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STUDIES ON THE INHERITANCE OF RESISTANCE TO DDT AND TO MALATHION IN THE MOSQUITO, *CULEX TARSALIS* COQ.

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A colony of *Culex tarsalis* Coq. highly resistant to DDT was collected from a log pond at Oak Ridge, Oregon, in 1956 and has been maintained at the Corvallis laboratory. Data on the amount of resistance to DDT present in the colony and on rearing techniques have been reported (Eddy *et al.* 1958).

A malathion-resistant strain of *tarsalis*, collected near Fresno, Calif., in 1957 is also being reared in the laboratory. The colony was about 100 times resistant to malathion at the time these experiments were undertaken and also about 2 times resistant to DDT. Initially it was about 75 to 80 times resistant to malathion and 5 times to DDT. The extent of cross-resistance to other insecticides present in this colony has been reported (Darrow and Plapp 1960).

In the present work the inheritance of insecticide resistance in these colonies was studied. Data are also presented on levels of cholinesterase (ChE) and aliphatic esterase (Ali-E) activity in the same colonies. Measurements of enzyme levels in resistant strains of insects have been of special interest since the finding that low levels of aliesterase are related to organophosphate resistance in the house fly (*Musca domestica* L.) (Asperen and Oppenoorth 1959, Bigley and Plapp 1960).

MATERIALS AND METHODS. Insects.—The DDT-resistant colony from Oak Ridge and the malathion-resistant colony from Fresno were the two resistant colonies used in these experiments. The insecticide-susceptible colony was obtained from the Rocky Mountain Laboratory, Hamilton, Mont., and has been reared for several years in the laboratory without exposure to insecticides. For the sake of brevity, the three colonies will be referred to as DDT-R, Mal-R, and Reg, respectively.

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The three colonies were maintained in the laboratory in separate rearing rooms. The methods employed in caring for them have been previously described (Eddy *et al.* 1958).

At the time of these experiments, levels of insecticide resistance in the DDT-R and Mal-R colonies had been maintained with little change for at least a year. In the Mal-R colony resistance was maintained by exposing fourth-instar larvae to 2 p.p.m. of malathion for 24 hours every third or fourth generation. Resistance in the DDT-R colony was maintained by pressuring fourth-instar larvae with 1.5 p.p.m. of DDT every second generation.

Genetic Technique.—Virgin females from the different colonies were obtained as newly emerged adults. They were collected within 24 hours of emergence. Previous work in the laboratory had shown that no mating took place until the females were at least 36 hours old (Darrow, unpublished data). Young males were obtained from the colonies at the same time the females were collected. Populations of 100 females were placed in approximately 1-cubic-foot cages with an equal number of males from one of the other colonies. All six possible crosses between the colonies were made. When the adults were 4 days old, the females were fed a blood meal by placing a young white mouse in the cage. Four days later a pan of water was placed in the cage. Maximum egg production occurred within 48 hours. Adults were fed sugar water at all times.

Larvae from the different crosses were reared routinely. Pupae of the F_1 generation were placed in cages and the emerging adults were allowed to mate to produce the F_2 generation. Several backcrosses were made in which females from the F_1 generation were mated with males from the parent colony which possessed the opposite character from the F_1 in regard to insecticide resistance.

Measurement of Resistance.—The susceptibility to DDT and to malathion of the parent colonies and of progeny from the various crosses was determined in tests with fourth-instar larvae. As far as possible 7-day-old larvae were used so that the tests could be completed before pupation took place, usually about 10 days after hatching. The two insecticides were tested with larvae at 6 concentrations, 0.01, 0.03, 0.1, 0.3, 1, and 3 p.p.m. These concentrations were sufficient to cover the complete range of susceptibility from the LC-50 with the most susceptible strain to nearly an LC-50 with the most highly resistant strain. The tests were conducted in 1-pint glass jars containing 250 ml. of water to which 20 larvae were added. The insecticides were then pipetted into the jars in either 0.3 or 1 ml. of acetone. Larvae were held at a temperature of about 25° C. Mortality determinations were made 24 and 48 hours later.

Determinations of Esterase Activity.—Measurements of ChE activity and of Ali-E activity were made by the colorimetric method of Hestrin (1949). Acetylcholine bromide at 2.5×10^{-3} M was the substrate employed for ChE measurements. Tributyrin at 1×10^{-3} M was used rather than methyl *n*-butyrate, the substrate employed in determining Ali-E activity in the house fly, because we found that esterase activity against methyl *n*-butyrate is not present in measurable amounts in *tarsalis*. Homogenates of larvae of the several colonies and crosses were prepared at a concentration of 10 insects per ml. One ml. of the homogenate, a ml. of pH 7.2 phosphate buffer, and a ml. of substrate were incubated at 37° C. for 15 minutes for esterase activity determinations. Details of the method have been described previously (Bigley and Plapp 1960).

RESULTS. The measurements of susceptibility to malathion are summarized in Table 1. The data show that the DDT-R and Reg colonies were equally susceptible to malathion with LC-50's of between 0.03 and 0.1 p.p.m. With the Mal-R colony, the LC-50 was slightly above 3 p.p.m. Mortalities obtained with F_1 larvae in the crosses in which a Mal-R parent was used

TABLE 1.—Mortalities of *Culex tarsalis* larvae after 24 hours of exposure to malathion in distilled water

Colony or cross	Generation	Mortality at indicated p.p.m. (%)					
		0.01	0.03	0.1	0.3	1.0	3.0
Regular	Parent	0	20	100	100	100	100
Malathion-R	"	0	0	0	0	0	26
DDT-R	"	4	22	100	100	100	100
Reg ♀ x Mal-R ♂	F_1	0	0	0	0	0	100
"	F_2	0	26	18	22	38	90
Reg ♀ x DDT-R ♂	F_1	6	62	100	100	100	100
"	F_2	0	88	100	100	100	100
Mal-R ♀ x Reg ♂	F_1	0	0	0	0	46	100
"	F_2	0	22	32	30	24	94
Mal-R ♀ x DDT-R ♂	F_1	0	0	0	2	60	100
"	F_2	0	24	20	26	20	92
DDT-R ♀ x Reg ♂	F_1	2	30	92	100	100	100
"	F_2	0	78	100	100	100	100
DDT-R ♀ x Mal-R ♂	F_1	0	0	0	0	64	100
"	F_2	2	12	14	24	34	94
(Mal-R ♀ x Reg ♂) ♀ x Reg ♂	Backcross	0	6	22	30	34	98

show that resistance is dominant over susceptibility. No evidence was obtained for sex-linkage since the F_1 larvae were resistant to malathion whether Mal-R males or females were used. However, the mortalities obtained at 1 and 3 p.p.m. in the F_1 were higher than those for the resistant parent colony, which indicated that resistance was not completely dominant.

The experiments with the F_2 generation were in agreement with a single gene hypothesis for inheritance of resistance. The expected 3:1, resistant:susceptible, ratios were obtained for all four crosses involving a Mal-R parent. Mortalities with F_2 larvae were lower than with F_1 larvae at 3 p.p.m. (92.5 percent average vs. 100 percent); thus further evidence was provided of incomplete dominance for the gene which conferred resistance.

However, in the backcross experiment, in which resistant F_1 females were mated with susceptible colony males, the expected 1:1 ratio was not obtained, but rather the same 3:1 ratio as in the F_2 generation. The same results were obtained when the experiment was repeated several months later. We do not know the reasons for the failure to obtain the expected ratio. It is possible that additive gene action or some modifiers of the major gene for resistance are involved.

The measurements of susceptibility to DDT are summarized in Table 2. The data show that some resistance to DDT was also present in the colony selected with malathion, since the LC-50 was between 0.03 and 0.1 p.p.m., whereas the figures were about 0.01 and 1 p.p.m. for the Reg and DDT-R colonies, respectively.

The bioassays of the F_1 generation showed that susceptibility to DDT was dominant over resistance. In all six crosses the mortalities obtained with F_1 larvae were nearly identical with those of the more susceptible of the two parents. That resistance was recessive to susceptibility was demonstrated in the F_2 generation where all tests showed that the larvae were less susceptible than their F_1 parents. The results of the backcross experiment were in agreement with those expected because the larvae were far less susceptible to DDT than were their F_1 parents.

From the data we concluded that in the strains tested, DDT resistance is recessive to susceptibility. The results are comparable to those discussed by Davidson & Jackson (1961) who showed that DDT resistance in mosquitoes is usually reces-

TABLE 2.—Mortalities of *Culex tarsalis* larvae after 24 hours of exposure to DDT in distilled water

Colony or cross	Generation	Mortality at indicated p.p.m. (%)					
		0.01	0.03	0.1	0.3	1.0	3.0
Regular	Parent	56	78	100	100	100	100
DDT-R	"	4	8	14	28	60	54
Malathion-R	"	8	12	76	92	100	100
Reg ♀ x DDT-R ♂	F_1	44	76	100	100	100	100
"	F_2	8	26	64	76	84	98
Reg ♀ x Mal-R ♂	F_1	26	90	98	100	100	100
"	F_2	10	84	94	100	100	100
DDT-R ♀ x Reg ♂	F_1	56	90	94	98	100	100
"	F_2	38	62	96	88	96	100
DDT-R ♀ x Mal-R ♂	F_1	30	24	78	98	100	100
"	F_2	14	30	62	84	96	100
Mal-R ♀ x Reg ♂	F_1	4	84	100	100	100	100
"	F_2	28	74	100	100	100	100
Mal-R ♀ x DDT-R ♂	F_1	4	2	90	100	100	100
"	F_2	24	22	64	94	84	98
(DDT-R ♀ x Reg ♂) ♀ x DDT-R ♂	Backcross	12	30	60	68	86	94

sive to susceptibility. The inheritance appears to be monogenetic although it is difficult to make the statement with finality from the data presented. In the present study the change in response in the DDT-R colony has been shown to be not so much a shift in the location of the dosage-mortality curve as a shift to a different type of response with a dosage-mortality curve having a lower slope. It is only on this basis that we can explain the fact that after several years of pressuring, individuals highly susceptible to DDT are still present in the DDT-R colony.

Of further interest is the fact that DDT resistance in the Mal-R strain is inherited in a different manner from malathion resistance in the same strain. This finding may be contrasted to results obtained when house flies have been pressured with organophosphates. Selection with organophosphates often induces a very high level of cross-resistance to DDT (March 1959). As demonstrated, no genetic relationship exists between the two types of resistance present in the *tarsalis* colony selected with malathion.

The measurements of ChE and Ali-E activity in the different colonies are summarized in Table 3. Levels of ChE were similar in Reg and Mal-R larvae. Surprisingly, ChE activity in the DDT-R colony was about twice as great as in the other colonies. Measurements of the F_1 progenies showed that inheritance of high levels of ChE was recessive to inheritance of low levels. Although inheritance of ChE levels is similar to the pattern of inheritance of DDT resistance, far more detailed studies are needed to demonstrate a causal relationship, and for the present, the similarity is merely noted.

TABLE 3.—Cholinesterase and ali-esterase activity in *Culex tarsalis* larvae

Colony or cross	Micromoles of substrate hydrolyzed per 15 minutes per 10 mosquito larvae	
	Acetylcholine (Che)	Tri *n*-butyrin (Ali-E)
Regular	0.725	1.68
Malathion-Resistant	0.65	1.88
DDT-Resistant	1.425	1.84
Reg ♀ x Mal-R ♂	0.50	1.68
Reg ♀ x DDT-R ♂	0.925	2.08
Mal-R ♀ x Reg ♂	0.65	1.68
Mal-R ♀ x DDT-R ♂	0.80	1.64
DDT-R ♀ x Reg ♂	0.675	1.64
DDT-R ♀ x Mal-R ♂	0.60	1.52

Ali-E activity as determined with the substrate tributyrin was similar in all colonies, and lowest in the Reg colony. Thus, unlike organophosphate-resistant house flies, inheritance of low levels of activity toward a substrate containing butyrate esters does not appear to be related to malathion resistance. Further evidence that the mechanism of resistance to malathion in *tarsalis* differs from that in the house fly has been indicated previously (Darrow and Plapp 1960). In that study it was shown that the pattern of cross-resistance to other insecticides in the Mal-R colony differed significantly from the typical pattern occurring in the house fly.

SUMMARY. 1. Experiments have demonstrated that resistance to malathion in the Fresno strain of *Culex tarsalis* is dominant over susceptibility.

2. DDT resistance, present at a high level in the Oak Ridge strain of *tarsalis* and at low levels in the Fresno strain, is recessive to susceptibility.

3. Cross-resistance to DDT in the strain selected with malathion appears to be caused by a separate factor and is not a result of selection with malathion as often happens with the house fly.

4. Quantitative changes in levels of either cholinesterase or ali-esterase are not related to malathion resistance, but high cholinesterase levels present in the Oak Ridge strain are inherited in the same manner as is resistance to DDT.

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