Genetics of Resistance of a Dimethoate-Selected Strain of Houseflies (*Musca domestica* L.) to Several Insecticides and Methylenedioxyphenyl Synergists

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At least five genes control or modify resistance to insecticides or synergists in the dimethoate-selected Danish strain $49r_2b$ of housefly (*Musca domestica* L.). On chromosome 2, gene *M* close to the marker *ar* controls resistance to malathion, malaoxon, and tetrachlorvinphos, gene *D*, about 20 map units from *ar*, controls resistance against dimethoate and several other organophosphate insecticides, and gene *Pb*, close to gene *D*, controls resistance to methylenedioxyphenyl synergists and synergized pyrethrum but not pyrethrum alone. The mechanism of resistance controlled by gene *D* is sesamex suppressible and confers stronger resistance against the phosphates than the corresponding phosphorothioates. On chromosome 5 gene *R5* controls another sesamex-suppressible mechanism of resistance to dimethoate, and on chromosome 3 gene *Pen* controls the mechanism, delaying entry of insecticides into houseflies, which intensifies the resistance conferred by the other genes. Retention of parathion resistance following the switchover of control from parathion to dimethoate was probably caused by the progressive disappearance of gene *a*, which conferred resistance to parathion but not dimethoate, and its replacement by gene *D*, which confers resistance to both dimethoate and parathion.

Dimethoate was introduced to control diazinon- and malathion-resistant houseflies in Denmark and the USA and for several years gave excellent results. In 1966, however, Keiding detected the first signs of resistance in Denmark (Keiding, 1967) and Hansens *et al.* (1967) detected them in the USA, and since then resistance to dimethoate has increased and is now widespread in Denmark (Keiding, 1973). Strong resistance was also reported recently on a few farms in California (Georghiou *et al.*, 1972). Although dimethoate resistance is not positively correlated with resistance to parathion (Keiding and Yasutomi, 1969), the dimethoate-selected strain $49r_2b$ resists parathion. Whether this resistance had been acquired by and retained after treatment of the field population with parathion or whether it is caused by the cross-resistance conferred by dimethoate resistance mechanisms(s) is uncertain.

Relatively little is known about the mechanism(s) of resistance to dimethoate in insects; Suplicy *et al.* (1972) recently suggested that it is probably caused by degradation of dimethoxon and is probably oxidative because Yasutomi and Keiding (1969) found that sesamex synergizes dimethoate against the resistant houseflies. In *Myzus persicae* Sulz., resistance to dimethoate is linked with strong carboxylesterase (E.C.3.1.1.1) activity (Needham and Sawicki, 1971) but the importance of this is not known.

This paper reports the results of the studies on the cross-resistance of the dimethoate-selected strain of housefly, $49r_2b$, to some insecticides, its genetics of resistance, and the possible reason why parathion resistance persists on selecting with dimethoate.

MATERIALS

Insects. *Parental Strains.* The following dimethoate-selected strains of houseflies (*Musca domestica* L.) were used.

Strain $49r_2b$ started with flies of wild phenotype, collected on 11/25/1970 by Keiding on farm 49, Northwest of Copenhagen, in an area where he had studied fly control since 1945 and where many compounds had been used (Table I).

The F_1 generation, after collecting from the field, was 120 times more resistant to dimethoate at LD_{50} and 400 times more resistant at LD_{90} than a susceptible strain (Keiding, 1971). F_2 and later generations were very resistant to fenthion, fenitrothion, trichlorphon, and iodophenphos. After collection, the strain was selected in the laboratory in Denmark at irregular intervals with dimethoate by dipping.

On receipt from Keiding (11.5.1971) the strain was only moderately resistant (×64) to dimethoate. It was selected intermittently as above, but more recently was selected at each generation either by applying dimethoate topically as measured drops or by dipping. Strong selection usually delayed egg laying by up to 10 days but delayed egg laying also accompanied strong resistance.

The strain is polygenic for resistance to dimethoate and other organophosphate insecticides and, in spite of strong selection, has remained very heterogeneous. Because of this heterogeneity, resistance decreases rapidly when selection is stopped, and even the greatest resistance obtained so far (Table II) is unlikely to represent the potential level of resistance the strain can achieve.

Strain $49r_2b$ not only resists insecticides but is also relatively resistant to anesthesia by ether, and the flies are exceptionally vigorous and fertile. Both larvae and adults eat more and tolerate starvation, crowding, or lack of water better than other strains in our laboratory. Strain $49r_2b$ has normal carboxylesterase activity.

Another dimethoate-resistant strain, 239fb, also obtained from Keiding, resisted dimethoate less than $49r_2b$ on receipt, but ultimately showed similar resistance. Some genetic results obtained with 239fb are included in this paper.

Strain 29 (Sawicki and Farnham, 1967) had, on chromosome 2, two genes derived from the diazinon-selected SKA strain of houseflies which control mechanisms detoxifying organophosphorus insecticides. Gene *g* (Oppenoorth *et al.*, 1972) controls glutathione *S*-ethyl transferase, which converts diazinon to desethyldiazinon (Lewis and Sawicki, 1971). Gene *a* (Oppenoorth, 1959) controls a modified carboxylesterase (E.C.3.1.1.1) with phosphatase activity, which hydrolyzes paraoxon and diazoxon. Together, these detoxication mechanisms made strain 29 from 1.4 to 48 times more resistant to several organophosphorus insecticides than the susceptible strain (Sawicki and Farnham, 1967), but these mechanisms were ineffective against dimethoate, to which strain 29 was almost fully susceptible.

Strain 1,2,3,5. The multi-marker susceptible strain 1,2,3,5, used for bioassays and genetic work, was marked on chromosomes 1, 2, 3, and 5 with the recessive mutant markers *ac;ar;bwb;ocra*. It was bred by A. W. Farnham,

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Table I. History of Insecticidal Treatment on Farm 49[a]

1946–1952	DDT chlordane
1953–1955	Probably parathion strings and diazinon
1956	Chlordane and an experimental organophosphorus compound
1957	DDT and coumithoate
1958	Cetyl bromoacetate, DDT
1959	Parathion strips
1960	Diazinon
1961–1962	Naled paint, bait, and spray
1963	Dimethoate
1964	Fenitrothion
1965, 1967	Dimethoate
1968	Trichlorfon paint and bait
1969	Tetrachlorvinphos

[a] Keiding (1972).

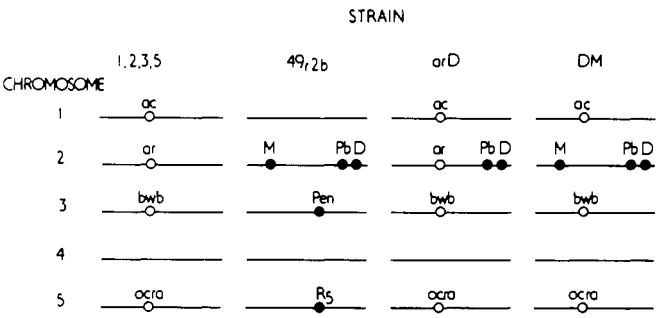

Figure 1. Schematic diagram of the genotype of the strains used: visible mutant markers, *ac* (curly wings), *ar* (aristopedia), *bwb* (brown body), *ocra* (ocra eyes); resistance genes, *D* (dimethoate R), *M* (malathion R), *Pb* (piperonyl butoxide R), *Pen* (delayed penetration), R_5 (resistance to dimethoate).

who outbred carbamate and pyrethrum knockdown resistance from the *ac;ar;bwb;ocra* SRS stock by outcrossing with the susceptible Cooper strain of wild phenotype.

The strains with chromosome 2 derived from strain $49r_2b$ were as follows. Strain *arD* was marked with the four mutant markers and carried on chromosome 2 marked with *ar*, the major gene of resistance to dimethoate gene *D*, derived from $49r_2b$. Strain *DM* marked on chromosomes 1, 3, and 5 carried on the unmarked chromosome 2 gene *D* and gene *M*, the major gene of resistance to malathion, both derived from $49r_2b$. Figure 1 shows the genotype of the strains 1,2,3,5, $49r_2b$, *arD*, and *DM*.

Insecticides. The following insecticides or additives were used: dimethoate, recrystallized from dry ether, des-*N*-methyldimethoate [*O,O*-dimethyl *S*-(carbamoylmethyl) phosphorodithioate], dimethoxon [*O,O*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolate], malathion, malaoxon, parathion, paraoxon, parathion methyl, dicrotophos, tetrachlorvinphos, Orthene, *O,S*-dimethylacetyl phosphoroamidothioate, pyrethrum extract (containing 25% of active ingredients), bioresmethrin, sesamex, piperonyl butoxides, TBTP (*S,S,S*-tributylphosphorotrithioate), and tributyltin acetate (TBTA).

METHODS

Rearing and Testing of Houseflies. Methods of rearing single pairs and mass cultures and test methods are described elsewhere (Sawicki and Farnham, 1968). For toxicity tests, 3–7-day-old female houseflies were each treated on the thorax with a 1-μl drop of insecticide dissolved in acetone using two replicates of 15 flies/dish. Most of the tests were repeated several times. Dead flies were counted after holding overnight at 20° after treatment with natural or synthetic pyrethroids, and at 29° after treatment with other insecticides. In some experiments flies were pretreated with 2 μg of sesamex, 1 μg of TBTP, or both 2–4 hr before treatment with insecticide. The LD_{50}'s estimated graphically from log-dose probit lines (ld-p) are in μg of poison/fly. Carboxylesterase activity was determined spectrometrically (van Asperen, 1962).

Location and Isolation of Some of the Genes of Resistance in Strains $49r_2b$ and 239fb. Each dimethoate-resistant strain ($49r_2b$ and 239fb) was crossed with the susceptible multi-marker strain 1,2,3,5.

Dominant resistance was allocated to individual chromosomes at the test-cross progeny stage with discriminating doses of dimethoate following the method described by Tsukamoto (1964). Each of the chromosome pairs of the resistance strain (except chromosome 4) was then isolated by substituting one of the marked pairs of chromosomes of the susceptible parent by a homologous pair from the resistant parent and each strain thus derived was tested for resistance to insecticides (Sawicki and Farnham, 1968). Lastly, one gene on chromosome 2 conferring resistance to organophosphate insecticides was separated from the other and the effect of these resistance genes was examined.

Identification and Isolation of Genes of Resistance on Chromosome 2. The replacement of chromosome 2 of the susceptible 1,2,3,5 strain marked with *ar* by the unmarked chromosome 2 of the resistance strains ($49r_2b$ and 239fb) using techniques described elsewhere (Sawicki and Farnham, 1968) failed to produce even one single pair of progeny homozygous for resistance to dimethoate. The main

Table II. Cross Resistance of Strain $49r_2b$ with and without Pretreatment with 2 μg of Sesamex/Fly

	Insecticide alone		Insecticide after pretreatment with sesamex		
	LD_{50} μg/fly	Resistance factor	LD_{50} μg/fly	Resistance factor	Synergistic factor
Dimethoate	1.20	120	0.20	34	6
Dimethoxon	5.60	785	0.18	36	31
Parathion methyl	0.56	24	0.24	11	2.3
Paraoxon methyl	2.0	74	0.13	~10	15
Parathion	0.45	28	0.19	13	2.4
Paraoxon	3.0	91	0.32	~20	6
Malathion[a]	>25.0	Not measurable	6.4	>400	Not measurable
Malaoxon[b]	>44.0	Not measurable	13.0	>860	>3.4
Dicrotophos[a]	13.0	>135	Not tested		
Tetrachlorvinphos[b]	~4.0	Not measurable	Not tested		
Orthene	1.5	160	Not tested		
Pyrethrum extract	0.26	None	0.040	46	6.5
Bioresmethrin	0.013	<3	0.0035	7	3.7
Piperonyl butoxide[a]	>100.0	Not measurable			
Sesamex	~85.0	8			

[a] Less than 50% killed by strongest dose. [b] Plateau at LD_{60}.

Table III. Cross Resistance of F_1 (1,2,3,5 × 49r_2b)

Insecticide	LD_{50} μg/insect, F_1 (1,2,3,5 × 49r_2b)	49r_2b
Dimethoate	0.45	1.2
Des-*N*-methyldimethoate	0.70	2.4
Dimethoxon	No kill at 0.75	
Malathion	20% survived, 60 μg/fly	

Table IV. Response of Females of 16 Phenotypes of the Test Cross Progeny Female 1,2,3,5 × Male F_1 (1,2,3,5 × 239fb)

Phenotype	% female killed by 0.035 μg of dimethoate/fly	Likely resistance factor[a]	Segregation of phenotypes	
			Female	Male
+;+;+;+	4	*D;Pen;R5*	52	48
ac;+;+;+	2	*D;Pen;R5*	47	50
+;*ar*;+;+	48	+;*Pen;R5*	41	43
+;+;*bwb*;+	26	*D*;+;*R5*	71	52
+,+;+;*ocra*	12	*D;Pen*;+	44	55
ac;ar;+;+	67	+;*Pen;R5*	37	46
ac;+;*bwb*;+	15	*D*;+;*R5*	41	39
ac;+;+;*ocra*	23	*D;Pen*;+	40	43
+;*ar;bwb*;+	67	+;+;*R5*	47	41
+;*ar*;+;*ocra*	92	+;*Pen*;+	48	59
ac;ar;bwb;+	90	+;+;*R5*	44	42
ac;ar;+;*ocra*	100	+;*Pen*;+	49	28
ac;+;*bwb;ocra*	36	*D*;+;+	41	25
+;*ar;bwb;ocra*	91	+;+;+	38	50
ac;ar;bwb;ocra	98	+;+;+	43	41

[a] *M* segregates with *D*.

Table V. Resistance in Strains with Individual Chromosome Pairs Derived from Strain 49r_2b

Strain	Chromosome pair isolated from 49r_2b	Resistance factor		
		Dimethoate	Dimethoxon	Parathion
1	1	1.4	1.7	1.2
2	2	11.0	2.8	7.4
3	3	1.7	1.0	3.8
4	4	1.0	1.0	1.0
5	5	3.8		2.5

cause of this failure was the heterozygosity of one or more resistance genes on chromosome 2 in both resistant parent strains.

To obtain homozygosity for resistance, the unmarked chromosome 2 of the resistant 49r_2b was introduced into the susceptible multi-marker 1,2,3,5 strain. Selection in turn with dimethoate, malathion, and malaoxon with sesamex ultimately yielded a strain homozygous for resistance on chromosome 2. This strain was called *DM*, after the first letter of each of the compounds against which resistance genes were most effective (*D*imethoate and *M*alathion) (Figure 1).

The genes of resistance were further separated after it was found that *ar*-marked F_2 flies of the cross (1.2.3.5 × *DM*) resisted dimethoate but not malathion. This indicated that resistance to dimethoate and malathion was controlled by different genes, gene *D* which confers strong resistance to dimethoate but not malathion, and gene *M*, which confers immunity to malathion but only little resistance to dimethoate. In the *ar*-marked flies of F_2 only gene *D* had crossed over (20% crossover). These flies were selected for several generations with dimethoate to yield strain *arD*, which was homozygous for strong resistance to dimethoate, lacked gene *M* for malathion resistance, and was marked with the marker *ar* (Figure 1).

Selection with Dimethoate of a Population Heterozygous for Gene *a* and Gene *D*. Field populations which develop resistance to dimethoate retain resistance to parathion or malathion. To determine if this is due to retention of the original parathion resistance mechanism or to its replacement on selecting with dimethoate by gene *D*, which confers cross-resistance to parathion, the following crosses and tests were done.

Strain 29, resistant to parathion because of genes *a* and *g* on chromosome 2, was crossed with strain *arD* homozygous for gene *D* and the marker *ar*, both on chromosome 2. Some of the F_1 flies were selected with dimethoate, and their progeny called *Sel* were selected five more times with dimethoate during the succeeding seven generations. The remaining F_1 flies were bred without selection and this substrain was called *Unsel*.

Resistance to parathion and dimethoate and the level of carboxylesterase in the two substrains was measured several times up to F_9.

Preparation of Derivatives of Dimethoate. Des-*N*-methyldimethoate was prepared by adding a solution of the sodium salt of dimethyl dithiophosphoric acid (33 g) in 50 ml of water dropwise under reflux for 1 hr to a solution of chloracetamide (15.5 g) in water (75 ml) layered with chloroform (75 ml). After refluxing for an additional 30 min, the chloroform layer was separated, and the aqueous phase was extracted three times with an equal volume of chloroform. The solvent was evaporated from the combined chloroform fraction *in vacuo* and the product recrystallized from dry ether. Identity was confirmed by nmr.

RESULTS

Cross Resistance of Strain 49r_2b. Table II shows cross resistance of strain 49r_2b at LD_{50} to several compounds with and without pretreatment with sesamex. The strain was homogeneous in its response to most of the compounds tested, but its response to malathion, malaoxon, and tetrachlorvinphos was heterogeneous and there was a plateau in the log-dose probit lines with these compounds at about 60% kill. The strain was very resistant to dimethoxon, Orthene, dicrotophos, dimethoate, paraoxon, and paraoxon methyl. It was much more resistant to the oxygen analogs than to the corresponding parent compounds. Sesamex synergized the oxygen analogs more than the corresponding parent compounds but did not eliminate resistance. This is probably because the strain resisted not only organophosphate insecticides but also methylenedioxyphenyl synergists.

Flies of strain 49r_2b pretreated with sesamex were very resistant to pyrethrum but only slightly to bioresmethrin and resistance to both compounds without pretreatment with synergist was slight or negligible. The strain was also resistant to knockdown by synergized or unsynergized pyrethrum.

Genetics of Resistance of Strain 49r_2b and 239fb. Bioassays of F_1 flies (49r_2b × 1,2,3,5) showed that resistance to dimethoate and its des-*N*-methyl analog was of intermediate dominance (Table III). Females of F_1 survived 0.75 μg of dimethoxon/female, indicating that the very strong resistance (>100) is either intermediate or dominant. Over 20% of F_1 females survived 30 and 60 μg of malathion/fly, demonstrating that only a small proportion of the population was homogeneous for dominance of resistance to this insecticide. The F_1 reciprocal crosses gave similar results, hence resistance is not sex-linked.

Bioassays of the test-cross progeny females 1,2,3,5 × F_1 males (1,2,3,5 × 239fb) showed that the "dominant effect" of resistance to dimethoate was greatest on chromosome 2, moderate on chromosome 5, very slight on chromosome 3, and negligible or absent on chromosomes 1 and 4 (Table IV). Tests on strains with the isolated chromosomes of strain 49r_2b, in which most of the resistance factors were heterozygous, confirmed these results (Table V).

Table VI. Cross Resistance of Strain *arD* with and without Pretreatment with 2 μg of Sesamex/Fly

	Insecticide alone		Insectidide after pretreatment with sesamex		
	LD_{50} μg/fly	Resistance factor	LD_{50} μg/fly	Resistance factor	Synergistic factor
Dimethoate	0.32	32	0.062	11	5
Dimethoxon	0.90	127	0.037	7	24
Des-*N*-methyldimethoate[a]	>0.50	>21			
Parathion methyl	0.14	9			
Parathion	0.093	6	0.021	<2	4
Paraoxon	0.14	4			
Malathion	1.4	4	0.80		4
Malaoxon	5.2	17	0.080	5	65
Dicrotophos	2.2	28			
Tetrachlorovinphos	0.062	2–3			
Orthene	0.10	~10			
Pyrethrum extract	0.50	<2	0.0083	9	60
Piperonyl butoxide	0.50	4			
Sesamex	19.0	2			

[a] Less than 50% killed by strongest dose.

Table VII. Cross Resistance of Strain *DM* to Some Insecticides

	Insecticide alone		Insecticide after pretreatment with sesamex		
	LD_{50} μg/fly	Resistance factor	LD_{50} μg/fly	Resistance factor	Synergistic factor
Malathion	No kill at 12.0 μg	Not measurable	No kill at 12.0 μg	Not measurable	Not measurable
Malaoxon	No kill at 11.0 μg	Not measurable	7.5/μg	>500	Not measurable
Tetrachlorvinphos	0.46	17			

Table VIII. Effect of Selecting with Dimethoate on Resistance to Parathion and Dimethoate in a Cross between a Dimethoate-Resistant (*arD*) and a Parathion-Resistant (29) Strain[a]

LD_{50} μg/ Strain	Dimethoate				Parathion			
	29	*arD*	*Unsel*	*Sel*	29	*arD*	*Unsel*	*Sel*
Generation								
P	0.015	0.34			0.28	0.10		
F_1 +			0.085	0.085			0.12	0.12
F_2 +			0.095	0.12			0.20	0.20
F_3 +			0.066	0.28				
F_4 +				0.43				0.15
F_5				0.32				0.14
F_6 +								
F_7 +								
F[c]			0.045	0.30			0.13	0.13

[a] Strain *sel* was selected with dimethoate at each generation marked with +. Strain *unsel* was bred without selecting with insecticide.

Only strains with chromosomes 2 and 5 resisted dimethoate and parathion, but resistance to dimethoate and parathion in strain 5 (*R5*) was eliminated by pretreatment with sesamex. *Pen*, the factor delaying penetration, was present in $49r_2b$ because strain 3 resisted tributyltin acetate (Plapp and Hoyer, 1968) but both *kdr* or *kdr*-0 genes were absent from chromosome 3 because strain 3 was fully susceptible to DDT.

As expected, there was no measurable resistance to any of the compounds tested in the strain in which chromosome 4 could have been derived from either parent or both.

Cross Resistance of Strain *arD*. Strain *arD* differed from its $49r_2b$ parent in being less resistant to most of the compounds tested, and in particular against malathion and tetrachlorvinphos, to which it was almost susceptible (Table VI). Strain *arD* resisted dimethoxon most, was moderately resistant to dimethoate, dicrotophos, and des-*N*-methyl dimethoate, and its resistance to other compounds was slight (less than ×10). Pretreatment with sesamex decreased but did not fully eliminate resistance and dimethoxon was synergized much more than dimethoate. The strain resisted slightly piperonyl butoxide and sesamex, and this is probably why it resisted synergized pyrethrum but not pyrethrum alone, and also why pretreatment with sesamex did not completely eliminate resistance to dimethoate or dimethoxon.

Cross Resistance of Strain *DM*. Only a little work was done on the cross resistance of this strain. Strain *DM* resisted dimethoate and Orthene and the methylene dioxyphenyl synergists as much as strain *arD*, but was very resistant, even more than $49r_2b$, to malathion and malaoxon, and resisted tetrachlorvinphos. Pretreatment with sesamex, TBTP, a carboxylesterase inhibitor (Plapp *et al.*, 1963), or a mixture of those compounds had no effect on the response of this strain to malathion. Although sesamex synergized malaoxon, resistance to this compound even after pretreatment with sesamex remained very strong (Table VII).

Effect of Selecting with Dimethoate on Resistance to Parathion and Dimethoate of a Population Heterogeneous for Genes *a* and *D*. The two substrains from the cross 29 × *arDM* were more vigorous and the flies were usually bigger than either parent. They were also more resistant to anesthesia with ether.

Table VIII shows the changes in response of the two

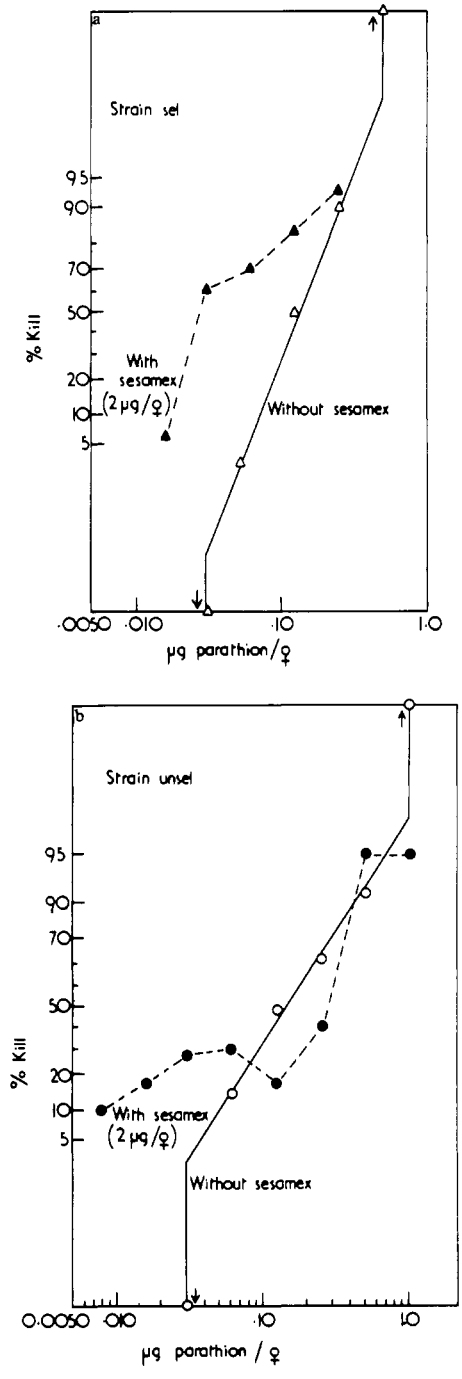

Figure 2. Effect of parathion with and without pretreatment with sesamex on two substrains of the progeny of a cross between two different organophosphorus-resistant strains (29 and *arD*). a, substrain selected with dimethoate. b, substrain unselected.

substrains of cross 29 × *arDM* to dimethoate and parathion. Resistance of the *ar* phenotype segregating in F_2 could not be measured because segregation was abnormal in both substrains and homozygotes for *ar* were rare. This is probably why resistance to dimethoate at F_2 was weak; most of the population was then either heterogeneous for *D* or lacked it. The cause of this abnormal segregation is not known because flies with *ar* bred readily when selfed. Flies of strain *Sel*, strongly resistant to dimethoate but lacking *ar*, were probably derived from individuals in which *D* and *ar* crossed over.

From F_4 onward strain *Sel* was 1.2–1.4 times more resistant to dimethoate and between 1.5 and 2 times more resistant to parathion than its *arD* parent. The reason for this is not known. Both substrains of 29 × *arD* retained moderate resistance to parathion but differed in having different proportions of each resistance mechanism. At F_8, strain *Sel* resisted dimethoate strongly, and its carboxylesterase activity was almost normal (3.7×10^{-7} mol α-naphthol/g fly/30 min), but as Figure 2a shows, at F_9 after two generations without selection, pretreatment with sesamex exposed between 30 and 40% of the flies that were still heterogeneous for gene *a* because these flies responded less to synergized parathion than flies of strain *arD*; gene *a* is antagonized by sesamex. At F_8 flies of the unselected strain had lost most of their dimethoate resistance and the carboxylesterase activity of 2.9×10^{-7} mol α-naphthol/g fly/30 min was intermediate between that of both its parents. Pretreatment with sesamex synergized parathion against 20–30% of the flies of this substrain, but sesamex antagonized the effect of parathion against the rest of the population (Figure 2b).

DISCUSSION

The strong resistance of strain $49r_2b$ to organophosphate insecticides and even to pyrethroids synergized with sesamex is the result of the action or interaction of resistance mechanisms controlled by at least five genes, of which three are on chromosome 2.

The two genes of resistance to organophosphate insecticides on chromosome 2, *viz.* genes *D* and *M*, probably control different biochemical or physiological systems of resistance because they confer discrete cross-resistance spectra and respond differently to sesamex. Gene *D*, which confers stronger resistance to the phosphates than to the corresponding phosphorothionates, may control mixed function oxidase(s) because it is inhibited by sesamex (Casida, 1970). Gene *M* is unlikely to be a modified carboxylesterase (Welling and Blaakmeer, 1971) because the activity of this enzyme to α-naphthyl acetate is normal and resistance to malathion is unaffected by pretreatment with TBTP. The cross resistance conferred by gene *M* had to be inferred from differences in cross resistance between strain *DM* and *arD*, which lacks *M*, because gene *M* has not yet been separated from gene *D*. The mechanism controlled by gene *M* probably confers little or no resistance to dimethoate because strains *arD* and *DM* respond to dimethoate similarly, but gene *M* is almost certainly responsible for most of the resistance to malathion because strain *arD*, which lacks gene *M*, is nearly susceptible to this insecticide. Both *D* and *M* confer strong resistance to malaoxon; *M* is probably responsible for the resistance to this compound when strain *DM* is pretreated with sesamex (Table VII).

Nothing is known about the nature of the mechanisms of resistance controlled by genes *D* and *M*. Dyte *et al.* (1970) have reported that the malathion-resistant CTC-12 strain of *Tribolium castaneum* detoxifies dimethyl phosphates but not dimethyl phosphorothionates by desmethylating the phosphate analogs, and a similar oxidative dealkylation was described in rats by Appleton and Nagatsugawa (1972).

Little is known about R_5 on chromosome 5 because it has not yet been characterized. When heterozygous, it confers only slight resistance to dimethoate and is sesamex-susceptible.

Resistance to methylenedioxyphenyl synergists is controlled by a gene called *Pb* which, in strain $49r_2b$, segregates with and is close to gene *D* on chromosome 2. It is, however, distinct from *D* because it is present in other resistant strains which lack gene *D*. Resistance to methylenedioxyphenyl synergists is the likely reason why flies of strain $49r_2b$ resist synergized pyrethrum, but not pyrethrum alone, and why sesamex does not eliminate fully resistance conferred by gene *D*. The history of insecticidal treatment of Form 49 (Table I) gives no clue either to the agent which selected this mechanism, the role it plays in resistance, or the nature of the mechanism conferring this resistance.

Gene *Pen* on chromosome 3 controls the mechanism that delays the entry of insecticides into houseflies. *Pen* confers little or no resistance to most insecticides when present by itself, but greatly increases the effect of some of the resistance mechanisms (Georghiou, 1971; Plapp and Hoyer, 1968; Hoyer and Plapp, 1971; Sawicki, 1971. *Pen* probably increases the resistance of the mechanism controlled by gene *D* to dimethoate (Table IV) even though differences in the penetration of dimethoate into flies having or lacking *Pen* are small. It is also likely to be responsible for the large difference in the resistance to tetrachlorovinphos between strain *DM* and $49r_2b$.

Resistance found in strain $49r_2b$ differs very much from the resistance which developed as a result of field application of parathion and diazinon. In Denmark, resistance to diazinon and parathion which developed in field populations was probably mainly caused by gene *a*, which decreases carboxylesterase activity toward α-naphthyl acetate. The observation that this esterase activity of strain $49r_2b$ which originally came from the field in Denmark was normal prompted the investigations into the effects of selecting with dimethoate on a strain which carried both gene *a* and *D*, as this approximately paralleled the situation which probably occurred in the field.

The results indicate that gene *a* progressively disappears by selecting with dimethoate, while resistance to parathion or diazinon (which occurs in both strains $49r_2b$ and *arD*) persists, but is now caused by gene *D*. The choice of strain *arDM* in which *D* was coupled with *ar* was probably unfortunate because previous work (Sawicki *et al.*, 1966) showed that *ar* segregates abnormally, which may partly explain not only why flies homogeneous for *ar* were rare, but also why gene *D* was rare in the unselected substrain of (29 × *arD*). However, dimethoate resistance in strain $49r_2b$ is probably still of the "young type" and has not yet stabilized; it was thus at a disadvantage against the "older" gene *a* resistance which has better fitness (Keiding, 1967). This is not so in the field anymore. Most recent reports (Keiding, 1973) indicate that resistance to dimethoate in houseflies on Danish farms has now become stabilized.

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