CROSS-RESISTANCE CHARACTERISTICS OF A MALATHION-TOLERANT STRAIN DEVELOPED IN *AEDES AEGYPTI*

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The control of *Aedes aegypti* by chlorinated hydrocarbons has encountered DDT-resistance in several parts of the Caribbean area, and dieldrin-resistance has also appeared in Puerto Rico. Fortunately the typical DDT-resistant strain from Trinidad proved to be only slightly less susceptible than normal to malathion (Fay, 1956; Abedi & Brown, 1960). But since malathion-resistance has developed in larvae of *Culex tarsalis* in California (Gjullin & Isaak, 1957), it became important to ascertain whether *Aedes aegypti* larvae would develop this resistance under selection pressure from malathion in the laboratory.

A strain originating from Penang, Malaya, and kindly supplied to us by the Army Chemical Center, Maryland, was submitted to malathion pressure for 8 generations in the laboratory. This strain was chosen because it had already shown itself capable of developing DDT-resistance under pressure, and thus probably had a greater genetic variability than most laboratory strains of *Aedes aegypti*. Selection pressures in excess of 90 percent mortality were applied with malathion to maturing larvae of the parental, F_2, F_4, F_6 and F_7 generations. The resulting larval susceptibility levels were assessed by the standard W.H.O. method (Brown, 1958).

The results are shown in Table 1. The tolerance levels to malathion increased steadily, to become 4 to 5 times the normal in the F_8 generation. Since a parallel non-selected strain showed during this period a slight drop in the LC_{50} from 0.30 down to 0.23 p.p.m. malathion, the difference between the selected and control strains was actually 6-fold.[1]

TABLE 1.—Larval LC_{50} levels in p.p.m. of the Penang strain of *Aedes aegypti* submitted to malathion pressure in the P_1, F_2, F_4, F_6 and F_7 generations

Generation	Malathion	Sevin	DDT	DDT*
P	0.30	1.93	0.08	0.08
F_1	0.62	—	—	0.10
F_2	0.62	2.05	1.08	0.82
F_3	0.68	3.7	—	—
F_4	0.82	—	—	—
F_6	1.05	4.4	—	—
F_7	1.28	—	1.8	—
F_8	1.40	9.1	2.5	—

* Repeat selection with malathion in the P & F_1 generations.

It was of interest to ascertain whether this malathion pressure induced a parallel cross-resistance to the carbamate Sevin and to DDT itself. It is seen in Table 1 that the cross-tolerance to Sevin increased slowly at first but later increased to 5 times the normal. The cross-resistance to DDT increased to over 30 times the normal, showing an abrupt rise between the F_1 and F_2 generations.

The cross-tolerance characteristics of the malathion-selected F_8 generation were then assessed with respect to other OP compounds, other carbamates, certain analogues of DDT, and the BHC-cyclodiene group of insecticides (Table 2). It was found that the tolerance of parathion and of diazinon had been scarcely increased by the malathion pressure. The carbamate insecticides Pyrolan and Dimetilan were insufficiently larvicidal; but it could be ascertained that the tolerance of isopropyl-phenyl-methyl-carbamate, a typical member of the group, increased much less than that of Sevin. A high cross-resistance had developed to DDD (TDE), indeed slightly more than to DDT, whereas with respect to methoxychlor there was only a 3-fold increase in tolerance.

This malathion-tolerant DDT-resistant strain showed little or no change from the normal in its susceptibility to dieldrin or heptachlor. However, the malathion selection did succeed in raising the BHC-tolerance of the Penang strain, which was initially rather high, by 2–3 times. It may well be asked what kind of malathion-resistance has been produced in this strain of *Aedes aegypti*. True malathion-resistance, such as was discovered in the *Culex tarsalis* of Fresno County, California, shows an increase in larval LC_{50} of about 30 times (Gjullin & Isaak, 1957). However, an increase in larval LC_{50} of 2–3 times proved sufficient to result in an observable resistance of *Aedes nigromaculis* to control with parathion in Kings and Tulare Counties (Lewallen & Brawley, 1958; Lewallen & Nicholson, 1959). On the other hand, a cross-resistance of 4 times the normal to EPN and 10 times the normal to malathion in the salt-marsh *Aedes* mosquitoes of Cocoa Beach, Florida, has passed without special comment (Keller & Chapman, 1953).

The question whether the results of malathion pressure here obtained represent increased vigor tolerance or true resistance can be illuminated not only by the cross-resistance data but also by the dosage-mortality regression lines of the successive generations. The criterion proposed by Hoskins & Gordon (1956) is that strains

[1] A subsequent selection in the F_8 did not change the LC_{50}, which remained stable at 1.40 in the unselected F_{10} and F_{11}.

TABLE 2.—Larval LC_{50} levels in p.p.m. of the normal Penang strain of *Aedes aegypti* and a malathion-resistant substrain in the F_8 generation

	Normal	Resistant		Normal	Resistant
Malathion	0.26	1.43	DDT	0.08	2.56
Parathion	0.035	0.055	Methoxychlor	0.14	0.40
Diazinon	0.12	0.17	DDD	0.32	14.0
Sevin	1.9	9.1	Dieldrin	0.028	0.034
Pyrolan	No kill up to 50		Heptachlor	0.023	0.023
Dimetilan	No kill up to 128		gamma-BHC	0.23	0.56*
3-Isopropylphenyl-N-methyl-carbamate				0.19	0.29

* F_7 Generation tested. † Kindly supplied by H. H. Moorefield, Boyce Thompson Institute.

on the way to true resistance show an initial flattening of the d-m line, whereas with vigor tolerance these lines stay parallel. It will be seen (Fig. 1) that the regression lines with malathion remain

With the cross-resistances to isopropyl-phenyl-methyl-carbamate, dieldrin, gamma-BHC and methoxychlor (Fig. 3), the d-m lines remain parallel or flatten slightly. With heptachlor there is no change in the

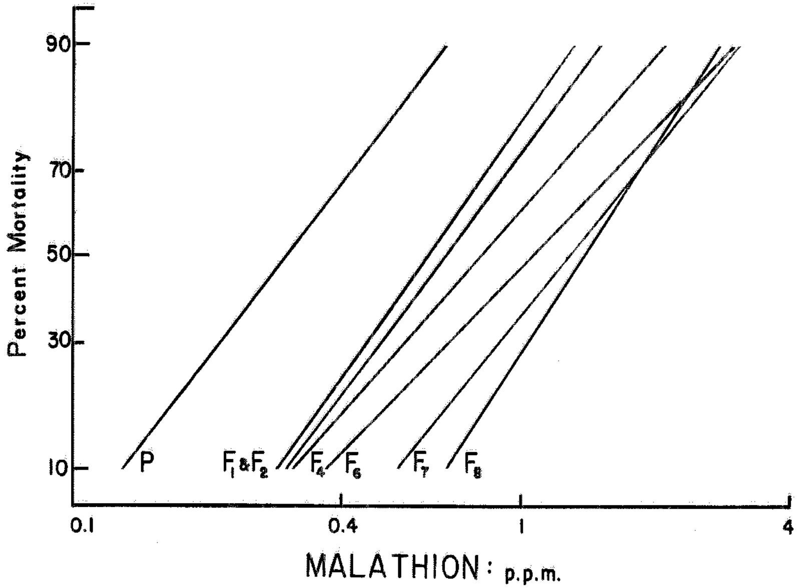

FIG. 1.—Dosage-mortality lines for malathion shown by successive generations.

roughly parallel in their movement towards resistance to the right, although there is a slight flattening between the F_4 and F_7 generations. The d-m lines for the cross-resistance to Sevin however stay almost completely parallel (Fig. 2), although there is a slight progressive decrease in slope.

line at all. With parathion the regression line flattened very slightly; but with diazinon it steepened, pivoting about the highest mortality, as if only the susceptible genotypes had been eliminated without any more tolerant genotypes appearing.

Most dramatic changes in the d-m lines appear with the development of cross-

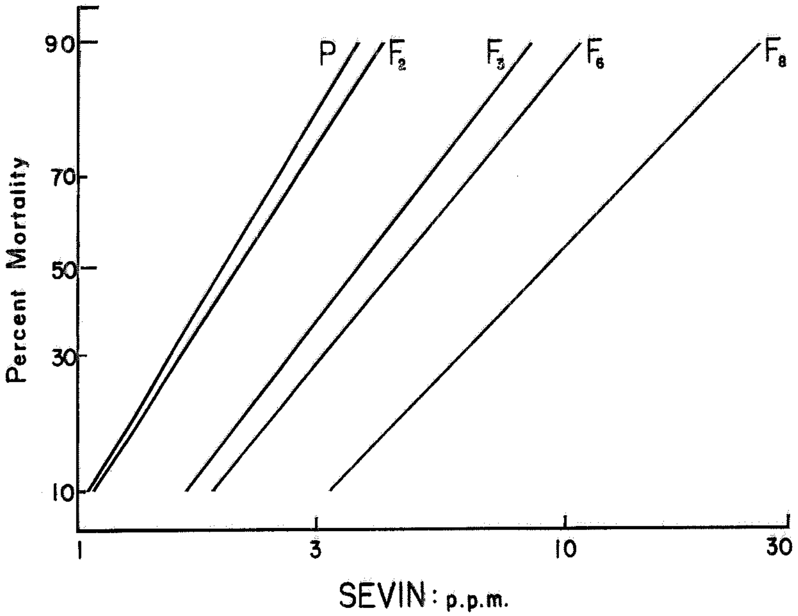

FIG. 2.—Dosage-mortality lines for Sevin shown by successive generations.

resistance to DDT (Fig. 4). These start with the abrupt steepening of the lower section in the F_1 generation. This is followed in the F_2 by a marked flattening of the upper part of the line, indicating the appearance of much more resistant types; another test on the same F_2 showed that all larvae in the sample had moved over to the resistant type with tolerances approaching 1 p.p.m. Subsequent pressure then moves the resistances beyond 1 p.p.m. by a marked flattening of the line again. A similar very flat line for the F_8 generation, indicating in effect that 50 percent or more of the sample is completely resistant, is also encountered with DDD.

Adults of the F_8 generation were tested for their resistance to DDT by the standard WHO test method. The mortality at the highest concentration (4 percent DDT) was only 16 percent. The normal strain before selection by malathion pressure had shown an LC_{50} of 1.5 percent DDT, and 16 percent mortality at 0.7 percent DDT. It may therefore be concluded that the DDT-resistance level of the adults of this malathion-selected strain had increased to approximately 6 times the normal. On the other hand, the adult LC_{50} to malathion had only increased from 1.8 to 2.2 percent.

DISCUSSION. This malathion-resistance induced in larvae of the Penang strain of *Aedes aegypti* is specific in that it does not extend to parathion and diazinon. Therein it resembles malathion-resistance in house flies, which Busvine (1959) and others have found to be separate from parathion- and diazinon-resistance. Malathion-resistance in *Culex tarsalis* also did not extend to parathion or other OP compounds (Gjullin & Isaak, 1957), and parathion-resistance in *Aedes nigromaculis* did not extend to malathion (Lewallen & Nicholson, 1959). The malathion-resistance in our *A. aegypti* is not specific in that it extends to Sevin; but therein it resembles the situation in house flies, in

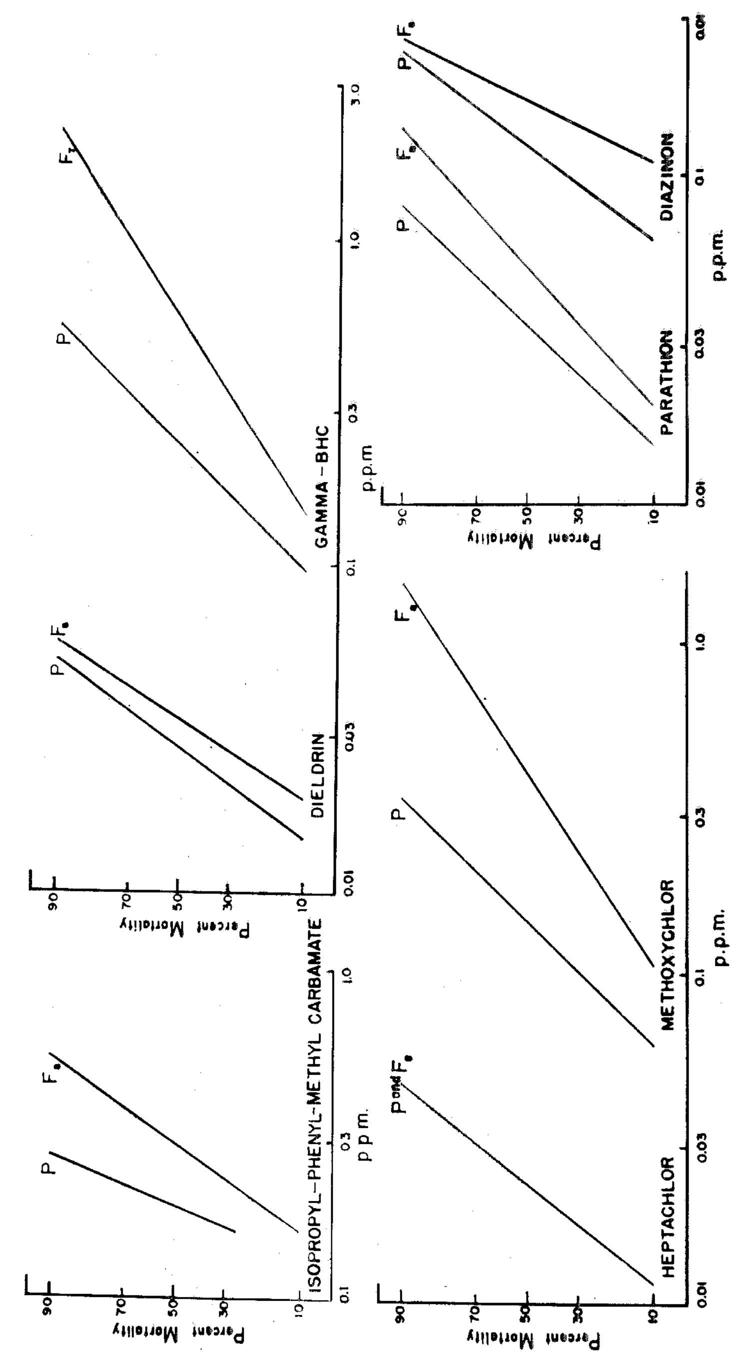

FIG. 3.—Dosage-mortality lines for gamma-BHC, dieldrin, heptachlor, parathion, diazinon and a carbamate shown by successive generations.

which parathion-resistant (Eldefrawi, Miskus & Hoskins, 1959), malathion-resistant (LaBrecque, Wilson & Smith, 1959) and diazinon-resistant (Forgash & Hansens, 1959) strains have been found to show cross-tolerance to Sevin.

The malathion-tolerance in *A. aegypti* also resembles that in house flies by involving a tremendous cross-resistance to DDT. This curiosity has been observed in many laboratory strains of house flies never exposed to DDT (Brown, 1958), which were also found to have developed the detoxifying enzyme DDT-dehydrochlorinase. In the Penang strain the ability to produce DDE was already well-developed before selection pressure was applied, and in the development of DDT-resistance by malathion pressure on this strain there was only a 40 percent increase in DDE production, about the same as developed under DDT pressure (Chattoraj & Brown, 1960). That this cross-resistance to DDT and DDD did not extend to methoxychlor is an argument against concluding that DDT-dehydrochlorinase is a mechanism for it. The malathion-resistance discovered in the Fresno strain of *Culex tarsalis*, on the other hand, was associated with no change from the normal in susceptibility to DDT (Gjullin & Isaak, 1957).

SUMMARY. Larvae of a strain of *Aedes aegypti* from Malaya, selected with mala-

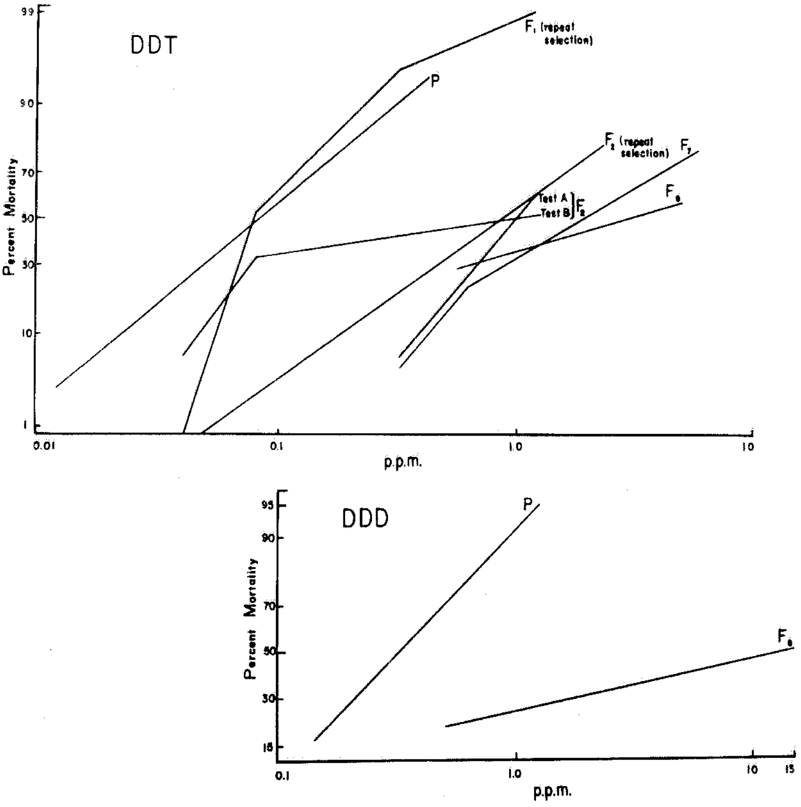

FIG. 4.—Dosage-mortality lines for DDT and DDD shown by successive generations.

thion for 8 generations, increased in malathion-tolerance by 5 times. They increased only slightly in their tolerance of parathion or diazinon. Their cross-resistance also increased by 5 times to Sevin but not to another carbamate insecticide. The tolerance of dieldrin, heptachlor and gamma-BHC increased slightly or not at all. They developed a cross-resistance in excess of 30 times the normal to DDT and DDD, but not to methoxychlor. Although the selection pressure was confined to the larvae, the adults also developed a 6-fold resistance to DDT.

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