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Genetic Analysis of Non-recessive Factors of Resistance to Diazinon in the SKA Strain of the Housefly (*Musca domestica* L.)*

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The recent allocation of many visible mutants to specific linkage-groups has made possible their use as genetic markers in the study of the inheritance of insecticide resistance. In most organophosphorus-resistant strains, resistance is controlled by a single gene on the V linkage-group responsible for resistance and low ali-esterase activity, but in some strains more than one factor is present. The present work was done to analyse the genetics of resistance to diazinon in a strain (the SKA strain) bred from two diazinon-resistant strains. Crosses between the SKA flies and four susceptible recessive marker strains, each marked on one linkage-group, followed by test-crosses with recessive markers and by bioassays of each cross, have shown that non-recessive factors for resistance to diazinon are present on the IV and V linkage-groups. The SKA strain genetically resembles its parents but differs by being considerably more resistant.

Resistance to organophosphorus insecticides was thought to be controlled in most strains of houseflies mainly by a single gene (Nguy & Busvine, 1960; Oppenoorth & van Asperen, 1961) on the V linkage-group [4] (Franco & Oppenoorth, 1962), which replaces ali-esterase *a* by a " phosphatase " able to degrade the toxic phosphates (Oppenoorth & van Asperen, 1960, 1961). However, Matsumura & Hogendijk (1964) reported that a " thionase " degrades parathion and diazinon into non-toxic metabolites, and Oppenoorth (1965) found that resistance in the F strain is partly caused by unknown factor(s) on the IV linkage-group. According to Tsukamoto & Suzuki (1965) the main resistance factor in the Hokoto strain is on the V chromosome (DZ), but factors on the IV and II chromosomes also contribute to resistance. Work on strains very resistant to diazinon—namely, the Rutgers (Forgash, Cook & Riley, 1962) and SKA strains—indicates that resistance is also partly caused by the decreased penetration of diazinon through the cuticle (Farnham, Lord & Sawicki, 1965), especially the exocuticle (Potter, 1965).

Because the SKA strain is derived from two organophosphorus-resistant strains and more than one factor seems to be responsible for its resistance (Farnham, Lord & Sawicki, 1965), the genetic factors involved were analysed by crossing the SKA flies with four susceptible recessive marker strains, each marked on one linkage-group, followed by test-crosses with the recessive markers and by bioassays of each cross. The analysis, which covered four of the five autosomes present in the housefly, revealed the presence of two incompletely dominant factors for resistance to diazinon.

MATERIALS

Six strains of *Musca domestica* L. were used:

(*a*) The SKA strain of wild phenotype, the result of a cross between two diazinon-resistant strains (Keiding 203a × Saccà a) and of continued selection by exposure to diazinon. This strain is very resistant to diazinon and to several other insecticides (Potter, 1964).

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[4] The numerical sequence of the linkage-groups in this paper is that of Milani (1961).

TABLE 1
MUTANT AND LINKAGE-GROUP OF MARKER STRAINS OF FLIES USED FOR CROSSES WITH SKA FLIES

Name of mutant	Symbol	Linkage-group
brown body	*bwb*	II
ali curve	*ac*	III
ochre-eyed	*ocra*	IV
aristapedia	*ar*	V

(*b*) The SRS strain (WHO Standard Reference Strain of *Musca domestica* L.), of wild phenotype. It is very susceptible to diazinon.

(*c*) Four closely related recessive marker strains, each carrying its marker on a distinct linkage-group (Table 1). The four strains are all susceptible to diazinon. To introduce a common genetical background, the four strains were established by out-crossing the recessive marker flies to SRS flies, recovering the homozygous recessives in the F_2, and repeating the out-crosses. Each recessive marker was out-crossed to SRS flies every second generation between 10 and 14 times and should have progressively lost most of its original genetic background and come to resemble the SRS strain.

After these out-crosses, regular colonies were established; when this work started each strain had already reached the 10th or 11th generation.

The insecticide was 99.6% pure diazinon (*OO*-diethyl(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate). The stock solution of diazinon in acetone was kept in a refrigerator and serial solutions were made immediately before each bioassay by diluting the stock solution with pure acetone.

REARING METHOD

The eggs, laid on cotton-wool soaked with milk, were collected daily for three or four days and were inoculated each day on freshly prepared larval medium of wheat-bran, milk and yeast. The larvae pupated in a 2-in (5-cm) layer of sand placed on top of the medium five days after inoculation; they were sieved and transferred into aluminium beakers, which were then placed in polystyrene containers (1000 ml) covered with a cloth. The emerging adults, immobilized with ether, were sexed twice daily (morning and late afternoon) and the sexed flies were placed in separate cages to prevent mating. The flies of each sex and phenotype were counted at each sorting. All the females, and the SRS flies of both sexes, while in the cages, were given water, sugar and milk; the males and all the flies sorted into dishes for bioassays (see below) were given water and sugar only.

SELECTION OF SKA FLIES

The emerging SKA flies were sexed twice daily and the sexes placed in separate cages. The flies were selected at each generation when 36 ± 12 hours old by the topical application of 1.0 µl of diazinon in acetone on the thorax, and for one cross (the repeat of the *ar* × SKA and its reciprocal) by dipping (Sawicki & Farnham, 1964) for 3 minutes in 60% aqueous acetone containing 0.040% (w/v) diazinon. Only flies that survived selection were used for crosses with the marker strains. Table 2 shows the details of these selections and the strain of the marker flies with which the survivors were crossed.

The SKA flies were mated with the susceptible flies at least two days after selection, because during the first 24 hours after selection contact with the SKA females was lethal to male marker flies. This was observed when *ocra* males were mated with the SKA females selected the day before with 10 µg diazinon per female by topical application on the thorax. All the males died while attempting mating; they showed signs of acute poisoning almost immediately after mounting, before mating. A fresh batch of *ocra* males introduced into the cage the following day, i.e., two days after selection, mated successfully and survived for several days. Mating in the reciprocal cross was normal, and the *ocra* females showed no signs of poisoning. Presumably the dose of diazinon on the thorax of the selected SKA females was still lethal to the highly susceptible *ocra* males one day after treatment, but not two days after. In the reciprocal cross,

TABLE 2
DETAILS OF SELECTION OF SKA FLIES BY TOPICAL APPLICATION FOR CROSSES WITH MARKER STRAINS

Dose per fly (µg diazinon)		Survivors (%) (SKA)		Strain of marker used for crossing with SKA survivors		
♀	♂	♀	♂			
10	6	67	84	♀ *ocra*;	♂ *ocra*;	♂ *bwb*
10	7	32	32	♀ *ac*;	♂ *ac*;	
8	6	76	67	♀ *ar*;	♂ *ar*;	♀ *bwb*

the *ocra* females survived probably because they were in light contact with the thorax of the selected SKA males only very briefly during mounting before mating.

GENETICAL METHODS

All the experiments were done with reciprocal mass-crosses with virgin flies three to five days old. Fifty flies of each sex were placed in the same cage and given water, milk and sugar *ad libitum*. Eggs were laid in dishes containing cotton-wool soaked with milk and the first batch was usually laid a day after mating.

Flies of the four marker strains were crossed with selected SKA flies in reciprocal crosses to give F_1 flies. The heterozygous F_1 flies were bioassayed for susceptibility to diazinon or were used for crosses. For the test-crosses, 50 F_1 heterozygous males of each reciprocal cross were crossed with 50 homozygous marker females. The progeny of each back-cross were bioassayed with diazinon, but the flies of each phenotype were treated separately. The F_2 flies were obtained only from one of each pair of reciprocal crosses, and were not bioassayed.

BIOASSAY METHOD

All tests followed the general design of probit assay (Finney, 1952). Flies three to five days old, immobilized with ether, were transferred from cages into 200-ml plastic containers (15 flies per dish) and a few hours later, or more often the next day, were immobilized again and treated topically on the thorax with a 1.0-μl drop of diazinon in acetone. Two replicates of 15 flies were treated at each concentration, and wherever possible the concentrations were spaced to cover the whole kill range. The flies affected were counted twice, 24 and 48 hours after treatment, but because the difference between the results was small, only the 48-hour results are reported. After sorting, all flies were fed with water and sugar only. The flies were treated and kept at temperatures varying between 19°C and 25°C.

TABLE 3

NUMBER OF FLIES OF BOTH SEXES OF F_1 GENERATION IN MASS-CROSSES BETWEEN MARKER AND SKA FLIES

Cross	No. of flies of each sex	
	♀	♂
♀ SKA × ♂ *bwb*	704	751
♀ *bwb* × ♂ SKA [a]	—	—
♀ SKA × ♂ *ac*	220	226
♀ *ac* × ♂ SKA [b]	467	571
♀ SKA × ♂ *ocra*	1 041	1 027
♀ *ocra* × ♂ SKA	637	605
♀ SKA × ♂ *ar*	761	751
♀ *ar* × ♂ SKA	902	826

[a] Flies of this cross were not sexed.

[b] Sex ratio significantly different from normal 1:1 ratio at P = 0.05.

TABLE 4

SEGREGATION OF PHENOTYPES IN F_2 OF CROSSES ♀ MARKER × ♂ SKA OR THEIR RECIPROCALS

Original cross	No. and sex of F_2 flies of each phenotype				Total number of F_2 of each phenotype	
	Marker		++ (wild)		Marker	++ (wild)
	♀	♂	♀	♂		
♀ SKA × ♂ *bwb* [a]	118	105	417	468	223	885
♀ *ac* × ♂ SKA [b]	56	57	200	142	113	342
♀ *ocra* × ♂ SKA [a]	76	88	375	338	164	713
♀ SKA × ♂ *ar*	91	134	320	325	225	645

[a] Segregation significantly different from 3:1 hypothesis at P = 0.05.

[b] Sex ratio significantly different from normal 1:1 ratio at P = 0.05.

GENETICAL RESULTS

F_1 progeny

All F_1 flies were of the wild phenotype. Table 3 gives the number of adults and the statistical significance of sex ratio in F_1 flies from reciprocal crosses between the four marker strains and the SKA flies. In all populations examined other than ♀ *ac* × ♂ SKA, the sex ratio approached normality. In this cross the sex ratio was normal in one batch of adults (60♀: 62♂) but in the other there was a large deficit of females (404 ♀: 509 ♂), possibly caused by overcrowding in the larval culture.

F_2 progeny

In the ♀ SKA × ♂ *bwb* and ♀ *ocra* × ♂ SKA F_2 crosses there was a deficit of marker flies, and segregation was significantly abnormal. The segregation of the phenotypes in the two other F_2 crosses was normal (Table 4).

Test-cross

The segregation of the phenotypes was abnormal in the test-cross ♀ *bwb* × ♂ F_1 (♀ SKA × ♂ *bwb*), where wild-type females considerably exceeded *bwb* females, and in the two *ocra* test-crosses, because there was a consistent deficit in the number of *ocra* flies of both sexes. The segregation of the phenotypes in the test-crosses of the other two marker strains was normal (Table 5).

TABLE 5

SEGREGATION OF PHENOTYPES IN PROGENY OF TEST-CROSSES BETWEEN F_1 MALES OF CROSS ♀ MARKER × ♂ SKA OR ITS RECIPROCAL AND HOMOZYGOUS MARKER FEMALES

Test-cross	No. of flies of each phenotype	
	Marker	++ (wild)
♀ *bwb* × ♂ F_1 (♀ SKA × ♂ *bwb*) [a, b]	587	678
♀ *bwb* × ♂ F_1 (♀ *bwb* × ♂ SKA)	395	418
♀ *ac* × ♂ F_1 (♀ SKA × ♂ *ac*)	848	899
♀ *ac* × ♂ F_1 (♀ *ac* × ♂ SKA)	117	101
♀ *ocra* × ♂ F_1 (♀ SKA × ♂ *ocra*) [a, b]	1 266	1 462
♀ *ocra* × ♂ F_1 (♀ *ocra* × ♂ SKA) [a, b]	1 287	1 796
♀ *ar* × ♂ F_1 (♀ SKA × ♂ *ar*)	948	874
♀ *ar* × ♂ F_1 (♀ *ar* × ♂ SKA)	920	914

[a] Segregation significantly different from normal 1:1 ratio at P = 0.05.
[b] Sex ratio significantly different from normal 1:1 ratio at P = 0.05.

BIOASSAY RESULTS

The F_1 flies were always tested together, using susceptible and resistant flies for a double control, and in order to have a single standard for susceptibility the SRS strain was used instead of the parent re-

TABLE 6A

RESULTS OF BIOASSAYS WITH F_1 FLIES OF CROSSES ♀ SKA × ♂ MARKER AND RECIPROCALS (FLIES WITHIN EACH PAIR OF RECIPROCAL CROSSES SIMILAR IN SIZE)

Cross	Sex	$LD_{50} \pm SE$ (μg diazinon/fly)	Slope (b) ± SE
♀ SKA × ♂ *bwb*	♀	1.62 ± 0.046	4.98 ± 0.67
	♂	0.60 ± 0.023	4.72 ± 0.66
♀ *bwb* × ♂ SKA	♀	1.20 ± 0.083	4.68 ± 0.65
	♂	0.55 ± 0.047	4.59 ± 0.94
♀ SKA × ♂ *ac*	♀	1.48 ± 0.13	4.66 ± 0.76
	♂	0.45 ± 0.039	4.38 ± 0.66
♀ *ac* × ♂ SKA	♀	1.41 ± 0.084	6.21 ± 0.89
	♂[a]	0.42 ± 0.12	5.21 ± 0.76
♀ SKA × ♂ *ocra*	♀	1.55 ± 0.089	6.63 ± 0.99
	♂	0.79 ± 0.053	4.85 ± 0.65
♀ *ocra* × ♂ SKA	♀	1.70 ± 0.11	5.11 ± 0.69
	♂	0.60 ± 0.039	5.56 ± 0.82
♀ SKA × ♂ *ar*	♀	2.34 ± 0.14	5.87 ± 0.82
	♂	0.78 ± 0.059	3.87 ± 0.46
♀ *ar* × ♂ SKA	♀	0.70 ± 0.12	4.33 ± 0.59
	♂	0.69 ± 0.049	4.66 ± 0.61

[a] χ^2 for heterogeneity significant at P = 0.05.

TABLE 6B

RESULTS OF BIOASSAYS WITH F_1 FLIES OF CROSSES ♀ SKA × ♂ MARKER AND RECIPROCALS (FLIES WITHIN EACH PAIR OF RECIPROCAL CROSSES DIFFERENT IN SIZE)

Cross	Sex	$LD_{50} \pm SE$ (μg diazinon/fly)	Slope (b) ± SE
♀ SKA × ♂ *ocra*	♀	1.51 ± 0.10	4.52 ± 0.57
	♂	1.07 ± 0.15	4.91 ± 1.43
♀ *ocra* × ♂ SKA[a]	♀[b]	0.78 ± 0.063	4.85 ± 0.78
	♂	0.43 ± 0.040	3.93 ± 0.63
♀ SKA × ♂ *ar*	♀	1.55 ± 0.15	4.62 ± 0.73
	♂	0.83 ± 0.048	6.89 ± 1.08
♀ *ar* × ♂ SKA[c]	♀	3.72 ± 0.29	5.55 ± 0.96
	♂	1.18 ± 0.084	6.60 ± 1.20

[a] Flies very small.
[b] χ^2 for heterogeneity significant at P = 0.05.
[c] Flies very large.

cessive markers. Except for the F_1 ♀ *bwb* × ♂ SKA and its reciprocal, which were crossed at different times, the progeny of reciprocal crosses were tested simultaneously to determine whether their ld-p lines[1] were similar. When they differed the tests were repeated. The ld-p lines and LD_{50} values within pairs of F_1 progeny from reciprocal crosses differed relatively little when the flies were of about the same size (Table 6A), but where they differed in size, the LD_{50} values were greatest in the progeny where the flies were biggest and this was independent of the direction of the cross (Table 6B). Weighted mean LD_{50} values and slopes were calculated for the SKA, F_1 and SRS flies (Table 9) to eliminate the variations between individual tests (Tables 6-8). The differences between the LD_{50} values of the reciprocal F_1 crosses were relatively small (Table 6A); a twofold difference between the lowest and highest values is to be expected in bioassays with houseflies. For this reason, weighted mean LD_{50} values and slopes were calculated for all tests on F_1 flies (Table 9).

TABLE 7
RESULTS OF BIOASSAYS WITH SKA FLIES

Generation	Sex	$LD_{50} \pm SE$ (μg diazinon/fly)	Slope (b) ± SE
F_1	♀[a]	11.48 ± 1.58	2.64 ± 0.53
	♂	4.27 ± 0.38	4.24 ± 0.69
F_2	♀	8.71 ± 0.68	3.78 ± 0.49
	♂	5.62 ± 0.37	5.96 ± 1.13
F_3	♀	14.79 ± 1.12	4.94 ± 0.86
	♂	2.04 ± 0.12	6.34 ± 0.96
F_3	♀	21.38 ± 3.34	3.06 ± 0.65
	♂	3.02 ± 0.20	5.04 ± 0.76
F_5	♀	9.12 ± 0.61	4.77 ± 0.63
	♂	2.63 ± 0.14	6.72 ± 0.99
F_5	♀	16.60 ± 1.72	3.64 ± 0.69
	♂	2.34 ± 0.20	4.12 ± 0.77

[a] χ^2 for heterogeneity significant at P = 0.05.

The weighted means (Fig. 1) show that ld-p lines of SRS and F_1 flies were completely separate, whereas those of F_1 and SKA flies slightly overlapped, mainly because in some tests the ld-p lines of SKA flies had a short "tail" below 10% kill. The heterozygotes were less resistant than the resistant parent strain, but close enough to allow overlapping between the most resistant F_1 flies and the least resistant SKA flies.

TABLE 8
RESULTS OF BIOASSAYS WITH SRS FLIES

Generation	Sex	$LD_{50} \pm SE$ (μg diazinon/fly)	Slope (b) ± SE
49	♀	0.031 ± 0.0019	9.26 ± 1.98
	♂	0.017 ± 0.00082	8.68 ± 1.48
50	♀	0.027 ± 0.0014	8.62 ± 1.45
	♂	0.013 ± 0.00078	6.21 ± 0.91
50	♀	0.034 ± 0.0019	9.02 ± 2.23
	♂	0.020 ± 0.0017	5.56 ± 0.86
51	♀[a]	0.017 ± 0.0094	7.24 ± 1.19
	♂	0.019 ± 0.0096	9.04 ± 1.52

[a] χ^2 for heterogeneity significant at P = 0.05.

EFFECT OF FOOD ON TOLERANCE OF DIAZINON BY HOUSEFLIES

Table 9 shows that the resistance indices differed in the two sexes, both in the F_1 and in the SKA strain. This apparently disagrees with occasional results obtained from bioassays on F_1 reciprocal crosses, which showed that there is no sexual difference in tolerance. There is now evidence suggesting that this discrepancy was caused by the absence of milk in the diet of the F_1 and SKA males. Because milk seems to make SRS males more resistant to diazinon, the resistance indices of the two sexes are not comparable.

SKA males also probably became considerably more susceptible to diazinon between the second and the fourth day after emergence, i.e., between sexing and bioassay (compare Tables 2 and 9), because they were deprived of milk. When given milk the SKA males remained resistant over the same period (Sawicki & Green, 1964). Therefore, the large difference of LD_{50} between the two sexes in the SKA strains (Table 9) could be explained by the lack of milk in the diet of the SKA males.

TEST-CROSSES

Fig. 2 and 3 show the ld-p lines of each phenotype segregating in the test-cross ♀ marker × ♂ F_1 (♀ marker × ♂ SKA) or its F_1 reciprocal.

[1] Log-dosage/probit regression line.

TABLE 9

WEIGHTED MEAN LD_{50} OF SRS, SKA AND F_1 FLIES [a]

Fly strain	Sex	Weighted mean $LD_{50} \pm SE$ (μg diazinon/fly)	Weighted mean slope (b) $\pm$ SE	Resistance index	Sex ratio of LD_{50} (♀/♂)
SRS	♀	0.028 ± 0.00090	8.17 ± 0.92	1.0	1.75
	♂	0.016 ± 0.000022	6.64 ± 0.54	1.0	
♀ SKA × ♂ marker	♀	1.64 ± 0.053	4.91 ± 0.36	59	2.38
	♂	0.69 ± 0.029	4.62 ± 0.29	43	
♀ marker × ♂ SKA	♀	1.48 ± 0.057	4.89 ± 0.34	53	2.69
	♂	0.55 ± 0.033	4.97 ± 0.64	34	
F_1 [b]	♀	1.58 ± 0.31	4.90 ± 0.25	56	2.43
	♂	0.65 ± 0.15	4.95 ± 0.19	41	
SKA	♀	12.74 ± 0.062	3.66 ± 0.26	455	4.26
	♂	2.99 ± 0.083	5.09 ± 0.34	187	

[a] Calculated from results given in Tables 6a, 7 and 8.
[b] Combined results of the two reciprocal crosses.

FIG. 1

WEIGHTED MEAN LD-P (LOG-DOSAGE/PROBIT) LINES OF SRS, F_1 AND SKA FLIES TREATED WITH DIAZINON

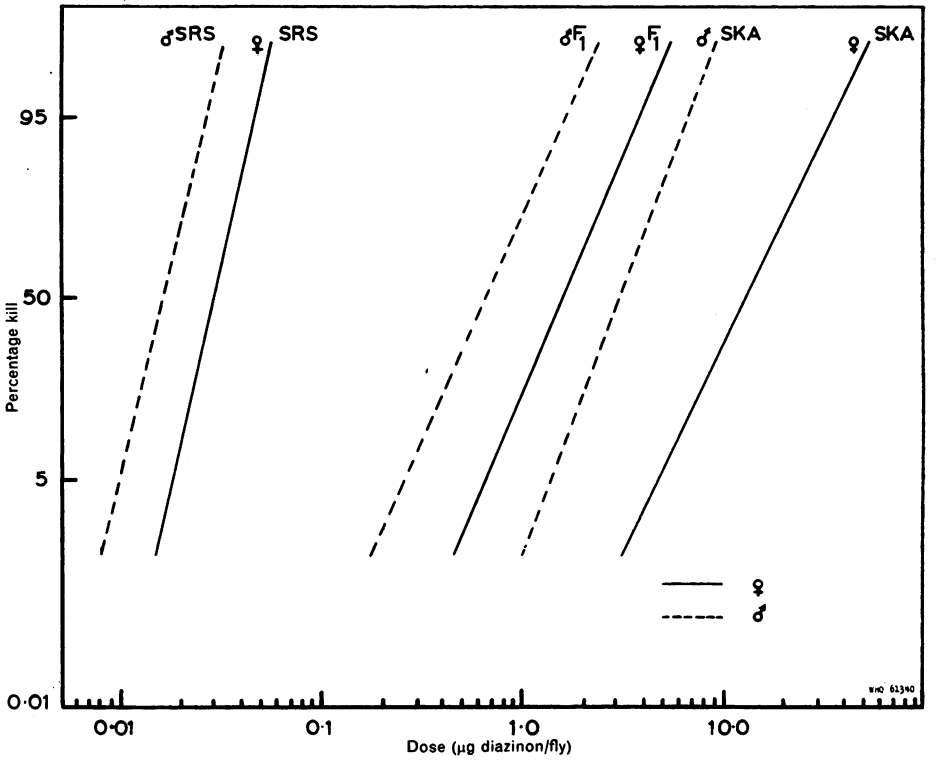
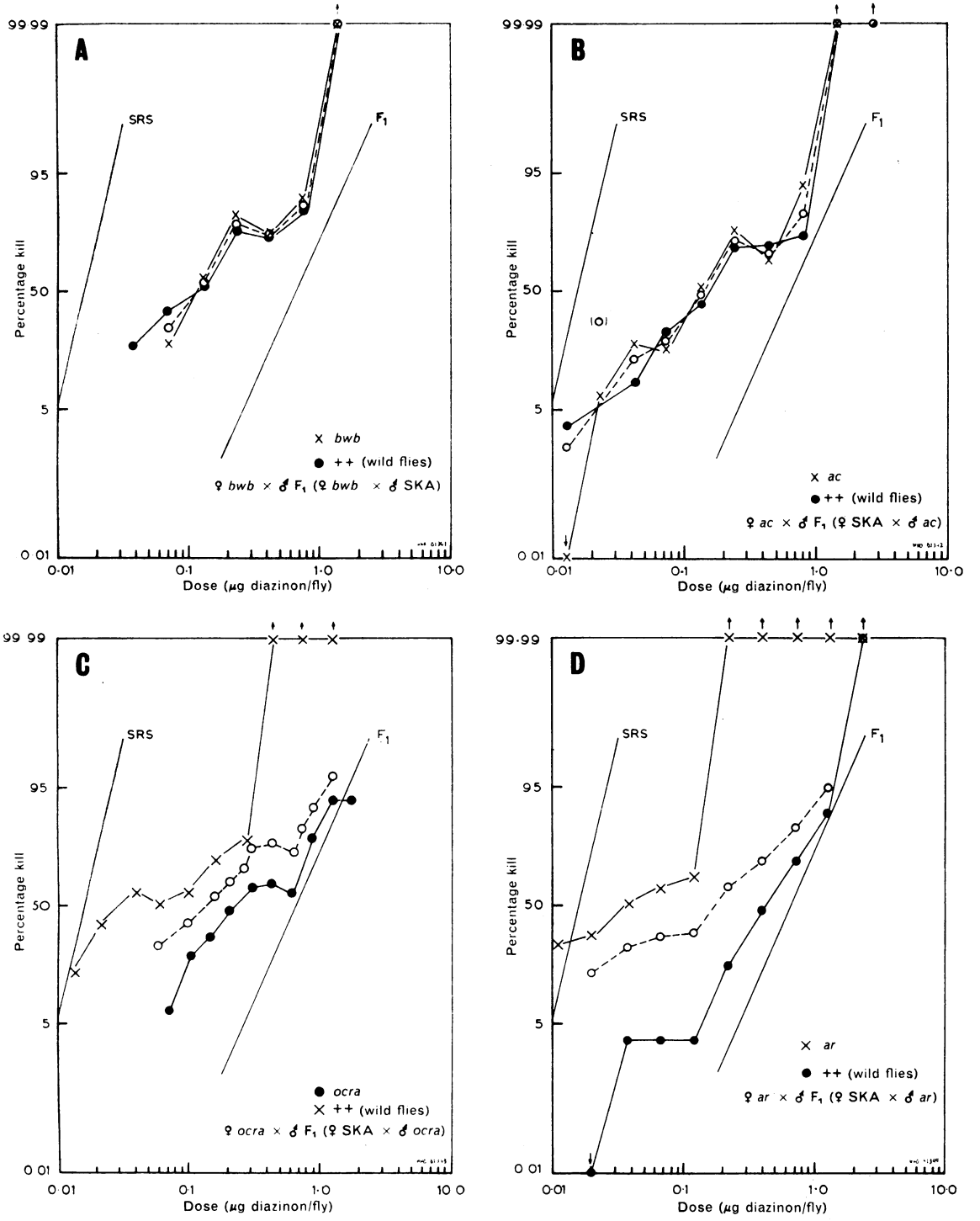


FIG. 2

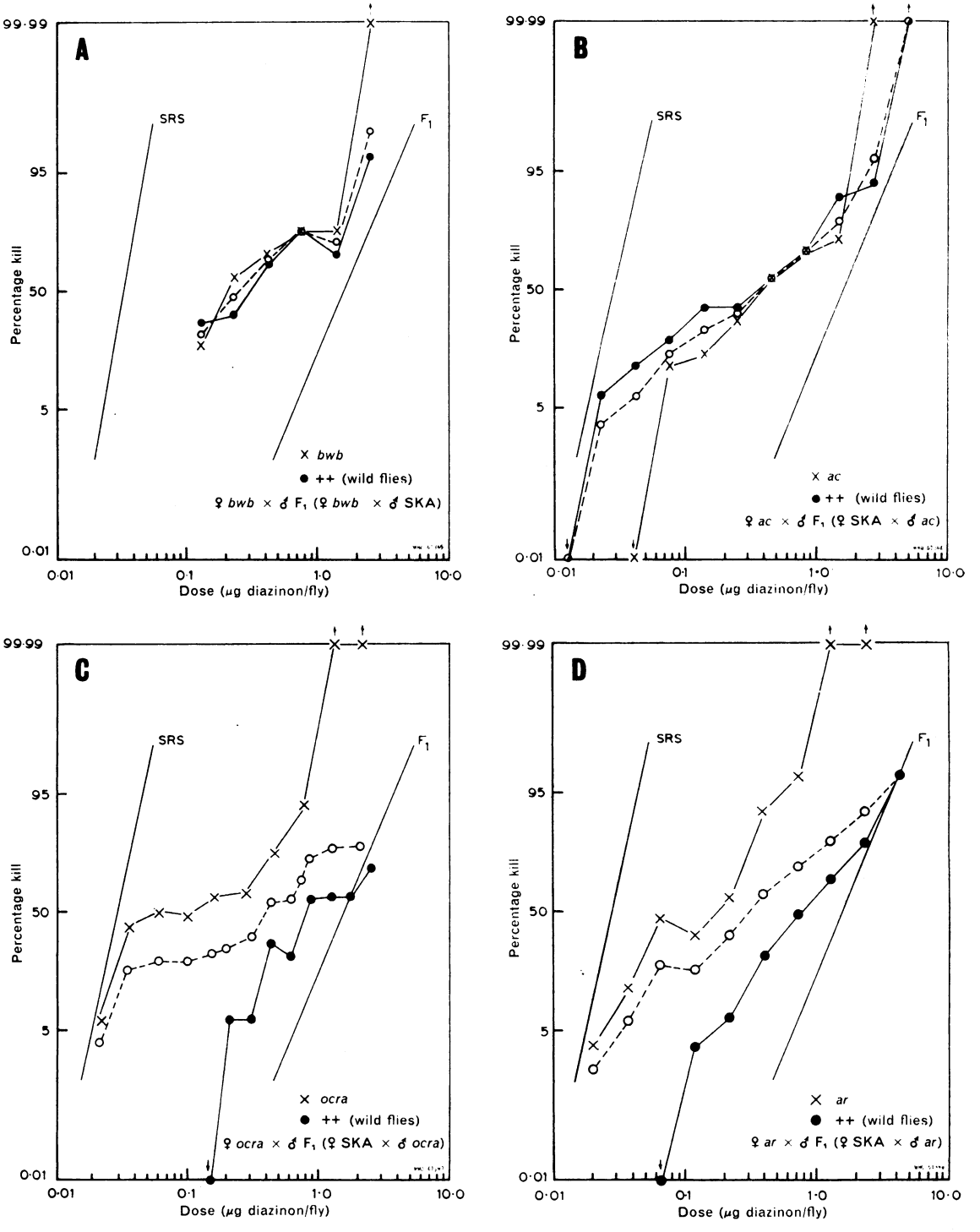
LD-P LINES OF FEMALE PROGENY OF TEST-CROSSES ♀ MARKER × ♂ F_1 (♀ MARKER × ♂ SKA) [a]



[a] **Dotted** line connecting white circles represents theoretical mean kill of the two phenotypes (marker and normal flies) at 1:1 ratio.

FIG. 3

LD-P LINES OF MALE PROGENY OF TEST-CROSSES ♀ MARKER × ♂ F_1 (♀ MARKER × ♂ SKA) [a]



[a] Dotted line connecting white circles represents theoretical mean kill of the two phenotypes (marker and normal flies) at 1:1 ratio.

(*i*) ♀ *bwb* × ♂ F_1 (♀ *bwb* × ♂ SKA)

Because the two test-crosses were rather infertile and the insects too few to provide adequate ld-p lines, the flies of the ♀ *bwb* × ♂ F_1 (♀ SKA × ♂ *bwb*) test-cross were used to obtain the useful range of concentrations with 15 or 20 flies per concentration, and the flies of the other test-cross were used to confirm the preliminary results using two replicates of 15 flies per concentration. The insects being too few, the ld-p lines covered a kill range from 100% to 20% only (Fig. 2 and 3). The ld-p lines of the two phenotypes in both sexes were similar.

(*ii*) ♀ *ac* × ♂ F_1 (♀ SKA × ♂ *ac*)

Only the progeny of the test-cross ♀ *ac* × ♂ F_1 (♀ SKA × ♂ *ac*) were tested because there were too few adults in the other test-cross. The differences between the ld-p lines of both phenotypes in the two sexes were small (Fig. 2B and 3B).

The results of the bioassays on flies from the *bwb* and *ac* test-crosses were similar. The kill-range of the females is represented by shallow ld-p lines, which cover the full range of doses affecting SRS and F_1 flies. The two inflexions at LD_{20}-LD_{25} and LD_{80} (Fig. 2A and 2B) correspond to doses killing, respectively, all SRS females and 50% of the F_1 females. The kills in males (Fig. 3A and 3B) were alike; there was a distinct plateau at about LD_{75} and some 50% of the males had tolerance levels intermediate between those of the SRS and F_1 males.

The responses of the mutant and wild-type flies of the progeny of these two test-crosses were almost identical. In both tests the position and shape of the ld-p lines of the two phenotypes were the same, and about 50% of both phenotypes had tolerance levels intermediate between those of the SRS and F_1 flies.

(*iii*) ♀ *ocra* × ♂ F_1 (♀ SKA × ♂ *ocra*)
♀ *ocra* × ♂ F_1 (♀ *ocra* × ♂ SKA)

Both phenotypes gave compound ld-p lines, each with a plateau at about LD_{50}, but the ld-p lines of each phenotype were at different dose ranges and the marker flies were more susceptible than the wild phenotypes (Fig. 2C and 3C).

(*iv*) ♀ *ar* × ♂ F_1 (♀ SKA × ♂ *ar*)
♀ *ar* × ♂ F_1 (♀ *ar* × ♂ SKA)

The ld-p lines of both phenotypes differed in dose range and shape (Fig. 2D and 3D). The marker flies were more susceptible than the flies of the wild phenotype.

The inflexions in the ld-p lines of the test-crosses with *ocra* and *ar* indicate that the flies of the mutant and normal phenotypes differed in their range of tolerances to diazinon. Each phenotypic class was compound in regard to susceptibility or tolerance to diazinon. Fully susceptible flies occurred only among the mutants; about half of the mutants of both sexes were as susceptible as the susceptible homozygous parent, and the tolerance levels of the other half reached only the LD_{50} of the F_1 hybrids. In contrast, flies of the normal phenotype had a range of tolerances only slightly wider than that of F_1, but clearly differed from F_1 by being more susceptible to smaller doses and by having a slight plateau at about LD_{50} to LD_{60}.

Genetic factors gave *ocra* and *ar* flies tolerance levels that were equal to the tolerance levels of only the most susceptible halves of the population of flies of the normal phenotype and the F_1 hybrids, whereas half the flies of the normal phenotype reached the greatest resistance of the F_1 hybrids.

The bioassays on the progeny of the four test-crosses show, therefore, that two of the chromosomes of SKA origin—namely, those carrying the genes bwb^+ and ac^+—can be fully replaced by their homologues from the susceptible strains without affecting the response to diazinon, whereas the two chromosomes of SKA origin that carry the normal alleles $ocra^+$ and ar^+ participate in the control of resistance and have similar quantitative and cumulative effects when heterozygous. Thus the test-crosses with susceptible marker flies gave three levels of tolerance—namely, 25% homozygous susceptible, 50% single heterozygous, and 25% double heterozygous, the last ones being like the F_1 flies. In the test-crosses with the markers *ocra* and *ar*, only half the mutants can be heterozygous for the factor(s) on the unmarked chromosomes, and thus are either susceptible or only slightly tolerant, whereas the normal flies must be either single or double heterozygotes, and are therefore either slightly tolerant or as tolerant as F_1 hybrids.

DISCUSSION

Four of the five autosomes were analysed for dominant or incompletely dominant factors of resistance to diazinon in the SKA strain. The plan used made it possible to recognize by the inspection of the progeny of the test-crosses the flies that received one of the two homologous chromosomes of a given pair from the SKA strain, i.e., flies of the normal phenotype, and flies that received both members of the

marked pair from the susceptible strain, i.e., flies of the mutant type. The two phenotypes can respond to the insecticide either in the same way or differently. When the tolerance levels of both phenotypes are similar, the investigated chromosome has no non-recessive resistance factors, but when the two phenotypes differ in their response to the insecticide, a linkage between the marker gene and the dominant resistance factor(s) is most likely, and the mutant phenotype should tend in susceptibility towards the marker stock, whereas the wild phenotype should tend towards F_1.

In the progeny of the test-crosses in which *bwb* and *ac* were used as markers for the II and III linkage-groups, respectively, the tolerances of the two phenotypes were similar and covered the whole tolerance range of the SRS and F_1 flies. There were therefore no dominant resistance factors on these linkage-groups. The ld-p lines showed heterogeneity in the distribution of resistance levels, indicating discontinuous levels of tolerance, unrelated to the recessive factors used as genetical markers, and the results of the two test-crosses agreed closely.

In the progeny of the test-crosses in which *ocra* and *ar* were used as markers for the IV and V linkage-groups, respectively, most of the mutant flies were more susceptible than their normal sibs, and the two phenotypic classes covered different parts of the full tolerance levels of SRS and F_1; half the mutants had the same tolerance as SRS flies and half the tolerance of the more susceptible half of F_1. The normal flies did not include any as susceptible as SRS flies and their tolerance range was slightly wider than the range of the F_1 hybrids, especially at the smaller dose range. The mutant flies therefore showed very clearly the presence of two toxicological levels (susceptible and slightly resistant), but a similar condition was hinted at, rather than shown, by the ld-p lines of the normal flies. This, and the good agreement between the results of the two test-crosses, suggests that there is only a small difference between single and double heterozygotes for resistance.

The evidence therefore indicates that the II and III linkage-groups do not have dominant or incompletely dominant factors of resistance, but that such factors are on the IV and V linkage-groups. *Ocra* and *ar* each segregate in opposition to one of two genetically independent incompletely dominant factors (or groups of factors) for resistance. Because resistance occurred in the heterozygous condition in only half of the mutant phenotype, which was homozygous for one specific chromosome derived from the susceptible strain, the resistance factor of flies of the mutant phenotype was located on the unmarked linkage-group. Thus resistance in *ocra* flies was caused by the factor segregating in opposition to *ar* and, inversely, resistance in *ar* flies was caused by the factor segregating in opposition to *ocra*. The wild phenotype progeny of both test-crosses were either heterozygous for the resistance factor on the linkage-group tested in the given cross or heterozygous for the resistance factors of both linkage-groups, i.e., of the same genotype as F_1. It is unlikely that there are dominant resistance factors on the VI linkage-group, because the discontinuity in the ld-p lines of *ocra* occurred at LD_{50} and there were only two inflexions, at LD_{25} and LD_{80}, in the ld-p lines of the test-crosses *bwb* and *ac*.

It seems, therefore, that there are only two partly dominant factors (or sets of factors) for resistance that confer individually a similar and rather small resistance to diazinon when heterozygous, and are partly cumulative in the double heterozygote. The results of the bioassays on F_1 reciprocal crosses indicate neither sex-linked nor cytoplasmic inheritance. However, diet and the size of the flies may strongly affect the response of the flies to diazinon.

The SKA strain was bred from two diazinon-resistant strains (Saccà a and Keiding 203a), which probably differ in their genetics of resistance. Nguy & Busvine (1960) suggest that resistance to parathion in the Saccà a strain is caused by a single, almost completely dominant gene; however, other factors may be present (Oppenoorth & van Asperen, 1961). The main factor, gene *a*, is on the V linkage-group (Franco & Oppenoorth, 1962; Hoyer, Plapp & Orchad, 1965). In the 203a strain (strain F of Oppenoorth), resistance to organophosphorus insecticides is partly caused by gene *a* and a factor on the IV linkage-group (Oppenoorth, 1965). Therefore, the SKA strain should have gene *a* from both Saccà a and Keiding 203a and additional factors from 203a. The need for continuous selection to keep the resistance of the SKA strain at its maximum and the occasional presence of a " tail " at the lower dose range indicate that the strain is still genetically heterogeneous and that the factors for resistance are cumulative.

Our results agree with the expectation that factors for resistance to diazinon would be found on the V linkage-group, as in both the parental strains, and on the IV linkage-group, as in the 203a strain. However, the SKA strain of mixed origin, and continuously selected, differs from the 203a strain by being

considerably more resistant, and further work is needed to establish whether there are additional non-dominant factors and also the role played by each factor (or sets of factors) and their effects when homozygous.

The F_2 generation was not bioassayed, because the results would have added little to those obtained for the test-crosses and did not justify the large amount of work that would have been involved. The nature of the resistance factors is not known, but the resistance factor of the V chromosome is most likely gene *a*, because the SKA strain, like most other organophosphorus-resistant strains, has little ali-esterase activity (Laudani & Grigolo, unpublished).

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RÉSUMÉ

La présente analyse génétique, portant sur quatre des cinq autosomes de la souche SKA de mouches domestiques (*Musca domestica* L.) résistantes au diazinon, visait à localiser les facteurs dominants ou incomplètement dominants de cette résistance. La souche SKA a été croisée avec quatre souches sensibles, chacune porteuse d'un marqueur génétique de caractère visible et récessif appartenant à un groupe spécifique de linkage. Grâce à ces caractères visibles, on a pu reconnaître dans les descendances des *test-crosses* les mouches qui avaient reçu un des deux chromosomes d'une paire donnée de la souche SKA, constituant le phénotype normal, de celles qui avaient reçu les deux membres de la paire marquée de la souche sensible, ou type mutant. Seules les mouches porteuses de marqueurs appartenant aux groupes de linkage IV et V avaient une sensibilité au diazinon différente du phénotype normal, démontrant ainsi que les facteurs non récessifs de résistance sont présents seulement sur ces deux groupes de linkage.

Le facteur de résistance du groupe de linkage V est vraisemblablement le gène *a*, responsable de la résistance et de la faible activité ali-estérasique dans la plupart des souches résistantes aux organo-phosphorés.

La souche SKA, obtenue à partir de deux souches résistantes au diazinon, ressemble génétiquement à ses parents mais en diffère par sa résistance plus importante. La nécessité d'une sélection continuelle pour maintenir cette résistance à un degré maximal indique que cette souche est encore génétiquement hétérogène et que les facteurs de résistance sont cumulatifs.

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