INHERITANCE OF CHLORDANE RESISTANCE IN THE GERMAN COCKROACH, Blattella germanica (L.)

by

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INTRODUCTION

Before the turn of the century the occurrence in insects of resistance to certain chemical poisons had been noted. During the first several decades of the twentieth century additional cases of insects exhibiting resistance to chemical poisons appeared following the more widespread use of such materials in insect control. In recent years this phenomenon had developed in a number of insects to such a degree that the control of these pests has become a serious problem in applied entomology.

Although insect resistance to insecticides was first encountered in the field of economic entomology it is now being studied in various other fields. This ability to withstand chemical poisons is of a very complex nature but complete understanding of its mechanism may be found in the combined findings from various research fields. Research in the fields of physiology, biochemistry and genetics may eventually lead to a clear understanding of this phenomenon. In each of these fields the greatest handicap is lack of adequate techniques. However, some aspects of resistance can be studied by means of the usual genetic procedures.

The work reported herein deals specifically with the inheritance of resistance to the insecticide chlordane in the German cockroach, Blattella germanica (Linn.). The interest of the author in resistance studies was aroused initially by the results of experiments with DDT-resistant roaches in which there was evidence to support a theory of

cytoplasmic factors as well as nuclear material being involved in the resistance. The chlordane-resistant strain of cockroach afforded an opportunity to determine if the mechanism of inheritance was similar to that of the DDT-resistant roaches. The former was a highly resistant strain of roach which developed in the field as a result of the sustained use of chlordane in roach control; whereas the DDT-resistant strain was experimentally developed in the laboratory.

REVIEW OF LITERATURE

Insects have been resisting man's efforts to control them for many years. With the advent of the newer chemical insecticides control seemed eminent; however, in recent years, there have been many cases of insects exhibiting a remarkable resistance to these insecticides. Although Melander's (17) "Can insects become resistant to sprays?" published in 1914 was probably among the first reports to appear in the literature, it appears that this phenomenon had been observed earlier by Smith (22).

In spite of this early beginning, resistance of insects to insecticides was relatively unexplored until the newer organic insecticides came into such extensive use. Since then many investigators have conducted research experiments on the various aspects of the problem. Two critical reviews have been prepared and published by Babers (1) and Babers and Pratt (2). A more recent review of the literature discussing the physiological, behavioristic and genetical aspects of insect resistance has been prepared by Grayson and Cochran (9).

The inheritance mechanisms of insect resistance to various chemical poisons is not well understood. While Mendelian principles seem to be involved the details vary in different insect species. Hough (12, 13), conducted the earliest investigations of this nature using the codling moth, Carpocapsa pomonella (Linn.). Reciprocal crosses were made between strains of codling moth which differed in

their ability to enter apples that had been sprayed with lead arsenate. The F_1 progeny from these crosses proved to be intermediate between the two parental strains in their ability to enter sprayed fruit. Progeny from back crosses were intermediate between the F_1 and the parental strain with which it was crossed. Hough reached no conclusion about the genetic mechanisms involved, but Brown (4) considered it as indicating autosomal multiplegene inheritance.

Investigations by Dickson (8) proved that the resistance to HCN fumigation exhibited by the California red scale insect, Aonidiella aurantii (Mask.), was inherited as a single gene, or group of closely linked genes, in the X-chromosome and is therefore sex-linked. This was confirmed by Yust et al. (25).

The literature on the inheritance of resistance to DDT in the house fly, Musca domestica (Linn.) gives varying conclusions. Reciprocal crosses between resistant and non-resistant strains made by Bruce and Decker (5) resulted in F_1 progeny intermediate in resistance to the two parental strains. They concluded that this probably indicates autosomal, multiple-gene inheritance. Harrison (10), however, found that reciprocal crosses produced F_1 progeny that were only slightly more resistant to DDT than the non-resistant parental strains. F_2 progeny of these crosses produced a ratio of 3 non-resistant flies to 1 resistant fly. This would indicate a one factor, autosomal inheritance with the factor for non-resistance incompletely dominant. A later study by Harrison (11) revealed that the character studied was

in reality resistance to "knock-down" rather than resistance to DDT-induced mortality. Her mortality studies revealed evidence of complex inheritance in houseflies. This agrees with Norton's (19) observations from reciprocal crosses showing that the F_1 progeny were midway in resistance between the resistant and non-resistant strains tested. F_1 backcrosses for a number of generations to the resistant parent type gave increased resistance. Similar F_1 backcrosses to the non-resistant parent type produced a decrease in resistance. Pimental et al. (21) obtained similar results but observed that the females influenced the progeny more than the males, yet concluded that there was no sex-linkage. On the other hand, Maelzer (16) concludes that the "Illinois" strain contains "weak" and "strong" individuals. Crosses involving "weak Illinois" individuals with non-resistant individuals gave high variability in the F_1 and F_2, with the F_2 progeny slightly more variable than the F_1. None of the F_2 progeny fell in the "strong Illinois" classification. However, a cross of "strong Illinois" individuals with non-resistant individuals gave a clear cut segregation in F_1 and F_2 progeny into "weak" and "strong" individuals. They suggest that the high resistance is due to a dominant gene superimposed on the partially resistant constitution of the "weak Illinois" individuals. In addition, Johnston et al. (14) consider the resistance to DDT by the housefly to be due almost entirely to cytoplasmic factors and that these factors are stimulated in the presence of DDT in the

environment to give resistance. This paper might be open to criticism in that the conclusions maybe considered too positive for the data obtained and because the autosomes were completely ignored in the discussion. Milani (18) concludes that "knock-down resistance" is inherited as monofactorial and might be linked to "kill-resistance" inasmuch as "knock-down resistant" segregants are also "kill-resistant"; the reverse is not true, as many "knock-down" sensitive flies are also "kill resistant".

The genetics of resistance in Drosophila is being investigated by a number of workers. Oppenoorth and Dresden (20) selected for a BHC-resistant strain from two wild and one laboratory strain. All three strains used in the experiment were equal in resistance with no increase in resistance obtained after 13 selections. Reciprocal crosses gave F_1 progeny whose resistance was about that of the resistant parent. They concluded that there was no cytoplasmic inheritance involved and that the resistance obtained was incompletely dominant. Crow (7) working with a DDT-resistant strain concluded that the resistance appears to be polygenic. Little, if any, loss of resistance was found after three years of no selection by DDT. Work reported by King (15) on D. melanogaster presents evidence for polygenic inheritance as based on data obtained from 2 lines of equally resistant DDT-resistant flies selected separately from the same stock. Reciprocal crosses between the two resistant lines gave F_1 progeny of the same resistance as the parent lines and

an F_2 with significantly lower resistance and greater variance. He concludes that the two lines have achieved resistance by consolidating different combinations of factors for resistance. Tsukamoto and Ogaki (24) found considerable resistance to DDT in some mutant strains of Drosophila, although they had never been exposed to the insecticide, suggesting that the DDT-resistance may exist originally rather than being acquired adaptively. Reciprocal crosses of resistant and non-resistant strains gave evidence that DDT-resistance was dominant, with no sex-linkage. Reciprocal backcrosses indicated one or a few major genes of DDT-resistance to be linked with and located near the vestigial gene on the second chromosome. It is not clear to the author as to whether they believe only a few major resistant genes compose the entire inheritance mechanism or that there are major genes complimented by lesser resistant genes.

The only reference in the literature considering the genetics of resistance to insecticides in the German Cockroach was that of Cochran et al. (6) for DDT-resistance. The data obtained gave evidence of the involvement of both chromosomal and extra-chromosomal factors. From the data it is suggested that the extra-chromosomal factor is dependent upon nuclear genes for its propagation.

The chlordane-resistant strain of German cockroach used in these experiments came originally from Corpus Christi, Texas. This strain is the result of commercial applications of chlordane applied in the field until it was no longer economically feasible to

maintain control of the roaches with this insecticide. The non-resistant roaches came from a laboratory strain which has not been exposed to an insecticide. Apparently the inheritance of resistance to chlordane in this insect species had not been previously studied.

MATERIALS

The cockroaches used in these experiments were obtained from stock cultures of chlordane-resistant and non-resistant strains currently being maintained in the Entomological Section of the Virginia Agricultural Experiment Station.

In rearing the roaches the following materials were used:

1. One-quart, glass jars.
2. One-gallon, glass battery jars.
3. Four-gallon, glass aquaria.
4. ¼-inch pressboard.
5. 4 x 8 inch copper, screen wire.
6. Round metal boxes measuring 1 7/8" diameter by 9/16" deep.
7. Cheese cloth.
8. Rubber bands.
9. Vaseline.
10. 20 cc watering syringe.
11. Dry commercial dog food.

In testing the roaches for resistance the following equipment was used:

1. Small mesh, cylindrical, screen wire cages measuring one inch in diameter and four inches in length.
2. Paper towel covered cork stoppers.
3. Centigrade thermometer.
4. Aspirator.

5. Constant-temperature water bath.

6. Interval timer.

7. Glass pipettes.

8. Glass graduates.

9. One-half gallon glass mixing jars.

10. Technical grade chlordane was used; this was dissolved in acetone to form stock solutions from which water suspensions of desired concentrations were prepared by volumetric measurement. The suspending agent, EMCOL H65A, was added in equal volume to the chlordane in order to facilitate good suspension at higher concentrations. Preliminary trials revealed that neither acetone nor the suspending agent exerted any deleterious effect upon the roaches at the concentrations employed in this study.

METHODS

I Rearing Technique:

Mass matings:

Each of the 1-gallon glass battery jars used as rearing chambers contained approximately 40 pairs of adult roaches and their progeny. In order to accommodate this large a number of individuals in each jar eight layers of ¼-inch pressboard were arranged in tiers and separated by four staples driven in the under side of each tier. Escape of the roaches was prevented by applying a thin film of vaseline about two inches wide on the inside rim or the jars. In addition, the jars were covered with cheesecloth held in place with rubber bands.

The food was placed loose in the bottom of the rearing chambers so that the roaches had free access to it. Water was provided in petri dishes placed on top of the layers of pressboard and was available at all times.

Shortly after hatching, the nymphs were transferred from rearing chambers to 4-gallon aquaria. Here, surface area was provided by eight tiers of ¼-inch pressboard measuring 7 x 8 inches. Food and water were placed on the upper tier.

To darken the interior and thus simulate natural conditions the aquaria sides were covered with brown wrapping paper.

A constant temperature room was not available for rearing purposes; hence, the roaches were reared at room temperature which

varied from 70 - 80 degrees Fahrenheit. Attempts were made to raise the relative humidity of the room by keeping water in pans at various points in the laboratory.

Pair matings:

Each of the one-quart, glass jars used as rearing chambers contained one pair of adult roaches. The adult roaches were transferred to a new rearing chamber after each egg case hatched. Small-mesh, copper screen wire was folded and placed in the jars to give more surface area, greater visibility, and support for the water boxes. Escape of roaches was prevented by applying a thin film of vaseline in a one-inch band on the inside rim of the jars. In addition, the jars were covered with cheesecloth held in place with rubber bands.

The food was placed loose in the bottom of the rearing chambers so that the roaches had free access to it. Water was provided in parafin coated metal boxes supported on the copper wire frame and was available at all times. A 20 cc syringe was used to facilitate watering the large number of rearing chambers; the needle was pushed through the cheesecloth covering and into the watering box, and then 10 cc of water was released.

To facilitate handling of the large number of jars and to conserve space, special trays were made to hold 21 jars each. The construction of the trays was such that they could be stacked one on top of the other and still have free circulation of air in and around

the jars.

Two constant temperature cabinets were used for rearing purposes in which the temperature varied from 29-31 degrees centigrade. The relative humidity in the cabinets was maintained between 50-60 per cent. This was accomplished by exposing a saturated aqueous solution of magnesium nitrate in open trays in the cabinets. Air was kept in constant circulation by means of a fan.

II Genetic Technique:

Mass matings:

Reciprocal crosses were made between chlordane-resistant and non-resistant strains of cockroaches. A reciprocal cross signifies, in this case, crossing females of the chlordane-resistant strain with males of the non-resistant strain and females of the non-resistant strain with males of the chlordane-resistant strain. In order to insure virgin females the sexes were separated before the adult stage was reached, and held separately until enough individuals were obtained to make the desired crosses.

The F_1 progeny from these crosses were inbred to secure an F_2 generation.

Pair matings:

Three strains of roaches were used. The first strain involved pair-matings between males and females of a chlordane-resistant culture. The second strain involved pair-matings between males and females of the non-resistant culture. The third strain was produced by crossing

females of the chlordane-resistant culture with males of the non-resistant culture.

In order to insure virgin females for all three strains the sexes were separated before the adult stage was reached. As they reached maturity males and females were paired and placed in individual rearing jars.

The parents in each set of crosses were reared in individual chambers, and were transferred to new rearing chambers shortly after each egg case hatched.

III Toxicological Procedure:

The order of resistance to chlordane of all strains was determined by treating known age adults from each strain with different concentrations of chlordane. The method of toxicological assay consisted of dipping the roaches in a water suspension of the toxicant at 30 degrees centigrade. The water suspensions of chlordane were prepared from acetone solutions by use of the suspending agent EMCOL H65A. The testing technique consisted of dipping the roaches in the toxicant for 20 seconds and then leaving them in the treatment cages for one hour following dipping before removal to recovery jars.

The sexes were treated separately in subsamples of approximately 30 insects each in the mass matings, and 5 to 10 in the pair-matings. Following treatment the roaches were placed in recovery jars which contained food and water. Observations for mortality were made at the end of 3, 6, and 10 days. Dead and moribund roaches were combined in the mortality counts. The criterion for considering a

roach as moribund was inability to exhibit active locomotion.

IV Statistical Procedure:

Mass matings:

The data obtained by treating the various strains of roaches at different concentrations of chlordane were plotted on logarithmic-probability paper and regression lines were fitted to the points by the method of least squares (Bliss, 3). Five to seven points were used to establish each line. For each concentration 50 to 200 insects, in subsamples of approximately 30, were used, and the tests were replicated two to five times.

Ninty-five per cent fudicial limits were determined for any lines that were considered close enough that they could be derived from genetically similar populations.

Chi-square values were calculated for all lines to determine how well the points fit each regression line.

Pair matings:

The data obtained from the treatment of progeny of pair-matings have been arranged in tables according to strain and sex. An index of resistance was calculated for the male and the female progeny of each parental pair in all strains. The index figure is the sum of the products obtained by multiplying the reciprocal of each concentration with the corresponding per cent mortality. Therefore, the greater the resistance the smaller the index figure and conversely, the larger index figures will indicate less resistance.

The data were plotted on regular graph paper. The scale for the progeny of the non-resistant strain is 50 times that for the progeny of the chlordane-resistant and X strains.

Correlation coefficients were determined for the males and females of each strain (Snedecor, 23).

RESULTS

The regression lines obtained by plotting the toxicological data are shown in figures 1 to 4. The progeny, male and female, from the cross involving the chlordane-resistant females and the non-resistant males are referred to as the X strain; and the progeny, male and female, from the cross involving the non-resistant females and the chlordane-resistant males are referred to as the Y strain.

The F_1 generation data show that the X- and Y-strain males and females are intermediate, with regard to resistance, between the two parental male and female types, respectively (figure 1 and 2).

The F_2 generation data show that the X- and Y-strain males and females have a wider range of resistance than the parental F_1 generation males and females, respectively (figure 3 and 4).

The points representing the mortalities at different concentrations did not vary significantly from any of the calculated regression lines, as indicated by the chi-square test.

The 95 per cent fiducial limits, for the X- and Y-strain males and females, respectively, of the F_1 and F_2 generations indicate that these regression lines could be coming from genetically similar populations (figures 5 and 6).

The toxicological data for progeny pair-matings within non-resistant and chlordane-resistant strains, and of chlordane-resistant females crossed with non-resistant males are presented in table 1 to 6.

The data from each of the three sets of pair matings are presented as per cent mortality of males and females (at the end of

10 days following treatment) for the various concentrations of chlordane. These data indicate a great variation in resistance to chlordane between the pairs within each strain.

Indices of the male and the female progeny from individual parental pairs have been plotted on regular graph paper (figures 7 and 8).

EXPLANATION OF FIGURE I

Figure 1 - Toxicity of chlordane to parental strains and F_1 progeny of non-resistant males crossed with chlordane-resistant females (X strain). Non-resistant males, A; F_1, X-strain males, B; F_1, X-strain females, C; and chlordane-resistant strain females, D.

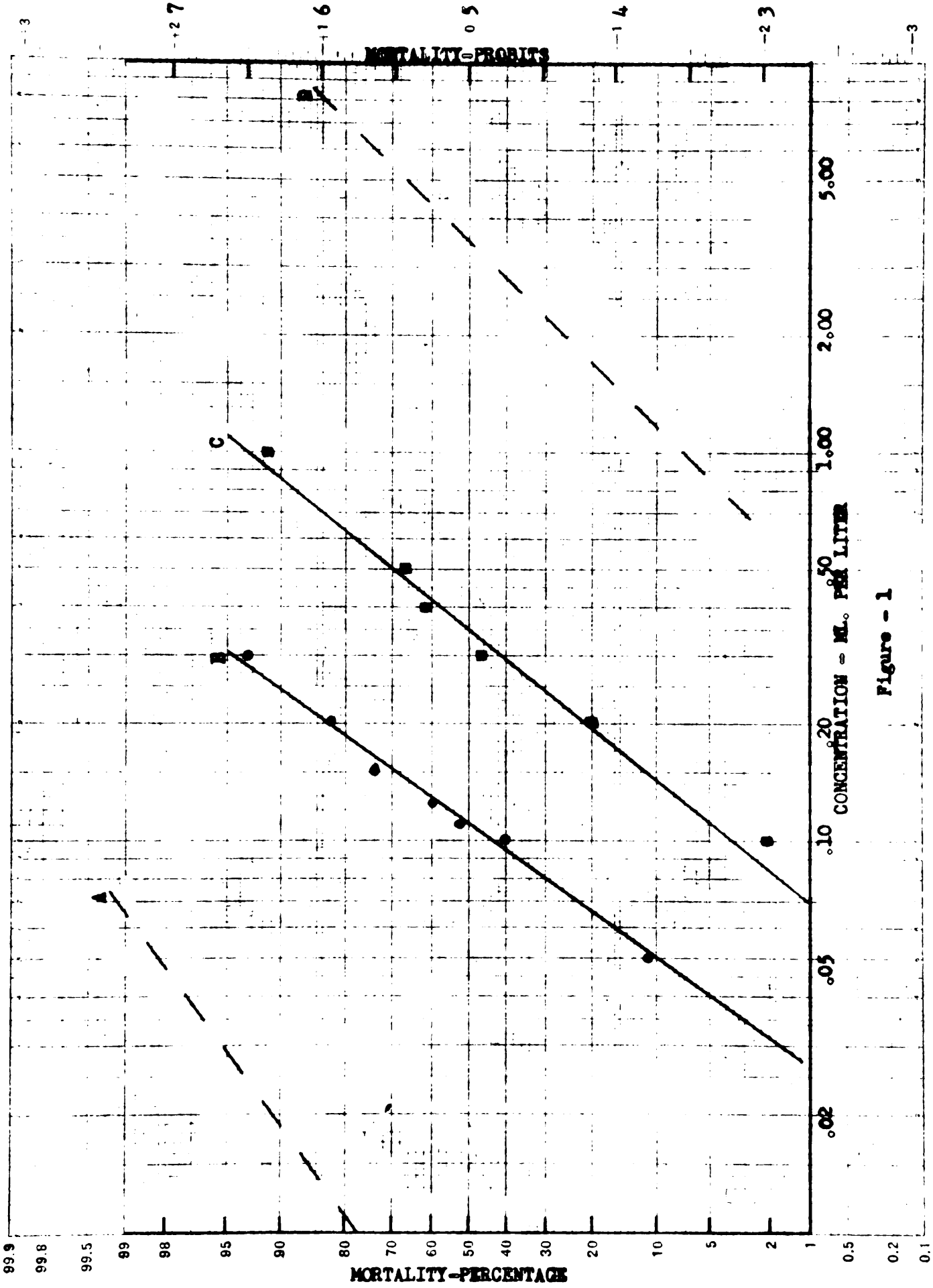

MORTALITY-PROBITS
+2.7
+1.6
0.5
-1.4
-2.3
-3
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
MORTALITY-PERCENTAGE
.02
.05
.10
.20
.50
1.00
2.00
5.00
CONCENTRATION = ML. PER LITER
A
B
C

Figure - 1

EXPLANATION OF FIGURE II

Figure 2 - Toxicity of chlordane to parental strains and F_1 progeny of non-resistant females crossed with chlordane-resistant males (Y strain). Non-resistant females, A; F_1, Y-strain males, B; F_1, Y-strain females, C; and chlordane-resistant strain males, D.

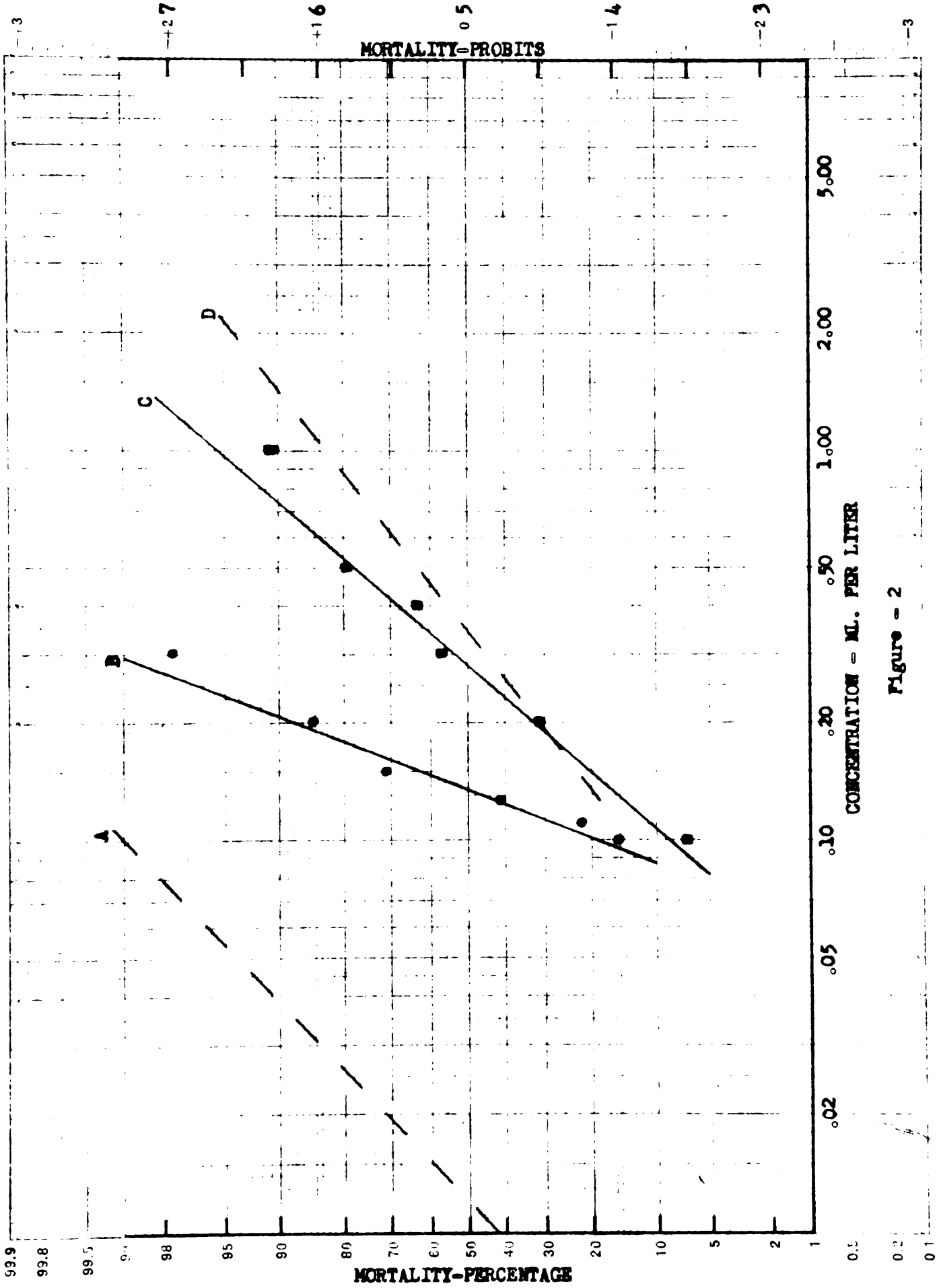

MORTALITY-PROBITS
+3
+2.7
+1.6
0.5
-1.4
-2.3
-3
A
B
C
D
5.00
2.00
1.00
.50
.20
.10
.05
.02
CONCENTRATION - ML. PER LITER
99.9
99.8
99.5
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
MORTALITY-PERCENTAGE

Figure - 2

EXPLANATION OF FIGURE III

Figure 3 - Toxicity of chlordane to the F_1 parents and F_2 progeny of the X strain. F_1, X-strain males, A; F_2, X-strain males, B; F_1, X-strain females, C; F_2, X-strain females, D.

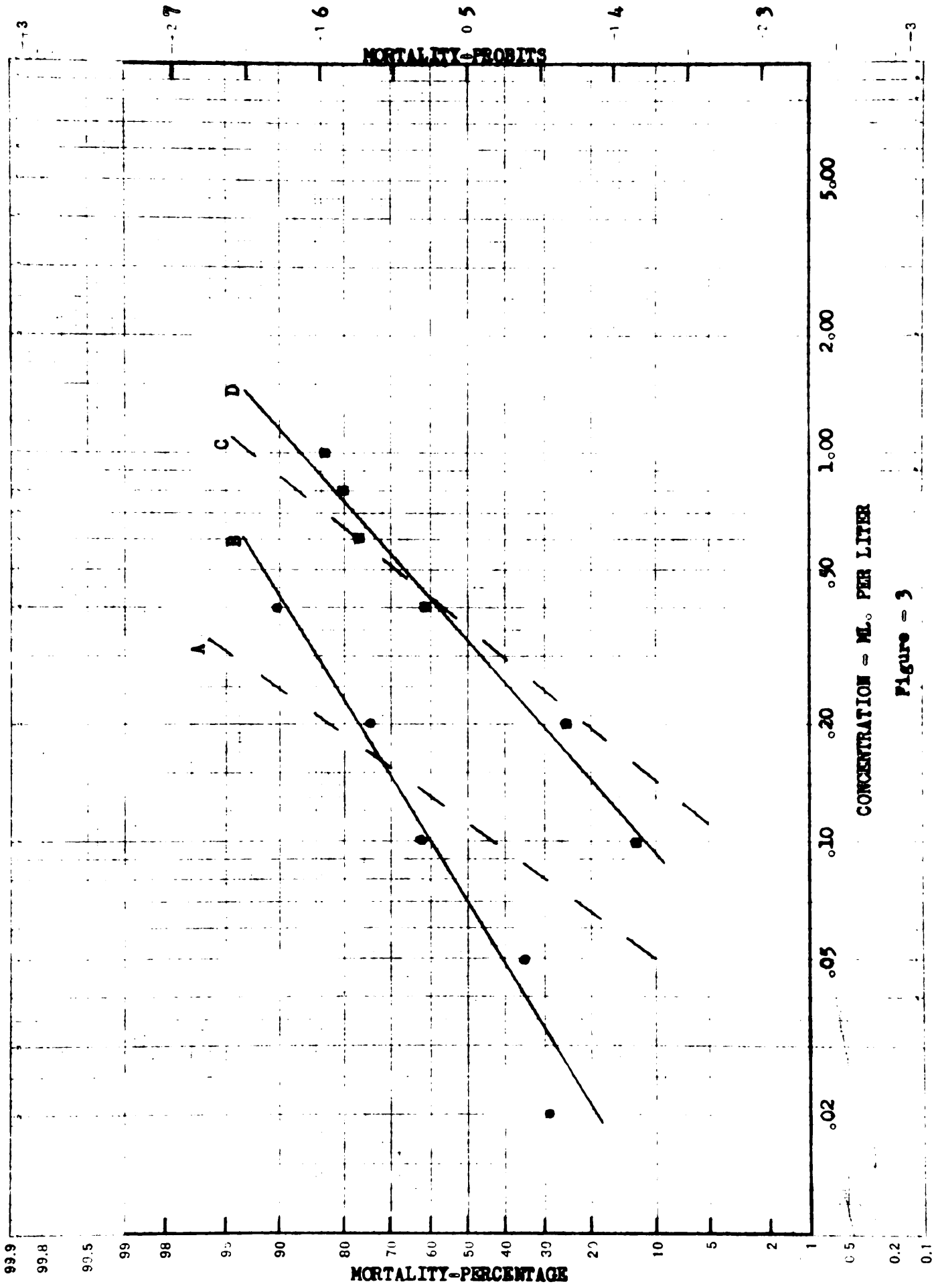

MORTALITY-PROBITS
MORTALITY-PERCENTAGE
CONCENTRATION = ML. PER LITER
A
C
D

Figure 3

EXPLANATION OF FIGURE IV

Figure 4 - Toxicity of chlordane to the F_1 parents and F_2 progeny of the Y strain. F_1, Y-strain males, A; F_1, Y-strain females, B; F_2, Y-strain males, C; F_2, Y-strain females, D.

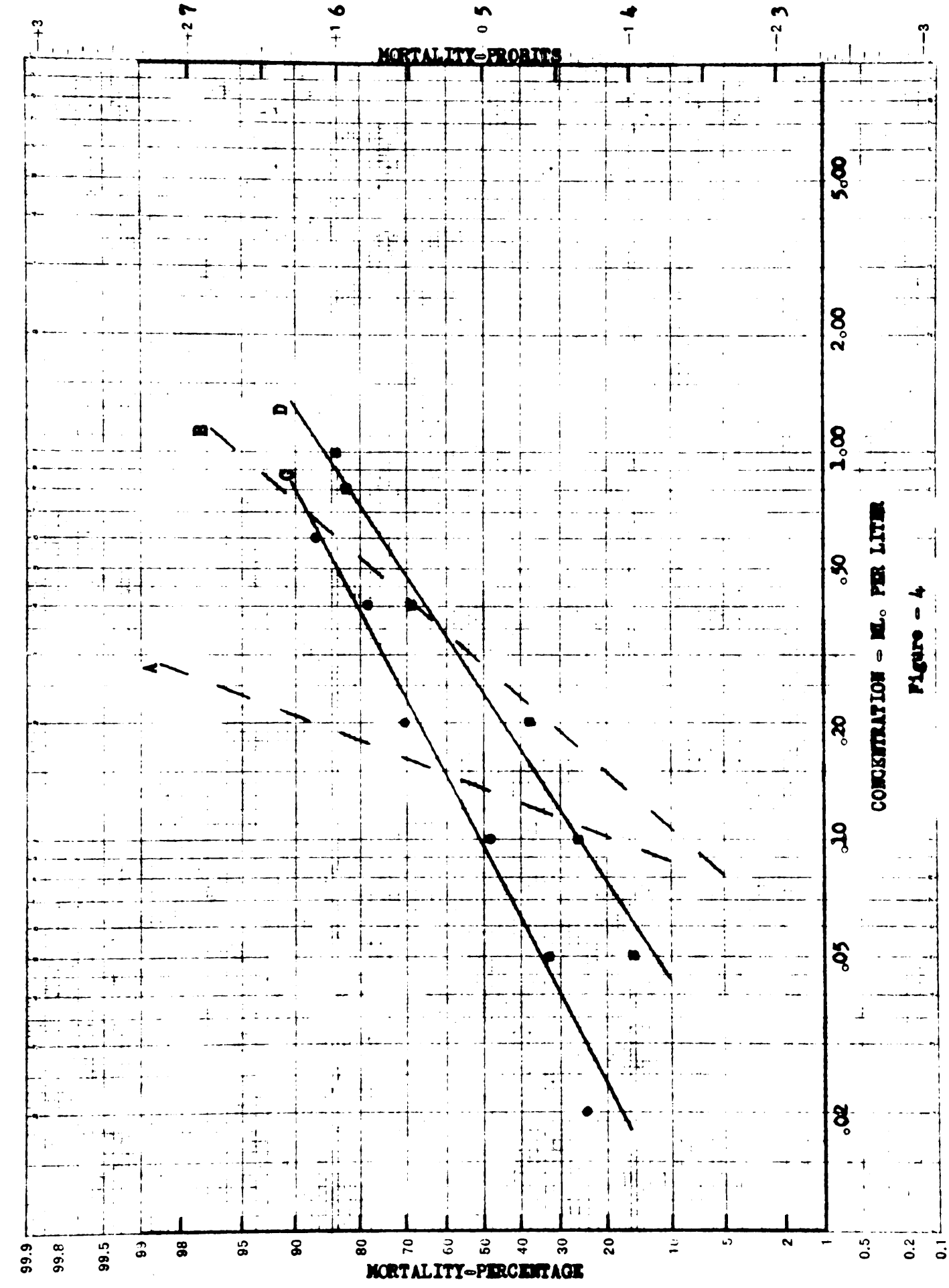

MORTALITY-PROBITS
+3
+2 7
+1 6
0 5
-1 4
-2 3
-3
A
B
C
D
5.00
2.00
1.00
.50
.20
.10
.05
.02
CONCENTRATION = ML. PER LITER
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
MORTALITY-PERCENTAGE

Figure - 4

EXPLANATION OF FIGURE V

Figure 5 - Fiducial limits of F_1 progeny of X and Y strains. F_1, Y-strain males, A; F_1, X-strain males, B; F_1, Y-strain females, C; and F_1, X-strain females, D.

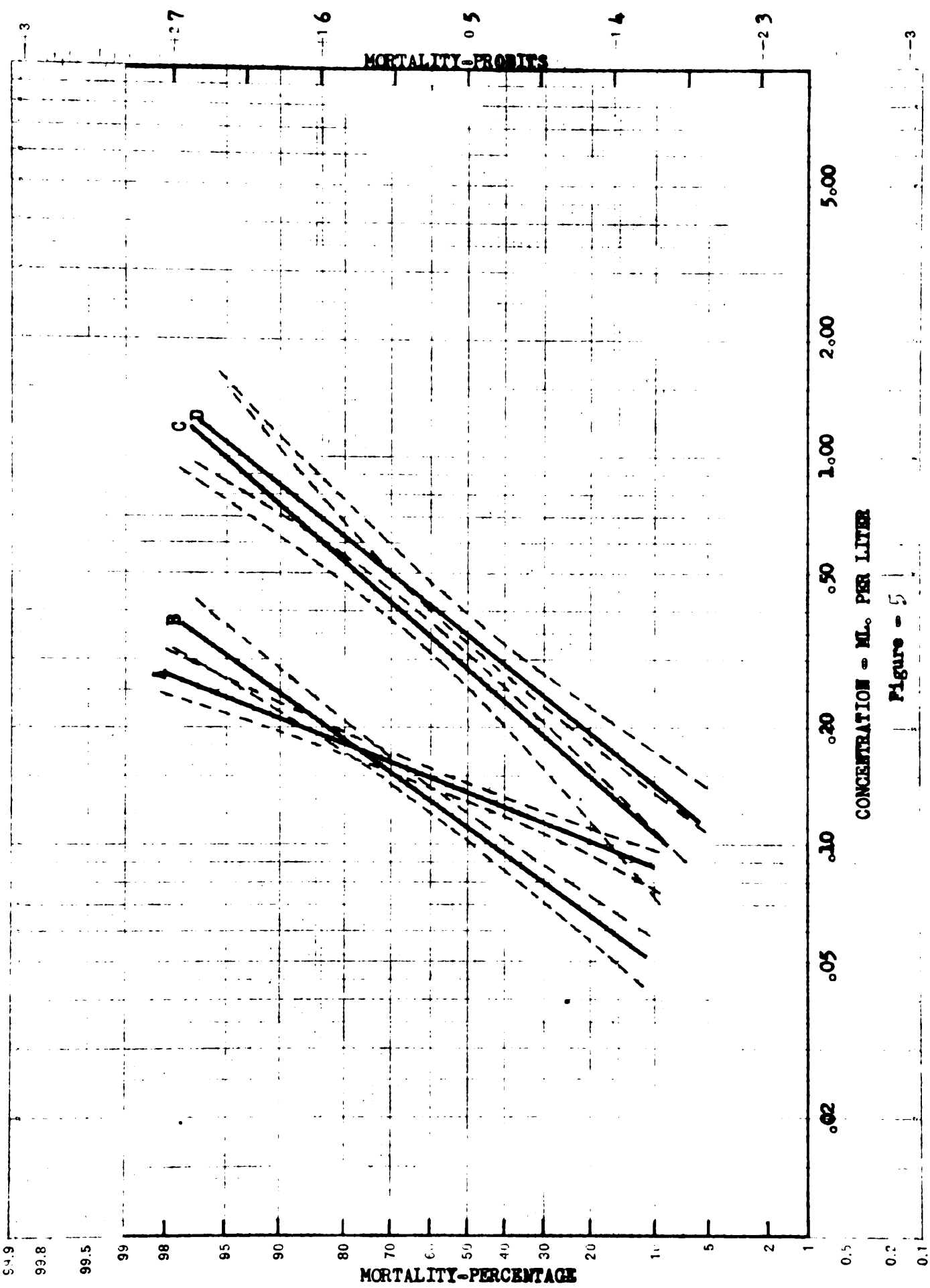
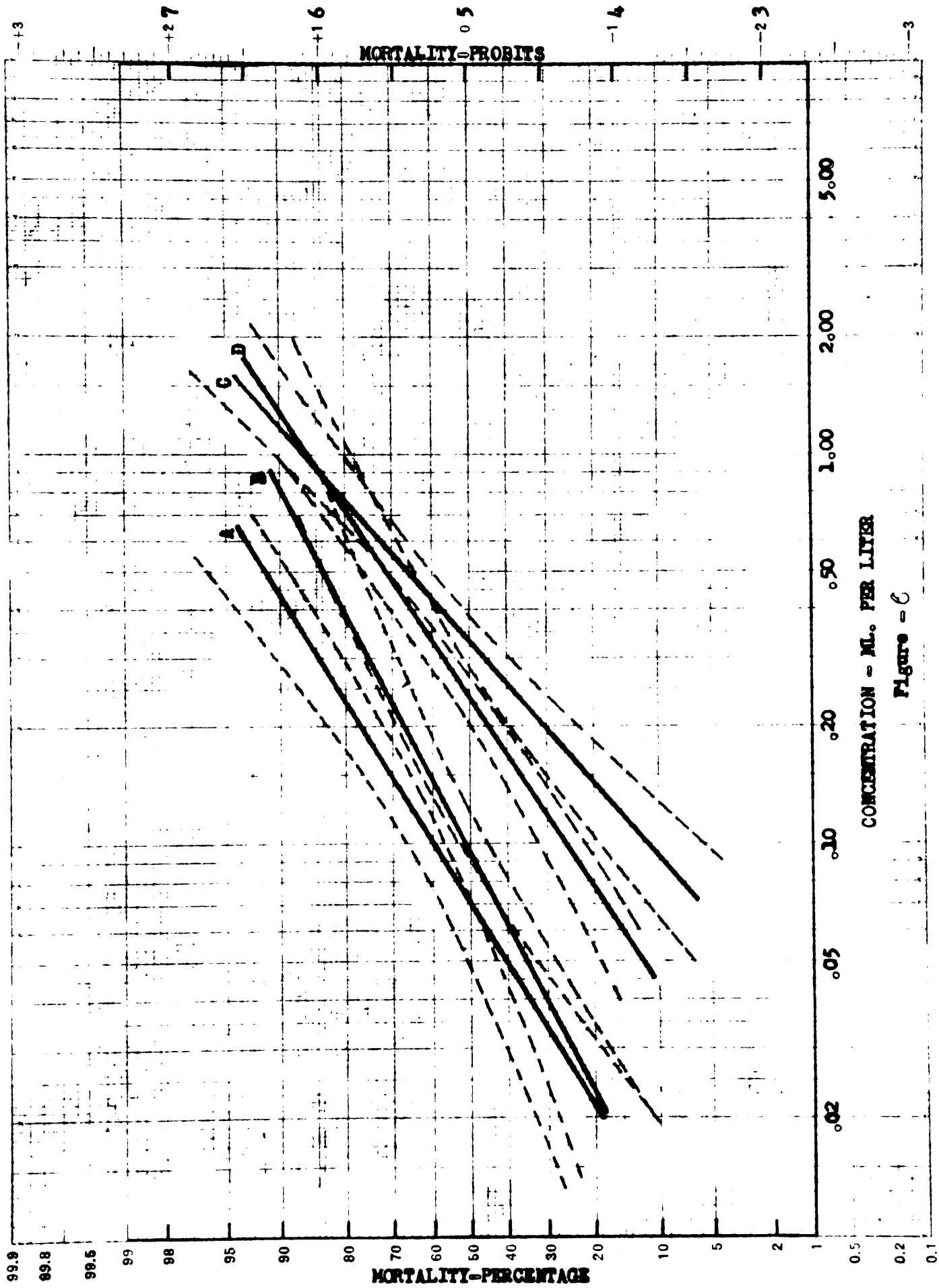

MORTALITY-PROBITS
+3
+2.7
+1.6
0.5
-1.4
-2.3
-3
5.00
2.00
1.00
.50
.20
.10
.05
.02
CONCENTRATION - ML. PER LITER
C
D
B
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
MORTALITY-PERCENTAGE

Figure - 5

EXPLANATION OF FIGURE VI

Figure 6 - Fiducial limits of F_2 progeny of X and Y strains. F_2, X-strain males, A; F_2, Y-strain males, B; F_2, X-strain females, C; and F_2, Y-strain females, D.


MORTALITY-PROBITS
+3
+2 7
+1 6
0 5
-1 4
-2 3
-3
A
B
C
D
5.00
2.00
1.00
.50
.20
.10
.05
.02
CONCENTRATION - ML. PER LITER
Figure 6
99.9
99.8
99.5
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0.5
0.2
0.1
MORTALITY-PERCENTAGE

EXPLANATION OF FIGURE VII

Figure 7 - Index of resistance of X-strain and chlordane-resistant strain progeny from pair matings. Each point represents the progeny of a single pair, and its position is determined from the male index value on the abscissa and the female index on the ordinate.

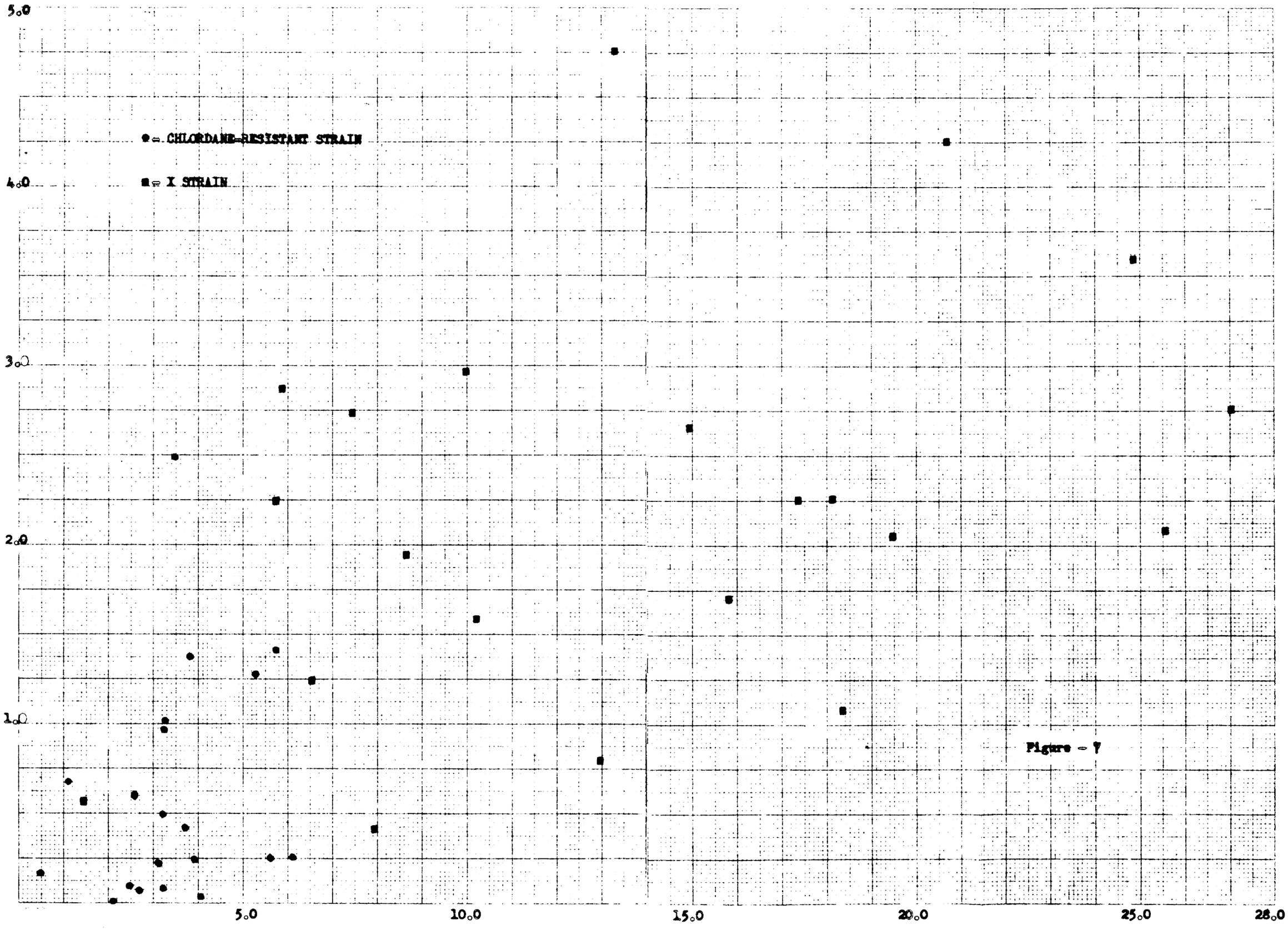

5.0
● = CHLORDANE-RESISTANT STRAIN
4.0
■ = X STRAIN
3.0
2.0
1.0
5.0
10.0
15.0
20.0
25.0
28.0

Figure - 7

EXPLANATION OF FIGURE VIII

Figure 8 - Index of resistance of pair mating progeny from non-resistant strain. Each point represents the progeny of a single pair, and its position is determined from the male index value on the abscissa and the female index on the ordinate.

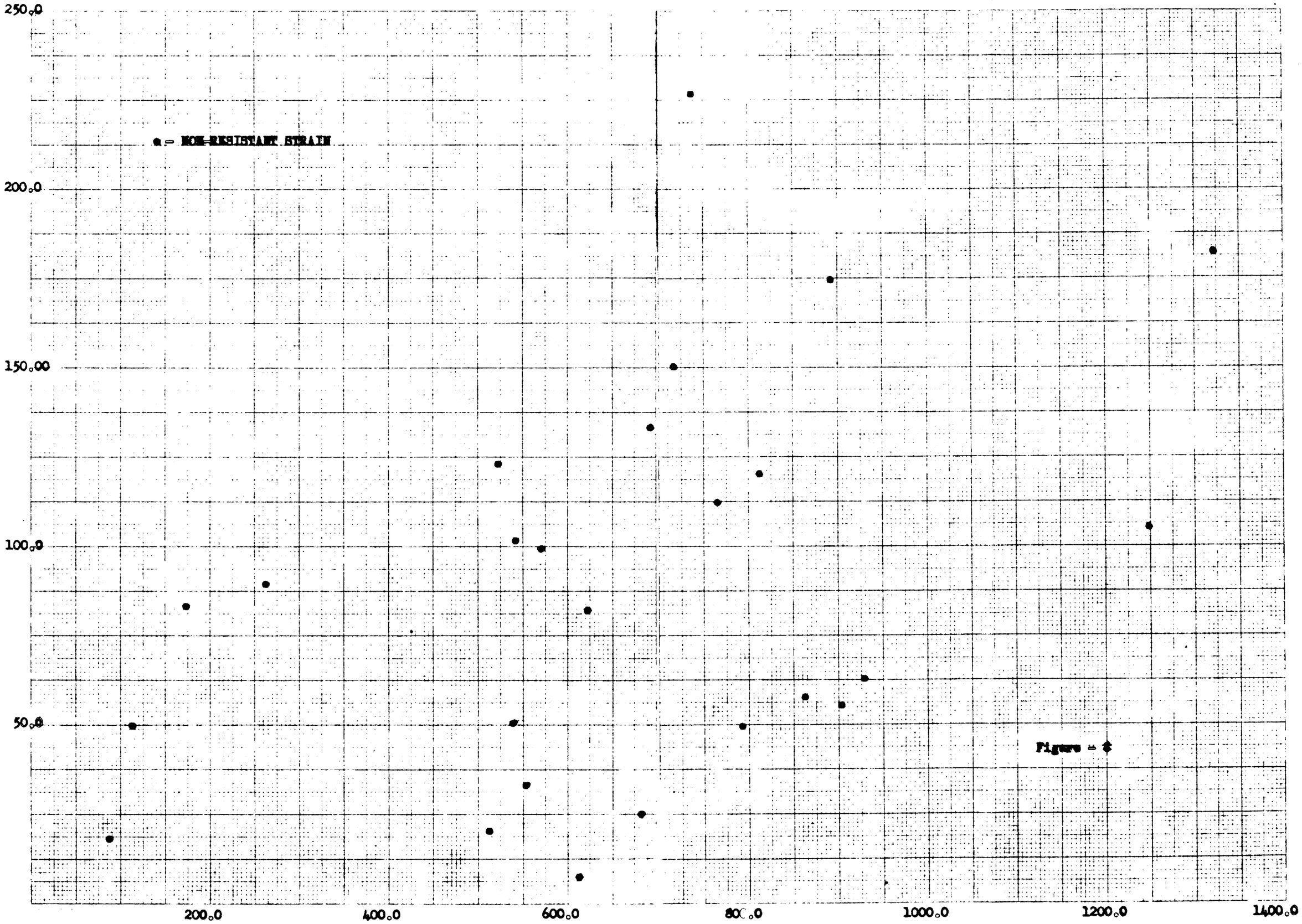

250.0
200.0
150.00
100.0
50.0
● = NON-RESISTANT STRAIN
200.0
400.0
600.0
800.0
1000.0
1200.0
1400.0

Figure 4

TABLE 1

Toxicity of chlordane at various concentrations* to male progeny of pair matings within non-resistant strain.

Pair Number	Percent mortality at the end of 10 days following treatment				Index** of Resistance
	.0015	.0025	.0035	.0045	
1	0	44	60	100	569.65
2	0	100	33	100	716.50
3	20	100	20	100	812.70
4	0	75	0	100	522.22
5	0	37	60	86	510.53
6	12	100	12	100	736.51
7	0	100	25	100	693.65
8	0	90	11	100	613.65
9	0	100	0	100	262.22
10	62	100	75	100	1249.84
11	0	90	0	80	537.78
12	9	91	82	92	862.72
13	12	100	67	100	893.65
14	17	86	40	100	793.84
15	25	100	40	100	903.17
16	0	80	25	67	540.31
17	0	0	0	50	111.11
18	0	29	0	25	171.55
19	0	100	0	100	622.22
20	0	100	50	100	765.07
21	44	91	33	80	929.39
22	62	100	100	100	1321.26
23	0	83	0	100	554.22
24	14	60	44	100	681.27
25	0	0	17	17	86.35

* Milliliters per liter.

** The sum of the products obtained by multiplying the reciprocal of each concentration with the corresponding mortality.

TABLE 2

Toxicity of chlordane at various concentrations* to female progeny of pair matings within non-resistant strain.

Pair Number	Percent mortality at the end of 10 days following treatment				Index** of Resistance
	.0070	.0100	.0200	.0300	
1	10	33	55	75	99.78
2	0	100	37	100	151.83
3	0	71	33	100	120.83
4	0	91	0	100	124.33
5	0	0	25	25	20.83
6	40	100	75	100	227.97
7	0	100	0	100	133.33
8	0	40	0	12	8.00
9	0	43	33	89	89.16
10	0	67	11	100	105.83
11	0	20	25	54	50.50
12	0	29	0	86	57.66
13	11	100	50	100	174.04
14	0	0	33	100	49.83
15	0	25	11	75	55.50
16	0	69	0	100	102.33
17	0	33	0	50	49.66
18	0	43	29	78	83.50
19	0	0	100	100	83.33
20	0	82	0	91	112.33
21	12	10	12	90	63.14
22	29	91	33	100	182.26
23	0	0	0	100	33.33
24	0	0	0	75	25.00
25	0	0	17	30	18.50

* Milliliters per liter.

** The sum of the products obtained by multiplying the reciprocal of each concentration with the corresponding mortality.

TABLE 3

Toxicity of chlordane at various concentrations* to male progeny of pair matings between chlordane-resistant females and non-resistant males.

Pair Number	Percent mortality at the end of 10 days following treatment				Index** of Resistance
	.080	.100	.120	.150	
1	45	0	0	100	12.29
2	25	86	86	100	25.56
3	0	0	0	22	1.47
4	14	100	100	71	24.82
5	12	33	11	44	8.65
6	25	33	0	57	10.23
7	20	0	25	29	6.52
8	10	0	0	93	7.45
9	7	0	0	75	5.87
10	10	0	20	75	7.92
11	40	33	0	75	13.30
12	82	0	14	100	18.09
13	50	20	0	100	14.92
14	89	33	80	89	27.02
15	0	0	0	86	5.74
16	33	50	58	100	20.63
17	37	36	45	80	17.31
18	44	100	80	100	28.83
19	14	25	67	89	15.77
20	20	0	33	71	9.98
21	36	44	33	100	18.32
22	20	67	43	100	19.45

* Milliliters per liter.

** The sum of the products obtained by multiplying the reciprocal of each concentration with the corresponding mortality.

TABLE 4

Toxicity of chlordane at various concentrations* to female progeny of pair matings between chlordane-resistant females and non-resistant males.

Pair Number	Percent mortality at the end of 10 days following treatment				Index** of Resistance
	.250	.400	.500	.700	
1	0	0	20	29	.81
2	0	33	27	50	2.08
3	0	0	29	0	.58
4	0	62	30	100	3.58
5	0	0	44	75	1.95
6	0	0	44	50	1.59
7	8	0	20	37	1.25
8	33	0	71	0	2.74
9	22	0	71	40	2.87
10	0	17	0	0	.42
11	64	0	67	60	4.76
12	11	0	64	37	2.25
13	8	0	91	36	2.65
14	0	0	78	83	2.75
15	0	0	54	82	2.25
16	11	43	75	86	4.24
17	11	12	43	44	2.23
18	33	23	78	73	4.50
19	0	0	33	73	1.70
20	8	0	61	100	2.97
21	0	0	44	14	1.08
22	17	0	50	25	2.04

* Milliliters per liter.

** The sum of the products obtained by multiplying the reciprocal of each concentration with the corresponding mortality.

TABLE 5

Toxicity of chlordane at various concentrations* to male progeny of pair matings within chlordane-resistant strain.

Pair Number	Percent mortality at the end of 10 days following treatment				Index** of Resistance
	.200	.400	.700	1.500	
1	0	17	0	14	.52
2	37	83	75	100	5.67
3	0	100	0	86	3.08
4	33	29	86	29	3.80
5	29	87	71	100	5.31
6	40	75	73	100	5.59
7	0	80	62	100	3.56
8	0	100	67	91	4.07
9	30	100	100	100	6.10
10	30	37	40	37	3.24
11	22	100	70	67	5.05
12	0	70	57	100	3.23
13	20	25	60	37	2.73
14	0	57	30	37	2.10
15	0	100	54	100	3.94
16	0	90	33	100	3.39
17	0	0	40	80	1.11
18	0	75	14	60	2.48
19	0	77	0	100	2.59
20	20	67	0	86	3.25

* Milliliters per liter.

** The sum of the products obtained by multiplying the reciprocal of each concentration with the corresponding mortality.

TABLE 6

Toxicity of chlordane at various concentrations* to female progeny of pair matings within chlordane-resistant strain.

Pair Number	Percent mortality at the end of 10 days following treatment				Index** of Resistance
	3.000	5.000	7.000	15.000	
1	0	29	60	29	.16
2	33	87	67	78	1.42
3	0	50	57	57	.22
4	37	17	78	0	1.37
5	33	75	14	37	1.29
6	0	100	12	67	.26
7	9	33	17	43	.42
8	0	0	20	29	.05
9	0	57	64	75	.26
10	29	0	33	17	1.02
11	0	14	36	14	.09
12	8	71	31	75	.50
13	0	0	25	44	.07
14	0	0	0	22	.01
15	0	89	0	100	.25
16	67	77	33	91	2.49
17	20	0	0	25	.68
18	0	10	10	91	.10
19	14	22	25	89	.61
20	25	27	27	73	.97

* Milliliters per liter.

** The sum of the products obtained by multiplying the reciprocal of each concentration with the corresponding mortality.

DISCUSSION

As stated earlier, the primary purpose of this investigation was to determine if the inheritance mechanism in the field-developed, chlordane-resistant strain of the German cockroach is similar to that found in the laboratory-developed, DDT- resistant strain.

In the DDT strain of cockroach the results gave evidence of a maternal factor for resistance (Cochran, Grayson and Levitan, 1952). This was demonstrated by the greater resistance exhibited by the F_1 males and females from DDT- resistant females crossed with non-resistant males than from the reciprocal of this cross. An analysis of the data obtained from reciprocal crosses between chlordane-resistant and non-resistant strains of roaches does not give any such evidence for a maternal factor. Calculations of 95 per cent fiducial limits for F_1 males and females, respectively, of X- and Y-strains do not show any significant difference (figures 5 and 6).

As in the DDT-resistant strain roaches, the possible involvement of sex-linkage is suggested by the greater resistance to chlordane exhibited by the females than by the males of the chlordane-resistant strain. This might be visualized as being due to the females having two factors for resistance, one on each X-chromosome, whereas the males have only one. According to this theory the F_1 females of the reciprocal crosses would be alike in their resistance

to the poison but the F_1 males would resemble their respective maternal parents with regard to resistance. However, since the males and females are different with respect to resistance the F_1 males would be expected to resemble the males of the strain from which their maternal parents came. The results obtained show that the F_1 males as well as the F_1 females are intermediate between their respective parental types (figures 1 and 2). This would indicate that resistance is probably not inherited as a simple, sex-linked factor.

Another mechanism for the inheritance of the resistance factors is by means of the autosomal chromosomes. This can best be demonstrated by consideration of the Y-strain, F_1 males. These roaches are the progeny from the cross involving non-resistant strain females and chlordane-resistant strain males. In this cross factors transmitted for resistance must come from the resistant male parent. Furthermore, the F_1, Y-strain males receive no sex chromosome from the paternal parent because male gametes containing an X-chromosome produce only females when united with female gametes. The F_1, Y-strain males do, however, receive a full set of autosomes from the paternal parent. Inasmuch as they were found to exhibit significantly greater resistance than the males of the non-resistant strain, it may be concluded that the resistance factors are transmitted on the autosomal chromosomes (figure 2).

If this conclusion is correct no significant difference in resistance would be expected between the F_1 of the two reciprocal crosses, inasmuch as the source of resistance would be the same for each. This expectation is borne out by the observations that the 95 per cent fiducial limits indicate no significant difference between the F_1, X- and Y-strain females (figure 5). This indicates that both F_1 strains may be genetically similar populations.

As the simplest hypothesis, we might consider that only a single locus is involved and that the genes for resistance and its allele for susceptibility are homozygous in the chlordane-resistant strain and the non-resistant strain, respectively. If so, the F_1 males and females of the X- and Y-strain crosses would be heterozygous and genetically identical in the two strains. Since the F_1 roaches are assumed to be heterozygous, the F_2 progeny, produced by mating F_1 males and females of each strain amongst themselves, would consist of varying genetypes and therefore would exhibit a wider variance of resistance to the insecticide. This should be reflected in F_2 regression lines of greater slope in the X- and Y-strain progeny as compared to the F_1 progeny (figures 3 and 4). The results permit this assumption to be true as no significant difference was found between the males and females, respectively, of the two strains (figure 6). The 95 per cent fiducial limits indicated the populations to be similar.

The assumption of homozygosity for resistance or lack of it in the two parental strains was further tested by making pair-matings and testing their individual progeny for resistance. The resulting data indicate great variation in resistance between individuals in each strain (tables 1 to 6). These results seem inconsistant with a simple, single-factor hypothesis. Instead, it would be more logical to assume that each strain consists of a multiplicity of phenotypes for resistance, and these probably reflect a multiplicity of genotypes.

The results obtained in these experiments seem to be consistant with the multiple-factor hypothesis. This assumes that there is a series of independent genes for a given quantitative trait and that these genes are cumulative in their effect. Dominance is usually absent, and the F_1 appears as a "blend" of the characters of the two parents. The data obtained apparently meet these requirements. The F_1 progeny of both strains are intermediate in resistance to the parental types without demonstrating any apparent dominance. The fact that there is a slope to the regression lines suggests varying genotypes in the F_1 individuals for resistance (figures 1 and 2). Crossing F_1 individuals within strains gave F_2 individuals varying in resistance from the non-resistant to the resistant parental types. This indicates the presence of a number of independent genes acting together for resistance with a cumulative effect.

The absence of clear groupings in the pair-mating progeny indices of the three strains may be additional evidence that many loci are involved. A feature of multiple-factor inheritance is a tendency toward continuous rather than stepped variation in the population (as, for example, in the character height in man).

The data obtained from the testing of progeny of the pair-matings suggest a number of additional points for discussion.

It was noted above that the correlation coefficient between male and female progeny of individual pairs of a given strain was highly significant in each case. This correlation between males and females for resistance to chlordane could be of great importance in the testing procedure of the German cockroach. At present, males and females are tested separately due to the fact that females exhibit much greater resistance to the poison than males. The correlations suggest that only one sex need be tested in future genetical experiments for resistance. The male is the sex suggested for most purposes, for two reasons. First, the concentrations of the insecticide used are lower and safer to handle. Second, the male is necessary for the detection of sex-linked factors. This would reduce the present testing procedure by half.

The observed correlation of males and females for resistance could be also a great aid in the selection of resistant individuals. Knowing that a correlation exists would allow the investigator to test male progeny of one group of pair-matings and save the females.

In a second group of pair-matings only the female progeny would be tested thus saving the males. The survivors of the tests would indicate which parental pairs were the source of greatest and most uniform resistance among the progeny. The untested male and female progeny of these highly resistant parental pairs could then be mated to produce a more resistant culture of roaches than that from which the parental pairs were obtained. This would enable the order of resistance to be increased more rapidly than it is by the present selection method. It could also produce considerable refinement in making crosses. By present methods no estimate can be made of the resistance phenotype of the parents in a cross, except the very general statement that they belong to "resistant strain" or "non-resistant strain". The saved siblings suggested above would have much better phenotype determinations. It might be very interesting, to determine, for example, whether the progeny of a cross between the most extremely susceptible members of the non-resistant strain and the most highly resistance members of the resistant strain would give the same results as crossing individuals of intermediate susceptibility with those of intermediate resistance.

An examination of the range of resistance found within each strain indicates that selection for resistance greatly reduces the variation between individuals. The non-resistant strain had the greatest range and the chlordane-resistant strain the least. The range in the X-strain progeny was between the other two strains,

as was expected from previous discussions. However, it is of interest to note that the X-strain roaches are not intermediate, but are closer to the chlordane-resistant strain and that the X-strain individuals of higher resistance overlap with the chlordane-resistant individuals of lower resistance (figures 7 and 8; tables 1 to 6). Overlapping occurs in both sexes.

The overlapping of resistance between X-strain individuals and chlordane-resistant strain individuals suggests the hypothesis that there may be dominance involved in some of the resistant factors. The presence of some dominant factors would account for the fact that some X-strain individuals demonstrate more than intermediate resistance and thus overlap with the chlordane-resistant strain at the lower resistance levels.

The difference between male and female resistance, noted previously has been found in all resistant and non-resistant strains in the laboratory. The experiments described do not throw any light on the mechanism for this difference. Apparently the females are physiologically capable of withstanding the effects of more poison than are the males.

CONCLUSIONS

On the basis of these experiments it appears that the following conclusions are justified:

1. The resistance which the strain of German cockroach used here has developed to chlordane is not inherited as a simple, sex-linked factor.
2. No maternal factor is involved in the resistance to chlordane.
3. The resistance to chlordane is primarily, if not solely, transmitted on the autosomal chromosomes.
4. A number of allelic gene pairs having duplicate and cumulative effects are involved.
5. The strains used in this experiment are composed of individuals genotypically heterogeneous with regard to the loci for resistance.
6. There is a high correlation between male and female siblings for resistance to chlordane.
7. Selection for resistance greatly reduces the variation between individuals.
8. Some of the genes involved in the resistance may exhibit dominance.

SUMMARY

1. The inheritance of resistance to chlordane in the German cockroach, Blattella germanica (L.), was studied by determining the toxicity of chlordane to the following strains of roaches: a) chlordane-resistant and non-resistant strains, b) F_1 and F_2 progeny of reciprocal crosses between two parental strains, c) progeny of pair matings within resistant and non-resistant strains, and from a cross between resistant females and non-resistant males.
2. The techniques employed in rearing the roaches and obtaining toxicological date on the different strains, as well as the genetical and statistical procedures, are described.
3. The toxicological data for the mass matings are presented in the form of regression lines.
4. The toxicological data for the pair matings are presented in the form of tables showing per cent mortality and index of resistance.
5. Appropriate statistical analyses were employed throughout the study.
6. Several conclusions are drawn relative to the inheritance of resistance to chlordane in the German cockroach.

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