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STUDIES ON INSECTICIDE RESISTANCE IN ANOPHELINE MOSQUITOS

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SYNOPSIS

This paper describes observations made both on laboratory colonies in London and on wild-caught material in the field of insecticide-susceptible and -resistant strains of *A. gambiae*, *A. sundaicus* and *A. stephensi*. The techniques used in assessing the susceptibilities of both larvae and adults are described and the effects of species, rearing, age, sex, feeding and ovarian development on susceptibility are discussed. The effect of continued selection by DDT in the laboratory of a presumably homogeneous susceptible strain of *A. stephensi* is then outlined and the phenomenon of "vigour tolerance" demonstrated.

Specific, monofactorially-inherited resistance is then described as witnessed with *A. gambiae* and *A. sundaicus*. *A. gambiae* shows a high degree of resistance to dieldrin and cross-resistance to the related cyclodiene compounds and gamma-BHC, but remains susceptible to DDT. Resistance is due to a single gene, partially dominant, the hybrid showing a moderate degree of resistance. *A. sundaicus* shows a moderate degree of resistance to DDT and cross-resistance to DDT analogues, but remains susceptible to dieldrin and gamma-BHC. Resistance is again due to a single gene but here it is virtually recessive and the hybrid is to all intents and purposes susceptible. The discriminating dosage technique for distinguishing the phenotypes in these species is described.

Resistance in other anophelines and in houseflies is discussed and suggestions made as to methods of detecting anopheline resistance in the field.

Resistance to chlorinated-hydrocarbon insecticides has been known among houseflies since 1946 and has now spread to almost every part of the world where these insecticides have been used on a large scale. Although this resistance was at first confined to DDT, change to the dieldrin-BHC group quickly brought about the appearance of strains resistant to all the chlorinated hydrocarbons. Attempts to control these multi-resistant strains by the use of organic phosphate insecticides have met with some success, but indications of failure in control are now becoming apparent.[16, 17]

Cases of resistance to the chlorinated hydrocarbons among anopheline mosquitos are, as yet, few and where resistant strains have appeared they are comparatively localized in their distribution. With one possible exception, all cases of anopheline resistance are confined to one or other of the two groups of chlorinated hydrocarbons, and change from the group to which they are resistant to the one to which they are susceptible has resulted in successful control. Resort has not yet had to be made to the less efficient organic phosphate insecticides.

A detailed study of resistance involves primarily the establishment of laboratory colonies of resistant and susceptible strains of the insect species, then a comparison of their susceptibilities to the range of insecticides, and finally a study of the manner of inheritance of the resistance by crossing the two strains. Such observations have now been completed on two species of anophelines, and preliminary field observations have been made on a third.

The account of these studies commences with details of techniques used in assessing the susceptibility of anopheline mosquitos to insecticides and the expected ranges of tolerance within homogeneous strains. A description is then given of the effect of continued selection by insecticide of a susceptible strain in an attempt to induce resistance in the laboratory. Finally, details are given of four cases of true insecticide resistance in three species of anophelines, the principles involved are discussed, and some suggestions are offered as to their application for the detection of resistance in the field.

Techniques Used in the Investigations

Most of the observations recorded here were made on laboratory-colony mosquitos reared in London. The rearing techniques described below apply in the main to these. In the field, mosquitos were tested as they were found.

Rearing techniques

For the careful comparison of the susceptibility of different species to insecticides it was apparent from the start of these investigations that standardization of the methods of rearing and maintaining laboratory colonies was all important. Substantial differences in the susceptibility of both larvae and adults were apparent between the larger, well-reared mosquitos and the smaller ones produced by overcrowded conditions and underfeeding. Table I gives some idea of the changes in susceptibility to DDT of *A. stephensi* adults with increased perfection of rearing methods. The lowest median lethal concentration (MLC) was recorded in 1955, when little attention was being paid to rearing methods.

Standardization of the larval-rearing technique has involved the standardization of larval density, type and temperature of water and type and

TABLE I. *A. STEPHENSI* : VARIATIONS IN SUSCEPTIBILITY OVER THREE YEARS

Year	Males	Unfed females	Females fed once
1955	0.9 (516)	0.46 (193)	1.7 (1848)
1956	1.8 (800)	2.9 (564)	1.9 (2166)
1957	1.4 (213)	2.4 (207)	

Median lethal concentrations (percentages in Risella oil) of DDT (1 hour's exposure) among laboratory-colony *A. stephensi* in 3 consecutive years. The numbers of mosquitos involved in the determination of the MLC's are given in parentheses.

quantity of food. As regards larval density, the larvae were not actually counted, but approximately equal numbers, as judged by eye, were reared per standard-size bowl in the same volume and surface area of water. The approximate density aimed at was 2 larvae per square inch of water surface at a depth of ½-1 inch.[a] The water used was ordinary tap-water maintained at a temperature of 25°C. Larval food consisted of two parts of dog-biscuit to one part of Bemax (a vitamin-protein-mineral mixture), all finely ground and passed through a 90-mesh sieve. Approximately the same quantity of food was added per bowl per day to larvae of the same instar and was increased as the larvae grew. In addition, except in the case of *A. stephensi*, a small piece of grass turf was provided in each bowl. This, it is thought, produces micro-organisms sufficient to nourish the very young larvae which find difficulty in assimilating the prepared food, fine though it is. *A. stephensi* could be reared successfully without this turf.

The adult mosquitos were allowed to emerge in 1-foot-cube[b] cages and were fed on either human or guinea-pig blood, usually 36 hours after emergence. Dilute glucose solution was provided in the cage from the time of emergence. The adult mosquitos were kept at a temperature of 26°C and a relative humidity of between 70% and 80%. Except for short periods during the day, they were kept in total darkness.

Determination of larval susceptibility to insecticides

The susceptibility to insecticides of larvae has been assessed by exposing 4th-instar larvae in insecticide-alcohol-water mixtures for a period of 24 hours, holding for a further period of 24 hours in clean water, and then recording the mortality. In these studies this has only been done with *A. stephensi* larvae and DDT.

[a] 1 inch = 2.54 cm; 1 square inch = 6.45 cm²

[b] 1 foot = 30.48 cm

Young 4th-stage larvae showing little or no pupation during the 24-hour exposure period were used for testing. Increasing age within the instar made some difference to susceptibility, as shown in Table II. Larvae showing considerable pupation during the exposure to insecticide showed a lower mortality than those showing little or no pupation.

TABLE II. *A. STEPHENSI* : EFFECT OF AGE WITHIN LARVAL INSTAR ON SUSCEPTIBILITY

Total larvae exposed	Percentage pupated during exposure	Percentage mortality (among larvae)
8930	0–20	59
989	21–40	42
558	41–60	49

Percentage mortalities among 4th-instar larvae of *A. stephensi* of differing ages when exposed for 24 hours to DDT in alcohol-water solution at a concentration of 0.5 p.p.m.

Exposure was carried out in glass vessels with a standard volume and surface area of water per larva. Small-scale exposures were done in 250-ml beakers containing 100 ml of test solution. Fifty larvae were added to each beaker. Larger-scale exposures were made in glass bowls in which 1100 ml of test solution gave the same depth and surface area of water as 11 of the beakers containing 100 ml; 550 larvae were added to each of these bowls. Thus the larval density was standardized at 10 larvae per square inch and 5 per cubic inch.[a]

Test solutions were made by diluting very small quantities of a standard alcoholic solution of insecticide in a large bulk of distilled water. The standard solution of DDT was 0.1% in 95% ethanol; 1 ml of this added to 1 litre of water was taken as a dilution of one part DDT per million. Other dilutions were made by varying the volume of stock solution added to a standard volume of water.

The larvae were exposed in the test solution at the same temperature as the water in which they were reared. They remained there for 24 hours without food. Transference of the larvae to the test solution was done with the aid of small nets, avoiding the transference of breeding water and larval food as far as possible. At the end of the 24-hour exposure, the larvae were sieved off through the nets, washed under a slow stream of tap-water (at room temperature) and transferred to clean tap-water in ordinary rearing bowls. Obvious live larvae were then immediately transferred to further clean water, and food was added to both recovery bowls. Larvae which pupated during the exposure period were counted and discarded (the

[a] 1 cubic inch = 16.4 cm^3

percentage pupating was recorded for comparison with the controls and with other tests). A final count of dead and live larvae was made 24 hours after the removal from the test solution. By this time there was little or no doubt as to whether the larvae were dead or alive. For controls or checks exactly the same procedure was adopted, using distilled water to which had been added the same quantity of alcohol as in the test solution. Check mortalities were negligible up to 5 ml of 95% ethanol in 1 litre of water.

The glassware used in the tests was cleaned by being left overnight in standard chromic acid cleaning solution, thoroughly rinsed in tap-water and then finally rinsed in distilled water. Contamination was tested for periodically by rearing susceptible larvae in the apparatus for periods of 24 hours or longer. Surprisingly little contamination was detected even though most of the glassware had been in constant use for at least a year.

Determination of adult susceptibility to insecticides

The technique used for the assessment of the susceptibility of adult anophelines to insecticides was that of Busvine & Nash.[5, 24] This technique involves confining mosquitos to surfaces treated with insecticides in oil solution for a given time and recording the mortality 24 hours after this exposure. Both DDT and gamma-BHC were used in their pure state; the dieldrin used was of 94% purity. The other insecticides involved were either in their pure state or of high technical grade.

Only minor departures were made from the Busvine & Nash technique. Mosquitos were gently blown into the exposure tube from a sucking-tube, and 10 to 15 per tube did not produce any significant handling mortality. For recovery chambers ½-pint,[a] unwaxed paper cups were used. Each cup was fitted with a net cover with a hole in it just large enough to admit the 1-inch diameter exposure tube. The hole was covered with a pad of cotton wool soaked in glucose solution as a source of nourishment during the 24-hour observation period. The use of these dispensable paper cups eliminated any risk of the contamination which may occur in recovery cages repeatedly used.

The use of volatile insecticides, e.g., gamma-BHC and aldrin, in this technique introduces the problem of the fumigant effect of the insecticide. It is conceivable that differing dosages of a volatile insecticide could, in a closed tube, produce similar mortalities from vapour effects. To reduce this possibility, open-ended tubes were used, the ends covered with mosquito-netting. With the mosquitos inside, these tubes were laid horizontally so that a free passage of air through them was available. Even though some of the mosquitos did settle on the untreated netting ends, an increase in kill with increasing dosage was nevertheless achieved. The use of open-ended tubes was also found necessary where long exposures, e.g., 18 hours, to non-

[a] 1 pint = 0.568 litres

volatile insecticides were made, as confinement in closed tubes for such long periods resulted in high control mortalities.

One unknown factor in this technique is the length of time an impregnated paper retains its toxicity. This persistence in toxicity must depend on the rate of evaporation of the oil solvent and also on the number of mosquitos allowed in contact with it. Throughout these investigations, papers were not as a rule used for more than one week after impregnation, and up to 100 mosquitos only were allowed contact with each paper during this period.

Exposures of laboratory-reared mosquitos were mainly done in daylight at the temperature and humidity at which the adult colonies were maintained, viz. 26°C. and 70%-80% relative humidity.

At the start of these observations, adult susceptibility tests among laboratory-reared mosquitos were confined to females fed on blood a few hours before exposure to the insecticide. This involved keeping the females for periods of 36 hours or more before feeding would take place readily, and discarding the males. Later the routine was adopted of using both males and females, usually on the day following emergence when they were at most about 20 hours old. Where week-ends and holidays intervened, emergences over the one or more days concerned were pooled and tested, the females still not having had a blood-meal but having been provided with glucose solution in the cage over the period before exposure. The effect of sex, age and feeding on mosquito susceptibility is discussed below.

In the field, wild-caught, blood-fed, half-gravid and gravid females were mainly used for testing, as these were the most easily caught, resting as they were inside houses and stables during the daytime. Exposures were done more or less " on the spot ", indoors.

Statistical treatment of data

The MLC of insecticide has been determined graphically by plotting a dosage-mortality curve on logarithmic-probability graph paper on which the data for homogeneous strains of mosquito species lie in a straight line. As control mortalities were almost negligible no correction of insecticide mortalities has been made. Numerous replicates at each dosage of each insecticide were the rule.

Natural Variations in Adult Susceptibility

Species differences

Results of the assessment of the susceptibility of the adults of many different species of anopheline mosquitos by the technique of Busvine & Nash in many different parts of the world have been summarized by Busvine [3] and more recently by the Malaria Section of the World Health

TABLE III. ANOPHELINE SUSCEPTIBILITIES : FIELD OBSERVATIONS

Insecticide	*A. gambiae*					*A. sundaicus*	*A. stephensi*
	Mauritius [3]	Tanganyika [3]	Lagos [11]	Ilaro [11]	Diggi *	Java **	Saudi Arabia †
DDT	0.7	0.7	0.6 (79)	0.6 (87)	0.8	0.5 (159)	1.8 (112)
Dieldrin	0.25	0.11–0.29			0.07	0.065 (206)	0.065 (164)
Gamma-BHC				0.006 (87)	0.004	0.008 (86)	

* J. A. Armstrong, C. D. Ramsdale & V. Ramakrishna, unpublished working document WHO/Mal/182 (WHO/Insecticides/52)

** G. Davidson, unpublished data, 1955

† R. H. Daggy, unpublished paper presented at the Third Conference of the Industrial Council for Tropical Health, 1957

Median lethal concentrations (percentage in Risella oil) of DDT, dieldrin and gamma-BHC for susceptible strains of *A. gambiae*, *A. sundaicus* and *A. stephensi* (1 hour's exposure) as recorded in the field by various authors. Where known, the numbers of mosquitos used to determine the MLC's are given in parentheses.

TABLE IV. ANOPHELINE SUSCEPTIBILITIES : LABORATORY COLONY OBSERVATIONS

Insecticide	*A. gambiae* (Lagos)*				*A. sundaicus* (Malaya)*		*A. stephensi* (India)*		
	males	unfed females	females fed once	females fed twice	males	unfed females	males	unfed females	females fed once
DDT	0.46 (230)	0.46 (481)	0.52 (187)	0.75 (103)	0.31 (306)	0.36 (355)	1.6 (1572)	2.8 (534)	1.6 (4014)
Dieldrin	0.052 (995)	0.072 (343)	0.066 (1431)	0.10 (75)	0.026 (105)	0.036 (165)		0.056 (143)	0.13 (388)
Gamma-BHC	0.0065 (450)	0.0085 (492)	0.0053 (478)	0.013 (74)	0.0019 (105)	0.0042 (142)		0.0085 (204)	0.0105 (131)

* Origin of species.

Median lethal concentrations (percentages in Risella oil) of DDT, dieldrin and gamma-BHC (1 hour's exposure) for three laboratory colonies of *A. gambiae*, *A. sundaicus* and *A. stephensi*. The numbers of mosquitos used to determine the MLC's are given in parentheses.

Organization.[25] The MLC's of the three insecticides, DDT, dieldrin and gamma-BHC, show surprisingly little variation in susceptible strains of different species. The ranges 0.5%-1% DDT, 0.05%-0.1% dieldrin and 0.005%-0.01% gamma-BHC cover the MLC's of most of the susceptible strains of the species listed in these reviews. In general the larger species, e.g., the *maculipennis* and *hyrcanus* groups, show slightly higher tolerances than the smaller ones, e.g., *sundaicus*.

Table III gives some of the MLC's recorded in the field for susceptible strains of the three species concerned in these studies. *A. stephensi* in Saudi Arabia shows a higher tolerance to DDT than either *A. gambiae* or *A. sundaicus*. This is apparent also in the laboratory colonies (Table IV). *A. gambiae* in Mauritius and Tanganyika shows a higher tolerance to dieldrin than the other two species, though a strain of *A. gambiae* from Northern Nigeria (Diggi) shows little difference. *A. gambiae* and *A. sundaicus* show similar tolerances to gamma-BHC. No figure is available for the susceptibility of susceptible *A. stephensi* to this insecticide in the field, but a DDT-resistant strain showed a MLC of the same order as the other two species (see Table XXIX).

In the laboratory colonies specific variations are more exaggerated (Table IV). *A. sundaicus* is much more susceptible to all three insecticides than the other two species. *A. stephensi* is similar to *A. gambiae* in its tolerance to dieldrin and gamma-BHC, but less susceptible to DDT.

Slight differences are apparent in susceptible strains of the same species from different areas. Two such strains of *A. gambiae* from different parts of Nigeria have been successfully colonized. One is from Northern Nigeria and has been maintained as a laboratory colony for some 15 generations only over a period of approximately one year. The other is from Lagos, some 600 miles [a] to the south, and has been maintained as a colony for 5 years. As shown in Table V, the Diggi strain is somewhat more susceptible to dieldrin and gamma-BHC than the Lagos strain.

TABLE V. *A. GAMBIAE*: SUSCEPTIBLE STRAIN DIFFERENCES IN TOLERANCE

Insecticide	Lagos		Diggi	
	males	unfed females	males	unfed females
DDT	0.46 (230)	0.46 (481)	0.56 (218)	0.48 (238)
Dieldrin	0.052 (995)	0.072 (343)	0.049 (204)	0.048 (315)
Gamma-BHC	0.0065 (450)	0.0085 (492)	0.0044 (158)	0.0024 (201)

Median lethal concentrations (percentages in Risella oil) of DDT, dieldrin and gamma-BHC (1 hour's exposure) for laboratory colonies of two susceptible strains of *A. gambiae* from Nigeria. The numbers of mosquitos used to determine the MLC's are given in parentheses.

[a] 1 mile = 1.6 km

Variations with age, sex, feeding and ovarian development

An analysis of the susceptibility to three insecticides of adults of the susceptible Lagos strain of *A. gambiae* with ages up to 7 days, according to sex and according to whether females were blood-fed or unfed is given in Table VI. Variations with age show no consistency and it is concluded that age has little effect within this short period of time and that such variations as do occur are the result of differences in the rearing of the adults. It will be noticed that variations are less marked in the females which have been fed on blood than in the unfed females and males. Males, on the whole, are more susceptible than females to all insecticides, as can be seen from a number of the tables included in this article. This difference is only slight, however, when males and unfed females are compared within 24 hours of emergence, and it is for this reason that most of the routine testing in these studies was done on both sexes on the day following emergence.

The effect of blood-feeding on the susceptibility of female mosquitos has been the subject of study by Hadaway & Barlow,[14] using the topical-application technique for susceptibility determination. Susceptibility was found to fluctuate in a regular manner through successive feeding cycles. Shortly after a blood-meal the female was only slightly less susceptible than she was in the unfed state; 24 hours after the blood-meal, when the ovaries

TABLE VI. *A. GAMBIAE* : EFFECT OF AGE ON SUSCEPTIBILITY

Insecticide and dosage	Age of mosquitos						
	1 day	2 days	3 days	4 days	5 days	6 days	7 days
	Males						
0.5 % DDT		50 (56)	78 (31)		38 (32)		
0.1 % dieldrin	52 (83)	99 (71)	76 (128)	84 (214)			
0.01 % BHC	77 (74)	95 (22)		68 (53)			
	Unfed females						
0.5 % DDT		33 (33)	72 (54)	89 (45)	33 (70)	28 (18)	
0.1 % dieldrin			75 (57)	76 (47)	74 (42)	48 (23)	
0.01 % BHC	84 (68)		59 (44)	37 (83)	17 (18)	33 (12)	57 (23)
	Females fed once						
0.5 % DDT		34 (29)		44 (34)			
0.1 % dieldrin	47 (89)	55 (128)	84 (87)	58 (125)			
0.01 % BHC		80 (71)	97 (63)	75 (80)			

Percentage mortalities among male and female *A. gambiae* (Lagos strain) of differing ages exposed for 1 hour to varying percentages of insecticides in Risella oil. The numbers of mosquitos exposed are given in parentheses.

were half developed, the tolerance to insecticide reached its peak, to decrease again with maturation of the eggs and oviposition.

Most of the fed females tested in the present studies were exposed only a few hours after feeding and their susceptibility is usually less than that of the unfed females, though not markedly so (see Table IV). Two notable exceptions are apparent: *A. stephensi* females are significantly more susceptible to DDT in the freshly-fed state that in the unfed state and *A. gambiae* females of the Lagos strain are more susceptible to gamma-BHC in the freshly-fed than in the unfed state.

The effect of a second feed appears to be to increase the tolerance of *A. gambiae* to the three main insecticides used. Again the females were exposed only a few hours after the second feed and this may indicate a difference in susceptibility with and without ovarian development accompanying the digestion of the blood. In this laboratory colony of *A. gambiae* two feeds are very often required before ovarian development to maturity occurs.

As a rule, field records show higher MLC's than those recorded here from laboratory colonies. This can be accounted for by the fact that most field observations are made on female mosquitos at the least susceptible stage in the gonotrophic cycle—namely, when they are half-gravid. Even so the differences between field and laboratory observations, even where these have been confined to unfed females and males, are not of great magnitude. Seldom is the field record more than twice that of the laboratory record.

One exception to this is *A. sundaicus*, for which laboratory records on unfed females and males show very much higher susceptibilities than have been recorded in the field. Nevertheless, that this species is one of the most susceptible of the anophelines is shown by a recent record in Burma by Delphin, who found a MLC of 0.31% DDT.[25]

Thus, on the whole, variations in susceptibility of the adults of homogeneous strains are not great and even the highest tolerances shown are far below the tolerances evident in true resistant strains, as will be shown later. As far as laboratory colonies are concerned, rearing methods and feeding of the females on blood appear to produce the greatest changes. Age, at least over a period of one week, and sex of the adults affect tolerance only to a minor degree.

Effect of Continued Selection by Exposure to Insecticide on Susceptibility of a Presumably Susceptible Strain of *Anopheles stephensi*

In 1955 a series of experiments was started to find out if resistance could be induced in a strain of *A. stephensi* by exposing it to sublethal dosages of DDT and rearing from the survivors. The strain originated in Delhi, India, and was sent to England in the form of eggs from a few females in 1947. It had been maintained as a laboratory colony ever since and had

never previously experienced insecticides. Three series of exposures were made:

(1) the " L " series, of 4th-stage larvae only in successive generations;
(2) the " A " series, of adults only in successive generations;
(3) the " LA " series, of larvae and adults in alternate generations.

Larval exposure series

Rearing was standardized in the way already described. Fourth-stage larvae were exposed to DDT in alcohol-water mixtures for 24 hours as already outlined. The selection dosage aimed at initially was one producing an approximate 50% mortality. Thus parent stock larvae surviving the MLC of the insecticide produced generation L i. L i larvae were again exposed to varied concentrations of DDT, the new MLC determined and a bulk exposure done at this concentration to produce generation L ii from the survivors and so on. From generation L xv onwards a higher selection dosage, nearer the LD_{90}, was employed. This resulted in so few survivors in some cases that insufficient numbers of the following generation were obtained for further exposure. Where this happened exposure was postponed until the following generation, i.e., a generation was missed. Finally, the twentieth generation in this larval exposure series was left unexposed and its susceptibility measured in the fourth and eighth generations following the last exposure.

While some L i larvae were used to produce generation L ii, others were reared to the adult stage for testing to see whether larval exposure alone produced resistance in the adults. These adults were, in fact, the offspring of mated adults reared from larvae surviving exposure to the insecticide.

Adult exposure series

Initially adult males and females emerging overnight were separated the following morning before there was much chance of fertilization. The sexes were then exposed separately to insecticide using the Busvine & Nash technique already described, and those surviving the MLC were allowed to mate and produce offspring. Successive generations were called A i, A ii, etc. For the first nine generations blood-fed females, fed about 36 hours after emergence, were used for these exposures, but from the tenth generation adults were exposed on the day following emergence and without feeding the females on blood. This eliminated the separation of males from females and the feeding of the females which was only possible two days after emergence. (The same procedure of adult exposure was adopted in the larval exposure series from generation L xviia onwards, in generation LA iva in the combined exposure series and in all the generations of a new adult series started from larval generation L xviia.)

Too high selection dosages, particularly among the male mosquitos, eventually resulted in the loss of this adult exposure series at the twelfth generation and a new adult exposure series was then started from the current larval generation, namely, L xviia. This new generation was itself lost after only two generations of exposure.

Larval and adult exposure series

Commencing with very large numbers of stock larvae it was found possible to expose the adults reared from larval survivors of insecticide exposure and produce sufficient numbers of generation LA i to repeat this procedure and produce generation LA ii. However, insufficient larvae were obtained in this generation to repeat this double exposure and a generation had to be missed to build up a colony sufficiently large to enable the procedure to be repeated. Exposure in alternate generations was also necessary to obtain the fourth generation when this series had to be abandoned because survivors were too few to create a further colony.

Results

Larval exposure series

The net result of larval selection by DDT over twenty generations has been a six-fold increase in the MLC—from 0.5 parts per million (p.p.m.) to about 3.0 (Table VII and Fig. 1). The increase has been quite gradual and the slope of the log-probit regression line has remained virtually unchanged. The effect of cessation of selection in the twentieth generation has been a slight reversion in susceptibility after eight generations without exposure. The greatest changes in mortalities have been at the lower dosages, e.g., 0.5 to 2.0 p.p.m., while at the higher concentrations a less marked fall in mortalities is apparent, e.g., 4.0 and 5.0 p.p.m.

That larval selection has resulted in increased tolerance to DDT in the adults of this larval exposure series is apparent from Table VIII. Here in 17 generations of exposure the MLC of females has increased somewhat less than three times. Marked changes in mortalities are apparent at all dosages, though again the slope of the log-probit curve has remained unchanged (Fig. 2). The few adults that were tested in the twentieth generation after it had remained unexposed to insecticide for four and eight generations showed no signs of increase in susceptibility.

Adult exposure series

Exposure of 11 successive generations of adult *A. stephensi* to DDT has also resulted in a significant change in tolerance to the insecticide, though the magnitude of this change cannot be definitely stated from the figures

FIG. 1. *A. STEPHENSI* LARVAL EXPOSURE SERIES : LARVAL MORTALITIES.

Comparison of Log-Probit Regression Lines of Parent Stock and Two Selected Generations

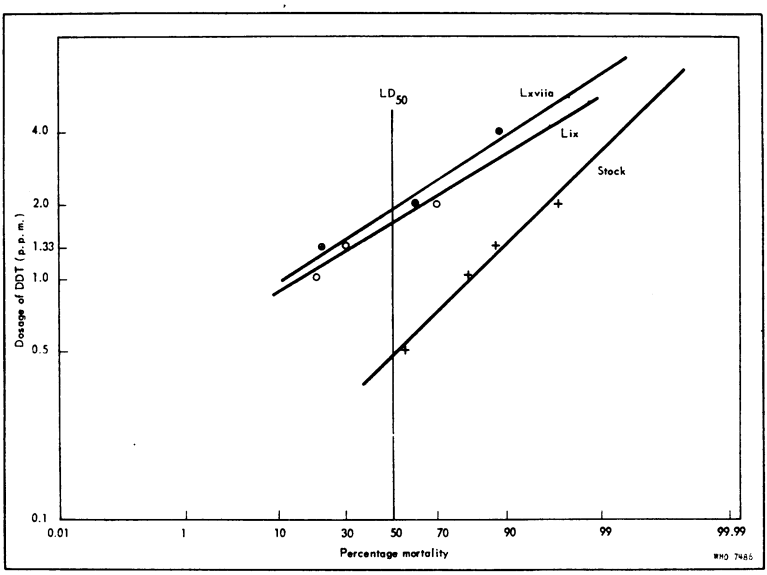


FIG. 2. *A. STEPHENSI* LARVAL EXPOSURE SERIES : ADULT FEMALE MORTALITIES

Comparison of Log-Probit Regression Lines of Parent Stock and Two Selected Generations

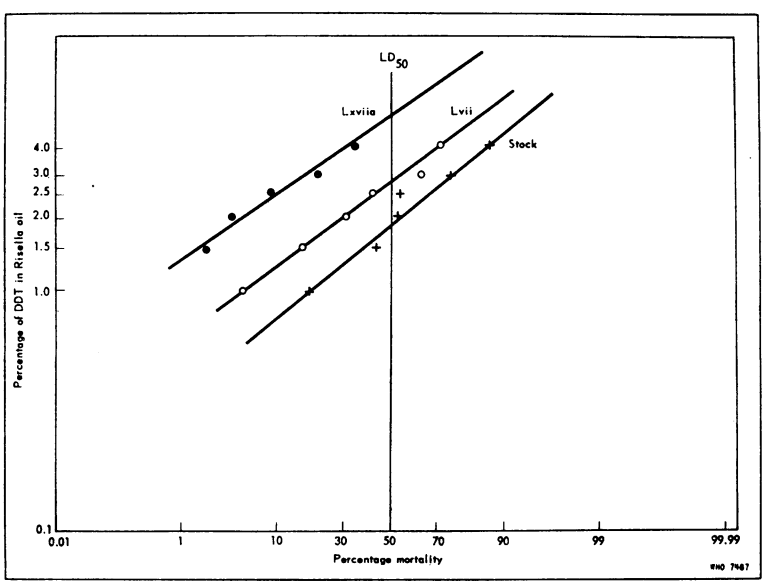


TABLE VII. *A. STEPHENSI* LARVAL EXPOSURE SERIES : LARVAL MORTALITIES

Generation	Concentration of DDT (p.p.m.)								Median lethal concentration (p.p.m.)
	0.5	0.67	1.0	1.33	2.0	3.0	4.0	5.0	
Stock	56 (10923)	44 (424)	81 (1839)	88 (1318)	97 (3648)		100 (924)		0.49
L iii	10 (154)	81 (364)	71 (408)						0.69
L iv	34 (373)	54 (288)	78 (1665)						0.64
L v			59 (1562)		95 (292)		100 (295)		0.90
L vi			27 (2344)	59 (1698)	84 (127)				1.28
L vii	7 (633)		59 (215)	48 (2092)	78 (308)				1.25
L viii			63 (151)	60 (151)	79 (3351)				0.88
L ix			20 (222)	30 (205)	70 (1835)				1.60
L x				37 (227)	58 (2256)		98 (249)		1.70
L xi				33 (222)	61 (1690)		96 (245)		1.70
L xii				45 (240)	74 (1872)		91 (248)		1.40
L xiii					56 (1582)				< 2.00
L xiv					63 (1601)				< 2.00
L xv					61 (1607)		91 (5137)		1.65
L xvi							82 (954)		?
L xviia *	9 (511)			22 (993)	61 (1117)		89 (6150)		1.90
L xviii							76 (530)	95 (3892)	3.25
L xixa *								96 (4094)	?
L xxd **						49 (360)	91 (386)		3.00
L xxh †	1 (550)				47 (550)		89 (148)		2.20

* Previous generations, namely, L xvii and L xix, not exposed ** L xx unexposed for 4 generations † L xx unexposed for 8 generations

Percentage mortalities, in successive generations, of 4th-stage larvae exposed to DDT in alcoholic solution for 24 hours at various concentrations. The numbers of larvae tested are given in parentheses.

TABLE VIII. *A. STEPHENSI* LARVAL EXPOSURE SERIES : ADULT FEMALE MORTALITIES

Generation	Percentage concentration of DDT in Risella oil						Median lethal concentration (%)
	1.0	1.5	2.0	2.5	3.0	4.0	
Stock	18 (623)	43 (950)	54 (759)	55 (521)	75 (569)	87 (583)	1.90
L i	29 (41)	65 (49)	74 (54)				1.35
L ii	48 (107)	72 (134)	79 (158)				1.05
L iii	19 (91)	58 (139)	73 (197)	42 (54)			1.45
L iv	11 (83)	27 (97)	37 (105)	35 (60)	56 (73)	82 (38)	2.50
L v	11 (94)	23 (95)	42 (92)	53 (85)	60 (106)	90 (97)	2.40
L vi	7 (106)	7 (77)	31 (106)	40 (80)	51 (49)	75 (49)	2.85
L vii	5 (158)	16 (177)	31 (180)	42 (171)	64 (210)	72 (185)	2.75
L viii	4 (169)	26 (179)	28 (190)	54 (150)	78 (137)	88 (171)	2.30
L ix	9 (84)	30 (98)	42 (131)	53 (102)	65 (120)	96 (112)	2.20
L x		37 (16)	55 (18)	74 (27)	95 (20)	95 (20)	1.80
L xi		9 (12)	43 (14)	45 (11)	22 (9)	70 (10)	2.60
L xviia*		2 (64)	4 (93)	9 (43)	21 (61)	34 (266)	5.40
L xxc**		7 (15)			27 (11)	0 (10)	?
L xxg†			0 (5)		30 (10)	0 (7)	?

* Generation L xvii not exposed
** Generation L xx unexposed for 3 generations
† Generation L xx unexposed for 7 generations

Percentage mortalities, in successive generations, of adult female *A. stephensi* exposed for 1 hour to filter-papers impregnated with DDT solution in Risella oil at various concentrations. The numbers of mosquitos tested are given in parentheses.

in Tables IX and X. By the eighth generation the MLC had doubled and continued to increase in succeeding generations to such an extent that the standard exposure time of one hour had to be doubled at the saturation level of DDT (4%) in Risella oil. Mortalities decreased considerably at all dosages and there has been a significant change in the slope of the log-probit line, especially among the males (Fig. 3 and 4).

Though these results would seem to indicate quite a marked increase in resistance to DDT through adult exposure, it should be pointed out that the survivors from the highest dosages (4% DDT for one and two hours), though capable of flight and feeding, had in many cases lost one or more of their legs as a result of the irritant effect of the insecticide in the small confines of a 3-inch × 1-inch tube and, judging from the difficulty in obtaining eggs in sufficient numbers for continuation of the series in the later generations, it is doubtful whether the normal rate of fertilization was maintained.

FIG. 3. *A. STEPHENSI* ADULT EXPOSURE SERIES : ADULT FEMALE MORTALITIES

Comparison of Log-Probit Regression Lines of Parent Stock and Sixth Selected Generation

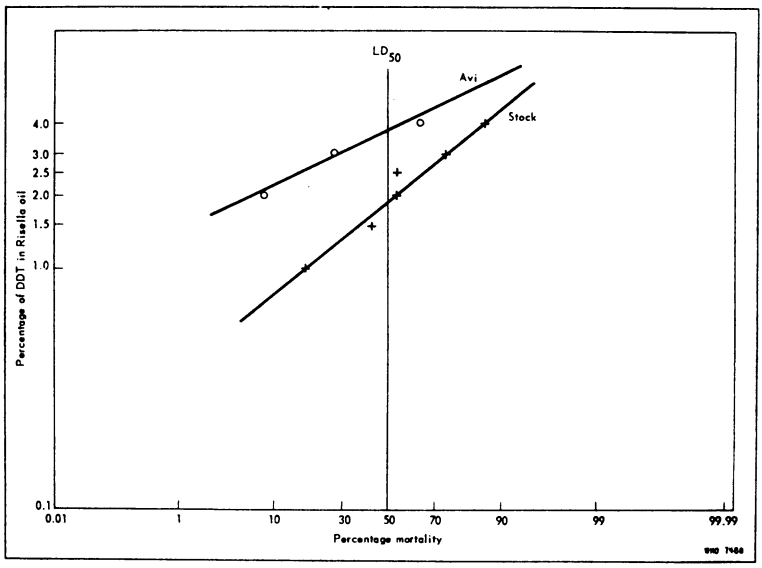


FIG. 4. *A. STEPHENSI* ADULT EXPOSURE SERIES : ADULT MALE MORTALITIES

Comparison of Log-Probit Regression Lines of Parent Stock and Eighth Selected Generation

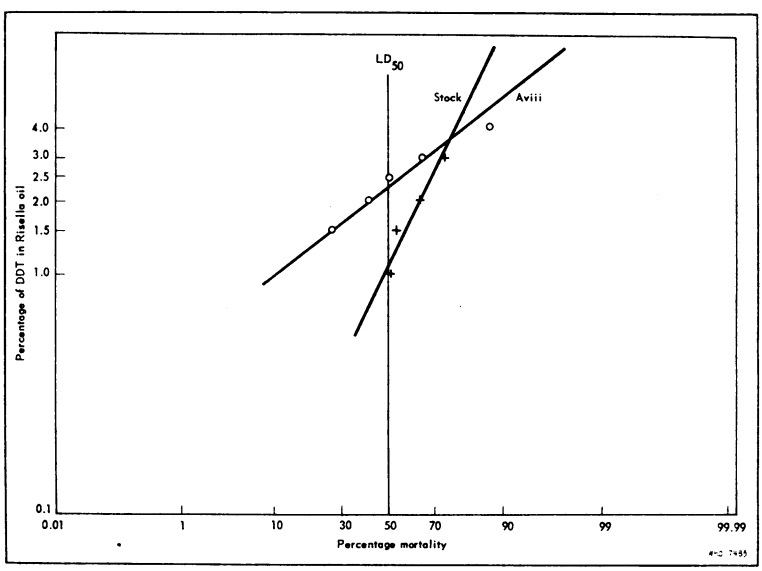


TABLE IX. *A. STEPHENSI* ADULT EXPOSURE SERIES : ADULT FEMALE MORTALITIES

Generation	Percentage concentration of DDT in Risella oil							Median lethal concentration (%)
	1.0	1.5	2.0	2.5	3.0	4.0 (1 hour)	4.0 (2 hours)	
Stock	18 (623)	43 (950)	54 (759)	55 (521)	75 (569)	87 (583)	100 (46)	1.90
A i		21 (14)	49 (162)					2.05
A ii				12 (16)	55 (274)			2.90
A iii					69 (360)			< 3.0
A iv					82 (404)			< 3.0
A v					52 (202)			< 3.0
A vi			8 (38)	3 (29)	27 (331)	65 (55)		3.90
A vii			34 (68)	47 (72)	36 (318)	84 (57)		3.20
A viii					25 (8)	54 (165)		< 4.0
A ix						20 (265)		> 4.0
A x							55 (590)	> 4.0
A xi							36 (128)	> 4.0

Percentage mortalities, in successive generations, of adult female *A. stephensi* exposed for 1 to 2 hours to filter-papers impregnated with DDT solution in Risella oil at various concentrations. The numbers of mosquitos tested are given in parentheses.

TABLE X. *A. STEPHENSI* ADULT EXPOSURE SERIES : ADULT MALE MORTALITIES

Generation	Percentage concentration of DDT in Risella oil								Median lethal concentration (%)
	0.5	1.0	1.5	2.0	2.5	3.0	4.0 (1 hour)	4.0 (2 hours)	
Stock	12 (164)	51 (539)	54 (182)	64 (159)	43 (80)	74 (108)	69 (71)	100 (46)	1.1
A i		44 (345)							> 1.0
A ii			57 (278)	75 (27)					1.4
A iii			61 (284)						< 1.5
A iv			53 (425)						< 1.5
A v			26 (300)			60 (81)			2.4
A vi				35 (332)	84 (19)				2.1
A vii				57 (590)	90 (61)	89 (65)	95 (62)		1.9
A viii			26 (19)	41 (34)	50 (42)	65 (553)	87 (136)		2.3
A ix							26 (312)		> 4.0
A x								93 (840)	?
A xi								80 (188)	?

Percentage mortalities, in successive generations, of adult male *A. stephensi* exposed for 1 to 2 hours to filter-papers impregnated with DDT solution in Risella oil at various concentrations. The numbers of mosquitos tested are given in parentheses.

In addition to the irritant effect, knockdown shortly after exposure remained significant throughout the series and invariably exceeded the 24-hour mortality. A comparison of the knockdowns and the kills of male and female *A. stephensi* at various dosages of DDT and in various generations of the adult exposure series is given in Tables XI and XII.

This is a very different picture from real DDT-resistance as witnessed with the same species in Saudi Arabia and with *A. sundaicus* both in the laboratory and in the field, where the resistant individuals are completely

TABLE XI. *A. STEPHENSI* ADULT EXPOSURE SERIES : KNOCKDOWN AND MORTALITY IN MALES

Generation	Percentage knockdown	Percentage kill	Total mosquitos
	1.5 % DDT		
Stock	75	52	130
A ii	70	57	278
A iii	75	65	167
A iv	65	55	245
A v	50	26	300
	2 % DDT		
Stock	92	60	93
A vii	89	41	181
A ix	49	26	310
	3 % DDT		
Stock	93	73	136
A v	98	61	81
A viii	83	64	387
	4 % DDT		
Stock	82	77	109
A viii	97	87	136
	4 % DDT (2 hours)		
Stock	100	100	46
A x	98	90	848
A xi	94	80	188

Percentage knockdowns and mortalities at varying percentages of DDT in Risella oil of *A. stephensi* males in successive generations of selection by DDT. Except where stated the exposure period was 1 hour. The knockdown was recorded within 1 hour of removal from the exposure tube, and the kill 24 hours after removal.

TABLE XII. *A. STEPHENSI* ADULT EXPOSURE SERIES : KNOCKDOWN AND MORTALITY IN FEMALES

Generation	Percentage knockdown	Percentage kill	Total mosquitos
	3 % DDT		
Stock	78	72	550
A ii	78	56	274
A iii	89	68	211
A iv	94	81	252
A v	60	42	252
A vii	74	42	134
	4 % DDT		
Stock	92	85	542
A viii	77	61	111
A ix	35	20	262
	4 % DDT (2 hours)		
Stock	100	90	46
A x	78	55	590
A xi	62	36	128

Percentage knockdowns and mortalities at varying percentages of DDT in Risella oil of *A. stephensi* females in successive generations of selection by DDT. Except where stated the exposure period was 1 hour. The knockdown was recorded witbin 1 hour of removal from the exposure tube, and the kill 24 hours after removal.

unaffected by one-hour exposures to the highest concentration of DDT in Risella oil.

The results of the smaller adult exposure series started from adults of the larval exposure series generation L xviia are recorded in Table XIII. They show that leaving one or two generations unexposed results in some loss of the tolerance gained.

DDT-selection of adults appeared to impart no increased tolerance among the larvae at least after seven generations of adult exposure. In the seventh generation the MLC had approximately doubled amongst both adult males and adult females. A comparison of mortalities among stock larvae and larvae of this seventh adult generation are given below. The observations were made at the same time.

	Concentration of DDT		
	0.5 p.p.m.	*1.0 p.p.m.*	*2.0 p.p.m.*
Stock	68% (172)	90% (184)	99% (200)
A vii	66% (169)	92% (179)	99% (200)

(The numbers of larvae tested are given in parentheses.)

TABLE XIII. *A. STEPHENSI* ADULT EXPOSURE SERIES : * ADULT MORTALITIES

Generation	Percentage concentration of DDT in Risella oil		
	4.0 (1 hour)	4.0 (1 ½ hours)	4.0 (2 hours)
Males			
L xviia	46 (282)		
A 1		68 (164)	96 (26)
A 2a * *			91 (572)
A 2b †	78 (215)		97 (233)
Females			
L xviia	34 (266)		
A 1		44 (233)	
A 2a * *			70 (429)
A 2b †	60 (189)		91 (195)

* A new adult exposure series started from the L xviia generation of the larval exposure series

** Generation A 2 was not exposed

† Generation A 2b was the offspring of unexposed A 2a

Percentage mortalities, in successive generations, of adult male and female *A. stephensi* exposed for 1 to 2 hours to filter papers impregnated with 4 % DDT in solution in Risella oil. The numbers of mosquitos tested are given in parentheses.

Larval and adult exposure series

Combined larval and adult exposure over four generations has increased the tolerance of both larvae and adults to the insecticide more rapidly than the separate exposures (Tables XIV and XV) if results are compared by generations. The larval MLC has increased some two-and-a-half times and the adult MLC about twice in only four generations of combined exposure.

It is concluded from these experiments with *A. stephensi* that continued exposure to DDT has resulted in the selection of a factor or a number of factors, other than a specific gene for resistance, imparting an increased tolerance to the insecticide. If a specific gene for resistance like the one established for *A. sundaicus* had been present in the original population, it is thought that it would have been selected out well within the number of generations involved in the series described. It is, for instance, possible that a strain with a thicker cuticle, less permeable to insecticide, has been selected, or possibly one with a larger fat body where the insecticide may be stored without reaching the nervous system. The term " vigour tolerance " has been coined by Hoskins & Gordon [15] for such changes, though there is little evidence that increased tolerance and vigour are necessarily associated. The significance of this increased intolerance after continued exposure of homogeneous strains is discussed elsewhere (see page 613).

TABLE XIV. *A. STEPHENSI* ADULT AND LARVAL EXPOSURE SERIES : LARVAL MORTALITIES

Generation	Concentration of DDT (p.p.m.)						Median lethal concentration (p.p.m.)
	0.5	0.67	1.0	1.33	2.0	4.0	
Stock	56 (10923)	44 (424)	81 (1839)	88 (1318)	97 (3648)	100 (924)	0.49
LA i	53 (729)	43 (309)	62 (2994)				0.60
LA ii			52 (6567)	84 (1701)	95 (243)		0.97
LA iiia *				78 (8262)			?
LA iva *				58 (2927)	94 (182)		1.25

* Generations LA iii and LA iv were not exposed, either as larvae or as adults.

Percentage mortalities, in successive generations, of 4th-stage larvae exposed to DDT in alcoholic solution for 24 hours at various concentrations. The numbers of larvae tested are given in parentheses.

TABLE XV. *A. STEPHENSI* LARVAL AND ADULT EXPOSURE SERIES : ADULT MORTALITIES

Generation	Percentage concentration of DDT in Risella oil								Median lethal concentration (%)
	0.25	0.5	0.75	1.0	1.5	2.0	2.5	3.0	
	Males								
Stock (not exposed as larvae)		12 (164)		51 (539)	54 (182)	64 (159)	43 (80)	74 (108)	1.10
Stock (survivors of larval exposure)	16 (19)	22 (222)	35 (490)	48 (162)					1.05
LA i ,,				38 (129)	71 (59)	78 (28)			1.18
LA ii ,,				50 (16)	43 (37)	76 (653)			1.30
LA iii						62 (324)			<2.0
LA iva						27 (166)			>2.0
	Females								
Stock (not exposed as larvae)				18 (623)	43 (950)	54 (759)	55 (521)	75 (569)	1.90
Stock (survivors of larval exposure)				46 (274)	48 (487)	75 (120)	81 (34)		1.50
LA i ,,					58 (149)	74 (39)	82 (112)	95 (19)	1.30
LA ii ,,				0 (19)	21 (19)	43 (58)		73 (650)	2.20
LA iiia*								63 (203)	<3.0
LA iva *								28 (341)	>3.0

* Generations LA iii and LA iv were not exposed, either as larvae or as adults.

Percentage mortalities, in successive generations, of adult male and female *A. stephensi* exposed for 1 hour to filter-papers impregnated with DDT solution in Risella oil at various concentrations. The numbers of mosquitos tested are given in parentheses.

Insecticide Resistance in *Anopheles gambiae*

A preliminary account of one resistant strain of *A. gambiae* which has appeared in Nigeria as the result of house-spraying with the insecticide dieldrin in a malaria-control project has already been given.[8] By testing the adults of this Ambursa strain it was shown to be some 800 times more resistant to dieldrin than the Lagos strain of the same species, a completely insecticide-susceptible strain. Though selected by dieldrin pressure only, this strain was found to be cross-resistant to the related cyclodiene chlorinated hydrocarbons, α-chlordane (10 000), β-chlordane (5000), aldrin (400), isodrin (140) and endrin (90) and also to gamma-BHC (25-32) (the figures in parentheses indicate the approximate degrees of resistance). It remained susceptible, though slightly more tolerant, to DDT however and also to the organic phosphate malathion and to pyrethrum (Table XVI). In its spectrum of resistance, therefore, this strain resembles very closely the dieldrin-BHC-resistant strains of other insects.[1, 22]

TABLE XVI. *A. GAMBIAE*: RESISTANCE SPECTRUM

Insecticide	Lagos (susceptible)	Ambursa (resistant)	Kano (resistant)
Dieldrin	0.052 –0.072 (2769)	>4.0 (1178)	>4.0 (180)
Aldrin	0.12 –0.26 (500)	>4.0 (36)	>4.0 (21)
Gamma-BHC	0.0053–0.0085 (1420)	0.17–0.21 (810)	0.26–0.32 (881)
DDT	0.46 –0.52 (898)	0.75–0.85 (615)	1.05–1.10 (237)
Malathion *	0.36 -0.73 (114)	0.7 –1.3 (79)	
Pyrethrum	0.5 (138)	0.72 (256)	

* In solution in olive oil

Ranges of medial lethal concentrations (percentages in Risella oil or olive oil) of various insecticides for one susceptible and two resistant strains of *A. gambiae* (1 hour's exposure). The numbers of mosquitos from which the MLC's have been derived are given in parentheses. They comprise males and females, the latter both fed and unfed.

On request being made for another susceptible strain of *A. gambiae* from an area nearer to the Ambursa resistant strain, eggs were received from Kano in Northern Nigeria some 300 miles to the east of Ambursa. Adults reared from these eggs were found, on exposure to the discriminating dosages, to be described later (see page 603), to be a mixed population with the following composition:

susceptibles . . . 16%,
hybrids 45%,
resistants . . . . 39%.

By exposing unmated adults to the higher of these discriminating dosages a pure resistant strain was immediately isolated and was found to be almost identical in its resistance pattern with the Ambursa strain though with a slightly higher resistance to aldrin (> 800) and gamma-BHC (38-49) (Table XVI). Inquiry has since revealed that the only insecticides ever used in Kano have been DDT and BHC, and these only in a small area around the airport.[11] This, then, is a second case of dieldrin-BHC-resistance in *A. gambiae* which has arisen completely independently of the Ambursa strain and through selection by BHC. The strain selected is nevertheless virtually identical with the dieldrin-selected Ambursa strain and the independent appearance of an identical single gene is therefore strongly indicated.

TABLE XVII. *A. GAMBIAE*: HYBRID SUSCEPTIBILITIES

Hybrid	Median lethal concentration (%)	
	dieldrin	gamma-BHC
Lagos ♂ × Ambursa ♀	1.7 (702)	0.044 (334)
Ambursa ♂ × Lagos ♀	2.0 (707)	0.045 (395)
Diggi ♂ × Ambursa ♀	1.7 (475)	—
Ambursa ♂ × Diggi ♀	1.6 (146)	—
Maidihini ♂ × Ambursa ♀	1.4 (117)	—
Lagos ♂ × Kano ♀	0.9 (448)	—
Kano ♂ × Lagos ♀	1.1 (507)	—
Diggi ♂ × Kano ♀	0.42 (119)	—
Kano ♂ × Diggi ♀	1.1 (219)	—
Maidihini ♂ × Kano ♀	1.0 (61)	—
Kano ♂ × Maidihini ♀	1.0 (36)	—

Median lethal concentrations (percentages in Risella oil) of dieldrin and gamma-BHC (1 hour's exposure) among various hybrid *A. gambiae* produced by crossing susceptible and resistant strains. The numbers of mosquitos from which the MLC's have been derived are given in parentheses. They comprise both males and females, the latter both unfed and fed.

By crossing resistant and susceptible strains of *A. gambiae* and determining the constitution of the offspring of backcrosses of the hybrids with the parent resistant and susceptible strains, the monofactorial inheritance of this type of resistance has now been established. This inheritance mechanism was initially worked out with the Ambursa and Lagos strains [9] and has since been confirmed with the Kano resistant strain and two other susceptible strains both originating about 20 miles away from Ambursa.

The hybrids from reciprocal crosses of the resistant and susceptible strains were found to be intermediate in their resistance (to dieldrin and

gamma-BHC, at any rate). They varied somewhat in their susceptibility according to the origin of the parent strains (Table XVII) but the MLC was almost invariably between 1% and 2% dieldrin (the one exception, Diggi ♂ × Kano ♀, is not an accurate figure as these hybrids showed virtually no kill at the lower discriminating dosage described below) and about 0.045% gamma-BHC. The hybrids were thus of the order of 20-30 times resistant to dieldrin and 10 times to gamma-BHC.

Exposure of susceptible, hybrid and resistant strains to a long series of dosages of dieldrin (Table XVIII) and gamma-BHC (Table XIX) then made possible the selection of two discriminating dosages of each insecticide, the lower of which killed all susceptibles but no hybrids or resistants,

TABLE XVIII. *A. GAMBIAE*: SUSCEPTIBILITY OF SUSCEPTIBLE, HYBRID AND RESISTANT STRAINS TO DIELDRIN

Dieldrin dosage (%)	Susceptible strain (Lagos)	Hybrid S♂ × R♀	Hybrid R♂ × S♀	Resistant strain (Ambursa)
0.025	0 (38)			
0.05	40 (484)			
0.1	69 (1204)	0 (29)	0 (20)	
0.15	92 (755)			
0.2	97 (536)		0 (46)	
0.33	100 (725)	0 (20)	0 (43)	
0.4	100 (140)			
0.5		4 (26)	13 (40)	
1.0	100 (39)	20 (133)	0 (37)	0 (35)
1.5		25 (53)	24 (72)	
2.0	100 (19)	47 (148)	47 (164)	0 (31)
2.5		76 (89)	59 (164)	
3.0		90 (134)	95 (82)	0 (34)
4.0		97 (119)	97 (225)	1 (1178)
4.0 (2 hours)		100 (22)		0 (21)
4.0 (5 hours)				11 (161)
4.0 (18 hours)				77 (436) *
Check (1 hour)	4 (623)	5 (102)	0 (58)	0 (110)
,, (5 hours)				0 (66)
,, (18 hours)				5 (191) *

* Fed females only

Percentage mortalities at varying percentages of dieldrin in Risella oil of a susceptible and a resistant strain of *A. gambiae* and their hybrids. The figures in parentheses are the numbers of mosquitos exposed and comprise both males and females, the latter both fed and unfed. All exposures were for 1 hour except where stated.

TABLE XIX. *A. GAMBIAE*: SUSCEPTIBILITY OF SUSCEPTIBLE, HYBRID AND RESISTANT STRAINS TO BHC

Gamma-BHC dosage (%)	Susceptible strain (Lagos)	Hybrid S♂ × R♀	Hybrid R♂ × S♀	Resistant strain (Ambursa)
0.005	41 (379)	0 (42)		
0.0075	79 (321)	0 (100)		
0.01	66 (691)	0 (41)		
0.02	91 (353)	0 (42)		
0.025	100 (97)	11 (37)	19 (88)	0 (51)
0.04	100 (123)			
0.05	100 (30)	48 (147)	49 (114)	6 (35)
0.067		96 (47)	83 (70)	
0.075		96 (103)	85 (123)	
0.1		100 (89)	100 (57)	6 (80)
0.15				33 (30)
0.2		100 (42)		47 (510)
0.25				66 (122)
0.33				91 (68)
Check	2 (350)	1 (118)	1 (87)	2 (188)

Percentage mortalities at varying percentages of gamma-BHC in Risella oil of a susceptible and a resistant strain of *A. gambiae* and their hybrids. The figures in parentheses are the numbers of mosquitos exposed and comprise both males and females, the latter both fed and unfed. All exposures were for 1 hour.

and the upper of which killed all hybrids (and susceptibles) but not resistants. These dosages were:

dieldrin: 0.33% for 1 hour and 4.0% for 2 hours;
gamma-BHC: 0.025% for 1 hour and 0.1% for 1 hour.

As the degree of resistance to BHC was not very high the discriminating dosages of this insecticide were not as perfect as those of dieldrin.

By the use of these discriminating dosages it was then possible to determine the phenotypic constitution of hybrid offspring and the offspring of the various backcrosses and hence the mode of inheritance of resistance.

All the crosses between the two resistant and three susceptible strains of *A. gambiae* produced vigorous hybrids, which survived over long periods and the females of which readily fed on blood and matured and laid numerous batches of eggs. In all but one of these crosses almost all the eggs laid were sterile, only an occasional one out of tens of thousands hatching to produce a larva. Examination of the testes of the male hybrids showed these to be in varying degrees of atrophy and an examination of the spermathecae of several hundred female hybrids showed no evidence of fertiliza-

tion. Hybrid sterility was first evident in the Lagos × Ambursa crosses and it was concluded that two geographical races of the species were concerned, since they originated some 600 miles apart. It was for this reason that susceptible strains from as near to the resistant strain as possible were obtained. One of these, the Maidihini strain, crossed with the Ambursa strain, again produced only sterile hybrids. The other, the Diggi strain, produced sterile hybrids when the cross, resistant female × susceptible male, was made but fertile hybrids when the cross, susceptible female × resistant male, was made. Furthermore, crossings of the two resistant strains and of two widely separated susceptible strains produced no hybrid sterility. Some association between sterility and resistance was thus indicated, coming down through the resistant female, but the precise nature of this association remains to be solved. The issue has recently been further complicated by the fact that the production of fertile hybrids by the cross mentioned has only been achieved once and repeated attempts since then have failed. Also it is difficult to see why the Diggi and Maidihini strains should differ in this respect as they are both near to one another (indications are that a common breeding-place serves both areas) and equidistant from Ambursa.

The fact remains that on one occasion offspring of interbred hybrids were obtained in large numbers and on exposure to the two discriminating dosages of dieldrin showed a constitution of 22% susceptibles, 53% hybrids and 25% resistants (Table XX), a ratio very near to the 1:2:1 ratio of simple Mendelian inheritance.

TABLE XX. *A. GAMBIAE* : F_2 GENERATION COMPOSITION

Dieldrin dosage (%)	Total mosquitos	Number dead	Percentage mortality
0.33 (1 hour)	♀ 526	113	21
	♂ 522	117	22
	Total: 1048	230	22
4.0 (2 hours)	♀ 171	124	73
	♂ 151	117	77
	Total: 322	241	75
Check	♀ 67	7	10
	♂ 70	1	1
	Total: 137	8	6

Details of mortalities among the offspring of interbred hybrid *A. gambiae* derived from the cross, Ambursa male × Diggi female, after exposure to the two discriminating dosages separating susceptibles, hybrids and resistants.

Because in most cases the hybrid male was sterile, backcrosses could only be made using the hybrid female (backcrosses with hybrid males were tried but only sterile eggs were obtained). Backcrosses with the susceptible parent produced offspring composed of 50% susceptibles, 50% hybrids and no resistants, while with the resistant parent they produced 50% hybrids and 50% resistants, but no susceptibles (Table XXI), as would be expected if a monofactorial mechanism is operating.

TABLE XXI. *A. GAMBIAE*: BACKCROSS OFFSPRING COMPOSITIONS

Hybrid	Parent male to which the hybrid female was backcrossed	Backcross offspring		
		susceptible	hybrid	resistant
	Dieldrin			
Lagos ♂ × Ambursa ♀	Lagos (SS)	55 (367)	45 (297)	
Ambursa ♂ × Lagos ♀	Lagos (SS)	48 (352)	52 (389)	
Ambursa ♂ × Lagos ♀	Ambursa (RR)		53 (368)	47 (328)
Lagos ♂ × Ambursa ♀	Ambursa (RR)		50 (416)	50 (408)
Lagos ♂ × Kano ♀	Lagos (SS)	41 (278)	59 (401)	
Kano ♂ × Lagos ♀	Lagos (SS)	53 (223)	47 (200)	
Maidihini ♂ × Kano ♀	Maidihini (SS)	41 (106)	59 (153)	
Kano ♂ × Maidihini ♀	Kano (RR)		55 (53)	45 (44)
	Gamma-BHC			
Lagos ♂ × Ambursa ♀	Lagos (SS)	50 (382)	50 (377)	

The percentage constitution of the offspring of various backcrosses between hybrid and parent susceptible and resistant strains of *A. gambiae* as determined by exposure to the discriminating dosages of dieldrin and gamma-BHC. The numbers in parentheses are the actual numbers of mosquitos involved. They comprise both males and females, the latter both fed and unfed.

(SS) = Susceptible

(RR) = Resistant

To complete the proof of the existence of a single factor for resistance the offspring of a backcross of hybrid females to susceptible males were exposed to the dosage killing susceptibles, the surviving unmated females were mated with resistant males and their offspring were exposed to the dosage killing hybrids. In both cases a 50% mortality resulted and the finally surviving females and males, mated with their opposite resistants, produced pure resistant strains. Thus the circle was completed.

Homogeneity of strains for the factor or factors being investigated is the basic essential in genetical work and its absence may be the reason for the complicated results of some studies on the inheritance of housefly resistance to insecticides. Homogeneity of the two strains of *A. gambiae* for the gene for resistance to insecticides is already virtually proved by the F_2

and backcross sort-outs already described. Further proof is given by the fact that neither strain has changed significantly in its resistance to dieldrin after one year's maintenance as a laboratory colony in the absence of any insecticide selection whatsoever. During this period some twenty generations of each strain have been passed through. It appears to be a feature of many so-called resistant strains of houseflies and other insects that in the absence of insecticidal pressure they show some reversion to susceptibility. This in itself indicates heterogeneity.

Further proof of the unalterable nature of the resistant gene in *A. gambiae* is given by the results of two series of experiments designed with purely practical considerations in mind.

TABLE XXII. *A. GAMBIAE*: EFFECT OF DDT-SELECTION OF RESISTANT STRAIN

Generation	Percentage concentration of DDT in Risella oil					
	1.5	2.0	2.5	2.67	2.75	3.0
Parent stock	93 (103)	100 (25)	100 (47)			100 (31)
I. Parents survived 1% DDT	23 (31)	70 (281)				
II. Parents survived 2% DDT			80 (505)			
III. Parents survived 2.5% DDT				100 (141)	100 (37)	99 (158)
IIc. Generation II unexposed for 3 generations	63 (505)	91 (130)				

Percentage mortalities, in successive generations, of adult male and female dieldrin-BHC-resistant *A. gambiae* (Ambursa strain) exposed for 1 hour to varying concentrations of DDT in Risella oil. The numbers of mosquitos tested are given in parentheses.

The first of these was carried out to see if DDT-resistance could be induced in this dieldrin-BHC-resistant mosquito. Unmated males and unfed females of the Ambursa strain were exposed to sublethal dosages of DDT and the survivors bred from, as in the adult exposure series in the *A. stephensi* experiments already described. The selection dosage was raised in successive generations. Table XXII gives the results of three generations of selection. Initially a quite marked drop in susceptibility occurred but a limit to the selection dosage was reached in the second generation at 2.5% DDT. When this second generation was left unexposed for three generations a marked reversion to the tolerance-level of the parent strain was already obvious. In this case of DDT-selection, unlike that of *A. stephensi* previously described, little difference was apparent between immediate knockdown and 24-hour kill (Table XXIII) and the surviving mosquitos did not appear irritated to any extent.

TABLE XXIII. *A. GAMBIAE*: DDT-SELECTION OF RESISTANT STRAIN (KNOCKDOWN AND MORTALITY)

Generation	Percentage knockdown	Percentage kill	Total mosquitos
1.5 % DDT			
Parent stock	96	94	113
I	23	23	31
IIc	56	63	505
2 % DDT			
Parent stock	100	100	36
I	74	70	281
IIc	92	91	130
2.5 % DDT			
Parent stock	100	100	47
II	80	80	505
2.67 % DDT			
III	100	100	141
2.75 % DDT			
III	100	100	37
3 % DDT			
Parent stock	100	100	45
III	99	99	158

Percentage knockdowns and mortalities at varying percentages of DDT in Risella oil of dieldrin-BHC-resistant *A. gambiae* (Ambursa strain) males and females in successive generations of selection by DDT. The exposure period was 1 hour. The knockdown was recorded 1 hour after removal from the exposure tube and the kill 24 hours after removal.

The purpose of the second experiment, carried out in a similar way, was to see if the tolerance to BHC could be raised, and for this the resistant strain from Kano was used. Again an initial sharp fall in susceptibility occurred but it was found impossible, in spite of repeated efforts, to raise the dosage level beyond 0.5% gamma-BHC (Table XXIV). Again relaxation of selection pressure resulted in a reversion towards the original tolerance-level of the parent strain and no change in the relation between knockdown and 24-hour kill was apparent (Table XXV).

The comparatively slight changes in tolerance-levels in these two resistant strains of *A. gambiae*, while supporting the contention of homogeneity, also show again the phenomenon of " vigour tolerance " already discussed in the case of the *A. stephensi* experiments.

TABLE XXIV. *A. GAMBIAE*: BHC-SELECTION OF RESISTANT STRAIN

Generation	Percentage concentration of gamma-BHC in Risella oil				
	0.25	0.33	0.4	0.5	0.6
Parent stock	34 (123)	70 (588)			
I: Parents survived 0.33% BHC		33 (76)	72 (797)	100 (49)	
II: Parents survived 0.4% BHC		41 (87)	64 (117)	82 (736)	
III (1): Parents survived 0.5 % BHC					97 (92)
IIa: Generation II unexposed for one generation		51 (256)	87 (414)	93 (559)	100 (122)
III (2): Parents (Generation IIa) survived 0.4 % BHC			69 (80)	96 (135)	
IV (1): Parents [Generation III (2)] survived 0.4 % BHC				96 (78)	
III (2) a: Generation III (2) unexposed for one generation	14 (57)	58 (33)	67 (418)	100 (13)	
IV (2): Parents [Generation III (2) a] survived 0.4% BHC				91 (197)	

Percentage mortalities, in successive generations, of adult male and female dieldrin-BHC-resistant *A. gambiae* (Kano strain) exposed for 1 hour to varying concentrations of gamma-BHC in Risella oil. The numbers of mosquitos tested are given in parentheses.

Insecticide Resistance in *Anopheles sundaicus*

A preliminary account of DDT-resistance in this species has already been given.[10] Strains of *A. sundaicus* resistant to DDT have appeared independently in at least four widely separated areas of the island of Java in recent years as a result of the use of DDT for malaria control. Surprisingly enough, however, no resistance has appeared in one area in the south of the island where systematic house-spraying with the same insecticide has been carried out for the past 4 years.[25]

The resistant strain of *A. sundaicus* was easily colonized in London with ordinary tap-water as rearing medium, despite the fact that in its natural habitat this species is a brackish-water breeder. To date, however, it has proved impossible to transport eggs of the susceptible strain of the same species from Java to London in a viable state and another susceptible strain from Malaya has had to be used instead. This strain proved much more difficult to colonize than the resistant strain.

Susceptibility tests on adults both in the field [a] and in the London laboratory have shown that, while the resistant strain is some forty times

[a] G. Davidson, unpublished data, 1955

TABLE XXV. *A. GAMBIAE*: BHC-SELECTION OF RESISTANT STRAIN (KNOCKDOWN AND MORTALITY)

Generation	Percentage knockdown	Percentage kill	Total mosquitos
	0.25 % gamma-BHC		
Parent stock	19	34	123
III (2)a	11	14	57
	0.33 % gamma-BHC		
Parent stock	53	70	588
I	28	33	76
II	38	41	87
IIa	48	51	256
III (2)a	61	58	33
	0.4 % gamma-BHC		
	61	72	783
II	68	64	117
IIa	88	87	414
III (2)	66	69	80
III (2)a	67	67	418
	0.5 % gamma-BHC		
I	100	100	49
II	75	83	709
IIa	92	93	559
III (2)	94	96	135
IV (1)	95	96	78
III (2)a	92	100	13
IV (2)	90	91	197
	0.6 % gamma-BHC		
III (1)	82	97	92
IIa	100	100	122

Percentage knockdowns and mortalities at varying percentages of gamma-BHC in Risella oil of dieldrin-BHC-resistant *A. gambiae* (Kano strain) males and females in successive generations of selection by gamma-BHC. The exposure period was 1 hour. The knockdown was recorded 1 hour after removal from the exposure tube and the kill 24 hours after removal.

more resistant to DDT than the susceptible strain, it is similar in its susceptibility to dieldrin, gamma-BHC and aldrin (Table XXVI). Further, exposure to three DDT-analogues showed that cross-resistance extends to these compounds (Table XXVII).

TABLE XXVI. *A. SUNDAICUS*: RESISTANCE SPECTRUM

Insecticide	Susceptible		Resistant	
	Java strain * (in Java)	Malaya strain (London colony)	Java strain * (in Java)	Java strain (London colony)
DDT	0.5 (159)	0.36 (355)	9.0 (165) **	>4.0 (168)
Dieldrin	0.065 (206)	0.036 (165)	0.08 (527)	0.03 (194)
Gamma-BHC	0.008 (86)	0.0042 (142)	0.0085 (151)	0.008 (102)
Aldrin	0.37 (101)		0.4 (171)	

* G. Davidson, unpublished data, 1955

** Interpolated from an extension of the logarithmic-probability graph

Median lethal concentrations (percentages in Risella oil) of various insecticides for the females of susceptible and resistant strains of *A. sundaicus* (1 hour's exposure). The numbers of mosquitos used to determine the MLC's are given in parentheses.

Crossing the two laboratory strains of *A. sundaicus* presented no difficulties and vigorous hybrids resulted which, however, were only slightly more resistant than the susceptible strain. It was not possible, therefore, to choose a discriminating dosage of the insecticide which would kill all susceptibles but no hybrids, as it was with dieldrin-resistant *A. gambiae*. All that was possible was to find a single discriminating dosage which killed almost all susceptibles and hybrids but hardly any resistants. The clear-cut discriminating dosages used in separating *A. gambiae* phenotypes could not be applied to *A. sundaicus* where the degree of resistance to DDT was so much lower and where the hybrid was virtually as susceptible as the susceptible strain. The single discriminating dosage selected was 2.5% DDT, though now it is considered that any dosage between this and 4% would be equally suitable.

TABLE XXVII. *A. SUNDAICUS*: CROSS-RESISTANCE TO DDT-ANALOGUES

Insecticide and dosage	Susceptible strain	Resistant strain	
	1 hour mortalities	1 hour mortalities	2 hour mortalities
1 % Methoxychlor	82 (73)	24 (38)	29 (28)
2 % Dichloro-diphenyl-dichloroethane (" Rhothane ")	98 (62)	3 (37)	15 (26)
4 % Diethyl-diphenyl-dichloroethane (" Perthane ")	100 (44)	16 (25)	37 (27)

Percentage mortalities among males and unfed females of the susceptible and resistant strains of *A. sundaicus* when exposed for 1 and 2 hours to three DDT-analogues in solution in Risella oil. The numbers of mosquitos exposed are given in parentheses.

Unlike most of the *A. gambiae* hybrids, *A. sundaicus* hybrids readily interbreed and produce viable offspring. The F_2 generations exposed to the discriminating dosage show an approximate 75% kill, the expected 3: 1 ratio of simple Mendelian inheritance with complete dominance of one character. Monofactorial inheritance has been confirmed by backcrossing the hybrids with the susceptible and resistant parent strains. The offspring of the backcross to the resistant parent showed approximately the expected 50% kill after exposure to the discriminating dosage. The offspring of the backcross to the susceptible parent showed an almost complete kill. Detailed results of mortalities among the two homozygous strains, their hybrids, hybrid offspring and all the possible backcross offspring are given in Table XXVIII.

To prove that mosquitos surviving the discriminating dosage were resistant, unmated F_2 males and females were exposed to 4% DDT and the survivors allowed to mate. Their offspring showed only a 4% mortality (among 90 mosquitos) when exposed to the same dosage.

Insecticide Resistance in *Anopheles stephensi*

House-spraying with DDT for the control of malaria transmitted by *A. stephensi* in some of the oases in the south-eastern part of Saudi Arabia was started in 1947 and produced an impressive reduction in the incidence of the disease for five years afterwards. From 1953 onwards, however, the disease returned in ever-increasing incidence and, with the finding of *A. stephensi* resting unharmed in the daytime on recently applied DDT deposits, resistance was suspected.[a] The writer was privileged to visit the area on behalf of the World Health Organization and preliminary susceptibility tests were made on *A. stephensi* from DDT-treated villages and from a small village where insecticides had never been used.[b] Resistance to DDT was confirmed and since that time more testing has been carried out.[a] A comparison of the MLC's of four insecticides for susceptible and resistant strains in the field and for the susceptible strain from India maintained as a colony in London is made in Table XXIX. Like *A. sundaicus*, the DDT-resistant strain of *A. stephensi* is susceptible to dieldrin, aldrin and gamma-BHC. The degree of resistance to DDT does not appear to be high from the figures so far recorded (some three to six times only), but the figures for the MLC's of the resistant strains have been interpolated from a log-probit regression line and may not be very accurate. Until similar mortalities are produced in both resistant and susceptible strains by exposures to actual possible concentrations of DDT in Risella oil the precise degree

[a] R. H. Daggy, unpublished paper presented at the Third Conference of the Industrial Council for Tropical Health, 1957

[b] G. Davidson, unpublished data, 1955

of resistance will not be found. A longer exposure period than one hour will of course be necessary.

That the population tested in 1955 was not a homogeneous one is indicated by the fact that significant mortalities occurred at quite low dosages of DDT. The actual detailed figures were:

Insecticide dosage (%)	*Mosquitos tested*	*Percentage mortality*
0.5	19	5
1.0	20	20
1.5	22	18
2.0	32	25
4.0	44	23
6.0	53	70
Check	30	3

(The 6% DDT is not a true dilution but was produced by diluting 4% with equal parts of the volatile solvent used in the Busvine & Nash test instead of the normal double quantity of solvent. How much of this DDT remained in solution in the Risella oil after evaporation of the solvent is not known.) What is interesting about these figures is the similar mortality produced by all dosages from 1% to 4% DDT. This may well indicate the susceptible and hybrid portion of the population and suggest that the discriminating dosage for this species is similar to that of *A. sundaicus*. If this is the case, the MLC of the resistant strain may be much more than the figure of 5% already given and the degree of resistance much higher than indicated.

The exact mode of inheritance of DDT-resistance in *A. stephensi* remains to be determined.

TABLE XXVIII. ***A. SUNDAICUS*:**

Percentage DDT in Risella oil	Susceptible strain (S)	Resistant strain (R)	Hybrid I R♂ × S♀	Hybrid II S♂ × R♀	Offspring of hybrid I	Offspring of hybrid II
0.25	44 (75)				37 (359)	
0.5	59 (148)		31 (39)	11 (36)	56 (120)	16 (43)
1.0	88 (130)	0 (63)	40 (25)	39 (70)	62 (76)	33 (45)
1.5	97 (96)		57 (30)	77 (68)	72 (57)	58 (33)
2.0	97 (119)	8 (84)	80 (54)	97 (123)	84 (70)	67 (45)
2.5	93 (86)	1 (78)	97 (100)	99 (236)	83 (199)	74 (304)
3.0	100 (18)	8 (71)	100 (27)		83 (115)	76 (78)
4.0	100 (81)	8 (383)	100 (27)		82 (136)	81 (59)
Check	3 (115)	4 (284)	9 (46)	4 (48)	4 (130)	2 (49)

Mortalities at varying percentages of DDT in Risella oil among susceptible, resistant, hybrid, interbred hybrid

Discussion

The general principles emerging from these studies on insecticide resistance in anopheline mosquitos may be enumerated as follows :

1. Two distinct types of resistance, each inherited by a monofactorial mechanism, exist:

(*a*) DDT-resistance, imparting cross-resistance to analogues of this chlorinated hydrocarbon but not to the less closely-related cyclodiene compounds nor to gamma-BHC. This type of resistance would appear to be of a lower degree than dieldrin-resistance and to be virtually recessive to normal susceptibility. Only two phenotypes are recognized by normal testing techniques.

(*b*) Dieldrin-resistance, imparting cross-resistance to the other cyclodiene chlorinated hydrocarbons and to gamma-BHC, but not to DDT. This type of resistance is very high to the cyclodiene compounds but comparatively low to gamma-BHC. Resistance in this case is partially dominant and three phenotypes can be recognized.

2. In addition to these two types of resistance, a general increase in tolerance to both groups of insecticides, and to other insecticides of entirely different groups, may be produced by prolonged exposures of apparently homogeneous strains to any of the insecticides. This applies both to susceptible strains and to strains possessing one of the two resistant genes already referred to. Thus a strain resistant to the dieldrin-BHC group of insecticides may show an increased tolerance to DDT, malathion and pyrethrum without having come into contact with these

INHERITANCE OF RESISTANCE

Backcross R♂ × hybrid I♀	Backcross hybrid I♂ × R♀	Backcross S♂ × hybrid I♀	Backcross hybrid I♂ × S♀	Backcross R♂ × hybrid II♀	Backcross hybrid II♂ × R♀	Backcross S♂ × hybrid II♀	Backcross hybrid II♂ × S♀
3 (30)				6 (47)	18 (40)	0 (20)	
				29 (24)	25 (8)	25 (20)	
				31 (26)	17 (6)	70 (20)	
				48 (23)	50 (8)	94 (16)	
	51 (51)	87 (266)	91 (102)	61 (397)	57 (279)	94 (273)	92 (74)
		89 (128)		74 (27)		91 (23)	
41 (27)		92 (133)		63 (79)	67 (33)	100 (28)	
0 (16)	0 (6)	2 (57)	0 (20)	3 (65)	11 (28)	2 (50)	13 (16)

offspring and backcross offspring of *A. sundaicus*. The numbers of mosquitos tested are given in parentheses.

TABLE XXIX. *A. STEPHENSI*: RESISTANCE SPECTRUM

Insecticide	Susceptible		Resistant		
			wild-caught, Saudi Arabia		
	wild-caught Saudi Arabia *	London colony (from India)	Al-Hasa Oasis		Qatif Oasis
			1955 **	1956 *	1956 *
DDT	1.8 (112)	1.6 (4014)	5.0 (190) †	8.0 (648) †	11.4 (143) †
Dieldrin	0.068 (164)	0.13 (388)	0.17 (68)	0.068 (586)	0.11 (116)
Gamma-BHC		0.0105 (181)	0.0125 (54)		
Aldrin		0.23 (203)	0.5 (38)		

* R. H. Daggy, unpublished paper presented at the Third Conference of the Industrial Council for Tropical Health, 1957

** G. Davidson, unpublished data, 1955

† Dosages interpolated from extension of logarithmic-probability graph

Medial lethal concentrations (percentages in Risella oil) of various insecticides for the females of susceptible and resistant strains of *A. stephensi* (1 hour's exposure). The numbers of mosquitos used to determine the MLC's are given in parentheses.

insecticides, or its tolerance to DDT may be increased by exposure to the insecticide. Also, an already resistant strain may have its tolerance to the insecticide to which it is already resistant raised by further exposure. However, this "vigour tolerance" as it has been called involves, as a rule, only a slight increase in tolerance. The mechanism governing this type of resistance is in all probability polygenic and the likelihood of selecting out a pure strain remote. This would perhaps explain the usual reversion to original tolerance level when selection is stopped.

That the DDT-resistant strains of both *A. sundaicus* and *A. stephensi* remain susceptible enough to dieldrin to be successfully controlled by the use of this insecticide has been amply borne out in the field in both Java and Saudi Arabia. Whether dieldrin-BHC-resistant *A. gambiae* will be controlled by DDT remains to be seen. Evidence of increased tolerance to the latter in the field was found in a recent survey of the area.[a] Some adults were found to survive 4% DDT. It seems possible that this imparted tolerance to DDT may be sufficient to prevent malaria control when a change is made to this insecticide, as evidence already exists that the mortality produced by DDT among *A. gambiae* in other parts of Africa is barely sufficient to intercept transmission,[19] because the irritant property of this insecticide causes many of the mosquitos to leave a treated surface before they pick up a lethal dose.[6]

Indications of a general increase in tolerance of *A. gambiae* to DDT, dieldrin and gamma-BHC without specific monofactorial resistance to

[a] J. A. Armstrong, C. D. Ramsdale & V. Ramakrishna, unpublished working document WHO/Mal/182 (WHO/Insecticides/52)

either of the two groups of insecticides involved are now apparent in the Ruzizi valley of the Belgian Congo where house-spraying with DDT has been carried out over a number of years.[25] Wild-caught mosquitos were found to survive 4% DDT (72% mortality) and 1% dieldrin (95% mortality), concentrations well above the LD_{100}'s recorded for susceptible strains of this species. A colony of this Ruzizi strain of *A. gambiae* has now been established in London from a large number of eggs from females surviving 2% DDT in the field. To all intents and purposes this London colony is a normal susceptible strain showing no survivals at 4% DDT or at 0.33% dieldrin.

A. quadrimaculatus is another species of anopheline in which a resistant strain has appeared in the Mississippi area of the USA. This strain is highly resistant to the dieldrin-BHC group of insecticides, but shows a normal susceptibility to DDT.[20]

The situation with regard to resistance in *A. sacharovi* in Greece is somewhat confusing. Resistance to DDT was first suspected in 1951, at which time a nation-wide antimalaria campaign had been in operation for five years, and an account of the findings was published in 1953.[18] Since then dieldrin, BHC and chlordane have been used to some extent and evidence of resistance to this group of compounds has appeared.[13] Most of the records [2] using the Busvine & Nash technique of assessment of adult susceptibility do not indicate the relatively high degree of tolerance found in *A. stephensi* and *A. sundaicus*. Further, there is evidence that these resistant strains of *A. sacharovi* are still being irritated by the insecticide to such an extent that malaria control is still being achieved.[4] This then might indicate "vigour tolerance" rather than the monofactorial type of resistance found in *A. stephensi* and *A. sundaicus*. Also, evidence is now appearing [26] that the susceptibility of adults of the *maculipennis*-complex may vary considerably seasonally. It has been found that the tolerance to DDT of hibernating females showing pronounced development of the fat body far exceeds those of normal active summer females. Perhaps some of the tests indicating increased tolerance were done on such hibernating females.

Recent findings by Hadjinicolaou in the Skála area of Greece now indicate a very high degree of resistance to DDT in *A. sacharovi* (the MLC has been calculated at 122% DDT, though 18% of mosquitos continue to be killed by 4% DDT, and 7% even by 0.5% DDT).[25] This, then, would seem to indicate true specific resistance rather than "vigour tolerance", though possibly the population tested was not homogeneous (cf. *A. stephensi* in Saudi Arabia).

Evidence of resistance to dieldrin and BHC in *A. sacharovi* has been produced by Busvine [a] and by Hadjinicolaou.[25] Busvine, testing small

[a] J. R. Busvine, unpublished data, 1955

numbers in September 1955, found a death-rate of zero at 0.5% dieldrin and only a 22% mortality at 1% dieldrin in a population showing only 14% mortality at 3% DDT. Hadjinicolaou records a MLC of 0.29% dieldrin (67% mortality at 0.4% dieldrin) and of 0.033% gamma-BHC (58% mortality at 0.04% gamma-BHC) among a population showing a MLC of 2.7% DDT (68% mortality at 4% DDT). Such figures are reminiscent of those of Holstein among *A. gambiae* in the Belgian Congo and may again indicate " vigour tolerance " rather than true resistance. The existence of a population of *A. sacharovi* containing specific resistance mechanisms for both groups of insecticides thus remains in doubt.

It has long been recognized that in general housefly resistance falls into the two main types of resistance to DDT and its analogues, and resistance to the dieldrin-BHC group of chlorinated hydrocarbons.[1, 22] However, flies resistant to the one group quite readily become resistant to the other group, resulting in multi-resistant strains. This would seem to indicate that the two genes for the two types of resistance very often occur in one and the same population.

One begins to wonder, however, how much of this cross-resistance to separate groups of insecticides is due to specific mechanisms and how much is due to " vigour tolerance " when it is recorded that selection of a multi-susceptible strain by diazinon (an organic phosphate) and by N, N-dimethyl-phenylcarbaminate raises the level of resistance to chlorinated hydrocarbons.[21]

The susceptibility of houseflies to insecticides has always been assessed by topical-application techniques. It is well known that the degree of resistance recorded by these techniques varies considerably with the solvent employed [2] and one wonders whether such artificial applications on the thorax (as they invariably are) are a valid and accurate means of assessing susceptibility.

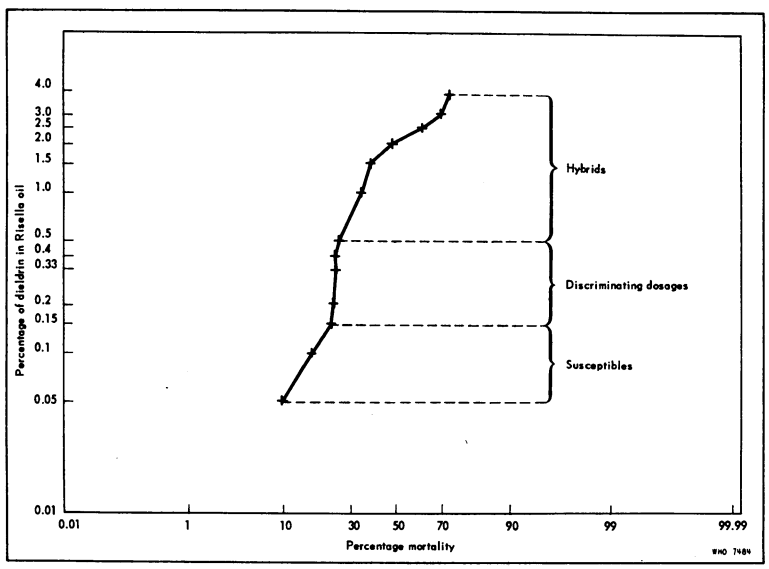
The inheritance of resistance in houseflies presents a most confusing picture.[4] Milani,[23] however, is of the opinion that many of the studies so far made can be interpreted as monofactorial.

The finding of a monofactorial inheritance in both dieldrin-BHC-resistance and DDT-resistance in two species of anophelines indicates the necessity for a change in the method of detecting resistance in the field. The present method of comparing the MLC of a particular insecticide in a mosquito population where resistance is suspected with that in a mosquito population where the insecticide has never been used may show only a small difference where susceptibles and hybrids predominate in the population. Thus in Northern Nigeria where the Ambursa resistant strain of *A. gambiae* came from, the degree of resistance to dieldrin was recorded as only eight times in 1955 [12] when the MLC was compared with that of a susceptible strain. A detailed analysis of the mortalities among the resistant population at four dosages of dieldrin showed significant mortalities between

0.5% and 4% dieldrin (1 hour's exposure), indicating the presence of both susceptibles and hybrids. It so happened that the mortalities recorded did fit a straight line on log-probit graph paper, and a MLC of 2% dieldrin was recorded. If many more mosquitos had been exposed to a larger series of dosages, from 0.05% to 4%, the regression line would probably have been found to possess a step at which an increase in dosage produced no increase in kill between approximately 0.2% and 1.0% dieldrin, the portion below the step representing susceptibles, and the portion above, hybrids. The second step separating hybrids from resistants cannot be graphically recorded with dieldrin at 1 hour's exposure as the resistant strain is not killed at all by a saturated solution of this insecticide in Risella oil. Fig. 5 shows a log-probit regression line based on some of the figures in Table XVIII, assuming a population composed of 25% susceptibles, 50% hybrids and 25% resistants.

FIG. 5. *A. GAMBIAE* : DISCRIMINATING DOSAGES OF DIELDRIN

Log-Probit Regression Line of a Mixed Population composed of 25% Susceptibles, 50% Hybrids and 25% Resistants, using the Mortalities recorded for the Lagos Strain and the Hybrid * Strain in Table XVIII



* Lagos ♂ × Ambursa ♀

Thus the suggested method for the detection of true monofactorial resistance is to establish first the LD_{100} of the insecticides in a susceptible population of the same species (preferably in an area close to that where resistance is suspected, but where insecticides have never been used). Mosquitos from the area where resistance is suspected should then be exposed

to these LD_{100}'s. Any survivors, even a single one, from these dosages should be immediately suspected as resistant individuals and attempts should be made to rear from them by getting the females to lay their eggs and rearing the larvae. This is comparatively easy to do if the original exposed mosquitos are females in a semi-gravid or gravid state. · The offspring should be exposed to the same dosage which their parents survived. If resistance is present and is of the type described for *A. gambiae* these offspring should show at least a 50% survival. With *A. gambiae* the hybrid shows some degree of resistance and 50% mortality would result if the offspring were from hybrid females previously mated with susceptible males. If the parent survivors were hybrid previously mated with hybrid the offspring would show a 25% kill only, and if they were purely resistant the offspring would show no kill at all.

If the resistance were of the type described for *A. sundaicus* the only survivors would be homozygous resistants (as the heterozygote is virtually as susceptible as the susceptible). However, if the resistant survivor had previously mated with a homozygous susceptible, the offspring being heterozygotes would be apparently susceptible, and only by interbreeding these offspring and producing an F_2 generation could homozygote resistants be detected (as shown by a 25% survival at the discriminating dosage). If the survivors had mated with heterozygotes the resulting mortality among the offspring would be 50%, and if with homozygote resistants, nil.

It has already been stated that the field population from which the Ambursa resistant strain of *A. gambiae* was derived was a mixed population comprising only some 17% homozygous resistants. Yet when eggs from this mixed population were dispatched to London, what was reared was a pure homozygous resistant strain and no selection by insecticides has ever been carried out on it. It is concluded that the long journey by road and air, occupying more than twice the normal period between oviposition and eclosion, resulted in a selection of the resistants in favour of the hybrids and susceptibles. In fact, only about 200 eggs hatched out of several thousand sent. This would suggest that the resistant strain is a hardy one.

Other evidence of increased hardiness in the resistant strains of anophelines also exists. In both *A. gambiae* and *A. sundaicus* the resistant strains have proved easier to rear and colonize in the laboratory than the susceptible strains. Indications of a longer life in the resistant strain were also found in *A. sundaicus* in Java. Measurement of the ampulla of the oviduct [7] of wild-caught females showed that 90% of 118 had oviposited at least once, and the natural daily mortality was assessed at 5%. Dissections of the susceptible strain showed evidence of only 63% of 99 females having oviposited, indicating a natural daily mortality of 20%. The superior longevity of the resistant strain was reflected in higher infection rates in mosquito and man in the area where it occurred.[a]

[a] G. Davidson, unpublished data, 1955

Evidence of " hybrid vigour " in both species is also apparent but not yet accurately assessed. As already described, most of the *A. gambiae* hybrid males are sterile, and some association between this sterility and resistance is suspected.

The significance of this is that if selection is stopped in a population of *A. gambiae* consisting of mainly resistants and hybrids, the population may well go on to pure resistance because:

(*a*) hybrid interbreeding which produces pure susceptibles may be reduced, and

(*b*) the resistant individuals may dominate in competition with the susceptibles.

In a population of *A. sundaicus* where no hybrid sterility is apparent continuation to resistance may still occur if the resistant strain is more hardy than the susceptible.

As regards the origin of resistance, it is presumed that this is by mutation in one chromosome, the carrier being heterozygous. The emergence of homozygotes is dependent on hybrid mating, the chances of this occurring being very remote in the absence of any selection of the population. In *A. gambiae* the chances would be further lessened if hybrid sterility is involved. These, then, could be explanations for the rarity of resistant individuals in unselected populations. The effect of selection by insecticides would be to increase the chances of hybrid mating, this increase being greater with *A. gambiae* where the heterozygote is itself resistant and almost certainly can survive field dosages of insecticides. With *A. sundaicus* the chances of hybrid mating will only be slightly increased as the heterozygote is almost as susceptible as the homozygote susceptible and most will be killed by field dosages of the insecticide. This might explain why DDT-resistance has always been much slower to appear in the field than was dieldrin-BHC-resistance in *A. gambiae* in Northern Nigeria.

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RÉSUMÉ

Les mécanismes de la résistance aux insecticides chez *A. gambiae*, *A. sundaicus* et *A. stephensi* sont étudiés et discutés dans cet article, d'après l'observation de ces espèces dans la nature et les expériences faites en laboratoire sur des souches d'élevage. Les données réunies ont conduit l'auteur aux conclusions suivantes:

Il existe deux types de résistance génétique monofactorielle: *a*) une résistance au DDT, avec résistance croisée aux substances chimiques analogues, mais non aux cyclodiènes et au HCH — caractère qui semble récessif par rapport à la sensibilité normale et s'observe chez *A. sundaicus*; *b*) une résistance à la dieldrine, avec résistance croisée aux autres cyclodiènes et au HCH, mais non au DDT — caractère partiellement dominant qui s'observe chez *A. gambiae*. Outre ces deux types de résistance, on constate une augmentation de la tolérance — attribuée à la vitalité de l'insecte — vis-à-vis des deux groupes principaux d'insecticides précités ainsi qu'à d'autres groupes encore. Cette tolérance peut être obtenue par une sélection consécutive à l'exposition prolongée aux insecticides de souches d'insectes apparemment homogènes. Elle disparaît en général lorsque l'on arrête le processus de sélection.

Les souches de *A. sundaicus* et *A. stephensi* résistantes au DDT ont conservé une sensibilité suffisante à la dieldrine pour que cet insecticide puisse être employé avec succès dans les campagnes antipaludiques. Il reste à voir s'il en sera de même dans le cas de *A. gambiae* et du DDT. Des expériences récentes semblent indiquer, dans certaine région du Congo Belge, une augmentation de la tolérance d'*A. gambiae* aux deux groupes d'insecticides. Mais cette tolérance ne s'est pas maintenue dans les souches élevées en laboratoire, issues de femelles résistantes dans la nature.

Abordant le problème de la résistance chez les mouches, l'auteur indique que les deux types de résistance génétique s'observent chez ces insectes, ce qui fait supposer que les deux gènes coexistent dans les populations de mouches. On se demande toutefois s'il ne s'agirait pas, en partie, d'une tolérance accrue, en rapport avec la vitalité de l'insecte.

L'auteur propose une nouvelle méthode permettant de déceler dans la nature la résistance vraie, de type monofactoriel, au sein d'une population, par croisements et étude de la descendance. Chez *A. gambiae*, la plupart des mâles hybrides provenant de croisements résistant × sensible sont stériles, et l'on devine une relation entre stérilité et résistance chez cet anophèle. Cette relation ne paraît pas exister chez *A. sundaicus*. L'auteur discute l'origine de la résistance et fournit une explication possible de la rareté des individus résistants dans les populations non sélectionnées de *A. gambiae*.

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