# INHERITANCE OF DDT-RESISTANCE INVOLVING THE Y-CHROMOSOME IN THE HOUSEFLY (MUSCA DOMESTICA L.)

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#### Summary

Selection for early maturation applied to a laboratory colony of Muscadomestica L. eliminated autosomally controlled DDT-resistance from both sexes, but a proportion of the males exhibited a genetically new type of resistance which was shown to be not transmitted through the females but to involve the Y-chromosome. By a single selection with DDT, applied to males only, the early-maturing strain was separated into two true-breeding strains homogeneous in both sexes with respect to DDT-tolerances, the one susceptible to DDT in both males and females, the other susceptible in females but showing at least an eightfold resistance to DDT in all its males.

# I. INTRODUCTION

Male insects have, in general, been found to be somewhat more susceptible to toxic substances than females of the same species. Of 59 comparisons between the sexes, reviewed by Busvine (1957), Brown (1958), and Nagasawa (1955), all but two show higher tolerances in the females to stomach or contact poisons. Even when body weight differences have been taken into account, there still remains in many cases a margin of higher resistance in the females. For example, females of *Drosophila* melanogaster Mg. were found to be 1.86 times as resistant as males to DDT applied topically in kerosene solution (Kerr 1954b). Correction for body weight reduced the ratio to 1.17 which, however, was still highly significant (P < 0.01).

Similar results have been obtained with a laboratory colony of the housefly, *Musca domestica* L., previously described by Kerr *et al.* (1957). In this the ratios of the  $LD_{50}$ 's (already corrected for body weight) for females and males ranged from 1.08 to 1.79 for DDT (mean of 22 tests, 1.33), 0.96 to 1.78 for gamma-BHC (mean of 26 tests, 1.34), 1.24 to 1.71 for allethrin (mean of 7 tests, 1.53), and 1.16 to 1.58 for diazinon (mean of 7 tests, 1.37). Two DDT-resistant strains (Kerr *et al.* 1957) derived from this colony by selection with DDT (strain D) or by selection for late maturation (strain L) showed ratios ranging from 1.14 to 2.51 (mean of 28 tests, 1.52) and 1.25 to 2.13 (mean of 6 tests, 1.61) respectively for DDT. Thus the increase in resistance resulting from selection by these two methods accentuated the difference between the sexes. All of the 56 tests with DDT showed higher tolerances in females than in males. In only one of the total of 96 determinations (a BHC test) was the ratio below unity.

This paper describes the selection, from the same colony, of a strain in which the males are approximately 8 times as resistant to DDT as the females, and the investigation of the mechanism of inheritance of this DDT resistance. As outlined

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in a previous brief account (Kerr 1960), the abnormally resistant males were discovered in a strain, designated as E, which was being selected for early maturation (i.e. rapid pre-adult development) by mass-rearing methods in the hope that this would produce a homogeneously DDT-susceptible strain.\* Selection was applied to six successive generations by breeding only from eggs laid by flies that emerged during the first 2 days of the emergence period (peak emergence usually occurring on the 4th day). It achieved the desired result in the case of females, which thereafter were homogeneously susceptible for as long as the selection for early maturation was maintained. But in the males there remained a high proportion which survived doses of DDT that killed all females.

#### II. MATERIALS AND METHODS

All rearing and testing were carried out in an air-conditioned laboratory maintained at  $78(\pm 2)^{\circ}$ F. The houseflies were reared by standardized procedures which followed closely those specified in the Peet–Grady method (Anon. 1943).

Puparia were collected from the cultures on the 10th day and, during emergence of the adults, were removed daily to a new cage, thus ensuring that the variation in age was not greater than 24 hr in any cage of test flies. The adults were fed on a 5% suspension of full-cream milk powder in water to which they had access continuously before testing.

Insecticide tests were carried out usually on flies from the maximum-emergence cage when they were 5–6 days old. Twenty flies of one sex were collected in a 4 by 1 in. vial containing carbon dioxide, and while still anaesthetized were weighed as a batch, and each dosed individually on the mesonotum with a volume of insecticide solution proportional to the batch weight. Each batch of 20 treated flies was transferred to a 6 by  $1\frac{1}{4}$  in. vial provided with a cotton-wool pad moistened with 10% sucrose solution and closed with a loose cotton-wool plug. Mortalities were determined 24 hr after treatment.

For obtaining dosage-mortality data a geometric series of test solutions of DDT (pp'-isomer) in odourless kerosene was prepared. The common ratio of the series was chosen so that not less than five nor more than seven successive concentrations spanned the mortality range. At each concentration the insecticide was applied to at least 40 flies (two batches) of the one sex by means of an optically graduated micropipette (Kerr 1951, 1954*a*). The volumes applied were of the order of 0.08 and  $0.13 \,\mu$ l for males and females respectively. These amounts of kerosene alone were practically harmless. In a series of 26 tests the highest mortality recorded for kerosene-treated males was 5%, that for females 7.5%. Mean mortalities were 1.93 and 2.02% respectively; thus, on the average, less than 1 fly in 40 could be reckoned as dying from causes other than the insecticide. In many tests the lowest insecticide dosage killed no flies, so that no correction for control mortality was required. When deaths occurred in controls, the observed mortalities for the insecticide dosages were adjusted accordingly by "Abbott's formula".

\* This was a reasonable expectation because selection in the opposite direction (i.e. for late maturation) had already given rise to a DDT-resistant strain (Kerr *et al.* 1957).

were then transformed to probits (Bliss 1935) and plotted against log dosage. Within the limits of sampling error, the probit values were usually arrayed linearly with respect to log dosage. These transformed data were analysed as outlined by Finney (1952) to give (1) the equation for the best-fitting regression line (hereafter called the ld-p line, this being a convenient abbreviation for log dosage-probit line suggested by Hoskins and Gordon (1956)); (2) the variance of the slope of this line; (3) the LD<sub>50</sub> and its fiducial limits at 95% probability; and (4) the  $\chi^2$  value for goodness of fit of the line to the data (which value indicates whether the treated flies were homogeneous or heterogeneous in their individual tolerances).

#### III. RESULTS

## (a) Composition of Original Colony

The original unselected colony is characterized by dosage-mortality curves of the type shown in Figure 1. Flattening of the curves in the region of high dosage indicates the presence of abnormally resistant individuals which in this particular generation comprised 17 and 19% of the male and female populations respectively. When these resistant flies are disregarded, the mortalities so adjusted are linearly arranged with respect to log dosage. The lines drawn through them are the ld-p lines fitted by the usual maximum-likelihood calculations. In both sexes the departure of the adjusted experimental points from their line is not significant ( $\chi^2_{(3)} = 0.97$  and 0.92 for males and females respectively), so that the data strongly indicate the susceptible flies of the population to be homogeneous in their DDT-tolerances. The equations for the lines are:

 $Y = -0.90 + 7.00 \ (\pm 1.05)x$  for males,

$$Y = -2 \cdot 41 + 7 \cdot 62 \ (\pm 1 \cdot 29)x$$
 for females,

where Y = mortality in probits and  $x = \log \text{ dosage of DDT}$  (dosage being expressed as  $\mu g/g$  of flies). In slope the lines are not significantly different at the 5% probability level ( $\chi^2_{(1)} = 0.14$ ), and the LD<sub>50</sub> for females is 1.35 times that for males, a ratio consistent with the mean value of 1.33 for 22 tests with DDT referred to earlier.

Thus the starting point in this selection experiment was the unselected colony as it stood in 1954, consisting, in both sexes, of a mixture of DDT-resistant flies and homogeneously susceptible flies.

## (b) Selection for Early Maturation

After six generations of selection for early maturation, DDT-resistant females were no longer detected. The relationship between dosage and mortality at this stage is given in Table 1. In females mortality increased to 100% with increasing dosage in a manner which indicated them to be homogeneous in their DDT-tolerances. The males of generation 6, however, were heterogeneous, about 12% of them being resistant to DDT.



Fig. 1.—Dosage-mortality curves for flies of the unselected colony (U) topically dosed with DDT. The curved lines flattening at 83 and 81% mortality of males and females respectively were fitted to the observed mortalities. The straight lines are the ld-p lines calculated for the 83 and 81% of the tested flies that were non-resistant. × Observed mortality (40 flies per point). • Mortality adjusted for 17% of resistant males or 19% of resistant females. Upward pointing and downward pointing arrows indicate 100 and 0% mortality respectively.

A year later the strain (E) was essentially the same in composition, as shown by the results obtained with generation 23 (Table 2), except that the resistant fraction in the males had increased to about 24%.

		TABLE 1						
DOSAGE-MORTALITY RELATIONS	SHIPS FOR FI	IES OF STR.	AIN E GENI	ERATION 6	TREATED W	ITH DDT		
40 flies per dosage								
Females*								
Dosage ( $\mu g/g$ of flies)	$4 \cdot 4$	<b>6</b> · 0	$8 \cdot 3$	$11 \cdot 5$	$15 \cdot 9$	$22 \cdot 0$		
Mortality (%)	$7 \cdot 5$	$25 \cdot 0$	$50 \cdot 0$	80.0	$92 \cdot 5$	100		
Males						· · ·		
Dosage ( $\mu g/g$ of flies)	$3 \cdot 1$	4 · 4	<b>6</b> · 0	8.3	$11 \cdot 5$	$15 \cdot 9$		
Mortality (%)	$22 \cdot 5$	$50 \cdot 0$	$72 \cdot 5$	$67 \cdot 5$	87.5	87.5		

\* Regression equation:  $Y = 0.10 + 5.38 \ (\pm 0.56)x$   $(\chi^2_{(4)} = 0.51)$ , where Y = mortalityin probits and  $x = \log$  dosage of DDT (dosage being expressed as  $\mu g/g$  of flies).

The failure to detect any resistant females when such an obvious proportion of resistant males existed was tentatively ascribed to sampling error and the primary aim to obtain a homogeneously susceptible strain by selection for early maturation

DOSAGE-MORTALITY REI	LATIONS	HIPS FOR F	LIES OF STR	AIN E GEN	ERATION 23	TREATED	WITH DDT		
40 flies per dosage									
Females*									
Dosage ( $\mu g/g$ of flies)	$2 \cdot 75$	$4 \cdot 05$	$5 \cdot 60$	$7 \cdot 40$	$10 \cdot 55$	14.75	$20 \cdot 10$		
Mortality (%)	$2 \cdot 5$	10.0	$17 \cdot 5$	$37 \cdot 5$	77 • 5	$97 \cdot 5$	100		
Males				· · · · · · · · · · · · · · · · · · ·					
Dosage ( $\mu g/g$ of flies)	$2 \cdot 15$	$2 \cdot 90$	$4 \cdot 15$	$5 \cdot 70$	$8 \cdot 25$	$10 \cdot 20$	$14 \cdot 45$		
Mortality (%)	$2 \cdot 5$	$7 \cdot 5$	$22 \cdot 5$	$25 \cdot 0$	<b>6</b> 0 · 0	$77 \cdot 5$	<b>75 · 0</b>		

TABLE 2

\* Regression equation:  $Y = 0.11 + 5.52 \ (\pm 0.57)x$   $(\chi^2_{(5)} = 6.04)$ , where Y = mortalityin probits and  $x = \log$  dosage of DDT (dosage being expressed as  $\mu g/g$  of flies).

was deemed to have been unsuccessful. Selection was discontinued at this stage and no further tests done until generation 46 (Table 3). The reappearance of a small proportion of resistant females and a diminution of the resistant fraction in the males to about 12% seemed to confirm this opinion.

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However, the experiment was repeated, with selection for early maturation re-applied from generation 68. Once again after six generations the females showed no indication of resistance (Fig. 2), and the males proved to be a mixture of DDT-resistant and normally susceptible types, this time in the proportion of about 3:1.

TABLE 3

40 flies per dosage									
Females									
Dosage $(\mu g/g \text{ of flies})$	$2 \cdot 8$	$4 \cdot 0$	$5 \cdot 6$	$8 \cdot 0$	$11 \cdot 2$	$16 \cdot 0$	$22 \cdot 4$		
Mortality (%)	$2 \cdot 5$	$42 \cdot 5$	<b>80</b> · 0	$95 \cdot 0$	$97 \cdot 5$	$92 \cdot 5$	$97 \cdot 5$		
Males									
Dosage ( $\mu$ g/g of flies)	$2 \cdot 0$	$2 \cdot 8$	$4 \cdot 0$	$5 \cdot 6$	$8 \cdot 0$	$11 \cdot 2$	<b>16</b> ·0		
Mortality (%)	$35 \cdot 0$	$55 \cdot 0$	$82 \cdot 5$	$92 \cdot 5$	80.0	$95 \cdot 0$	87 · 5		

The part played by cuticular penetration in the resistance of the males was examined at this stage by bypassing the cuticle and injecting DDT in peanut oil into the flight muscles at an average dosage of 20  $\mu$ g DDT/g of flies. Forty males each received 0.12  $\mu$ l, and 40 females 0.20  $\mu$ l of the test solution. All the females died,



Fig. 2.—Dosage-mortality curves for females and males of strain E generation 74 topically dosed with DDT. × Observed mortality (40 females per point). ● Observed mortality (40 males per point). Upward pointing arrow indicates 100% mortality.

but 85% of the males survived without developing symptoms of DDT poisoning. Thus the resistance of the males was an internal phenomenon and not simply due to exclusion or restriction of entry of DDT by the cuticle. The distinct flattening of the mortality curve (Fig. 2) for males at about 20% mortality and extending over several dosage increments pointed to the possibility of separating the two types of males by means of selection with differentiating doses of DDT.

### (c) Selection for DDT-resistance and DDT-susceptibility in Males

In the following generation (75) the sexes were separated before feeding and within 24 hr of emergence, thus ensuring the virginity of the females. The males were treated when 4–6 days old, in batches of 20, at a dosage of  $32 \mu g$  DDT/g of flies, each male thus receiving almost three times as much DDT (in proportion to body weight) as the amount which had proved lethal to all females of the strain. The total number dosed was 955 of which 727 (76%) survived. About half of these survivors were mated *en masse* with a similar number of the virgin females and a new strain, EY, started with the eggs laid.

A sample of 425 of the resulting adult males (EY generation 1) was tested in the same way. Survival was  $95 \cdot 5\%$ . The mortality of  $4 \cdot 5\%$  indicates that a few of the resistant males having the lowest DDT-tolerances were killed by the test dosage. A dosage lower than  $32 \,\mu g/g$  could therefore have been used for selection, but it was necessary to be certain that all non-resistant individuals were eliminated, and the loss of a few resistant males was considered to be unimportant. The test on strain E (Fig. 2) indicated that a dosage of  $11 \cdot 2 \,\mu g/g$  would probably have been sufficient. The strain was then cultured without any further selection with DDT.

In generation 76 of strain E, selection with DDT was applied in the opposite direction to give a strain ES, susceptible in males as well as in females. Sixty-one newly emerged males were collected singly and each one caged with 10 virgin females. Matings were observed to commence on the following day. On the 5th day the male from each cage was tested with DDT at a dosage of  $11 \cdot 2 \mu g/g$ . Fifty-two of them survived, and the females with which they had been caged were discarded. It was assumed that the nine males which died were DDT-susceptible, and eggs were pooled and cultured from the nine cages which had contained these males. Samples of the progeny (ES generation 1) were tested with DDT, each fly being weighed and dosed at the rate of  $11 \cdot 2 \mu g/g$  body weight. All of 184 females tested were susceptible, but of 174 males tested 3 were surviving at 24 hr without symptoms of DDT toxicosis; they also survived a second dose  $(13 \cdot 5 \ \mu g/g)$  and were therefore deemed to be resistant. Thus the first attempt to rid the strain of resistant males in a single selection failed, presumably because one (or more) of the nine male parents was a resistant one that had died during the test from causes other than DDT poisoning, and had thus been classed erroneously among the susceptibles.

In the second attempt (next generation) 32 male parents were classified correctly as DDT-susceptible. None of their progeny in a sample of 600 males and 300 females survived a dose of  $11 \cdot 2 \ \mu g$  DDT/g and similar tests on three successive generations also confirmed the absence of resistant flies from the strain. A dosage-mortality test (Table 4) carried out on generation 7 showed both sexes to be homogeneous in their DDT-tolerances which all lay within a narrow range of dosage below  $11 \cdot 2 \ \mu g/g$ .

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On the same day, strain EY was tested in a similar manner for comparison. These results are also given in Table 4. Both sexes were indicated to be homogeneous  $(\chi^2 \text{ values not significant})$  in DDT-tolerances. EY females closely resembled ES flies, their tolerances lying below  $11 \cdot 2 \mu g/g$ . The males of EY all survived a dosage of  $11 \cdot 2 \mu g/g$ , and the slope of their ld-p line was not significantly different from that of ES males, so that the ratio of the  $LD_{50}$ 's  $(32 \cdot 28/3 \cdot 33)$  for the males of the two strains may be taken as indicating that EY males were approximately 10 times as resistant

AND STRAIN EY GENERATION 8 TREATED WITH DDT								
40 flies per dosage								
ES females								
Dosage ( $\mu g/g$ of flies)	$2 \cdot 0$	$2 \cdot 8$	$4 \cdot 0$	$5 \cdot 6$	$8 \cdot 0$	$11 \cdot 2$		
Mortality (%)	$2 \cdot 5$	$37 \cdot 5$	$75 \cdot 0$	$92 \cdot 5$	100	100		
$LD_{50}$ ( $\mu g/g$ of flies)	$3 \cdot 28$ (3	$3 \cdot 28 \ (3 \cdot 00 \ \text{and} \ 3 \cdot 52)^*$						
Regression equation <sup>†</sup>	Y = 1	$28 + 7 \cdot 22$ (	$\pm 0.85$ )x	$\chi^2_{(3)} = 2 \cdot 47$				
ES males				- ///	-			
Dosage ( $\mu g/g$ of flies)	$2 \cdot 0$	$2 \cdot 8$	$4 \cdot 0$	$5 \cdot 6$	$8 \cdot 0$	$11 \cdot 2$		
Mortality (%)	$10 \cdot 0$	$32 \cdot 5$	$70 \cdot 0$	$87 \cdot 5$	100	100		
$LD_{50}$ (µg/g of flies)	$3 \cdot 33 \ (2 \cdot 97 \ \text{and} \ 3 \cdot 56)^*$							
Regression equation <sup>†</sup>	Y = 1.	$94 + 5 \cdot 87$ (	$\pm 0.70)x$	$(\chi^2_{(3)} = 1 \cdot 2)$	1)			
EY females								
Dosage ( $\mu g/g$ of flies)	$2 \cdot 0$	$2 \cdot 8$	$4 \cdot 0$	$5 \cdot 6$	$8 \cdot 0$	$11 \cdot 2$		
Mortality (%)	$2 \cdot 5$	$5 \cdot 0$	$40 \cdot 0$	$85 \cdot 0$	100	100		
$LD_{50}$ ( $\mu g/g$ of flies)	$4 \cdot 20$ (3	$4 \cdot 20 \ (3 \cdot 91 \ \text{and} \ 4 \cdot 54)^*$						
Regression equation <sup>†</sup>	Y = -	$Y = -0.05 + 8.10 \ (\pm 0.96)x  (\chi^2_{(3)} = 6.91)$						
EY males								
Dosage ( $\mu g/g$ of flies)	$11 \cdot 2$	$16 \cdot 0$	$22 \cdot 4$	$32 \cdot 0$	$44 \cdot 8$	$64 \cdot 0$		
Mortality (%)	0	10.0	$17 \cdot 5$	$45 \cdot 0$	$75 \cdot 0$	$97 \cdot 5$		
$LD_{50}$ ( $\mu g/g$ of flies)	$32 \cdot 28$ (2	$29 \cdot 44$ and $3$	$(5 \cdot 39)^*$					
Regression equation <sup>†</sup>	Y = -	$3 \cdot 03 + 5 \cdot 32$	$(\pm 0.58)$	$x (\chi^2_{(4)} = 3$	·43)			

 Table 4

 comparison of dosage-mortality relationships for flies of strain ES generation 7

 and strain EY generation 8 treated with ddt

\* Fiducial limits at 95% probability.

† Where Y = mortality in probits and  $x = \log \text{ dosage of DDT}$  (dosage being expressed as  $\mu g/g$  of flies).

as ES males to DDT applied to the mesonotum in kerosene solution. The slopes of the ld-p lines for EY females and EY males are the only ones in this whole comparison which differ significantly from each other at the 5% level ( $\chi^2_{(1)} = 6 \cdot 2$ ). Hence no simple value can be derived from this test for the relative resistance of males and females of EY, but comparisons may still be made at specified mortality levels, e.g. the ratio of the LD<sub>50</sub>'s is approximately 8.

The evidence of these tests strongly suggested that the females played no part in the transmission of this type of DDT-resistance to male progeny. Had they participated it is difficult to see how the single selections (for resistance and susceptibility) applied only to males could have led successfully to the establishment of the two true-breeding strains EY and ES. The implication was, therefore, that the DDT-resistance of EY males was determined directly by a Y-chromosome factor or indirectly, like sex in some species of *Drosophila*, by the balance between the non-homologous parts of X and Y and the rest of the karyotype. These alternative mechanisms would be rather difficult to differentiate experimentally.

### (d) Genetic Tests

Since the mechanism of inheritance of DDT-resistance indicated above was unprecedented, further evidence was sought by carrying out reciprocal crosses between the strains EY and ES. The levels of DDT-tolerance in the strains at the starting point of this test are shown in Figure 3. It is clear that the tolerances of males and females of ES and females of EY were all below  $11 \cdot 2 \mu g$  DDT/g, while those of EY males were all above it. This dosage was therefore chosen as the discriminating dose for progeny testing.

(i) Cross 1: ES Females × EY Males.—A sample of 140 females was collected from the maximum-emergence cage of ES generation 7 before feeding and within 24 hr of emergence, thus ensuring virginity. These were caged with 140 males from the corresponding cage of EY generation 8. Eggs were cultured on the 6th day, pupae collected 9 days later, and daily emergence cages of  $F_1$  adults obtained. When 4–6 days old, all normal-sized and apparently healthy males were tested individually with the discriminating dose of DDT. Of 773 tested, 745 (96.4%) survived, a result closely comparable with the 95.5% surviving in the test on EY generation 1. The mortality (3.6%) was considered too small to have any genetic significance. Most of it occurred in flies from the maximum-emergence cage which contained about 1000 flies including females. The mortalities of tested males from the earlier and later cages, which were far less crowded, were 0 and 2% respectively, figures not exceeding the normal expectation for solvent-treated controls. It was considered, therefore, that flies that died in the test had done so from causes other than DDT poisoning.

Obviously the resistance of the  $F_1$  males could not have been transmitted through the female parents since these were from strain ES, the males of which had been exclusively susceptible for 7 generations.

(ii) Cross 2: EY Females  $\times ES$  Males.—The same procedure as for cross 1 was employed except that 500 virgin EY females were mated with 500 ES males. A total of 1257 F<sub>1</sub> males tested individually with the discriminating dose of DDT all died. These tested males had already mated with the F<sub>1</sub> females which were then used to breed an F<sub>2</sub> generation. The discriminating dose of DDT killed all the 894 F<sub>2</sub> males tested.

The failure of this cross to produce any resistant males in either the  $F_1$  or  $F_2$  generation would convincingly refute any suggestion that EY females were able to transmit either a dominant or a recessive gene for resistance which expressed itself only in males. The results strongly suggested that the EY females did not possess any resistance factor to pass on to their male progeny. However, it was necessary

to demonstrate that the female progeny of cross 2 had neither lost nor gained anything which would prevent them producing male offspring when mated with Y-type resistant males, and thus to demonstrate that such a loss or gain was not the reason for none of the male progeny of cross 2 being resistant.



Fig. 3.—Dosage-mortality curves for females and males of strain EY generation 8 and strain ES generation 7 topically dosed with DDT.
× Observed mortality (40 females per point). ● Observed mortality (40 males per point). Upward pointing and downward pointing arrows indicate 100 and 0% mortality respectively.

(iii) Backcross:  $F_2$  Females (cross 2) × EY Males.—A sample of 500 virgin  $F_2$  females of cross 2 were caged with 600 EY males of generation 11. After mating, 480 of these EY males were used in a dosage-mortality test to redetermine DDT-tolerance levels which had not been checked for three generations of this strain. Figure 4 shows the experimental points (after adjustment for 5% mortality in the controls) and the calculated ld-p line. The departure of the points from the line is

not significant at the 5% level ( $\chi^2_{(4)} = 6.75$ ), so that the data indicate the EY males to be still homogeneous in their DDT-tolerances. The regression equation is

$$Y = -6 \cdot 28 + 6 \cdot 99(\pm 0 \cdot 67)x,$$

where Y and x have the same meanings as previously. The slope of the line is not



Fig. 4.—Dosage-mortality curves for male parents of backcross (strain EY generation 11) and male and female  $F_1$  progeny topically dosed with DDT.  $\times$  Observed mortality after adjustment for mortality in control (40 females per point).  $\bullet$  Observed mortality after adjustment for mortality in control (80 males per point). Upward pointing and downward pointing arrows indicate 100 and 0% mortality respectively.

significantly different from that for generation 8. The  $LD_{50}$ ,  $41 \cdot 1 \mu g/g$ , is somewhat greater than in generation 8 ( $32 \cdot 3 \mu g/g$ ), and there seems to have been a general upward shift of tolerances, which, however, does not amount to more than one dosage increment. Thus the actual male EY parents used in the backcross were shown to be the normal Y-type resistant males characteristic of this strain.

Dosage-mortality data were obtained for both female and male  $F_1$  progeny of the backcross. The data after adjustment for 2 and 1% mortality respectively in the controls, are shown in Figure 4 with their ld-p lines, the equations of which were calculated as:

$$Y = -0.33 + 5.82 \ (+0.70)x$$
 for females  $(\chi^2_{(4)} = 5.7)$ ,

and

$$Y = -4 \cdot 81 + 5 \cdot 30 \ (\pm 0 \cdot 61) x$$
 for males  $(\chi^2_{(3)} = 1 \cdot 4)$ ,

where Y and x have the same meanings as previously. The  $\chi^2$  values for goodness



Fig. 5.—Dosage-mortality curves for females and males of strain EY generation 12 topically dosed with DDT. × Observed mortality after adjustment for 2% mortality in control (40 females per point). ● Observed mortality after adjustment for 2% mortality in control (40 males per point). Upward pointing arrow indicates 100% mortality.

of fit indicate both sexes to be homogeneous in tolerances. Since the slopes are not significantly different at the 5% level ( $\chi^2_{(1)} = 0.3$ ), the relative resistance of the sexes is indicated by the ratio of the  $\text{LD}_{50}$ 's which is 8.6.  $\text{LD}_{50}$ 's and (in brackets) their fiducial limits at 95% probability are 71.0 (65.0 and 76.5) and 8.25 (7.53 and 9.06)  $\mu g/g$  for males and females respectively. Thus the  $F_1$  males of the backcross were typically Y-type resistants like their male parents, practically none of them being killed by a DDT-dosage just sufficient to kill all their non-resistant sisters. It is interesting to note that although tolerances seem to have undergone a general upward shift during the course of this experiment (cf., for example, the  $\text{LD}_{50}$ 's above with those for EY generation 8 in Table 4), the Y-type resistant males maintained a more than eightfold resistance as compared with their sisters.

The remainder of the  $F_1$  males of the backcross were divided into two equal groups of 428, one group being treated with the discriminating dose of DDT dissolved

in odourless kerosene as usual, the other group with the kerosene alone. The mortality in the DDT group was 0.93% and in the kerosene alone group 1.87%. Thus the corrected mortality due to DDT was zero, and all the males were therefore DDT-resistant.

The backcross results demonstrate that there was nothing in the female progeny of cross 2 to prevent them reproducing normally when mated with DDT-resistant males. Hence the complete absence of resistant individuals in the  $F_1$  and  $F_2$  generations of cross 2 may be taken as proving conclusively that the females of strain EY do not transmit resistance.

Without exception, the results of the reciprocal-cross experiment verify that the sex-limited DDT-resistance of EY males is determined directly by a Y-chromosome factor or indirectly by the balance between the non-homologous parts of X and Y and the rest of the chromosome set.

(iv) Stability Check on Strain EY.—At the conclusion of the genetic test, the DDT-tolerances of strain EY were re-examined. The results (Fig. 5) indicated that both sexes had remained homogeneous ( $\chi^2_{(3)} = 0.2$  for females,  $\chi^2_{(4)} = 1.8$  for males), and that their tolerance ranges were still completely separated from each other. The equations for the ld-p lines were calculated as:

and

$$Y = -2.60 + 10.13 \ (\pm 1.40)x$$
 for females,  
 $Y = -7.06 + 6.65 \ (\pm 0.86)x$  for males.

In slope the lines were significantly different at the 5% level  $(\chi^{2}_{(1)} = 4.5)$ , so that, as in the previous test on generation 8 of the strain, no simple value could be derived for the relative resistance of the sexes. However, comparisons could be made at stipulated mortality levels. The LD<sub>50</sub>'s and their fiducial limits were calculated to be 65.2 (59.5 and 71.0) and 5.62 (5.24 and 6.01)  $\mu$ g/g for males and females respectively. At all mortality levels above 0.1% the resistance ratio for the sexes, i.e. the ratio of equitoxic doses, exceeded 8.

Comparing the results for generations 8 and 12, the regression lines for males are not significantly different in slope  $(\chi^2_{(1)} = 1 \cdot 7)$ , nor are those for the females  $(\chi^2_{(1)} = 1 \cdot 4)$ . The LD<sub>50</sub> for males increased during the four generations by a factor of 2  $\cdot 0$ , whereas the corresponding factor for females was 1  $\cdot 3$ . It is not clear why the tolerances of males increased more than those of females. The point to be stressed, however, is that both the susceptible females and the resistant males showed a similar trend in tolerance variation over the period of the genetic test, and this suggests that the upward shift in tolerances shown by the progeny of cross 2 was perhaps no more than the "normal" variation to be expected from generation to generation in these strains.

#### IV. DISCUSSION

The derivation of the strains selected and the outcome of the genetic tests applied in this investigation are summarized diagrammatically in Figure 6.

The lack of information on the genetic activity of the Y-chromosome in M. domestica is a serious handicap to the understanding of the origin of this unique mechanism of inheritance of DDT-resistance. In the absence of cytological evidence it would appear reasonable tentatively to regard this trait of DDT-resistance confined to certain males as due either to the presence of a new allele at a specific locus on the Y or to a gain (or, less likely, a loss) of chromatic material by the Y. For many genera-



Fig. 6.—Diagram showing the derivation from the unselected laboratory colony of *Musca domestica* of two homogeneous strains, ES (in which both sexes are non-resistant to DDT) and EY (in which the females are non-resistant and the males DDT-resistant), and also the reciprocal cross and backcross tests which proved the holandric inheritance of the DDT-resistance of strain EY males. Sectors of the male and female symbols are blackened to represent the proportions of the populations found to be DDT-resistant. The broken horizontal lines represent selection "screens" through which the flies were "passed".

tions the Y-type DDT-resistant males have coexisted with the normally susceptible males, so that, if a gain or loss of chromatin by the Y were involved, it would need to be such as not to impair fertility.

The Y-chromosome in Diptera has generally been regarded as relatively inert genetically, consisting predominantly of heterochromatin with few or no genes.

However, Tate (1947) found a genetic locus concerned with eye colour on the Ychromosome of Calliphora erythrocephala, and there are some genes or at least specific regions in the Y-heterochromatin of D. melanogaster (see Goldschmidt 1955) and D. buscki (Krivshenko 1950) which are concerned with male fertility and development. Bristle size in D. melanogaster (Stern 1927) and certain colour traits in the fish Lebistes reticulatus (Schmidt 1920) and the beetle Phytodecta variabilis (Zulueta 1925) also involve the Y-chromosome. Gates (1946) listed 14 abnormal conditions in man for which there is evidence of Y-linkage, but Stern (1957) pointed out that the evidence for complete Y-linkage was inconclusive in all 17 reported cases in man. These species (excepting C. erythrocephala) appear to be the only ones in which holandric inheritance has been observed. Thus the DDT-resistance confined to males in the strain of M. domestica described here is thought to be the first record for this species of a major characteristic, outside of sex and fertility, being determined by a Y-chromosome factor.

#### V. References

ANON. (1943).-Peet-Grady method. Blue Book Soap Sanit. Chem., 1943.

- BLISS, C. I. (1935).—The calculation of the dosage-mortality curve. Ann. Appl. Biol. 22: 134-67.
- BROWN, A. W. A. (1958).—"Insecticide Resistance in Arthropods." (Monograph Series No. 38.) 240 pp. (World Health Organization: Geneva.)
- BUSVINE, J. R. (1957).—"A Critical Review of the Techniques for Testing Insecticides." 208 pp. (Commonwealth Institute of Entomology: London.)
- FINNEY, D. J. (1952).—"Probit Analysis." 318 pp. (Cambridge Univ. Press.)
- GATES, R. G. (1946).—"Human Genetics." 1518 pp. (The Macmillan Company: New York.)
  GOLDSCHMIDT, R. B. (1955).—"Theoretical Genetics." 563 pp. (University of California Press: Berkeley.)
- HOSKINS, W. M., and GORDON, H. T. (1956).—Arthropod resistance to chemicals. Annu. Rev. Ent. 1: 89-122.

KERR, R. W. (1951).-Adjuvants for pyrethrins in fly sprays. C.S.I.R.O. Aust. Bull. No. 261.

- KERR, R. W. (1954a).—A method for the topical application of small measured doses of insecticide solutions to individual insects. Bull. Ent. Res. 45 (2): 317–21.
- KERR, R. W. (1954b).—Variation with age in the susceptibility to DDT and the respiration rate of male and female *Drosophila melanogaster* Mg. Bull. Ent. Res. 45(2): 323-8.
- KERR, R. W. (1960).-Sex-limited DDT-resistance in houseflies. Nature 185: 868.
- KERR, R. W., VENABLES, D. G., ROULSTON, W. J., and SCHNITZERLING, H. J. (1957).—Specific DDT-resistance in houseflies. *Nature* 180: 1132–3.
- KRIVSHENKO, J. (1950).—On the genetical activity of the Y-chromosome in Drosophila buscki. Genetics 35: 119.
- NAGASAWA, S. (1955).—Studies on the biological assay of insecticides. XXXVII. Bull. Kyoto Univ. Inst. Chem. Res. 33(6): 241-56.
- SCHMIDT, J. (1920).—Racial investigations. IV. The genetic behaviour of a secondary sexual character. C.R. Lab. Carlsberg 14: 1-12.
- STERN, C. (1927).—Ein genetischer und zytologischer Beweis für Vererbung im Y-Chromosom von Drosophila melanogaster. Z. indukt. Abstamm.-u. VererbLehre 44: 187–231.
- STERN, C. (1957).—The problem of complete Y-linkage in man. Amer. J. Hum. Genet. 9: 147-66.
- TATE, P. (1947).—A sex-linked and sex-limited white-eyed mutation of the blow-fly (Calliphora erythrocephala). J. Genet. 48: 176-91.
- ZULUETA, A. DE (1925).—La herencia ligada al sexo en el coleoptero Phytodecta variabilis. Eos, Madr. 1: 203-31.