (200)	45 (200)	
1	4/2	9 (519)	64 (2971)	30 (1264) [c]
8	4/2	67 (100)		18 (101) [d]

[a] The numbers of larvae tested are shown in parentheses.
[b] Exposures were to 100 p.p.m. DDT in all cases.
[c] After first selection.
[d] After second selection.

TABLE 10

24-HOUR MORTALITIES IN ONE-DAY-OLD ADULT MALE AND FEMALE *A. STEPHENSI* OF THE SELECTED F_2 STRAIN (PRODUCED FROM THE SELECTION OF LARVAE) COMPARED WITH THE PARENT RESISTANT STRAIN [a]

Generation	Exposure time (hours)	Males		Females	
		Total tested	Percentage mortality	Total tested	Percentage mortality
Parent resistant strain					
	1	487	18	336	9
	2	411	51	256	29
F_2 selected strain					
1	1	45	18	105	8
5	2	115	53	54	26
9	1	95	35	57	12
12, 13, 15	1	116	22	51	2

[a] Exposure was to 4% DDT in Risella oil for 1 or 2 hours.

enhanced susceptibility of DDT-resistant *A. stephensi* to malathion is also shown by DDT-resistant *A. sundaicus*. There are differences, however. *A. sundaicus* shows a higher degree of resistance than *A. stephensi* and the factor for resistance is virtually recessive in the former species, i.e., the heterozygote is almost as susceptible as the susceptible strain. These differences, however, may be solely due to the fact that the susceptible strain of *A. stephensi* from India used for comparison and crossing with the resistant strain shows what is considered to be an abnormally high tolerance to DDT. Moreover, this strain has been maintained as a laboratory colony since 1947 and has shown changes in tolerance over the years (Davidson, 1958). It would have been more valid to use a DDT-resistant strain and a susceptible strain of *A. stephensi* from the same part of the world for the observations here described.

So far as the detection of DDT-resistance in the field in these two species is concerned, it would appear that survival of adults after exposure to 4% DDT in Risella oil for one hour, and of fourth-stage larvae to 50 p.p.m. (*A. sundaicus*) or 100 p.p.m. (*A. stephensi*) DDT for one hour, using the Elliott larval test, would give very strong indications of the presence of resistant individuals but would not, where there were very few survivors, rule out the possibility of these being susceptibles at the extreme range of susceptibility, especially with *A. stephensi*. Confirmation of resistance would then come from the rearing and testing of offspring of these survivors which, if resistant individuals, would normally show a significantly lower mortality than the parent population from which they were selected.

The low degree of resistance in *A. stephensi* would suggest that some mortality of the homozygous resistant individuals as well as the heterozygotes must occur in the field. To verify this, adults of the three genotypes were exposed to mud blocks (made from soil from Babate in Tanganyika), sprayed with a DDT-wettable powder at a dosage of 200 mg per square foot (2g/m²) for varying times and at varying intervals after spraying. The results are summarized

TABLE 11

PERCENTAGE MORTALITIES AMONG MALE AND FEMALE SUSCEPTIBLE, HYBRID AND RESISTANT *A. STEPHENSI* EXPOSED FOR VARYING TIMES TO MUD BLOCKS SPRAYED WITH DDT AT 2 g/m²

Age of residue (weeks)	Exposure time (minutes)	Genotype [a]	Number exposed	Percentage mortality 24 hours after exposure
1	30	SS	48	75
		RS	165	62
		RR	27	22
1	60	SS	22	100
		RS	29	90
		RR	65	25
1	120	RR	24	83
2	60	SS	113	97
		RS	56	4
		RR	104	14
2	120	RS	81	48
		RR	23	52
3	60	SS	30	73
		RS	89	26
3	120	RS	79	72
		RR	49	12

[a] S = factor for susceptibility; R = factor for resistance; RS = resistant ♂ × susceptible ♀.

in Table 11, and show significant kills in all three genotypes at short exposure times in the first week after the blocks were sprayed. The rapid decline in mortalities in the second and third weeks after spraying is attributable to the sorptive qualities of the mud used (Barlow & Hadaway, 1955). The *A. sundaicus* genotypes similarly exposed showed mortalities of the same order.

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RÉSUMÉ

Une souche *d'Anopheles stephensi* résistante au DDT et à ses analogues, a été isolée parmi d'autres provenant de l'Irak. Elle était 12 fois plus résistante qu'une souche sensible, de la même espèce, provenant de Dehli (Inde). Ces deux souches étaient cependant sensibles aux insecticides du groupe de la dieldrine. Une certaine concentration a été choisie comme critère de différenciation entre les deux souches homozygotes: pour les larves (test d'Elliott) exposition à 100 p.p.m. de DDT/Ethanol en solution aqueuse pendant 1 heure, suivie de 5 heures de récupération; pour les adultes (test de Busvine & Nash) exposition d'1 heure à 4% de DDT dans le Risella. Ces tests ont été effectués sur des larves au stade IV, de 7–10 jours, et sur des adultes d'un jour, à jeun.

On a effectué des croisements, et les hybrides, les F_2 et les croisements de retour, ont été soumis à la concentration précitée. Il a été établi que la résistance est un caractère monofactoriel, ainsi que l'ont prouvé la sélection d'une souche homozygote résistante de la F_2, et la sélection répétée d'un hybride d'un croisement de retour avec la souche sensible, par 6 croisements de retour successifs. Le pourcentage de mortalité à la concentration critique n'a jamais dépassé celui du premier croisement de retour.

Sous l'effet d'une pression sélective persistante du DDT, les deux souches d'anophèles ont produit une souche présentant une tolérance physiologique, qui a disparu lorsque a cessé la pression sélective, ce qui indique que des facteurs secondaires sont en jeu dans ces phénomènes.

La résistance au DDT chez *A. stephensi* peut être comparée à celle de *A. sundaicus*. Ces deux souches ont une résistance faible, génétiquement monofactorielle, qui s'étend aux analogues du DDT, mais pas à la dieldrine. Ce facteur est complètement récessif chez *A. sundaicus*, mais ne l'est que partiellement chez *A. stephensi*.

Des adultes des trois génotypes de *A. stephensi* (resistant, sensible, et hybride résistant × sensible) ont été exposés, sur des blocs de boue séchée, traités à raison de 6 g de DDT par m^2 environ. Des pourcentages de mortalité significatifs ont été observés pour les trois génotypes; il est donc possible qu'il y ait une certaine mortalité des résistants, dans les conditions naturelles. En outre, la majorité des hybrides est tuée par cette concentration. Ces deux faits peuvent expliquer pourquoi la resistance au DDT apparaît plus lentement que la résistance à la dieldrine.

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