# INHERITANCE OF DDT RESISTANCE IN A LABORATORY COLONY OF THE HOUSEFLY, *MUSCA DOMESTICA*

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[*Manuscript received October* 10, 1969]

*Summary*

Strains of the housefly *Musca domestica* L., derived by selection from the Canberra laboratory colony established in 1939, were examined genetically and cytologically to determine their composition, in respect to resistance to DDT, and the modes of sex determination and inheritance of this resistance.

DDT resistance was found to be determined by an incompletely dominant allele of a gene in chromosome II that confers the ability to metabolize DDT to DDE. In flies of the normal karyotype, $2n = 12$: $XX$ females and $XY$ males, resistance is inherited independently of sex, but in atypical $XX$ males, lacking the $Y$-chromosome, the male determinant is linked with presumably the same resistance allele in one of the chromosome II homologues. The resistance gene in this homologue is thus obligatory for, and confined to, atypical males, but on rare occasions is inherited by a female, indicating that the linkage with the male determinant is not completely stable.

Atypical males were not detected in the unselected colony. They were brought into prominence by selection either for early adult emergence or for DDT resistance. Under DDT selection pressure, the progression of one strain towards homozygosity for the resistance allele was retarded slightly by the complete replacement of $XY$ males by the atypical $XX$ type, and the strain was still heterogeneous after 200 generations of selection.

Each of 10 lines propagated from single homozygous resistant pairs became heterogeneous when mass-reared in the absence of DDT. Instability of the resistance gene may therefore have been a factor opposing selection in the strain under DDT pressure.

Rare $XXY$ males and $XXX$ females among flies of the normal karyotype, and rarer $XO$ and $XXX$ males among the $XX$ males of atypical strains, were considered to be products of non-disjunctions. No $XO$ females were found.

## I. INTRODUCTION

The colony of the housefly investigated has been reared under laboratory conditions at Canberra since 1939 when the initial field collection of flies was made at nearby dairies. Its breeding stocks have never been exposed to insecticides. The flies of this unselected colony are predominantly insecticide-susceptible, the $LD_{50}$ for DDT being about 5 $\mu$g/g in males and about 7 $\mu$g/g in females. However, the ease with which DDT-resistant strains have been selected from the colony indicated the presence of a gene or genes for resistance in the population prior to the application of selection (Kerr *et al.* 1957), and analyses of dosage–mortality data for earlier generations dating back to 1952 showed a varying degree of heterogeneity that was due to a small,

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fluctuating proportion of DDT-resistant individuals in the colony. This proportion generally tended to be lower in early-emerging than in late-emerging flies, and was decreased or increased considerably by selection for early or late emergence respectively. But prolonged selection by this means alone did not eliminate resistance from the "early" strain E (Kerr 1960, 1961), nor susceptibility from the "late" strain L.

The susceptibility factor still persisted in a DDT-selected strain D (Kerr *et al.* 1957) after 200 successive generations of culturing in medium containing sufficient DDT to kill all non-resistant individuals in the larval stage. Whenever this strain was cultured without DDT, some non-resistant females were found among the flies produced.

Continued co-existence of resistant and non-resistant types in the colony suggested that neither type has a significant selective advantage under normal conditions of rearing in the laboratory. But there also seemed to be some protective mechanism which prevented the extinction of both the resistance and the susceptibility factors under severe conditions of selection.

Genetic tests were carried out with some of the derived strains to study the mode of inheritance that had enabled both the DDT-resistance factor and the susceptibility factor to survive the prolonged selection pressures against them. These tests were supplemented by cytological examinations of metaphase chromosomes in larval ganglia and pupal testes.

## II. Methods

The following strains of flies, each derived by selection from the colony, were used in genetic tests or examined cytologically:

D: Resistant to DDT. Continuously reared in medium containing DDT at 320 p.p.m. which kills all non-resistant individuals in the larval stage. Flies for testing with DDT were obtained from cultures specially set up without DDT in the medium.

ES: Homogeneously non-resistant. Isolated from strain E.

EM: Females homogeneously non-resistant, males homogeneously resistant by virtue of linkage of the male-determining and DDT-resistance factors. Isolated from strain E. Formerly named EY (Kerr 1960, 1961).

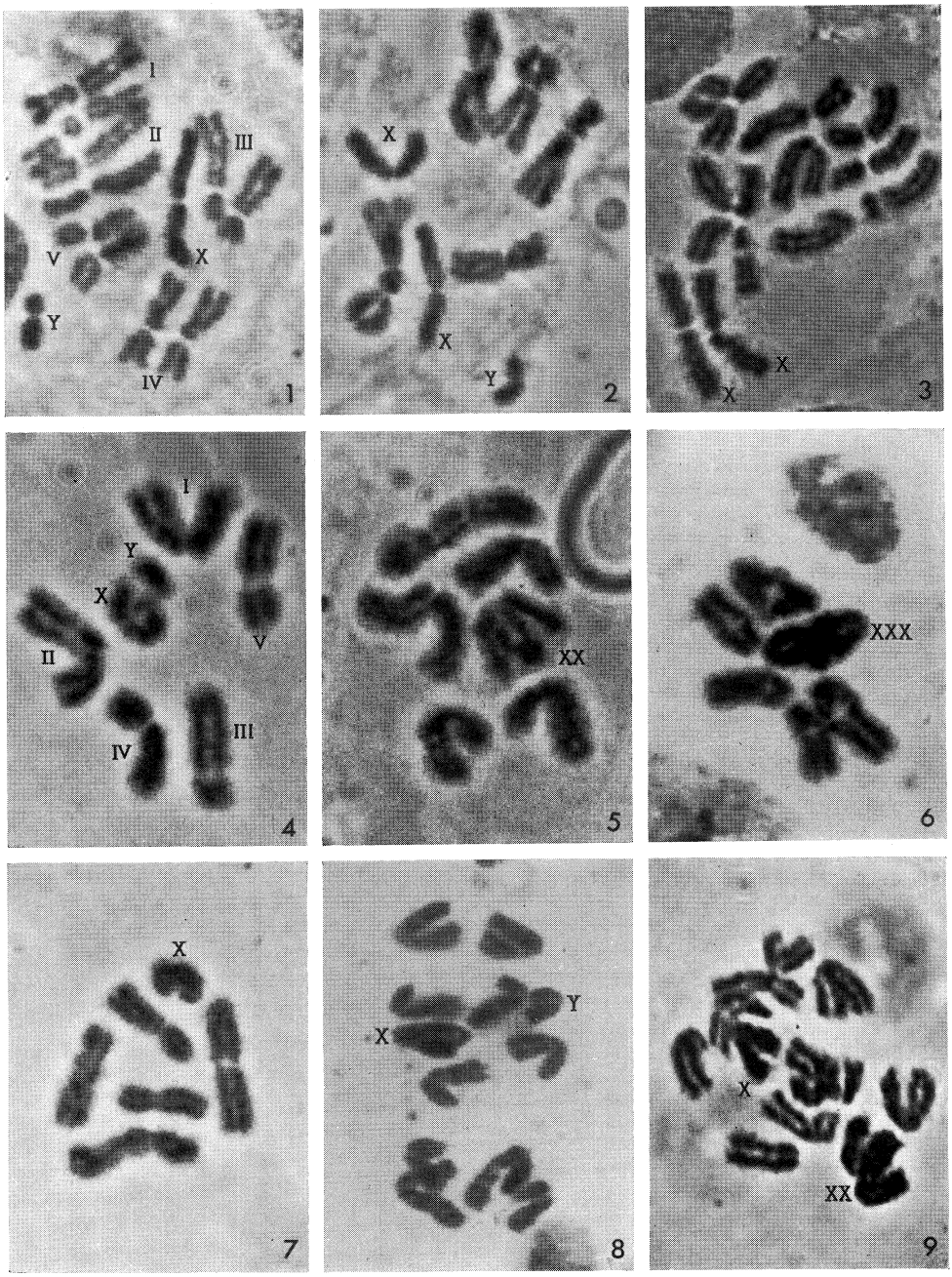
E: Females homogeneously non-resistant, males a mixture of EM and ES types. Continuously selected for early adult emergence.

L: Heterogeneous, majority of males and females resistant. Continuously selected for late adult emergence.

Rearing and testing were carried out in a laboratory controlled at 25·5 (±1)°C. The larval culture medium was a mixture of bran, powdered whole milk, and water. Adult flies were fed on 5% powdered whole milk in water.

Virgins for crossing experiments were obtained by withholding food from newly emerged flies and separating the sexes within 16 hr of emergence.

Flies for testing with DDT were collected in batches of 20 of the one sex from cages of flies 5–6 days old. Each batch was anaesthetized with carbon dioxide, weighed, and treated by applying to the mesonotum of each fly a measured dose of DDT in odourless kerosene or in a 3 : 1 mixture of odourless kerosene and dioxan. The volume of solution applied to each fly of the batch was directly proportional to the batch weight (5·4 μl/g), and the required dosages of DDT within the tolerance ranges of the strains were obtained by using appropriate concentrations forming geometric series. Dosages (μg DDT/g of flies) are thus independent of variations in batch weight. Each batch of treated flies was held, for a 24-hr mortality determination, in a 16 by 3 cm vial with a cotton wool pad moistened with 10% sucrose solution at the bottom, and a dry cotton wool stopper.




Figs. 1–9.—Chromosomes of *Musca domestica*. *1–3*, metaphase in larval ganglion of: *1*, typical *XY* male, $2n = 12$ (homozygous DDT-resistant line 7); *2*, atypical *XXY* male, $2n = 13$ (strain ES); *3*, atypical *XX* male, $2n = 12$ (strain EM). *4–7*, meiotic metaphase I in testis of: *4*, *XY* pupa (strain ES); *5*, *XX* pupa (strain D); *6*, *XXX* pupa (strain EM); *7*, *XO* pupa (strain D). *8* and *9*, meiotic anaphase I in testis of: *8*, *XY* pupa (strain ES); *9*, *XXX* pupa (strain EM).

Mortality, on a probit scale (Bliss 1935), was plotted against log dosage. When appropriate, the data were analysed to give the equation for the best-fitting regression line, the variance of the slope of this line, the $LD_{50}$ and its fiducial limits at 95% probability, and the $\chi^2$ value for goodness of fit of the line to the data as an indication of whether the treated flies were homogeneous or heterogeneous in their tolerances to DDT.

Comparisons between calculated slopes and $LD_{50}$'s were made by $\chi^2$- and $t$-tests respectively. The relative resistance of a strain or progeny compared with another is expressed first as the ratio of their $LD_{50}$'s and then, in parentheses, by the ratios of their $LD_1$'s and $LD_{99}$'s in that order, thus taking into account the differences in range of tolerances that were indicated by the differing slopes of the regression lines. When the comparison is with the non-resistant ES flies, the relative resistance value is the resistance factor (RF) for the strain or progeny, and, in the tables, $RF_{50}$, $RF_1$, and $RF_{99}$ are the values of the resistance factor obtained at the 50, 1, and 99% mortality levels respectively.

Testes were dissected from pupae, fixed in aceto-ethanol, stained with aceto-orcein, and squashed between microscope slides and cover glasses for phase-contrast microscopic examination of the chromosomes at certain stages of spermatogenesis; similar preparations were made of ganglia from third-instar larvae for examination of neuroblast chromosomes at metaphase (see Figs. 1–9).

## III. Results

### (*a*) *Cross I: D Females × ES Males*

This cross was made with 500 virgin females of strain D mated collectively with 500 ES males. The maximum emergence batch of $F_1$ flies was sampled for dosage–mortality tests on both sexes when the flies were 5–6 days old. All eggs laid during the following night by the remainder of the $F_1$ females were thoroughly mixed, by shaking in water, and sampled for culturing the $F_2$. The $F_2$ flies were sampled and tested in the same way. A dosage–mortality test was done on flies from each of the parent strains at the time of the $F_1$ tests.

The data obtained for the parent strains [Figs. 10(*a*) and 10(*b*)] showed that the DDT tolerances of the ES flies were all below 11·2 μg/g of flies, whereas those of D flies were mostly, if not all, above 22·4 μg/g. The resistance factor for strain D was 19·1 (15·6–23·3) in males and 21·1 (16·7–26·8) in females.

In the $F_1$ tests the observed mortalities, plotted in Figures 10(*a*) and 10(*b*), showed that the majority of the $F_1$ flies were resistant, but that some non-resistant flies were present, as indicated by the mortalities at low dosages. Since strain D was known to be heterogeneous, by the production of some non-resistant flies whenever the selection pressure was removed from it, the presence of some non-resistant flies in the $F_1$ was to be expected. The best estimate of the proportion of non-resistants obtainable from the data by reiterative analysis was 15% in males and 7% in females. The calculated mortalities among the remaining resistant $F_1$ flies fitted very closely the regression lines drawn in Figures 10(*a*) and 10(*b*) ($\chi^2_{(5)} = 1{\cdot}1$ for males, $\chi^2_{(4)} = 2{\cdot}3$ for females). Thus in both sexes the resistant $F_1$ flies appeared to be homogeneous. Their resistance factors were: males 12·8 (13·4–12·1), females 14·4 (13·0–16·0). Hence the resistance of $F_1$ flies was somewhat more than half that of D flies, and their tolerances were approximately five times closer to those of D than to those of ES flies. The resistance inherited from D females was clearly dominant, but incompletely so.

Mortalities in the $F_2$ flies [Figs. 10(*a*) and 10(*b*)] showed the presence of both parental types, since some of the flies were killed by dosages lethal to non-resistant

flies but sublethal to the resistant $F_1$ flies, and, at the other extreme, some flies survived dosages greater than the $F_1$ tolerances. There was also a marked discontinuity in tolerance distribution as indicated by the virtually constant mortality over a threefold change in dosage. The discontinuity coincided fairly well in males, and more precisely in females, with the gap between the tolerances of the non-resistant ES and the resistant $F_1$ flies, and showed the absence of types intermediate in tolerance between the non-resistant and $F_1$ types.

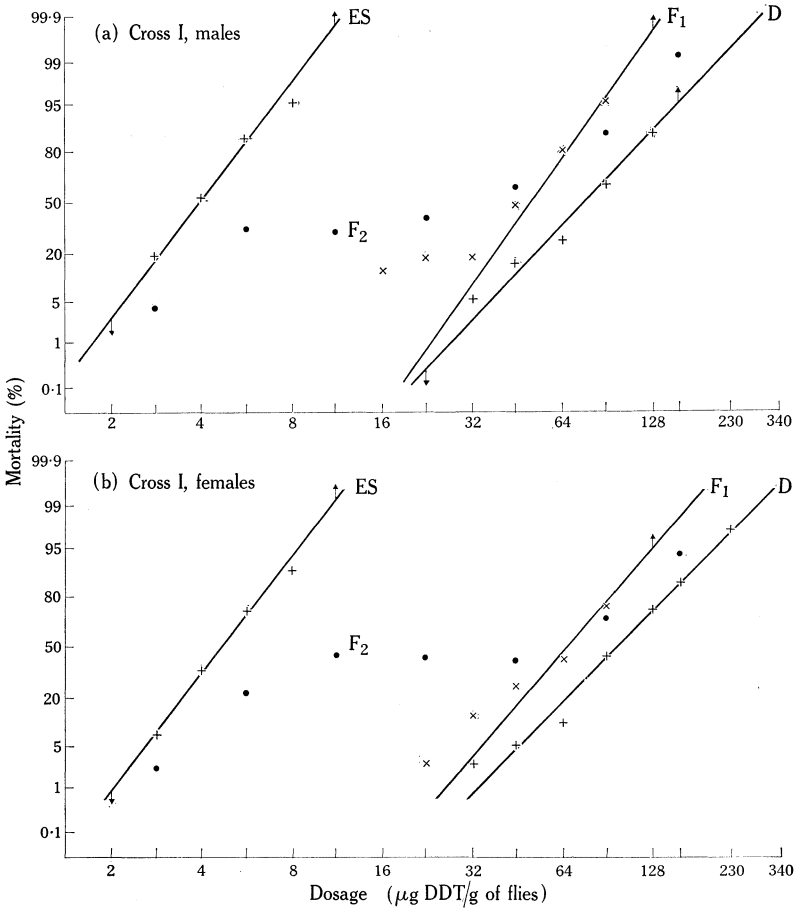



Fig. 10.—Dosage–mortality relationships for male (*a*) and female (*b*) flies of strains ES and D and of the $F_1$ and $F_2$ of cross I (D females × ES males). Plotted points are observed mortalities: 40 flies per point for ES, D and $F_1$, 160 per point for $F_2$. The $F_1$ regression line fits the mortalities corrected for 15% (*a*) and 7% (*b*) of non-resistants.

These features of the $F_2$ results all pointed to a single gene mode of inheritance of resistance from strain D females. The experiment was not designed to prove the mode of inheritance quantitatively by accurate assessment of segregation ratios in the $F_2$. Such ratios could not be derived from the percentages of non-resistants in

the $F_2$ test samples, partly because the samples were inadequate for this purpose, but mainly because the $F_1$ flies that produced them contained an unknown proportion of non-resistants.

The observed and analytical results of cross I are given in Table 1.

TABLE 1

OBSERVED MORTALITIES AND DERIVED DATA FOR CROSS I FLIES (D FEMALES × ES (MALES) TREATED WITH DDT

| | | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ES | D | $F_1$ | $F_2$ | ES | D | $F_1$ | $F_2$ |
| Number tested per dosage | | 40 | 40 | 40 | 160 | 40 | 40 | 40 | 160 |
| Numbers killed* | | 0 | 0 | 5 | 6 | 0 | 1 | 1 | 4 |
| | | 7 | 2 | 7 | 52 | 3 | 2 | 5 | 35 |
| | | 21 | 6 | 7 | 50 | 14 | 4 | 10 | 70 |
| | | 34 | 10 | 19 | 61 | 29 | 17 | 17 | 68 |
| | | 38 | 24 | 32 | 94 | 36 | 29 | 30 | 66 |
| | | 40 | 35 | 38 | 139 | 40 | 34 | 40 | 108 |
| | | — | 40 | 40 | 159 | — | 39 | — | 150 |
| Non-resistants (%) | | 100 | 0 | 15 | 34 | 100 | 0 | 7 | 42 |
| Regression parameters† | $a$ | 1·06 | −4·91 | −6·95 | | 0·65 | −5·08 | −5·54 | |
| | $b$ | 6·55 | 5·26 | 7·00 | | 6·50 | 5·06 | 5·77 | |
| | | (±0·71) | (±0·53) | (±1·09) | | (±0·70) | (±0·51) | (±0·70) | |
| Regression line fit | $\chi^2$ | 2·4 | 6·1 | 1·1 | | 1·6 | 3·9 | 2·3 | |
| | D.F. | 4 | 5 | 5 | | 4 | 5 | 4 | |
| Resistance factor | $RF_{50}$ | | 19·1 | 12·8 | | | 21·1 | 14·4 | |
| | $RF_1$ | | 15·6 | 13·4 | | | 16·7 | 13·0 | |
| | $RF_{99}$ | | 23·3 | 12·1 | | | 26·8 | 16·0 | |

* At dosages in ascending series as indicated by plotted points in Figures 10(*a*) and 10(*b*).

†For the equation $Y = a + bx$, where $Y$ is mortality in probits and $x$ is log dosage of DDT (dosage being expressed as μg/g of flies).

### (*b*) *Cross II: D Males × ES Females*

This, the reciprocal cross to that made in the previous experiment, was carried out by a procedure that was otherwise identical. The results are given in Table 2 and Figures 11(*a*) and 11(*b*).

Flies of the two parent strains showed almost the same ranges of DDT tolerances as before, with regression slopes not significantly different in the two experiments, and $LD_{50}$'s not significantly different either in ES males, ES females, or D females. D males showed a marginally significant change in $LD_{50}$, by a factor of 1·14, which, however, is only of the order of generation to generation fluctuations in tolerance level, and is of no genetic significance here. The resistance factors calculated for strain D were 22·9 (20·0–26·3) for the males and 22·5 (18·4–27·6) for the females, which are in fair agreement with the estimates in the previous experiment.

In the $F_1$ flies all the males and 92% of the females were resistant. Their resistance factors were calculated to be 12·3 (12·7–11·9) for the males and 14·8 (14·4–15·1) for the females, and their tolerances were not significantly different (see Table 3) from those of the resistant $F_1$ flies of cross I. Thus the resistance inherited from the D males in cross II was quantitatively the same as that from the D females in cross I.

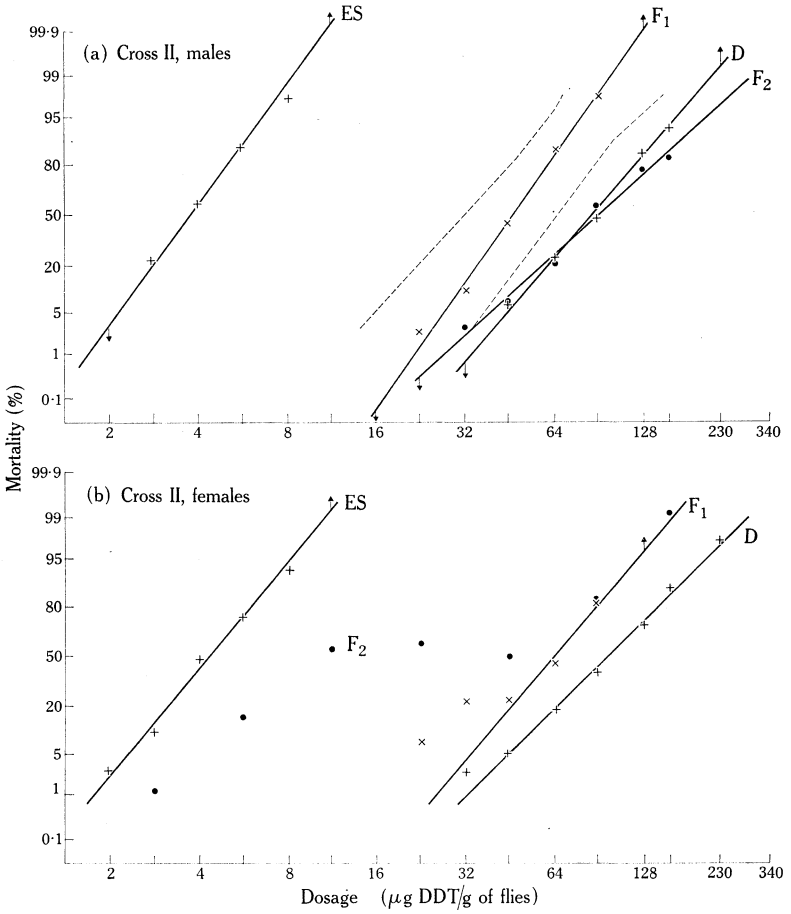



Fig. 11.—Dosage–mortality relationships for male (*a*) and female (*b*) flies of strains ES and D and of the $F_1$ and $F_2$ of cross II (D males × ES females). Plotted points are observed mortalities: 40 flies per point for ES, D, and $F_1$, 80 (*a*) and 160 (*b*) per point for $F_2$. In (*a*) the broken lines define the tolerance band of strain EM males (see text). In (*b*) the $F_1$ regression line fits the mortalities corrected for 8% of non-resistants.

The $F_2$ females were about 54% non-resistants, the remainder resistants with tolerances not exceeding those of the resistant $F_1$ females. $F_2$ males were all resistant, with a tolerance distribution that spanned the tolerances of D males and most of the $F_1$ males. Analysis showed no significant difference between $F_2$ and D males either in regression slope ($\chi^2_{(1)} = 3·2$) or in $LD_{50}$ ($t = 0·07$), but highly significant differences

TABLE 2

OBSERVED MORTALITIES AND DERIVED DATA FOR CROSS II FLIES (D MALES × ES FEMALES) TREATED WITH DDT

| | | Males | | | | Females | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | ES | D | $F_1$ | $F_2$ | ES | D | $F_1$ | $F_2$ |
| Number tested per dosage | | 40 | 40 | 40 | 80 | 40 | 40 | 40 | 160 |
| Numbers killed* | | 0 | 0 | 0 | 0 | 1 | 1 | 3 | 2 |
| | | 9 | 3 | 1 | 2 | 4 | 2 | 9 | 25 |
| | | 23 | 9 | 4 | 6 | 19 | 7 | 9 | 88 |
| | | 35 | 19 | 18 | 16 | 30 | 16 | 18 | 94 |
| | | 39 | 34 | 34 | 44 | 37 | 28 | 33 | 81 |
| | | 40 | 37 | 39 | 61 | 40 | 35 | 40 | 132 |
| | | — | 40 | 40 | 66 | — | 39 | — | 159 |
| Non-resistants (%) | | 100 | 0 | 0 | 0 | 100 | 0 | 8 | 54 |
| Regression parameters† | $a$ | 1·09 | −6·15 | −6·77 | −4·01 | 1·19 | −4·71 | −5·55 | |
| | $b$ | 6·76 | 5·75 | 7·06 | 4·60 | 6·01 | 4·89 | 5·85 | |
| | | (±0·70) | (±0·54) | (±0·78) | (±0·35) | (±0·63) | (±0·47) | (±0·76) | |
| Regression line fit | $\chi^2$ | 1·6 | 1·5 | 1·1 | 4·1 | 1·3 | 1·7 | 7·4 | |
| | D.F. | 4 | 5 | 5 | 5 | 4 | 5 | 4 | |
| Resistance factor | $RF_{50}$ | | 22·9 | 12·3 | 23·9 | | 22·5 | 14·8 | |
| | $RF_1$ | | 20·0 | 12·7 | 16·5 | | 18·4 | 14·4 | |
| | $RF_{99}$ | | 26·3 | 11·9 | 34·6 | | 27·6 | 15·1 | |

* At dosages in ascending series as indicated by plotted points in Figures 11(*a*) and 11(*b*).

† For the equation $Y = a + bx$, where $Y$ is mortality in probits and $x$ is log dosage of DDT (dosage being expressed as μg/g of flies).

TABLE 3

COMPARISONS BETWEEN CROSSES I AND II

| Strain or Progeny | Sex | Regression Slope | | | $LD_{50}$ (μg DDT/g of flies) | | |
|---|---|---|---|---|---|---|---|
| | | Cross I | Cross II | $\chi^2_{(1)}$ | Cross I | Cross II | $t$ |
| ES | Male | 6·55 | 6·76 | 0·04 | 4·00 | 3·79 | 0·96 |
| | Female | 6·50 | 6·01 | 0·28 | 4·66 | 4·31 | 1·32 |
| D | Male | 5·26 | 5·75 | 0·42 | 76·3 | 86·8 | 2·04* |
| | Female | 5·06 | 4·89 | 0·06 | 98·6 | 96·9 | 0·27 |
| $F_1$ | Male | 7·00 | 7·06 | 0·002 | 51·0 | 46·5 | 1·40 |
| | Female | 5·77 | 5·85 | 0·006 | 67·1 | 63·6 | 0·80 |

* Significant ($P < 0·05$). All other $\chi^2$ and $t$ values indicate no significant difference between cross I and cross II.

between $F_2$ and $F_1$ males in both slope ($\chi^2_{(1)} = 8 \cdot 2$) and $LD_{50}$. The relative resistance of $F_2$ males to $F_1$ males was $1 \cdot 95$ ($1 \cdot 30$–$2 \cdot 92$).

The complete absence of non-resistant males in the $F_1$ and $F_2$ indicated a linkage between resistance and maleness, such as that already found in strain EM, derived from the same colony. It also indicated that all the males of strain D had this linkage. The linkage accounted adequately for the differences found between the $F_2$ progenies of crosses I and II.

The result for $F_2$ females was close to that expected from the mating of non-resistant males with females heterozygous for a dominant resistance gene, i.e. a 50 : 50 ratio of non-resistants to resistant heterozygotes. Dilution of the mated females with 8% of non-resistants, as found experimentally in the $F_1$ females, would, under conditions of random mating and equal fitness of genotypes, distort the ratio to 54 : 46. This is even closer to the ratio of non-resistant to resistant females found in the $F_2$, as shown in Figure 11(*b*) by the mortalities at dosages lethal to non-resistants but sublethal to resistant flies. The clear-cut separation of the $F_2$ females into predictable proportions of only two phenotypes, i.e. non-resistants and hybrid types like the resistant $F_1$ females, was convincing evidence that only one primary gene was involved in the inheritance of DDT resistance from D males to female progeny. The result also showed that, as far as their female progeny were concerned, the $F_1$ males behaved genetically the same as would non-resistant males. Hence the $F_1$ males were indicated to be heterozygous only for whatever gene(s) conferred their male-limited resistance, and in this respect they were analogous to, if not identical with, the males of strain EM.

The broken lines drawn in Figure 11(*a*) define the extremities within which lie all nine of the regression lines obtained so far for strain EM males of various generations. Between these extremities the regression line for the $F_1$ males is positioned almost centrally, and analysis showed no significant difference either in slope or $LD_{50}$ between the $F_1$ line and the mean line for EM males. A similar comparison between EM males and the resistant $F_1$ males of cross I also showed no significant differences. It was evident that these three groups of heterozygous resistant males had virtually identical mean tolerances and tolerance ranges, and thus that the resistance gene(s) linked with the male determinant in EM and D males conferred the same degree of resistance as did the resistance gene of the D females. Obviously the simplest explanation of this equality would be that one and the same gene was involved in the two modes of inheritance. Experiments that provided some evidence on this question are described later.

The composition of the $F_2$ males of cross II could not be resolved in quite the same way as that of the females. All were resistant, and their tolerances merged to such an extent that no evidence of heterogeneity was obtained in analysis of the data. However, their tolerances extended across the $F_1$ range and beyond, and it can be inferred that they were composed of two types, both having the male determinant linked with resistance inherited from the $F_1$ males. One type had no additional resistance, and therefore showed the same tolerances as the $F_1$ males; the other type had additional resistance which could only have been acquired from their resistant $F_1$ female parents. Thus the gene for resistance inherited by females from D males was passed back to some of the $F_2$ males, and this free exchange between the sexes leaves little room for doubt that it was the same gene as that transmitted by D females to both sexes in cross I.

While these two unrefined crossing experiments provided considerable information about the inheritance of DDT resistance in the colony, they were deficient in their ability to separate resistant genotypes and to give accurate estimates of segregation ratios which are generally considered desirable for the proof of modes of inheritance. The following backcross experiment was designed to circumvent these deficiencies and to test the validity of the conclusions drawn from the simple crosses.

### (c) *Crosses III and IV, Backcrosses A and B*

The backcross experiment was started with two reciprocal crosses, involving mass matings of 300 virgin D females with 300 ES males (cross III) and the same numbers of D males with virgin ES females (cross IV). Virgin $F_1$ flies, 300 of each sex, in both crosses were dosed with DDT at 11·2 $\mu$g/g to determine the proportions of, and eliminate, non-resistants. This procedure provided more accurate estimates of the non-resistants than those obtained in crosses I and II, and also gave batches of pure $F_1$ hybrids for backcrossing. Further $F_1$ flies were tested with serial dosages of DDT to determine their dosage–mortality relationships. The results are given in Table 4.

TABLE 4

OBSERVED MORTALITIES AND DERIVED DATA FOR $F_1$ FLIES OF CROSSES III (D FEMALES × ES MALES) AND IV (D MALES × ES FEMALES) TREATED WITH DDT

| | | Males | | Females | |
|---|---|---|---|---|---|
| | | Cross III | Cross IV | Cross III | Cross IV |
| Number tested per dosage | | 40 | 40 | 40 | 40 |
| Numbers killed* | | 5 | 0 | 3 | 3 |
| | | 8 | 2 | 4 | 4 |
| | | 11 | 10 | 9 | 7 |
| | | 29 | 27 | 18 | 19 |
| | | 38 | 39 | 34 | 32 |
| | | 40 | 40 | 39 | 39 |
| | | | | 40 | 40 |
| Non-resistants (%) | | 14·8 | 0 | 8·5 | 9·3 |
| Regression parameters† | $a$ | −9·99 | −8·66 | −7·79 | −8·22 |
| | $b$ | 8·52 | 7·87 | 7·02 | 7·20 |
| | | (±1·42) | (±0·87) | (±0·97) | (±1·01) |
| Regression line fit | $\chi^2$ | 0·99 | 0·76 | 0·96 | 0·32 |
| | D.F. | 3 | 4 | 4 | 4 |
| $LD_{50}$ ($\mu$g/g of flies) | | 57·5 | 54·3 | 66·4 | 68·8 |
| Test for difference in $b$ | | $\chi^2_{(1)} = 0·15$ | | $\chi^2_{(1)} = 0·02$ | |
| Test for difference in $LD_{50}$ | | $t = 0·85$ | | $t = 0·52$ | |

* At dosages in ascending series as indicated by plotted points in Figures 12(*a*) and 12(*b*).

† For the equation $Y = a + bx$, where $Y$ is mortality in probits and $x$ is log dosage of DDT (dosage being expressed as $\mu$g/g of flies).

The significant percentages of non-resistant flies present in all groups of $F_1$ flies except the males of cross IV confirmed that some of the males and females of the parent

strain D were heterozygous, and the absence of non-resistants from the latter group showed again that the resistance determiner in such heterozygous D males is always passed on to the male progeny.

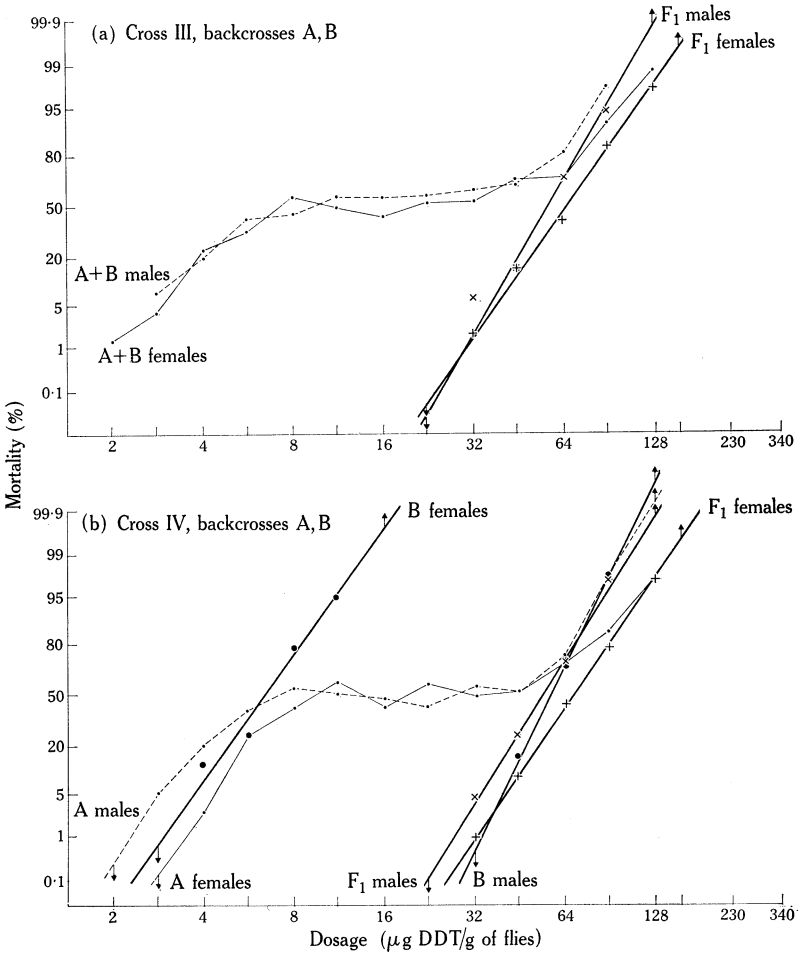



Fig. 12.—(*a*) Dosage–mortality relationships for $F_1$ males and females of cross III (D females × ES males) and for pooled male and pooled female progenies of backcrosses A ($F_1$ females × ES males) and B ($F_1$ males × ES females). Plotted points for $F_1$ males and females are mortalities obtained from 40 flies per dosage after correction for 14·8 and 8·5% of non-resistants respectively, those for the pooled backcross progenies are observed mortalities in 80 flies per dosage. (*b*) Dosage–mortality relationships for $F_1$ males and females of cross IV (D males × ES females) and for male and female progenies of backcrosses A ($F_1$ females × ES males) and B ($F_1$ males × ES females). Plotted points for $F_1$ females are mortalities obtained from 40 flies per dosage after correction for 9·3% of non-resistants. All other plotted points are observed mortalities in 40 flies per dosage.

After the corrections for non-resistants present, the $F_1$ data showed no evidence of heterogeneity, and, as in the previous experiments, there were no significant differences between the crosses in the tolerance distributions of either the male or the female $F_1$ flies, as shown in Table 4 by the non-significant values of $\chi^2$ and $t$. Corrected mortalities for the resistant $F_1$ flies are plotted in Figures 12(*a*) and 12(*b*) with their

best-fitting regression lines. A comparison between these $F_1$ flies and their counterparts in crosses I and II revealed that the resistance level had not changed significantly.

In each cross, the virgin $F_1$ females surviving the discriminating dose were backcrossed *en masse* with an equal number of ES males (backcross A), and the surviving $F_1$ males similarly backcrossed with virgin ES females (backcross B). This gave eight groups of backcross progeny defined by their sex, parentage, and grandparentage as in Table 5. Each group of progeny was tested separately with 13 serial dosages of DDT spanning the tolerance ranges of resistant and non-resistant flies. The eight columns of Table 5 give the numbers killed in each batch of 40 per dosage.

TABLE 5

MORTALITIES PRODUCED IN BATCHES OF 40 FIRST-GENERATION PROGENY OF BACKCROSSES BY TREATMENT WITH DDT

| Dosage ($\mu$g/g) | Backcross IIIA* | | Backcross IIIB† | | Backcross IVA‡ | | Backcross IVB§ | |
|---|---|---|---|---|---|---|---|---|
| | Males | Females | Males | Females | Males | Females | Males | Females |
| 2·0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 2·8 | 3 | 0 | 3 | 3 | 2 | 0 | 0 | 0 |
| 4·0 | 6 | 11 | 10 | 8 | 8 | 1 | 0 | 5 |
| 5·6 | 16 | 13 | 17 | 14 | 16 | 10 | 0 | 10 |
| 8·0 | 17 | 19 | 19 | 26 | 22 | 17 | 0 | 31 |
| 11·2 | 22 | 18 | 23 | 21 | 21 | 24 | 1 | 38 |
| 16·0 | 19 | 17 | 25 | 17 | 19 | 17 | 1 | 40 |
| 22·4 | 21 | 24 | 24 | 18 | 17 | 23 | 0 | 40 |
| 32·0 | 25 | 22 | 23 | 20 | 22 | 20 | 0 | 40 |
| 44·8 | 26 | 23 | 25 | 30 | 21 | 21 | 6 | 40 |
| 64·0 | 30 | 27 | 34 | 27 | 30 | 28 | 27 | 39 |
| 89·6 | 37 | 34 | 40 | 39 | 39 | 34 | 39 | 40 |
| 128·0 | 40 | 40 | 40 | 39 | 40 | 39 | 40 | 40 |

* $F_1$ females (D females × ES males) × ES males.

† $F_1$ males (D females × ES males) × ES females.

‡ $F_1$ females (D males × ES females) × ES males.

§ $F_1$ males (D males × ES females) × ES females.

(i) *Backcrosses IIIA and IIIB*

In the four progeny groups from cross III there were no significant differences at the 5% level between the sexes ($\chi^2_{(9)} = 5{\cdot}5$ for IIIA, 13·8 for IIIB) or between the combined sexes of IIIA and IIIB ($\chi^2_{(9)} = 5{\cdot}6$). This permitted the pooling of all mortalities at the three dosages, 11·2, 16·0, and 22·4 $\mu$g/g, between the tolerance ranges of non-resistants and $F_1$ hybrids, in order to obtain the best estimate of the segregation ratio. The mean mortality for the 12 batches so treated was 51·9 ($\pm$0·8)%, and since this was based on an aggregate sample of 480 test flies, it can be regarded as a reasonably accurate measure of the proportion of non-resistants present in the backcross populations derived from cross III. It may be noted that mortality among the resistant flies due to causes other than DDT was not taken into account in deriving this estimate. Such "control" mortality has never been found to exceed 2% in samples of 400 or more flies tested in the same manner but without DDT in the solvent applied.

Had it been measured and corrected for in this experiment, the estimate of the proportion of non-resistants obviously would have been even closer to the expected 50%.

The data for the IIIA and IIIB progenies were pooled according to sex and plotted in Figure 12(*a*), with linking lines between consecutive points solely for the purpose of identification. Fitting of a calculated curve to the plotted points was not necessary, since the lay-out of the points quite adequately indicated the two-component nature of the population, the relative proportion of the components, and the tolerance ranges which identified the components as non-resistants and heterozygous resistants of the $F_1$ hybrid type. Since crossing over does not occur as a normal, regular event in the chromosomes of males of *Musca domestica*, the fact that the heterozygous $F_1$ male flies used for backcross IIIA transmitted the resistance gene to half of their male and half of their female progeny, and not exclusively to all of the latter, proved that the resistance gene inherited by them from the D females was in an autosome, not in the *X*-chromosome.

(ii) *Backcross IVA*

This backcross gave almost identical results to those of IIIA and IIIB, as shown in Table 5 and Figure 12(*b*). There was no significant difference between the IVA male and female progenies ($\chi^2_{(9)} = 12{\cdot}3$), and the combined sexes did not differ significantly from the pooled IIIA and IIIB progenies ($\chi^2_{(9)} = 9{\cdot}5$). The mean mortality for the six batches tested at the three "plateau" dosages was $50{\cdot}4\ (\pm 1{\cdot}2)\%$, thus indicating the same 1 : 1 segregation ratio as in cross III.

(iii) *Backcross IVB*

The IVB progeny, being derived from D males, were expected to have the male-determinant linkage and therefore to be composed exclusively of resistant males and non-resistant females. The data used to obtain the regression lines [Fig. 12(*b*)] for these flies showed no sign of heterogeneity ($\chi^2_{(3)} = 0{\cdot}2$ for males, $\chi^2_{(4)} = 3{\cdot}3$ for females), and indicated that the females were all non-resistant, and the males all resistant with tolerances identical to those of the $F_1$ males (Table 6). This seemingly

TABLE 6

COMPARISON BETWEEN $F_1$ AND B PROGENIES OF CROSS IV

| Sex | Regression Slope | | | $LD_{50}$ ($\mu$g DDT/g of flies) | | |
|---|---|---|---|---|---|---|
| | $F_1$ | B | $\chi^2_{(1)}$ | $F_1$ | B | *t* |
| Male | 7·87 | 10·19 | 2·2 | 54·3 | 57·4 | 1·1 |
| Female | 7·20 | 6·97 | 0·03 | 68·8 | 6·45 | h.s. |

perfect fulfilment of the prediction was slightly marred, however, by the survival of one female among the 280 tested at dosages above the tolerance range of non-resistants (Table 5). The non-conforming individual also survived a dose of 16 $\mu$g DDT/g applied on the following day, and thus proved to be resistant. Hence it could not be altogether ignored. Two deaths in the 360 males tested at dosages sublethal to

resistant flies (Table 5) were probably due to natural causes or accidental injury, because this mortality rate (0·6%) is lower than the "control" mortality rate that has to be expected in a sample of this size (Kerr 1961).

The backcrossing of the IV $F_1$ males with ES females was, of course, analogous to the normal breeding of strain EM in which the females are non-resistant and the males have resistance linked with the male determinant. By transposing the EM extremity lines of Figure 11(*a*) to Figure 12(*b*), it was found that all the plotted mortalities and the 95% fiducial limits to the $LD_{50}$'s of both the $F_1$ and B males lay within this EM tolerance band. So also did those of cross III $F_1$ males in Figure 12(*a*). The indications from these comparisons were therefore the same as those from the similar comparisons made in crosses I and II.

Apart from the anomaly of the non-conforming female in the IVB progeny, the results of the backcross experiment either verified or complemented in a genetically consistent manner the findings of crosses I and II. The combined information revealed, to the following extent, the genetic composition of strain D flies with respect to DDT resistance and the modes of inheritance of this resistance:

(1) the resistant females of strain D were a mixture of two genotypes, homozygous and heterozygous respectively for an incompletely dominant resistance allele of a single autosomal gene;

(2) the D males possessed, in one homologue of the same autosome, a resistance-conferring factor limited to males by virtue of a linkage with the male determinant. In the other homologue they either had or did not have the resistance allele of the females;

(3) males heterozygous for either the resistance allele or the male-limited resistance factor had DDT resistance of the same magnitude and equal to the male-limited resistance in strain EM which had a common origin with strain D.

The likelihood that the same gene determined these resistances was increased by the results of a DDT-metabolism study carried out by Nelson (unpublished data, 1963) who found that EM males metabolized DDT in the same manner as resistant females, by dehydrochlorination to DDE.

The origin of the resistant female in the IVB progeny could not be deduced with certainty. However, the consideration of similar events in the following experiment pointed to a likely explanation.

### (*d*) *Stability Tests on Strain EM*

After this strain was isolated in 1958, it remained stable for 16 generations without further selection, producing non-resistant females and DDT-resistant males. In generation 21 a few resistant females were found. It was purified by destroying the females, testing the males with a dose of DDT lethal to non-resistants, and mating the survivors with virgin ES females, for two generations. Regular monitoring tests on about 60 females in each generation occasionally revealed a resistant individual. A second purification became necessary in 1962, and after that the strain was propagated by the purification procedure in each generation. Despite this a resistant

female was found on four occasions, several generations apart. In view of the small numbers monitored, the "transfer" of the normally male-limited resistance in this strain to a female, though clearly uncommon, may not have been a particularly rare event.

One of these females laid fertile eggs. Its female progeny were a mixture of non-resistants and resistants, thus showing it to have been heterozygous for whatever resistance gene(s) it had acquired. Its resistant female progeny were mated with ES males, and the line was continued by repeating this procedure in each generation, to maintain the resistance in the heterozygous state. Tests of all the females in generations 2 and 3 gave the ratio of non-resistants to resistants as 19 : 20 and 131 : 116, neither of which values is significantly different from 1. In a sample of 500 females from generation 11, the ratio was 196 : 304, which is significantly less than 1 [$\chi^2_{(1)} = 23$]. Thus there was no evidence that normal crossing over in the females raised the proportion of non-resistants to a level above 50%, and consequently no evidence for more than one gene determining the "transferred" DDT resistance.

The reciprocal backcross, heterozygous males × ES females, was made at generation 19. Both male and female progeny were a mixture of non-resistants and resistants, which showed that the transferred resistance gene was in an autosome, not in the *X*-chromosome. The ratio of non-resistants to resistants in a sample of 320 tested was 170 : 150, which is not significantly different from 1 [$\chi^2_{(1)} = 1 \cdot 3$].

TABLE 7

TEST FOR THE INVOLVEMENT OF HOMOLOGOUS OR DIFFERENT CHROMOSOMES IN THE TRANSFER OF NORMALLY MALE-LIMITED DDT RESISTANCE TO FEMALES

Mortality in female progenies treated with DDT at 16 μg/g

| | Female Progeny* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I | J |
| No. killed | 0 | 0 | 0 | 0 | 0 | 0 | 64 | 55 | 68 | 87 |
| No. not killed | 73 | 97 | 52 | 65 | 46 | 82 | 0 | 0 | 0 | 0 |

* $F_1$ males (from the cross EM males × "transfer" line females) × ES females.

To determine whether the resistance gene released from the male-determinant linkage was in the same or a different chromosome pair the following experiment was performed. EM males were mated *en masse* with virgin heterozygous females of the transfer line to give $F_1$ males all possessing the male-limited resistance gene either with or without the transferred resistance gene. Each of 10 $F_1$ males was mated with three virgin ES females, and the females of the progenies produced were tested with DDT at a dosage of 16 μg/g which discriminated between non-resistants and resistants. The expectation was that, if the same chromosome pair were involved, some of the female progenies would be all resistants, whereas, if a different chromosome pair had received the transferred gene, some would be 1 : 1 mixtures of non-resistants and resistants. In both cases, the remainder of the female progenies would be all non-resistants, and therefore of no discriminative value. The results (Table 7) for

10 progenies showed that the transferred gene had remained in the same chromosome pair. It seemed possible that the event which caused the transfer was a simple crossover, which is somewhat rare in males, but the other product expected of a crossover, namely a male (or males) with the non-resistance allele linked to the male determinant, would have been impossible to detect with a DDT test.

### (e) *Homozygous Resistant Lines*

The richest source of homozygous resistant females was strain D in which the estimated frequency of this type was about 0·8. Since apparently all the males of this strain were abnormal in having the linkage between resistance and the male determinant, a similar source of normal homozygous resistant males had to be created. This was done by crossing D females with ES males, backcrossing the resistant $F_1$ males with D females, and then eliminating with a discriminating dose of DDT all non-resistants from the breeders in subsequent generations. In three generations of such DDT selection the frequency of homozygous flies increased to about 0·7, so that, thereafter, there was a better than 50% chance that a male from this source and a D female would be a homozygous pair.

Single-pair matings were then made, and the resulting progenies reared as separate lines. All, or a maximum of 40, of the adult flies of the first generation, and 200 of the second generation of each line were tested with DDT at 22·4 μg/g. Lines such as 1 and 2 in Table 8, which had had both or one of the parents heterozygous

TABLE 8

TESTS FOR HOMOZYGOSITY AND STABILITY IN SINGLE-PAIR DDT-RESISTANT LINES

Flies treated with DDT at 22·4 μg/g

| Single-pair Line | Mortality (%) in Generation: | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 8 | 14 | 16 | 17 | 18 | 23 | 24 | 27 | 29 | 32–37 | 46 |
| 1 | 22·5 | | | | | | | | | | | | | |
| 2 | 0 | 7·8 | | | | | | | | | | | | |
| 3 | 5·0 | 1·0 | | 0·5 | | | | | | | | | 21·8 | |
| 4 | 0 | 0·5 | | 0·3 | | | | | | | | | 16·5 | |
| 5 | 0 | 1·0 | | | 0·8 | 0·8 | | | | | | | 20·3 | |
| 6 | 0 | 0 | | | | 2·0 | | | | | | | 43·8 | |
| 7 | 0 | 0·5 | 1·2 | 2·0* | 1·8 | | | 2·5 | 4·0 | 3·0 | | | | 26·0 |
| 8 | 2·5 | 1·0 | | | | 2·0 | | | | | | | 28·8 | |
| 9 | 2·5 | 1·0 | | | | | 2·8 | | | | | | 70·0 | |
| 10 | 0 | 1·0 | | | | | 2·5 | | | | | | 23·0 | |
| 11 | 0 | 1·0 | | | | | | | 1·3 | 5·3 | | | 38·0 | |
| 12 | 0 | 0·5 | | | | | | | | | 2·3 | 1·8 | 5·8 | |

* In a similar sample of these flies, treated with the solvent only, the mortality was 1·8%.

were detectable by mortalities of the order of 25% in the $F_1$ or 6% in the $F_2$ generation respectively, and were destroyed. Those in which 95% or more of the $F_1$ and 99% or more of the $F_2$ flies survived the test were considered to have stemmed from a homozygous pair and were continued.

In tests on samples of 400 flies of subsequent generations a mortality not greater than 2% was considered as indicating no change in the line, since mortalities up to this level had been recorded in the same number of "control" flies treated with the solvent only. Line 7 was tested at fairly regular intervals, but it proved impractical to examine the other lines to the same extent. The information obtained, though meagre, indicated that seven lines were stable to generation 8, five to generation 14, three to generation 16, and one to generation 23. All of the lines eventually became heterogeneous, with significant, and in some cases quite high, proportions of non-resistants, as shown in Table 8 by the mortalities in the last test of each line. In all cases the proportion was of the same order in males and females. The rise in frequency of non-resistants to such levels clearly indicated an effective advantage of the non-resistance allele over the resistance allele in the absence of any selection pressure by DDT.

It was considered that the cause of the breakdown of these homozygous lines was "reverse" mutation of the resistance gene to a non-resistance allele. This could not be proved, but the only alternative was contamination by a "foreign" fly possessing the non-resistance allele, and, in view of the stringent precautions taken to safeguard the lines from contamination, it was highly improbable that such an accident occurred in every one of the 10 lines studied. The number of flies reared per generation from the $F_3$ onwards was more than 2000 in each line, so that the production per line before the earliest indication of the presence of non-resistants was of the order of 20,000–50,000 flies. The chance of reverse mutation having occurred in populations of this order of magnitude did not seem too unlikely.

### (*f*) *Resistance in the Unselected Colony*

The proportion of DDT-resistant flies in the unselected colony was determined in four of the generations before selections were started on subcultures. After a lapse of several years, during which the colony was maintained without any intentional selection while the strains derived from it were being investigated, the proportion of resistants was again determined.

The results, in Table 9, showed that, although the proportion of resistants varied considerably from generation to generation, it was not significantly different in males and females of any particular generation. As the proportion was not greater in males than in females, there was no evidence that the unselected colony contained males with sex-limited resistance either before or after the derivation of the two strains, D and EM, in which the males were exclusively of this type.

### (*g*) *Cytological Observations*

The various types of flies examined cytologically and genetically or both are listed in Table 10, with their sex chromosomes and genotypes with respect to the gene for DDT resistance indicated. The chromosomes were readily distinguishable from each other (Figs. 1–9). The *X*-chromosome and the much shorter *Y*-chromosome were usually stained more intensely by the orcein than were the five pairs of autosomes each of which was identifiable by its relative length, the position of the centromere, and by certain well-defined bands of non-staining material (the so-called secondary constrictions).

However, the small numbers produced showed quite clearly (Table 11), by the presence of aristapedia in every female and its absence in every male, that the male determinant was in chromosome II. The marker strain failed to adapt to the local rearing conditions, so that the experiment could not be repeated, but adequate confirmation of the above finding was obtained by classifying the large numbers of vigorous $F_2$ and $F_3$ progeny produced from the surplus of $F_1$ males and females. As indicated in Table 11, no *ar* males were produced in either generation. According to expectation, some of the females were not *ar* type, and the proportions of each of the other marker types were also lower than in the backcross progeny.

TABLE 11

CLASSIFICATION OF PROGENIES DERIVED FROM THE CROSS MARKER STRAIN FEMALES × EM MALES

| Flies | No. Examined | | Marker Type | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *ac* | | *ar* | | *bwb* | | *ocra* | |
| | Female | Male | Female | Male | Female | Male | Female | Male | Female | Male |
| Backcross progeny | 25 | 33 | 5 | 12 | 25 | 0 | 9 | 19 | 8 | 10 |
| $F_2$ | 268 | 314 | 82 | 81 | 106 | 0 | 64 | 82 | 41 | 58 |
| $F_3$ | 2398 | 2292 | 368 | 362 | 490 | 0 | 529 | 487 | 328 | 302 |

The four markers were eventually recombined by the mating of suitable types selected or derived from the $F_2$ and $F_3$ progenies to form a new strain composed of $XX$ females homozygous for the four marker genes and $XX$ males homozygous for *ac*, *bwb*, *ocra*, heterozygous for *ar*, and hemizygous for the male determinant.

Tests with DDT showed that all the females of the new strain were non-resistant and all the males resistant, as in strain EM from which it was derived. It was concluded, therefore, that the DDT resistance gene was in chromosome II.

## IV. DISCUSSION

The majority of the flies of the Canberra laboratory colony conformed to the karyotype generally considered normal for *Musca domestica* (Stevens 1908; Metz 1916; Perje 1948; Boyes and Naylor 1962; Wagoner 1967). The five pairs of autosomes and the sex chromosomes, $XX$ in females, $XY$ in males, morphologically matched those photographed by Hiroyoshi (1964), and were similar in relative lengths and arm ratios to those of the strains examined by Boyes, Corey, and Paterson (1964).

A small proportion of the flies of one of the strains selected from the colony were $XXX$ females and $XXY$ males, the latter being identified by testis chromosomes. The $Y$-chromosome was clearly epistatic over $XX$. This finding indicates the $Y$-chromosome as the site of the male determinant in normal males of the colony.

Heterogeneity in tolerances to DDT has apparently been a feature of the unselected colony for a long time. When the first tests with DDT were done in 1952, DDT-resistant flies were already coexisting at readily detectable frequency with

non-resistant flies, and have apparently continued to do so throughout a further 276 generations of rearing in the absence of any intentional selection pressure. While the possession of resistance is of no advantage under these conditions, it certainly does not appear to have been disadvantageous to any significant extent.

All the evidence obtained from tests with DDT and cytological examinations (Tables 9 and 10) indicates that the flies of the unselected colony, whether resistant or non-resistant, remained true to karyotype. If any deviations from the karyotype, such as those found in selected strains, occurred in the unselected colony, they were either short-lived or confined to an undetectably small minority of the flies.

The ratio of resistants to non-resistants varied from generation to generation depending on the relative proportions of eggs contributed (to the random samples taken for culturing) by early-emerging and late-emerging flies, the late emergers being predominantly resistants (Kerr *et al.* 1957). Selection for late emergence (strain L) appears to have done nothing more than increase this ratio, since no chromosomal changes were found (Table 10). However, the reverse selection, for early emergence, not only reduced the gene pool by eliminating the normally inherited DDT resistance, but evidently modified it to an extent which enabled *XX* type resistant males and non-resistant flies with the supernumerary *X*-chromosome to become established in strains E and ES respectively where they coexisted with flies of the normal karyotype for many generations after the selection pressure had been removed.

The finding that DDT resistance in the colony, and strains derived from it, is determined by an allele of a gene in chromosome II, which confers the ability to metabolize DDT by dehydrochlorination to DDE, indicates close affinities with one of the common types of resistance that have occurred in *Musca domestica* in other countries. Lichtwardt (1964), Tsukamoto and Suzuki (1964), Oppenoorth (1965*a*, 1965*b*) and Grigolo and Oppenoorth (1966) found that the dominant gene for DDT resistance (variously named *R*, *D-ase*, $R^{DDT}$) in strains originating in the United States of America and Japan was located in chromosome II. Oppenoorth's work proved that this gene caused the production of the enzyme DDT-dehydrochlorinase, discovered by Sternburg, Kearns, and Moorefield (1954), and indicated the possibility that different alleles of this gene have been selected in strains with various activities of the enzyme. At present it seems likely that the DDT resistance gene of the Canberra colony is allelic with $R^{DDT}$. However, the similarity with other strains ends here, for two reasons. First, no indication was obtained that the colony contains any other gene for resistance, such as the *kdr* and *kdr-O* genes found in chromosome III by Milani and Travaglino (1957) and Milani and Franco (1959) or the chromosome V factor of Oppenoorth (1965*b*). Second, the *XX* males of strains D and EM have the male determinant linked with their resistance in chromosome II, which is a new site for a male determinant. Other *XX* males examined by Franco, Lanna, and Milani (1962) and Hiroyoshi (1964) have the male determinant in the *bwb* linkage group, which is chromosome III.

Before the present cytological examinations were made, it was tentatively assumed that the male-limited DDT resistance factor isolated in strain EY (Kerr 1960, 1961) was located in the *Y*-chromosome. When the *Y* was found to be absent, the strain name EY was no longer tenable and was changed to EM. In the meantime Tsukamoto, Baba, and Hiraga (1961) had suggested a translocation between the *Y*

and chromosome III as an explanation of the male-limited resistance in this strain. Hiroyoshi (1964) and Oppenoorth (1965*c*) also considered this possibility. Whether or not the loss of the *Y* was due to a translocation, it is clearly established that chromosome II (not III) acquired a male determinant epistatic over both *XX* and *XXX* in strains D and EM.

The discovery of both *XO* and *XXX* males among the *XX* males of strains D and EM strongly suggests that these rare types were the complementary products of non-disjunction of the *X*'s. Hiroyoshi (1964) found only *XO* and *XX* males in his TY-Furen and TY-Akashi strains, and proposed a formula whereby the relative frequency of *XO* and *XX* males in a population could be used to estimate the age, in generations, of the translocation of the *Y*-chromosome. An attempt to date the loss of the *Y*-chromosome in the Canberra colony by this procedure would be pointless in view of the likelihood that *XO* males can be regenerated by non-disjunction of the *X*'s, and this would be so for any similar population in which the presence of both *XO* and *XXX* males provides evidence that non-disjunction may be occurring.

The fact that resistant males of the same type ($XX,R^M$) were brought into prominence in strains D and E by different methods of selection suggests that they did not appear independently in these strains but had a common origin in the unselected colony, although their presence there was not detected. In the absence of DDT their resistance would confer no conceivable advantage. However, in an environment containing sufficient DDT to kill non-resistants their advantage over the normal *XY* males is obvious, because, unlike the latter, they do not produce any non-resistant male progeny.

Without postulating any difference in reproductive capability between the two types of males, it can be shown by analysis of genotype frequencies in this selection and mating system that some degree of replacement of $XY,R$ males by $XX,R^M$ males is consequential to the selection with DDT against non-resistants. However, in such a system, the rate of replacement declines as the frequency of the non-resistance allele is reduced by the selection, and the degree of replacement achieved depends upon the relative frequencies of $R$ and $R^M$ at the start of selection. For example, computer calculations showed that, with arbitrary starting frequencies of $R$ and $R^M$ in the ratios 1 : 1, 8 : 1, and 200 : 1, the frequency of $XX,R^M$ males in the selected population would approach end-point values of only about 0·35, 0·1, and 0·005 respectively, as the non-resistance allele approached extinction.

Some additional selective force must have operated in strain D to complete the replacement while the non-resistance allele still survived. Since the females generally mate only once, there is probably an effective selective advantage to males that reach sexual maturity early, and the finding that $XX,R^M$ males were favoured by selection for early emergence (strain E), but not by selection for late emergence (strain L), suggests that, in the selection of strain D, the replacement of $XY,R$ males by $XX,R^M$ males may have been accelerated by such a mating precedence.

There was a reasonable expectation that the elimination of all non-resistants prior to mating would have reduced the frequency of the non-resistance allele in strain D to a level at which, in the limited-sized population, extinction of the allele became probable. But although the selection was maintained for 200 generations, and the numbers of flies per generation occasionally dropped to a few hundred, strain D

did not become homozygous for the resistance gene. Two reasons can now be advanced as to why the selection was less effective than anticipated. First, the replacement of $XY$ males by the $XX,R^M$ type made resistance to DDT obligatory in the males. Thenceforth selection was less drastic, since it operated only in females, and the frequency of the non-resistance allele in males lagged a generation behind the frequency in the selected females. Second, the finding that all of the homozygous resistant single-pair lines eventually "reverted" suggests an appreciable rate of "reverse" mutation of the *R* allele to a non-resistance form. Further critical examination of the question of "reverse" mutation is needed. At the present stage the only type of genetic instability that has been examined in any detail is the breaking of the linkage between the male determinant and the *DDT* resistance gene, which may be due to a low-frequency crossing over in males, similar to that reported by Sullivan (1961), Franco, Lanna, and Milani (1962), and Hiroyoshi (1964), or to the detachment and loss of the male determinant from chromosome II. In strain D, where the resistance gene is present in both sexes, an occasional breakdown of the linkage with the male determinant would be of no consequence because the gene has the same effect in the linked and unlinked states.

## V. Acknowledgments

I am indebted to Mrs. H. M. Abbey and Mrs. M. A. Shearer for their skilful handling of the rearing, selection, and testing procedures, and to Mrs. A. M. Nelson for permission to refer to her unpublished findings on the metabolism of DDT. A supply of insecticide-free bran for the culture medium was kindly arranged by Mr. H. J. Moss of the Bread Research Institute of Australia. The World Health Organization provided a grant for part of the work and arranged the despatch of marker strain pupae from the Istituto di Zoologia, Pavia, Italy.

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