Bull. Org. mond. Santé }
Bull. Wld Hlth Org. } 1961, 24, 519-526

Genetical Studies on Dieldrin-Resistance in *Aëdes aegypti* and its Cross-Resistance to DDT

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A strain of Aëdes aegypti *was recently found in Puerto Rico which proved to be resistant to both DDT and dieldrin. This paper reports on genetical studies of this strain to determine whether a single entity is involved or two distinct resistances. Tests carried out by repeated back-crossing combined with selection pressure from DDT and dieldrin and by crossing with a strain with marker genes indicate that a single entity is responsible (dieldrin-resistance with cross-resistance to DDT) and that its genetic factor is located on chromosome 2 at a distance of 25 from locus "yellow".*

DDT-resistant populations of *Aëdes aegypti* are now of frequent occurrence in the Caribbean region (Brown, 1960), but they are characteristically still susceptible to γ-BHC or dieldrin. However, Fox [3] has recently discovered a DDT-resistant population at Isla Verde airport, near San Juan, Puerto Rico, that is also highly resistant to dieldrin.

The purpose of this investigation was to ascertain whether the dieldrin-resistance and the DDT-resistance were the same entity or were two separate resistances. Therefore the Isla Verde strain was repeatedly back-crossed with a susceptible strain while under selection pressure from either dieldrin or DDT, according to the method of Wright (1952). This method had proved effective in the hands of Busvine (1953), who separated BHC-resistance from DDT-resistance in the housefly by a combination of back-crossing and BHC pressure for four generations. An opportunity to associate the resistance with a particular chromosome in the genome of *Aëdes aegypti* was offered by the use of a susceptible strain carrying marker genes on chromosomes 2 and 3, with which the Isla Verde strain was crossed, and the hybrid was back-crossed with the marker strain.

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[3] Communication to the World Health Organization, 1959, by I. Fox, reported in *WHO Information Circular on Insecticide Resistance*, No. 18.

MATERIAL AND METHODS

The Isla Verde strain was obtained from the University of Notre Dame, Indiana, USA, shortly after it had been subcultured there from San Juan, where it had first been colonized in the laboratory for six months. Its DDT-resistance is about 200 times, and its dieldrin-resistance about 300 times, the level of the susceptible Guelph strain (Table 1). It is also resistant to γ-BHC, but not to organophosphorus compounds or the carbamate Sevin.

The Guelph strain is a highly susceptible and homogeneous subcolony obtained from a single

TABLE 1
LARVAL LC_{50} LEVELS (p.p.m.) OF THE ISLA VERDE STRAIN AND THREE SUSCEPTIBLE STRAINS

Insecticide	Isla Verde	*blt, y*	Guelph	Penang
DDT	1.05	0.028	0.005	0.08
Methoxychlor	0.21	—	—	0.14
Dieldrin	2.3	0.019	0.007	0.028
γ-BHC	1.9	—	0.053	0.23
Malathion	0.38	—	0.21	0.26
Parathion	0.035	—	—	0.035
Diazinon	0.07	—	—	0.12
Sevin	2.5	—	—	1.9

TABLE 2
SUSCEPTIBILITY LEVELS OF PARENTS, HYBRIDS, AND OFFSPRING FROM BACK-CROSSES AFTER SELECTION OF HYBRIDS WITH DDT OR DIELDRIN

Generation		DDT		Dieldrin	
		LC_{50}	Slope	LC_{50}	Slope
P	Isla Verde	1.05	1.5	2.3	1.3
	Guelph	0.005	3.3	0.007	3.1
F_1	♂ Isla Verde × ♀ Guelph	0.17	1.6	0.28	2.2
	♀ Isla Verde × ♂ Guelph	0.17	2.2	0.28	2.3
1st back-cross	After DDT pressure				
	♂ (♂ I.V. × ♀ G.) × ♀ Guelph	0.056	1.8	0.062	2.3
	♀ (♂ I.V. × ♀ G.) × ♂ Guelph	0.057	2.6	0.075	2.2
	♂ (♀ I.V. × ♂ G.) × ♀ Guelph	0.051	2.1	0.074	1.8
	♀ (♀ I.V. × ♂ G.) × ♂ Guelph	0.057	2.6	0.079	1.8
	After dieldrin pressure				
	♂ (♂ I.V. × ♀ G.) × ♀ Guelph	0.048	2.1	0.155	2.0
	♀ (♂ I.V. × ♀ G.) × ♂ Guelph	0.050	4.0	0.165	2.0
	♂ (♀ I.V. × ♂ G.) × ♀ Guelph	0.046	2.4	0.170	1.8
	♀ (♀ I.V. × ♂ G.) × ♂ Guelph	0.068	2.4	0.168	2.0
2nd back-cross	After DDT pressure				
	♂ (♂ hybrid × ♀ G.) × ♀ Guelph	0.050	2.5	0.100	2.2
	♀ (♂ hybrid × ♀ G.) × ♂ Guelph	0.056	2.1	0.100	2.1
	After dieldrin pressure				
	♂ (♂ hybrid × ♀ G.) × ♀ Guelph	0.040	3.0	0.165	2.7
	♀ (♂ hybrid × ♀ G.) × ♂ Guelph	0.042	2.6	0.175	2.4
3rd back-cross	After DDT pressure				
	♂ (♂ hybrid × ♀ G.) × ♀ Guelph	0.067	2.4	0.105	2.0
	After dieldrin pressure				
	♂ (♂ hybrid × ♀ G.) × ♀ Guelph	0.052	1.7	0.160	3.3
4th back-cross	After DDT pressure				
	♂ (♂ hybrid × ♀ G.) × ♀ Guelph	0.067	2.8	0.105	2.6
	After dieldrin pressure				
	♂ (♂ hybrid × ♀ G.) × ♀ Guelph	0.055	2.7	0.148	1.8

female taken from the Orlando strain at the Entomology Laboratory, Canada Department of Agriculture, Guelph, Ontario.

The *blt,y* strain is a susceptible subcolony derived from the Trinidad resistant strain at the University of Notre Dame, Indiana, but containing the mutant *black-tarsus* associated with chromosome 3 and the mutant *yellow* (larvae) associated with chromosome 2 (Craig & Gilham, 1959).

Larvae were reared on a diet of 10 parts brewer's yeast powder, 1 part blood albumin, and 0.1 part streptomycin. Individual adults were obtained by isolating the pupae in vials over a plug of moist cotton wool. Mating was then effected by placing 100-150 adults of each sex and type in a cage of a capacity of 3.5 cubic feet (about 110 litres). Reciprocal crosses were made both in the P and the F_1 generations, resulting in four different first back-cross groups.

Susceptibility levels of larvae and adults to DDT or dieldrin were determined by the respective standard WHO test methods. The LC_{50} values were derived from dosage-mortality regression lines fitted by eye; the slope of the regression lines was expressed as the change in probits per tenfold change in dosage (Hoskins & Gordon, 1956). Selection pressure was applied by exposing 25 larvae in 250 ml of water for 24 hours to a dose of insecticide that gave 80%-90% mortality.

RESULTS

The F_1 offspring of the reciprocal crosses between the Isla Verde and the Guelph strains (Table 2) were identical with each other with regard to their DDT- and dieldrin-resistance, which was intermediate between that of their parents.

Either group of hybrid offspring, after being exposed to selection pressure from DDT or from dieldrin, was back-crossed with the Guelph strain, the reciprocal crossing giving four first back-cross groups under DDT pressure and four first back-cross groups under dieldrin pressure. Since there were no significant differences between each of the four groups in their susceptibility to DDT or dieldrin, only two of the groups in each case were chosen to produce the second back-cross generation. These reciprocal conditions proving identical, only one of the group in each case was chosen to produce the third back-cross, i.e., the one in which there was a succession of back-crossing hybrid males with Guelph females.

The results may be expressed as the resistance ratios to DDT and to dieldrin in each generation (Table 3); the ratio between dieldrin-resistance and DDT-resistance is also calculated. It will be seen that the DDT-resistance was not maintained under DDT pressure at a materially higher level than under dieldrin pressure. The dieldrin-resistance stabilized at a higher level under dieldrin pressure than under DDT pressure, but the ratio of dieldrin-resistance to DDT-resistance did not increase during the four generations of selection. This evidence indicates a single inseparable resistance to DDT and dieldrin rather than two separate types of resistance.

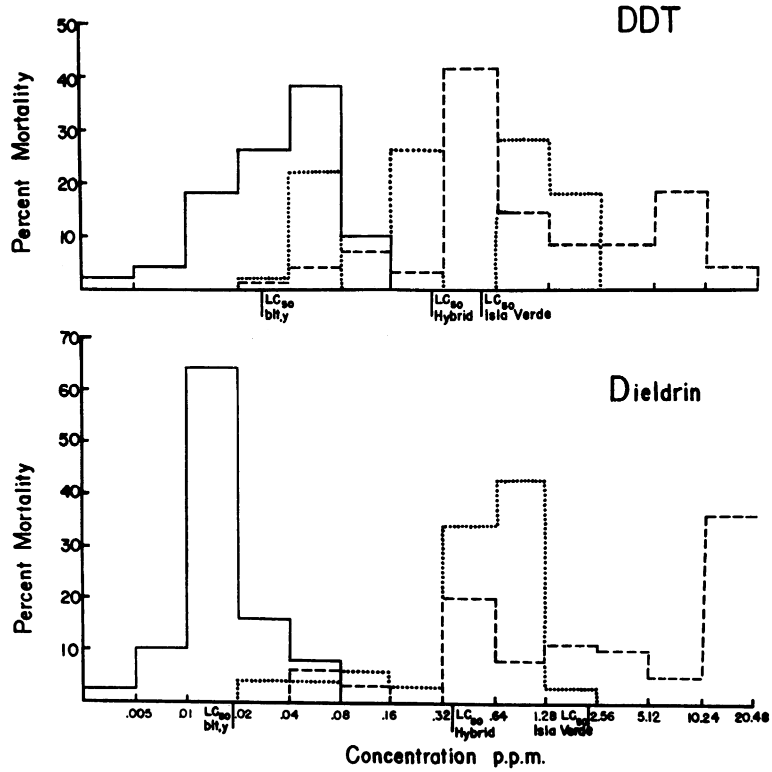
Crossing experiments were then conducted between the Isla Verde strain and the *blt,y* marker strain to determine which marker gene and chromosome was associated with the resistance. The F_1 hybrid generation Isla Verde × *blt,y* was obtained and the yellow larvae (54%) and black-tarsus adults (3%) were culled from it; the remaining F_1 material thus were heterozygotes in which the Isla Verde chromosomes contributed exclusively the wild-type alleles (++, or banded-tarsus, gray) of these marker genes. The LC_{50} of the hybrids for DDT was

TABLE 3

RESISTANCE RATIOS OF EACH GENERATION COMPARED TO THE GUELPH SUSCEPTIBLE STRAIN, AND RATIOS BETWEEN DIELDRIN-RESISTANCE AND DDT-RESISTANCE

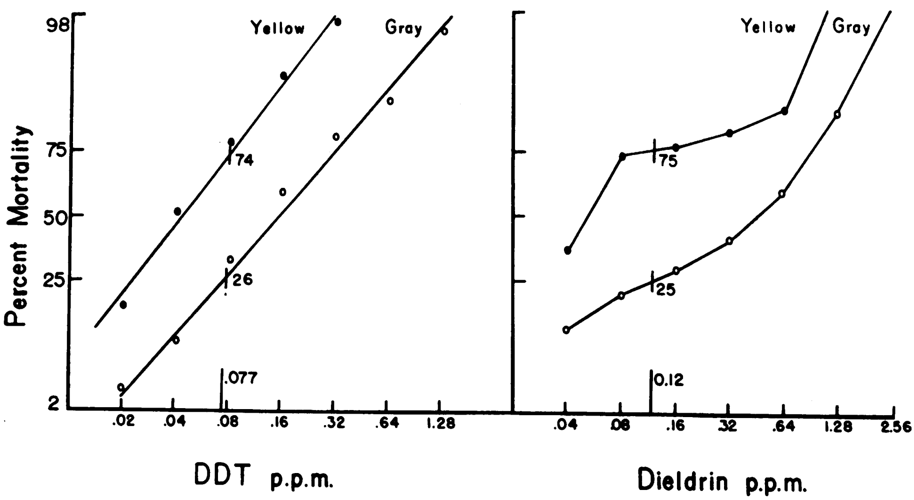
Generation	DDT		Dieldrin		Ratio
	LC_{50}	Resistance ratio	LC_{50}	Resistance ratio	$\left(\frac{LC_{50}\ \text{dieldrin}}{LC_{50}\ \text{DDT}}\right)$
Guelph P	0.005	1	0.007	1	1.42
Isla Verde P	1.05	210	2.30	328	2.18
F_1	0.17	34	0.29	40	1.65
After DDT pressure					
1st back-cross	0.056	11	0.062	9	1.17
2nd back-cross	0.050	10	0.100	14	2.00
3rd back-cross	0.067	13	0.105	15	1.56
4th back-cross	0.067	13	0.105	15	1.56
After dieldrin pressure					
1st back-cross	0.048	10	0.155	22	3.22
2nd back-cross	0.040	8	0.165	24	4.12
3rd back-cross	0.052	10	0.160	23	3.08
4th back-cross	0.055	11	0.148	21	2.69

FIG. 1. FREQUENCY DISTRIBUTION OF SUSCEPTIBILITY TO DDT AND DIELDRIN OF ISLA VERDE STRAIN, *blt,y* STRAIN AND THEIR HYBRID



----- = Isla Verde strain. ——— = *blt, y* strain. . . . . . = Hybrid.

FIG. 2. DOSAGE-MORTALITY RELATIONSHIPS TO DDT AND DIELDRIN FOR YELLOW AND GRAY LARVAL PHENOTYPES OF ALL FOUR GROUPS OF THE BACK-CROSS (ISLA VERDE × *blt,y*) × *blt,y*

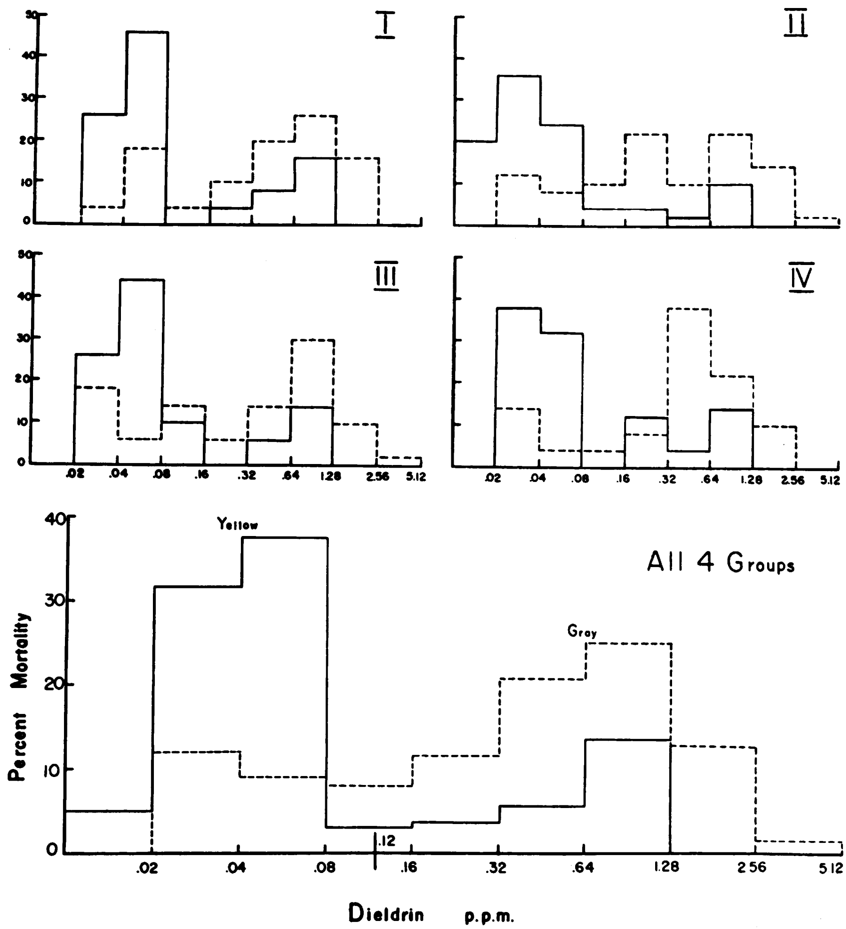


0.27 p.p.m., as compared with 0.028 and 1.05 p.p.m. for the two parents; and for dieldrin it was 0.37 p.p.m., as compared with 0.019 and 2.3 p.p.m. (Fig. 1). The frequency distributions of susceptibility levels indicate that the Isla Verde was not a pure strain for resistance, but it was used as it stood because it was uncertain at the time whether DDT or dieldrin selection pressure should be applied to purify it. These hybrids were then back-crossed with the *blt,y* homozygotes. Since reciprocal crosses were performed in both the P and F_1 generations, four first back-cross groups were obtained.

The first back-cross larvae appeared as an equal mixture of yellow (*y*/*y*) and gray (+/*y*) types. From each of the four groups, 800 yellow and 800 gray larvae were taken, and half of each category were tested with dieldrin and half with DDT. The dosage-mortality regression lines for dieldrin (Fig. 2) show the greater dieldrin-resistance to be associated with the gray larvae, but there is a marked inflection in the lines for both yellow and gray larvae in the range of dosage between 0.8 and 1.6 p.p.m. When these results are plotted as a frequency distribution of susceptibility (Fig. 3), it becomes evident that there is a strong segregation in this region, some quarter of the gray and three-quarters of the yellow larvae being susceptible to doses below this level. The cumulative regression line (Fig. 2) for all four groups together

FIG. 3

FREQUENCY DISTRIBUTION OF SUSCEPTIBILITY TO DIELDRIN OF LARVAE FROM THE FOUR GROUPS OF THE BACK-CROSS (ISLA VERDE × *blt,y*) × *blt,y*



shows that the diagnostic dosage of 1.2 p.p.m. segregates 25% of the gray larvae and 75% of the yellow larvae as being susceptible to it. This diagnostic level is also valid in separating the *blt,y* strain from the F_1 hybrid (Fig. 1), provided it is remembered that the F_1 may contain a few susceptible homozygotes due to probable impurities of the Isla Verde strain. The results therefore indicate that dieldrin-resistance is linked with locus *y* on chromosome 2, with 25% crossing over.

The DDT-resistance tended to stay associated with the dieldrin-resistance and with the gray larvae (Table 4), in that the ratio between the LC_{50} values for dieldrin and DDT remained at the approximate values of 1.8 whether the larvae were gray and mainly resistant or yellow and mainly susceptible. The dosage-mortality regression lines for DDT (Fig. 2) show the greater DDT-resistance to be associated with the gray larvae. With this insecticide the regressions are linear and the frequency distributions (Fig. 4) are normal and show no segregation. Indeed, the frequency distributions of the *blt,y* strain and the hybrid show a considerable overlap (Fig. 1). However, it is possible to derive a diagnostic dosage from the two regression lines (Fig. 2); this is 0.077 p.p.m., to which 24% of the gray larvae and 76% of the yellow larvae are susceptible. The diagnostic dosage for DDT as well as for dieldrin categorizes each of the four individual groups satisfactorily (Table 5). The results confirm the

FIG. 4

FREQUENCY DISTRIBUTION OF SUSCEPTIBILITY TO DDT OF LARVAE FROM THE FOUR GROUPS OF THE BACK-CROSS (ISLA VERDE × *blt,y*) × *blt,y*

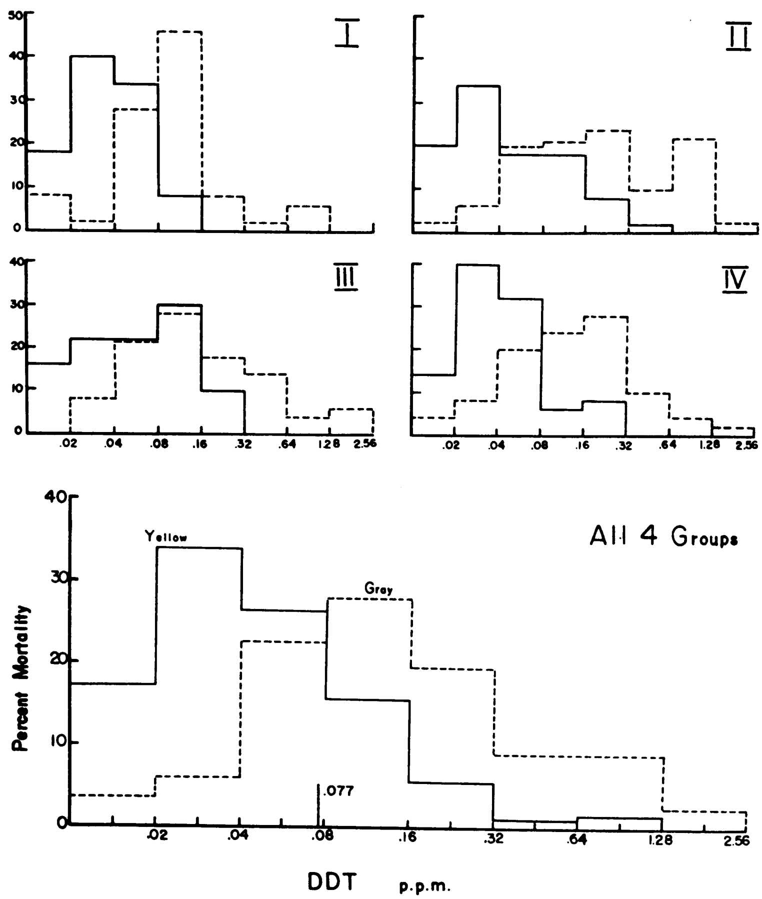


TABLE 4

LC$_{50}$ LEVELS (p.p.m.) FOR YELLOW AND GRAY LARVAE TO DDT AND DIELDRIN, AND RATIOS BETWEEN DIELDRIN-RESISTANCE AND DDT-RESISTANCE

	Group [a]				Average
	I	II	III	IV	
Yellow larvae:					
DDT	0.0365	0.0465	0.052	0.049	0.046
Dieldrin	0.086	0.084	0.088	0.086	0.086
Ratio $\left(\frac{\text{Dieldrin}}{\text{DDT}}\right)$	2.35	1.80	1.70	1.75	1.86
Gray larvae:					
DDT	1.08	1.65	1.75	1.40	1.47
Dieldrin	2.75	2.84	2.35	2.65	2.65
Ratio $\left(\frac{\text{Dieldrin}}{\text{DDT}}\right)$	2.55	1.72	1.34	1.89	1.81

[a] Cross I : ♀ (♂ Isla Verde × ♀ *blt*, y) × ♂ *blt*, y
Cross II: ♂ (♂ Isla Verde × ♀ *blt*, y) × ♀ *blt*, y
Cross III: ♀ (♂ *blt*, y × ♀ Isla Verde) × ♂ *blt*, y
Cross IV: ♂ (♂ *blt*, y × ♀ Isla Verde) × ♀ *blt*, y

TABLE 5

PERCENTAGE COMPOSITION OF THE FIRST BACK-CROSS OFFSPRING WITH RESPECT TO DIAGNOSTIC DOSAGE OF DIELDRIN AND DDT

	Group				All groups
	I	II	III	IV	
Dieldrin					
Resistant to 0.12 p.p.m.	72	83	76	70	75
Susceptible to 0.12 p.p.m.	24	25	31	20	25
DDT					
Resistant to 0.077 p.p.m.	79 [a]	71	70	76	74
Susceptible to 0.077 p.p.m.	36 [a]	23	24	28	26

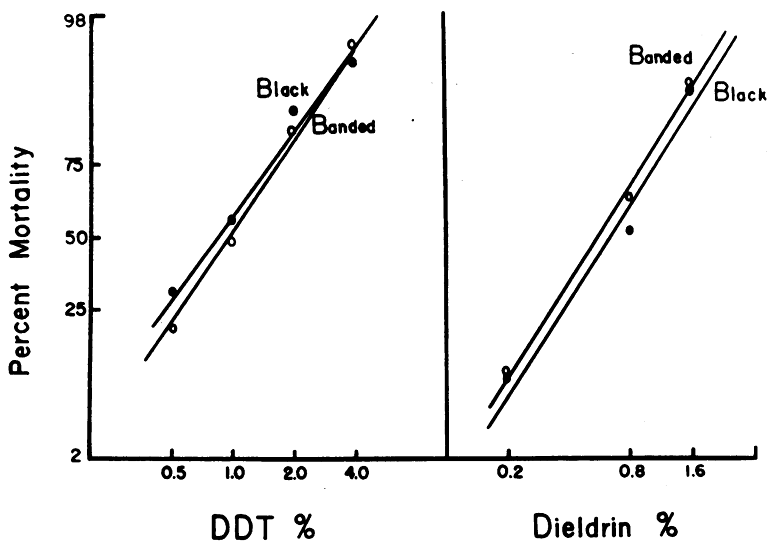
[a] If the diagnostic dosage is taken as 0.055 p.p.m. for this group I, the categorization becomes 75: 25.

opinion that the DDT-resistance is essentially a cross-resistance characteristic of the dieldrin-resistance, and is associated with chromosome 2 at a cross-over distance of 24 or 25 from locus *y*.

If the resistance of dieldrin and DDT is associated with *y* on chromosome 2, it should show independent assortment with *blt* on chromosome 3. After the

FIG. 5

DOSAGE-MORTALITY RELATIONSHIPS TO DDT AND DIELDRIN FOR BLACK-TARSUS AND BANDED-TARSUS ADULT PHENOTYPES FOR THREE GROUPS OF THE BACK-CROSS (ISLA VERDE × *blt,y*) × *blt,y*



larval tests, in three out of the four groups sufficient adults were obtained to allow tests of susceptibility levels. The numbers of females tested were respectively: black-tarsus, 115, 147 and 154; banded-tarsus (wild type), 107, 131 and 178. The dosage-mortality regression lines obtained (Fig. 5) show that the susceptibility levels of the two categories were virtually identical, the LC_{50} figures for DDT being 0.86% and 0.98%, and for dieldrin 0.49% and 0.53% by the adult test. There is evidently no association of the resistance with the normal banded-tarsus type; it shows independent assortment with locus *blt*, and is therefore not located on chromosome 3.

DISCUSSION

These investigations indicate that the dieldrin-resistance in this strain of *Aëdes aegypti* from Puerto Rico is unusual in that it has a cross-resistance to DDT. It thus offers a sharp contrast to the dieldrin-resistance of *Anopheles gambiae* (Davidson, 1956), *A. quadrimaculatus* (Mathis et al., 1956) and *Psorophora confinnis* (Mathis et al., 1955), which did not extend to DDT. It resembles, however, the dieldrin-resistance recently discovered in *Aëdes taeniorhynchus* on Cockspur Island, Georgia, USA, which shows a high cross-resistance to DDT (Schoof, 1959).

The genetic segregation of the dieldrin-resistant heterozygotes from the susceptible homozygotes is so well marked that it is logical to suspect that the resistance is monofactorial in origin. However, this matter will eventually be settled when experiments become possible with a pure resistant strain and marker strains with a representative array of mutant genes.

ACKNOWLEDGMENTS

The authors are deeply indebted to Dr G. B. Craig, jr, Assistant Professor, Department of Biology, University of Notre Dame, Indiana, for kindly supplying us with eggs of the Isla Verde strain and the *blt,y* marker strain; to Mr L. Burgess, Canada Department of Agriculture, for stock of the Guelph strain; and to Dr Austin Kerr, Pan American Health Organization, Washington, D.C., for making the Isla Verde strain available for study. This investigation was financed by a grant from the World Health Organization, Geneva.

RÉSUMÉ

Les populations d'*Aëdes aegypti* résistantes au DDT, qui se rencontrent assez fréquemment dans les Antilles, restent en général sensibles au HCH gamma et à la dieldrine. Une souche cependant a été découverte, qui est résistante non seulement au DDT mais à la dieldrine.

Les auteurs se sont proposé d'étudier du point de vue génétique s'il s'agissait d'une seule ou de deux formes de résistance. Cette souche d'*Aëdes* a été soumise à des croisements avec une souche sensible, sous la pression sélective de la dieldrine ou du HCH. Cette méthode avait permis à d'autres chercheurs de dissocier la résistance au DDT de la résistance au HCH chez la mouche. Grâce à la présence, dans la souche sensible d'*Aëdes* de gènes marqueurs il a été possible de préciser sur quel chromosome était fixé le facteur résistance.

Ayant décrit la méthode employée, les auteurs exposent leurs résultats et concluent que la résistance à la dieldrine, chez la souche à l'étude, est insolite, étant une résistance croisée avec le DDT. Elle ne correspond nullement à celle que l'on observe chez *Anopheles gambiae* ou *A. quadrimaculatus*, qui ne s'étend pas au DDT. Elle ressemble en revanche à la résistance croisée (dieldrine-DDT) constatée chez *Aëdes taeniorhynchus* en Géorgie, USA.

La dissociation génétique des hétérozygotes résistants à la dieldrine à partir des homozygotes sensibles est si nette que l'on est en droit de penser que la résistance est monofactorielle. Toutefois cette hypothèse demande à être vérifiée.

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